INTRODUCTION

The majority of autoimmune diseases in adults and children are considered complex in aetiology, with risk conferred by both genes and the environment ***(Huang, 2012)***. Systemic lupus erythematosus (SLE) is one of the major rheumatic diseases of childhood, which often lead to serious morbidity and mortality ***(Huang et al., 2004).***

It is a systemic autoimmune disease characterized by involvement of multiple organ systems and the production of a variety of antibodies directed to self-components ***(Shirai and Hirose, 2006)***.

High-mobility group box-1 (HMGB1) is a nuclear protein that binds DNA and modulates chromosomal architecture. Once released into the extracellular space, after cell death or upon activation, HMGB1 acts as a danger associated molecular pattern or as an alarmin and stimulates inflammatory and immunological activities that include cytokine production, chemotaxis, cell proliferation, angiogenesis and cell differentiation ***(Silva de Souza et al., 2013).***

Previous studies reported that serum HMGB1 could be a biomarker of SLE activity in adults, especially in patients with lupus nephritis ***(Abdulahad et al., 2012; Zickert et al., 2012)***.

AIM OF THE WORK

The aim of this study is to evaluate the expression of serum high-mobility group box-1 (HMGB1) protein in patients with pediatric SLE in relation to disease characteristics and activity in an attempt to investigate its potential role as a biomarker of SLE activity.

SERUM HIGH-MOBILITY GROUP BOX-1

High-mobility group box 1 protein (HMGB1) is a highly abundant and conserved protein that has important biological activities inside as well as outside the cell ***(Andersson and Tracey, 2011).*** Inside the nucleus, HMGB1 interacts with double stranded deoxyribonucliec acid (DNA) and histones to determine chromatin structure and regulate key processes such as transcription ***(Rovere-Querini et al., 2007)***. Outside the cell, HMGB1 acquire a new identity to serve as an alarmin ***(Harris et al., 2012)***. It induces both migration and activation of dendritic cells (DCs) and enhances antigen-specific immune responses that favor T helper (Th) 1 polarization ***(Urbonaviciute et al., 2008)***.

Concept of an alarmin:

Alarmins are endogenous molecules that are passively released from necrotic cells upon infection or tissue injury or are rapidly secreted by stimulated leukocytes or epithelia ***(Bianchi, 2009)***.

Mammalian organisms have evolved diverse systems to recognize certain molecules as ‘danger signals’ and respond quickly to life-threatening events, including infection and trauma ***(Muller et al., 2001).*** These danger signals can arise from exogenous which is called pathogen associated molecular pattern (PAMPs) as well as endogenous which is called damaged associated molecular pattern (DAMPs) sources and

can induce innate and adaptive immune responses ***(Bianchi, 2007).***

The alarmins family comprises *HMGB1, S100 proteins (A8,A9, A12, B), heat shock proteins (60,70), β-defensin, cathelicidin and lactoferrin* ***(Mease, 2011).*** The HMGB1 is probably the best characterized alarmin ***(Yang et al., 2012).***

Discovery and structure of HMGB1:

HMG proteins were first discovered in 1973 as nuclear proteins with rapid migration in electrophoretic gels, a property leading to their name ***(Goodwin et al., 1973)***.

HMGB1 protein is a highly conserved protein of 215 amino acids that is universally expressed in nuclei where it binds to DNA. The protein can be divided into three separate domains: the *A box, B box* and *acidic tail*. *A box* serves as a competitive antagonist for HMGB1 and inhibits HMGB1 activity. A- and B-boxes are important for its DNA binding functions and are positively charged, whereas the tail is negatively charged, giving the molecule a bipolar charge **(Figure 1)** ***(Štros, 2010).***

