Recent advances in inflammatory markers. — HMGB1 and TREM-1 —

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This review describes recent advances in studies of the inflammatory markers, high mobility group box 1 (HMGB1) and triggering receptor expressed on myeloid cells-1 (TREM-1). HMGB1 is a ubiquitous nuclear protein that is widely distributed among mammalian cells, passively released from necrotic cells and actively released from stimulated inflammatory cells. Released HMGB1 can bind to RAGE and/or TLRs and elicit inflammatory responses through the production of pro-inflammatory cytokines and chemokines. Extracellular levels of HMGB1 are increased in patients with various diseases including sepsis, cancer, ARDS, DIC, RA, acute coronary syndrome, heart failure and atherosclerosis. Thus, HMGB1 might function as an endogenous immune adjuvant and play a crucial role in the development of various inflammatory diseases. TREM-1 is a cell surface receptor expressed on phagocytes that can amplify inflammatory responses initiated by TLRs. Although a natural ligand for TREM-1 has not been identified, agonistic antibodies against TREM-1 can promote synergistic enhancement of TLR-mediated inflammatory responses. Furthermore, the expression of TREM-1 and soluble TREM-1 is increased in sepsis. These findings indicate that HMGB1 and TREM-1 might be useful inflammatory markers for the diagnosis and monitoring of various inflammatory diseases.

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Introduction

Inflammatory markers of various inflammatory diseases have been investigated for diagnostic and differential diagnostic purposes, to assess disease activity, to predict relapse, and to monitor therapeutic effects. Several inflammatory markers, including C reactive protein (CRP), erythrocyte sedimentation rate (ESR), and serum amyloid A (SAA), have been identified and CRP is most widely applied in the clinical setting as an inflammatory
High mobility group box 1 (HMGB1)

High mobility group box 1 (HMGB1) protein was identified 30 years ago as a non-histone nuclear protein with high electrophoretic mobility\(^1\). HMGB1 protein comprises 215 amino acids composed of two 80 amino-acid domains referred to as ‘HMG boxes A and B’. The “A” and “B” box of HMGB1 can interact with DNA, leading to distortion and bending of the double helix\(^2\). The amino and carboxyl termini of the protein are enriched with basic and acidic residues, respectively, and both structures are highly conserved among species\(^3\)(Fig. 1). HMGB1 is a ubiquitous nuclear protein that is widely distributed among mammalian cells. However, tissue HMGB1 distribution differs with significant amounts being expressed in the thymus, lymphoid tissues, testis, and neonatal liver\(^8\).

HMGB1 is a DNA binding protein that can promote the maintenance of nucleosomal structures and the regulation of gene transcription in mammalian cells. Furthermore, HMGB1 can regulate DNA recombination as well as repair, replication and gene transcription through the action of internal repeats of positively charged N terminal domains\(^5\). Furthermore, Wang et al. recently demonstrated that HMGB1 released from necrotic cells into the extra-cellular milieu could elicit inflammatory responses through the cellular activation of macrophages\(^10\). Although the precise mechanism remains unknown, HMGB1 is also actively released into the extracellular milieu by various types of cells including activated macrophages/monocytes, pituitary, endothelial cells, neutrophils, epithelial cells, dendritic cells, smooth muscle cells, and erythroleukemic cells\(^10\). This finding was turning point in the understanding of HMGB1. Thereafter, several studies found that HMGB1 could play a crucial role in various inflammatory diseases including sepsis, atherosclerosis\(^4\) and arthritis\(^5\). Following its release from necrotic cells, HMGB1 binds to the cell-surface receptor RAGE (the receptor for advanced glycation end products), which results in activation of the transcription factor NF-\(\kappa\)B and mitogen-activated protein kinases (MAPK). Toll-like receptor-2 (TLR-2) and TLR-4 are possible HMGB1 receptors. Interaction between HMGB1 and RAGE and/or TLRs might elicit various cellular responses including chemotaxis, cellular movement and the production of various pro-inflammatory cytokines such as TNF-\(\alpha\), IL-1\(\beta\), IL-1\(\alpha\), and IL-6\(^{14-20}\). HMGB1 released from the nucleus of activated macrophages or necrotic cells could act as an endogenous immune adjuvant through the activation of dendritic cells, macrophages and T-cells\(^21\). Moreover, HMGB1 might also modulate the regeneration and repair of injured tissue by stimulating progenitor cells\(^22\).

We recently discovered novel evidence indicating a pivotal role of endothelial cell thrombomodulin (TM) in the elimination of circulating HMGB1. Originally identified as a specific receptor that can convert thrombin from a procoagulant to an anticoagulant protease, TM can also bind and neutralize HMGB1 activities \(\textit{in vivo}\)\(^23\). Based on these findings, we postulated that endothelial TM regulates the extra-cellular concentration of HMGB1 by eliminating HMGB1 from inflammatory foci.

