Abstract. Objective: As a late mediator of inflammation, high mobility group box 1 protein (HMGB1) amplifies the inflammatory responses to tissue injury and infection by inducing and extending the production of proinflammatory cytokines. The aim was to investigate whether HMGB1 mediates such effects by affecting the production of anti-inflammatory mediators.

Materials and methods: The murine macrophage RAW 264.7 cells were stimulated with 0.5 µg/ml of LPS and the levels of HMGB1, TNFα, IL-1β, IL-10 and TGF-β1 in the culture supernatants were measured by ELISA. Also, the mRNA expression for IL-10 and TGF-β1 was assessed by RT-PCR.

Results: LPS induced HMGB1 release at 8 h and reached a peak at 48 h. Significant (p < 0.05) production of TNFα, IL-1β, IL-10 and TGF-β1 was seen after 8 h. However, while the levels of TNFα and IL-1β remained elevated, IL-10 and TGF-β1 release markedly declined by 24 h after stimulation. When cells were stimulated in the presence of conditioned medium derived from a 24 h LPS-stimulated culture, the production of TNFα and IL-1β was increased while IL-10 and TGF-β1 release and mRNA transcripts were decreased. A neutralizing anti-HMGB1 antibody added to the conditioned media reversed these responses.

Conclusions: HMGB1 modulates the inflammatory cascade in activated macrophages by inducing proinflammatory, while suppressing anti-inflammatory responses.

Key words: HMGB1 – Inflammation-macrophages

Introduction

High mobility group box 1 protein (HMGB1) is an abundant nuclear and cytoplasmic protein that was first identified as a non-histone chromatin-associated factor that facilitates gene transcription [1–3]. Recently, HMGB1 was recognized as an inflammatory mediator of delayed endotoxin lethality and lung injury [4, 5]. HMGB1 is secreted by monocytes/macrophages activated with LPS or proinflammatory cytokines [6, 7] and induces the release of proinflammatory mediators from these cells [8]. Once released, HMGB1 potently stimulates macrophage functions [9]. It is also released from necrotic cells to trigger inflammation [10]. Previous studies have shown that HMGB1 induces the release of the proinflammatory cytokines TNFα and IL-1β by human monocytes [4] and that intratracheal administration of HMGB1 induces lung injury while anti-HMGB1 antibodies reduces acute lung inflammation [11].

Monocytes/macrophages play a pivotal role in coordinating the immune and inflammatory response to infection and tissue injuries. Macrophages in the airways, for example, are the predominant immune effector cells responsible for both enhancing and suppressing the inflammatory responses [12, 13]. In addition to the production of proinflammatory mediators such as TNFα and IL-1β, and HMGB1, activated macrophages also produce anti-inflammatory cytokines such as IL-10 and TGF-β1. These regulatory cytokines have suppressive or enhancing effects (depending on the context) on the immune and inflammatory responses [14], including pulmonary inflammation [15].

This study aimed to investigate the relationship between pro- and anti-inflammatory responses in LPS-activated macrophages. The results demonstrate that HMGB1 plays a pivotal role in macrophage inflammatory responses by modulating the production of inflammatory mediators.

Material and methods

Cell culture

The murine monocyte/macrophage-like cell line, RAW 264.7, was obtained from American Type Culture Collection (Manassas, VA). Cells were cultured at 1 × 10^6 cell/ml in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 2 mM glutamine, 10% fetal bovine serum, and 1% Streptomycin/Penicillin. Cells were plated in six-well culture plates and were left unstimulated or stimulated with 0.5 µg/ml...
of LPS (Sigma, St. Louis, MO) for 8, 12, 24, or 48 h. In one experiment, cells were cultured for 8 h in fresh medium alone or fresh medium containing 20% (v/v) of conditioned medium obtained from a 24 h LPS-stimulated culture. Anti-HMGB1 antibody or isotype-matched control antibody (Abcam, Cambridge, MA) was added at 1 µg/ml to some cultures that contained conditioned medium. Cultures were maintained at 37 °C under 5% CO2 atmosphere.

Enzyme-Linked Immunosorbent Assay (ELISA)

To determine the production of inflammatory mediators in activated macrophages, RAW 264.7 cells were stimulated with LPS as described above and culture supernatants were collected and assayed for HMGB1, TNFα, IL-1β, IL-10, and TGF-β1 using ELISA kits, according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN). Briefly,