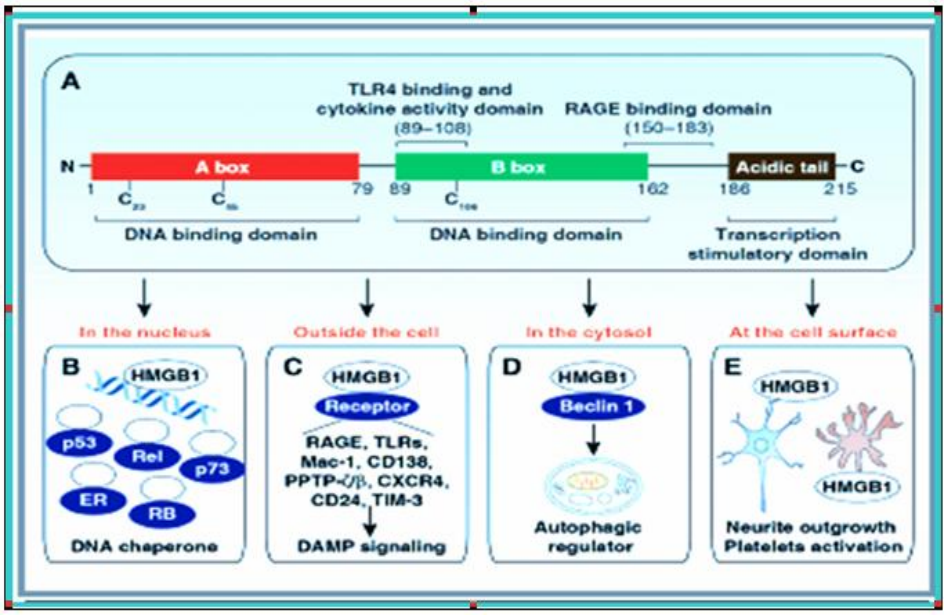

Figure (1): Structure and function of the HMGB1 protein. *(A)* HMGB1 is composed of the A box, B box and C tail domains. (B) As a DNA chaperone, nuclear HMGB1 participates in DNA replication, recombination, transcription and repair. In particular, HMGB1 interacts with and enhances the activities of a number of transcription factors, including p53(tumor protein), p73(tumor suppressor protein), the retinoblastoma protein (RB), members of the Rel/NF-κB family and estrogen receptor (ER). (C) Once released, HMGB1 binds to various receptors to activate DAMP signaling involved in multiple cellular processes. (D) Cytoplasmic HMGB1 protein binds with beclin 1 to induce autophagy. (E) Membrane HMGB1 promotes neurite outgrowth and platelet activation. ***CXCR*** *(chemokine-X-motif),* ***Mac-1(****macrophage-1 antigen****),* ***RAGE*** *(receptor for advanced glycation end product),* ***TIM*** *(T-cell immunoglobulin and mucin domain),* ***TLRs*** *(toll-like receptors)* ***(Chen et al., 2013).***

Translocation of HMGB1:

HMGB1 can be liberated from the cell into the extracellular space, where it acts as a DAMP and thus as alarmin. Translocation from the nucleus to the cytoplasm occurs through acetylation of lysine residues, where the protein is concentrated in vesicles for secretion ***(Scaffidi et al., 2002; Kazama et al., 2008)***. The extracellular release of HMGB1 can

occur either passively or actively by secretion. More importantly, the manner of release determines posttranslational modifications of released HMGB1. Passive release of HMGB1 usually results from leakage due to damaged cell membranes. Active release by secretion occurs when immune cells are exposed to, for instance, PAMPs, tumor necrosis factor (TNF)-α or lipopolysaccharide (LPS) via non classical pathways ***(Andersson et al., 2000; Youn et al., 2008)***.

Release of HMGB1:

The protein can be released from cells in three different ways **(Figure 2):**

1. From cells of the innate immune system such as monocytes, macrophages and DCs that are activated by inflammatory cytokines or LPS ***(Wang et al., 1999; Scaffidi et al. 2002; Tang et al., 2011)***. This activity has also been demonstrated in other cells: hepatocytes, endothelial cells, glial cells and neurons ***(Tang et al., 2011)***.

2. Necrotic cells will leak HMGB1 upon cell damage and permeabilized cell membrane ***(Scaffidi et al., 2002)***.

3. Apoptotic cells that escape clearance by macrophage phagocytosis and reach late apoptosis (secondary necrosis) can leak HMGB1 to the extracellular milieu ***(Bell et al., 2006; Andersson and Rauvala, 2011)***. HMGB1 will either be oxidized or tightly bound to chromatin ***(Scaffidi et al., 2002; Urbonaviciute et al., 2009)***.