1) Pathological role of HMGB1

HMGB1 is actively released by stimulated monocytes or macrophages and is also passively released by damaged or necrotic cells. Released molecules potentially elicit inflammatory responses in the setting of various inflammatory diseases through the production of a variety of inflammatory cytokines and chemokines including TNF-\(\alpha\), IL-1\(\alpha\), IL-1\(\beta\), IL-1RA, IL-6, IL-8, MIP-1\(\alpha\) and MIP-1\(\beta\)\(^{10,25}\)(Fig. 2).

2) HMGB1 assay system

Wang et al. first determined circulating HMGB1 levels in the blood using Western blotting in 1999\(^1\). However, this technique has limited application because the experimental procedure is
complex. An enzyme linked immunosorbent assay (ELISA) system was subsequently developed that is versatile and applicable to high throughput\cite{26,27}. We recently established a specific ELISA that can detect HMGB1 within a concentration range of 0.2 to 100 μg/L, with CV ranging from 2.9% to 4.9% and the inter-assay CV ranging from 4.8% to 8.5%. This ELISA does not detect serum HMGB1 in normal healthy volunteers.

3) Pathological roles of serum HMGB1 on various diseases

Extracellular HMGB1 might function as an endogenous immune adjuvant and play a crucial role in the development of various diseases through the production of various inflammatory mediators. Accumulating evidence indicates that circulating HMGB1 is a useful inflammatory marker of various diseases (Table 1).

(1) Cancer

The expression of HMGB1 is up-regulated in various malignancies including colorectal cancer, hepatoma, breast cancer, pancreatic cancer, and melanoma and RAGE is expressed in various transformed and tumor cells. Necrotic and damaged tumor cells release HMGB1 that potentially elicits local inflammatory responses around tumor cells and promotes tumor cell growth. Inflammatory cells migrating to the tumor also actively excrete HMGB1. Therefore, interaction between HMGB1 and the receptor (RAGE and/or TLRs) via autocrine and/or paracrine mechanisms might contribute to the migration and growth of tumor cells\cite{28-32}. Yasuda et al. recently found enhanced circulating HMGB1 levels and detected the protein in serum samples from 26 patients with progressive cancer. Furthermore, serum levels of HMGB1 closely correlate with tumor markers such as CEA and CA19-9 (Yasuda A, unpublished results). Circulating HMGB1 levels are significantly enhanced in cancer patients with anorexia or with anticancer drug-induced adverse effects. These findings indicate that HMGB1 is essentially involved in the development of systemic damage in cancer patients, and that circulating HMGB1 might be a useful prognostic marker of progressive malignancies. Figure 3 shows the clinical course and serum HMGB1 level in a patient with gastric cancer. These findings provide a new insight into the mechanisms underlying the pathological roles of HMGB1 in tumor progression.

(2) Sepsis

Kobayashi et al. recently found that serum HMGB1 levels are significantly enhanced in patients with lethal septic shock compared with those of surviving patients (p = 0.01), and are also closely associated with the prognosis of sepsis (Kobayashi M,

Table 1 HMGB1 levels in biological samples

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sample source</th>
<th>HMGB1^b</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal volunteers</td>
<td>SF</td>
<td>ND</td>
<td>34</td>
</tr>
<tr>
<td>Cancer</td>
<td>S</td>
<td>2.2 ± 5.3</td>
<td>35</td>
</tr>
<tr>
<td>Sepsis (survived)</td>
<td>P</td>
<td>4.3 ± 1.8</td>
<td>NP</td>
</tr>
<tr>
<td>Sepsis (not survived)</td>
<td>P</td>
<td>13.0 ± 4.8</td>
<td>NP</td>
</tr>
<tr>
<td>ALI/ARDS</td>
<td>P</td>
<td>13.7 ± 12.1</td>
<td>34</td>
</tr>
<tr>
<td>DIC (survived)</td>
<td>P</td>
<td>1.7 ± 5.7</td>
<td>35</td>
</tr>
<tr>
<td>DIC (not survived)</td>
<td>P</td>
<td>16.5 ± 11.6</td>
<td>35</td>
</tr>
<tr>
<td>RA</td>
<td>SF</td>
<td>54.1 ± 73.0</td>
<td>15</td>
</tr>
<tr>
<td>OA</td>
<td>SF</td>
<td>12.0 ± 17.7</td>
<td>15</td>
</tr>
<tr>
<td>ACS</td>
<td>S</td>
<td>2.2 ± 0.9</td>
<td>27</td>
</tr>
<tr>
<td>Heart failure</td>
<td>S</td>
<td>5.1 ± 2.5</td>
<td>NP</td>
</tr>
<tr>
<td>Trauma</td>
<td>P</td>
<td>6.5 ± 13.1</td>
<td>35</td>
</tr>
<tr>
<td>Anemia</td>
<td>P</td>
<td>4.6 ± 11.3</td>
<td>35</td>
</tr>
</tbody>
</table>

