A Direct Inhibitor of HMGB1 Cytokine

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HMGB1 and the recently described interleukin-33 (IL-33) are abundant chromatin-associated nuclear factors with potent proinflammatory cytokine activities. In this issue of *Chemistry & Biology*, Mollica et al. [1] report the identification of the first direct small-molecule inhibitor of HMGB1.

Cytokines play a major role in a wide range of inflammatory, infectious, and autoimmune diseases. In 1999, K.J. Tracey and colleagues discovered that the abundant chromatin-associated protein HMGB1 is secreted by activated macrophages during inflammation, and plays a critical role as a late mediator of lethal endotoxemia and sepsis [2]. Since this initial report, the cytokine activity of HMGB1 has been confirmed by many groups and HMGB1 has now been proposed to be a crucial mediator in the pathogenesis of many diseases including sepsis, arthritis, and cancer [3, 4]. The identification of HMGB1 inhibitors or antagonists is therefore of great therapeutic interest.

IL-33, the most recent addition to the IL-1 family, is a potent proinflammatory cytokine that induces production of Th2-associated cytokines IL-4, IL-5, and IL-13, both in vitro and in vivo [5]. Surprisingly, IL-33 has also been described as an abundant chromatin-associated nuclear factor, which associates with mitotic chromosomes in living cells and with interphase chromatin in the nucleus of endothelial cells in vivo [6, 7]. IL-33 therefore constitutes a second example of a chromatin-associated cytokine.

There are a number of different approaches that can be used to target chromatin-associated cytokines (Figure 1). These include production of biological agents, (i.e., function-blocking antibodies, peptide antagonists, and soluble receptors), as well as synthesis of small-molecule chemical inhibitors. HMGB1-specific antibodies have been shown to protect mice against endotoxin and sepsis lethality [2, 8]. Similar protective effects were observed with HMGB1 A box peptide, a competitive antagonist of HMGB1 cytokine activity [8]. Antibodies against HMGB1 or recombinant A box peptide have also been found to ameliorate the symptoms of collagen-induced arthritis [9]. Humanized anti-HMGB1 monoclonal antibodies that are currently under development may therefore find applications in both acute and chronic inflammatory diseases. Although neutralizing antibodies and antagonistic peptides for IL-33 are not yet available, a soluble IL-33 (ST2) receptor has been generated. It has exhibited therapeutic efficacy in several experimental disease models in vivo, including collagen-induced arthritis [10] and allergic asthma associated with Th2 responses [11].

In addition to the biological agents, there are several small-molecule chemical compounds that have been used to inhibit HMGB1 proinflammatory activities in vivo. These pharmacological agents, which belong to the class of cytokine-release inhibitory drugs (CRIDs) and include ethyl pyruvate [12], cholinergic agonists nicotine and acetylcholine [13], stearoyl lysophosphatidylcholine [14], and steroid-like pigment tanshinone IIA [15], have been shown to interfere specifically with HMGB1 release from the nucleus into the extracellular space, without affecting its mRNA or protein levels [12, 13]. In contrast, many other steroidal drugs (such as dexamethasone and cortisone) and nonsteroidal anti-inflammatory drugs (such as aspirin, ibuprofen, and indomethacin) failed to inhibit HMGB1 extracellular release significantly [15]. Mechanistic studies with endotoxin-activated monocytes and macrophages revealed that HMGB1 CRIDs block cytoplasmic translocation of HMGB1 [13–15], the first step in its extracellular secretion [16]. The HMGB1 CRIDs have shown impressive efficacy in animal models of lethal endotoxemia and sepsis [12–15] and the protective effects occurred in therapeutically achievable, safe doses [12, 15], supporting the therapeutic potential of these inhibitors in HMGB1-mediated human inflammatory diseases. Unfortunately, nothing is known yet about the mechanisms of IL-33 release from chromatin [6] and IL-33 CRIDs are not yet available.