S, serum; P, plasma; SF, synovial fluid; ND, not detected; NP, not published. * ng/ml; mean ± SE.
Triggering receptor expressed on myeloid cells-1 (TREM-1)

This novel cell surface receptor is located on neutrophils and monocytes. Human TREM-1 consists of an extracellular region of 194 amino acid residues (aa), a membrane-spanning domain of 29 aa and a short cytoplasmic tail of 5 aa. The extracellular Ig-like domain contains the motif DxGxYxC, which corresponds to the V-type Ig-domain. The Ig domain is connected to the transmembrane region by a 60-aa portion containing three N-glycosylation sites. The spanning region contains a Lys residue, which forms a salt-bridge with an Asp residue of the trans-

pression of HMGB1 is also increased in an experimental animal model of RA. These studies suggest that HMGB1 facilitates rheumatoid inflammation through the induction of pro-inflammatory cytokines.

(6) Acute coronary syndrome (ACS) and heart failure

Patients with acute coronary syndrome and detectable serum HMGB1 levels (>0.3 ng/ml) at the time of discharge from hospital tend to develop exacerbated cardiac disorders within one month (p < 0.0001)\(^{(27)}\). Patients with heart failure and higher levels of serum HMGB1 (>0.5 ng/ml) at the time of admission to the ICU as well as at discharge from hospital also tend to develop significantly for exacerbated heart failure (p = 0.0015 and p = 0.0064, respectively) (Ishi J, unpublished results).

(7) Other diseases

Several lines of evidence indicate a close relationship between serum HMGB1 levels and various diseases such as falciparum malaria\(^{(36)}\), thermal injury\(^{(37)}\), Alzheimer’s disease\(^{(38)}\), psychiatric diseases\(^{(39)}\), hemorrhagic shock\(^{(40)}\), hepatitis\(^{(41)}\), and atherosclerosis\(^{(42)}\). Levels of HMGB1 are high in human atherosclerotic lesions, which is notable, particularly because atherosclerosis has been recently identified as an inflammatory disease.

4) Future directions

Investigation of the biological properties of HMGB1 and establishment of a specific assay system for HMGB1 have provided novel evidence that HMGB1 can play a crucial role in inflammation, tissue repair, tissue destruction and carcinogenesis. Since HMGB1 is a ubiquitous nuclear protein, its extracellular levels increase in response to various stimuli. Therefore, HMGB1 might be a useful inflammatory marker for various disease conditions and not be limited to one specific disease. Further investigation should evaluate disease mechanisms involving circulating HMGB1, establish system of HMGB1 monitoring and apply anti-HMGB1 neutralizing antibodies as a therapeutic strategy against various diseases.

unpublished data). Sunden-Cullberg et al. also documented similar results\(^{(33)}\). Therefore, HMGB1 might be a critical laboratory marker that can be used to monitor the progress of sepsis. Wang et al. demonstrated that HMGB1 is released from activated macrophages and functions as a late mediator of lethal endotoxemia\(^{(11)}\). Thus, neutralizing antibodies against HMGB1 might confer a protective therapeutic advantage against septic lethality.

(3) Acute lung injury (ALI) / Acute respiratory distress syndrome (ARDS)

Intra-tracheally administered HMGB1 could elicit acute inflammatory lung injury mediated by neutrophil infiltration, edema formation, and increased production of cytokines. Levels of HMGB1 in plasma as well as in lavaged fluid are increased in patients with ALI/ARDS (disease group n = 21 vs. control group n = 10; p < 0.05)\(^{(44)}\).

(4) Disseminated intravascular coagulation (DIC)

Hatada et al. recently found a correlation between circulating HMGB1 levels and severity of DIC. Plasma HMGB1 levels in 201 patients with DIC admitted to the ICU were significantly higher among those with, than without DIC (14.05 ± 12.56 ng/ml vs. 1.17 ± 3.88 ng/ml; p < 0.001), and were also associated with DIC (Y = 2.34X-1.52; R = 0.586, p < 0.001) and SOFA (Y=1.49X-0.65; R = 0.572, p < 0.001) scores\(^{(35)}\).

(5) Rheumatoid arthritis (RA)

Levels of HMGB1 are increased in the synovial fluid of patients with RA (54.1 ± 73.0 ng/ml) compared with those of patients with osteoarthritis (OA) (12.0 ± 17.7 ng/ml)\(^{(35)}\). The ex-

Fig.3  Time course of serum HMGB1 in a patient with progressive cancer
Forty-eight year-old female patient with inoperable gastric carcinoma and ovarian metastasis was treated with oral Fluoropyrimidine (TS-1; anticancer drug). Serum HMGB1 was determined by ELISA.
membrane domain of DAP12, allowing TREM-1 to associate with its adaptor protein\(^{35,40}\) (Fig. 4).

1) TREM-1 as an amplifier of inflammatory responses

Infection with organisms such as gram-positive and -negative bacteria, as well as fungi, significantly enhances cellular TREM-1 expression on neutrophils, monocytes and macrophages, whereas TREM-1 expression on inflammatory cells in patients with noninfectious inflammatory disorders such as psoriasis, ulcerative colitis and vasculitis caused by immune complexes remains low\(^{45}\). Lipoteichoic acid- and lipopolysaccharide (LPS)-mediated activation of Toll-like receptors (TLRs) both upregulate TREM-1 expression. Although natural ligands for TREM-1 remain unknown, targeting of TREM-1 with agonist monoclonal antibodies increases the production of various proinflammatory cytokines including TNF-\(\alpha\), IL-1\(\beta\), GM-CSF, and inhibits the anti-inflammatory cytokine IL-10\(^{46}\). Furthermore, a combination of agonist monoclonal antibodies and LPS synergistically enhances the production of pro-inflammatory cytokines by monocytes and macrophages, indicating that TREM-1 can amplify inflammatory responses initiated by TLRs.

Cleavage of the extracellular portion of TREM-1 on the membrane of activated neutrophils and monocytes results in TREM-1 shedding. Soluble TREM-1 (sTREM-1) is detectable in various body fluids and it functions as a decoy receptor for TREM-1 ligand. A recombinant form of sTREM-1 inhibits the lethal effects of LPS in vivo\(^{47}\) (Fig. 4).

2) TREM-1 in sepsis

Passini et al. demonstrated that the surface of peripheral neutrophils obtained from septic patients express high levels of TREM-1\(^{48}\). Levels of sTREM-1 are also increased in serum samples from patients with sepsis. Notably, TREM-1 expression on neutrophils is not enhanced in patients with non-infectious systemic inflammatory response syndrome (SIRS). Taken together, these data indicate that membrane expression of TREM-1 is induced on neutrophils and monocytes/macrophages and that circulating sTREM-1 levels also increase during sepsis.

3) Diagnosis of infection based on sTREM-1

The involvement of TREM-1 in infection indicates the diagnostic value of plasma sTREM-1 especially in distinguishing sepsis from severe systemic non-infectious inflammation. Gibot et al. recently determined plasma sTREM-1 levels at admission in critical patients with suspected infection. Plasma levels of C-reactive protein (CRP), procalcitonin (PCT), and sTREM-1 were significantly higher in patients with sepsis than with systemic inflammatory response syndrome (SIRS). Plasma sTREM-1 levels appeared to be the most useful parameter in differentiating patients with sepsis from those with SIRS. Median plasma sTREM-1 levels upon admission were 0 ng/ml (range, 0 to 144 ng/ml) in non-infected patients and 149 ng/ml (range, 30 to 428 ng/ml) in patients with sepsis (\(p<0.001\))\(^{47}\) (Table 2). These findings indicate that sTREM-1 is a useful and sensitive marker of infection and inflammation.

4) TREM-1 as a therapeutic target molecule

Bouchon et al. demonstrated that a TREM-1 blockade established using a fusion protein containing the murine TREM-1 extracellular domain and the human immunoglobulin G (IgG1) Fc portion (mTREM-1/IgG1) protects mice against LPS-induced shock, as well as against microbial sepsis caused by live Escherichia coli\(^{45}\) or CLP\(^{47}\). Inhibition of TREM-1 signaling could reduce, at least in part, the production of pro-inflammatory
cytokines and protect septic animals from severe bacterial infection.

5) TREM-1 ligand

Crystallographic analyses using antibody-equivalent complementary determining region loops (such as T-cell receptors, CD8 and cytotoxic T-lymphocyte associated antigen-4) can predict TREM-1 recognition sites. However, a natural ligand for TREM-1 has not been identified48,49).

6) Future directions

We recently demonstrated that PGE₂ induces the expression of both TREM-1 and sTREM-1 in macrophages. This finding sheds new light on the role of PGE₂ as a regulator of the inflammatory response to microbial infection. The increased expression of cellular TREM-1 as well as sTREM-1 in various inflammatory diseases indicates that TREM-1 is a useful inflammatory marker. Further investigations should be directed toward assessment of the pathophysiological roles of TREM-1 and sTREM-1 in various inflammatory diseases. Such studies might help to elucidate the precise role of TREM-1 expression in inflammation and provide evidence that could lead to new strategies for treating inflammatory diseases.

### References


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