In this issue of *Chemistry & Biology*, Mollica et al. [1] report the identification of glycyrrhizin, a natural anti-inflammatory and antiviral triterpene in clinical use, as a novel pharmacological inhibitor of HMGB1 cytokine activities. The authors show that glycyrrhizin, which is produced by the licorice plant *Glycyrrhiza glabra*, inhibits the chemotactic and mitogenic activities of HMGB1. Importantly, they demonstrate that glycyrrhizin binds directly to HMGB1 and identify its binding surface on HMGB1 using state of the art NMR chemical shift difference mapping. They also provide binding constants and three-dimensional models of the glycyrrhizin:HMGB1 complex. Surprisingly, despite the fact the glycyrrhizin binding sites on HMGB1 (helix 1 and 2 in the HMG boxes) partially overlap with the DNA binding sites (Figure 1), glycyrrhizin interferes only mildly with the binding of HMGB1 to DNA in living cells.

These results are important because they convincingly demonstrate that glycyrrhizin is a direct inhibitor of HMGB1 and that its mechanism of action is different from those of HMGB1

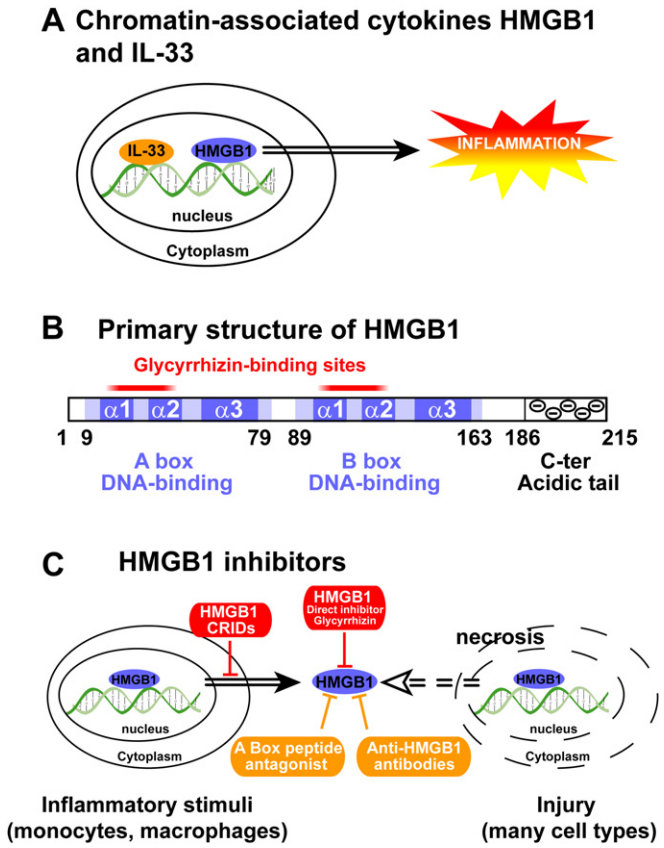

Figure 1. Chromatin-Associated Cytokine HMGB1 and Strategies to Inhibit Its Proinflammatory Activities

(A) HMGB1 and IL-33 are abundant chromatin-associated nuclear factors that possess potent proinflammatory cytokine activities. The identification of HMGB1 and IL-33 inhibitors is therefore of great therapeutic interest.

(B) Human HGMB1. The two DNA-binding HMG boxes (A and B boxes) and the acidic C terminus are indicated. The B box mediates HMGB1 cytokine activities. In contrast, the A box is a competitive antagonist of HMGB1 extracellular functions. Glycyrrhizin binds to two homologous sites in the A and B HMG boxes [1].

(C) Strategies for HMGB1 inhibition. Small-molecule inhibitors include the cytokine release inhibitory drugs (CRIDs) and the direct inhibitor glycyrrhizin. Biological agents include anti-HMGB1 antibodies and the recombinant A box peptide antagonist. Glycyrrhizin and the biologics can inhibit HMGB1 released by both activated macrophages and necrotic cells. In contrast, HMGB1 CRIDs only block the release of HMGB1 from activated monocytes/macrophages.

CRIDs. CRIDs block HMGB1 release from the nucleus into the extracellular space, but do not bind directly to HMGB1 and thus can't block its extracellular functions. In contrast, glycyrrhizin does not interfere with HMGB1 release, but can directly inhibit its extracellular cytokine activities. Interestingly, biologically active HMGB1 has been shown to be passively released by necrotic or damaged cells, in addition to being secreted by activated macrophages [17]. Glycyrrhizin, which binds directly to HMGB1, can potentially block HMGB1 released into the extracellular space by the two distinct mechanisms, while HMGB1 CRIDs only interfere with the release of HMGB1 by activated inflammatory cells (Figure 1). Direct small-molecule inhibitors of HMGB1, such as glycyrrhizin, may therefore exhibit greater therapeutic potential than HMGB1 CRIDs.

Of note, glycyrrhizin is commonly used in Japan to treat patients with chronic hepatitis and in a related study, M.E. Bianchi and colleagues have shown that glycyrrhizin reduces liver disease in a mouse model of hepatitis B by interfering with HMGB1-induced recruitment of neutrophils and other inflammatory cells in the liver [18]. The inhibitory effects of glycyrrhizin in this model were similar to those of recombinant A box peptide, further supporting a direct inhibitory effect of glycyrrhizin on extracellular HMGB1.

The NMR chemical shift difference method used by Mollica and colleagues proved to be very useful for mapping the two glycyrrhizin binding sites on HMGB1 (DNA binding HMG boxes A and B) and for modelling glycyrrhizin interaction with these sites [1]. The affinity of glycyrrhizin for HMGB1 is relatively modest ($K_d \sim 150\ \mu M$) and will need to be further improved for any therapeutic application. The three-dimensional molecular model of the glycyrrhizin:HMGB1 complex provided by the authors is very likely to be useful in this respect. Hopefully, it will open the way to the design of new glycyrrhizin derivatives with improved HMGB1 binding and anti-inflammatory properties in vivo. Finally, it will be interesting to see whether a similar approach can be applied to the identification of small-molecule inhibitors targeting IL-33. A direct chemical inhibitor of IL-33 would undoubtedly be of great pharmaceutical interest for the therapy of human inflammatory diseases.

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Chemical Tools from Biology-Oriented Synthesis

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Leßmann et al. present a chiral natural-product-derived library of α,β-unsaturated δ-lactones obtained through stereoselective synthesis on a solid support [1]. Phenotypic screening of this compound collection provided new modulators of cell cycle progression and of viral cell entry with high hit rates.

In this issue of *Chemistry & Biology*, Waldmann and colleagues report on the discovery of new modulators of cell cycle progression and viral entry through cellular screening of a natural-product-derived compound library [1]. This library was built around a α,β-unsaturated δ-lactone scaffold, which is a frequently encountered structural motif in biologically active natural products (Figure 1). Screening was conducted using fluorescence-based cellular assays that scored for effects on the actin or microtubule cytoskeleton, or, in a second case, for inhibition of cellular entry of the vesicular stomatitis virus (VSV). Remarkably, several compounds with activities in the low μM concentration range could be identified in both assay systems, in spite of a library size of only 50 compounds. Compared to conventional HTS campaigns, this is a spectacular hit rate. Two of the compounds that scored positive in the cell cycle progression assay were subsequently demonstrated to inhibit tubulin polymerization in vitro, thus linking the activity observed at the cellular level to a specific molecular target (although other targets may still contribute to the overall cellular phenotype).

Based on this short factual summary, the paper would appear to describe what nowadays might be called a "typical" chemical biology project, namely the screening of a library of low-molecular-weight synthetic compounds in fluorescence-based, phenotypic cellular assays followed (sometimes) by the analysis of hit compounds in target-specific systems. However, the paper is not just a "typical" (yet important) manifestation of modern chemical biology research. It is particularly notable for the conceptual framework that forms the basis for library design and synthesis and for Waldmann's research in general [2, 3]. The dissection of the overall concept underlying Waldmann's approach to the identification of new tool compounds for chemical biology research and new lead structures for drug discovery reveals three essential ingredients (apart from the use of powerful screening technology), whose combination now has been demonstrated repeatedly to enable the efficient identification of potent and specific inhibitors of various biological targets [2, 3].

The first of these ingredients is the idea that natural products represent prevalidated lead structures for chemical biology and medicinal chemistry research, due to an inherent propensity for protein binding. In other words, natural products are good starting points for the discovery of ligands for virtually any protein, as they have evolved to do precisely this, bind to proteins, either during their own biosynthesis or through their involvement in the modulation of a vast array of