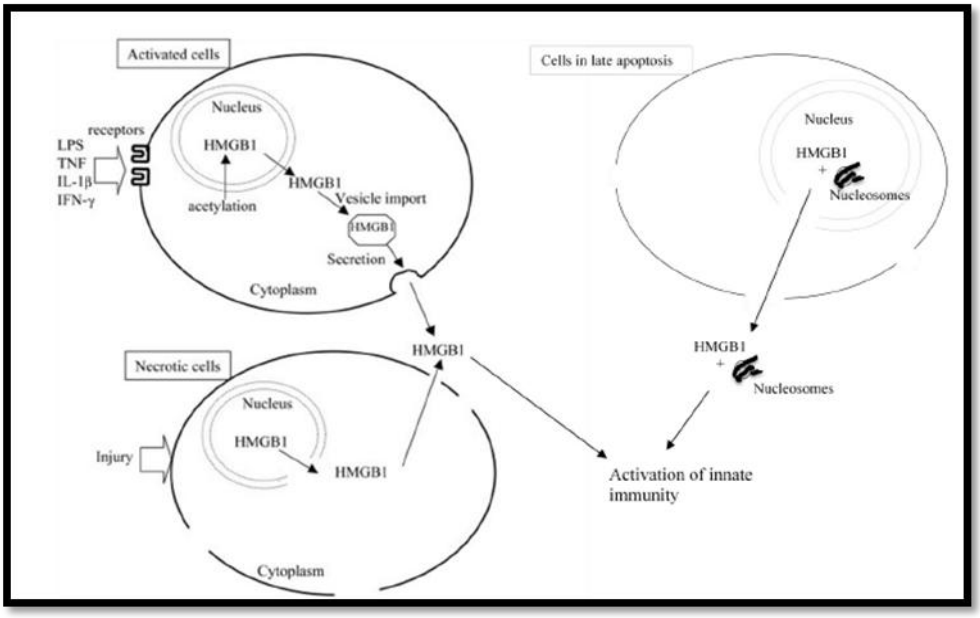

Figure (2): Extracellular release of HMGB1: Upper left: Active release from stimulated cells of the innate immune system. Lower left: Passive release from necrotic or damaged cells. Right: Passive release from cells in late apoptosis. HMGB1 is oxidized and tightly bound to chromatin ***(Yang et al., 2005)***.

1.Release of HMGB1 during cell activation:

To function as an alarmin, HMGB1 must transit from an intracellular location to the extracellular space. Following cell activation, HMGB1 undergoes post-translational modifications, including phosphorylation, acetylation, and methylation that modify its charge ***(Bonaldi et al., 2003).***

Once HMGB1 is modified, its interaction with chromatin diminishes. Eventually, HMGB1 translocates to the cytoplasm, where it can enter the endosomal compartment. The exit of HMGB1 from the cell occurs via a non-conventional secretory mechanism as cell activation proceeds ***(Ito et al., 2007).***

In addition to LPS, proinflammatory mediators as well as TLR ligands can trigger the release reaction. Thus, interferon-γ (IFN-γ), IFN-α/β, and nitric oxide can all induce externalization of HMGB1 ***(Wähämaa et al., 2007).***

2. Release of HMGB1 during cell death:

Recent studies have expanded categorization of immunologically relevant cell death forms, although all likely lead to HMGB1 release and impact the pathogenesis of inflammatory and autoimmune disease **(Figure 3)** ***(Magna and Pisetsky, 2014).***

- **Necrosis:**

Necrosis is a form of accidental cell death induced by chemical or physical trauma ***(Andersson and Harris, 2010).*** HMGB1 protein can readily move outside the cell, when membrane integrity is lost during necrosis. Importantly, since necrotic cells lacking HMGB1 fail to induce cytokine production ***(Rovere-Querini et al., 2004)*** some studies suggested that HMGB1 is a dominant immune player during cell death ***(Beyer et al., 2012; Venereau et al., 2012).***

- **Apoptosis:**

Apoptosis is programmed cell death that can occur in both physiological and pathological conditions ***(Nagata et al., 2010).*** If left unperturbed or uncleared, apoptotic cells can enter a phase called late apoptosis or secondary necrosis that includes membrane breakdown and the release of intracellular content,

linked to the pathogenesis of systemic lupus erythematosus (SLE) ***(Nagata et al., 2010)***. The most characteristic antibodies in these settings are directed to DNA or histones, the partners of HMGB1 in the nucleus, and are considered the result of an immune response to persistent dead cell remains, with DAMPs serving as autoadjuvants ***(Kruse, 2010).***

- **Pyroptosis:**

Pyroptosis is considered to be a specialized form of regulated cell death of macrophages and dendritic cells. Like apoptosis, pyroptosis involves nuclear changes, with DNA condensation and cleavage resulting from the activity of an unidentified nuclease ***(Miao et al., 2011).*** Pyroptosis has been demonstrated to be an important pathway for active HMGB1 release driven by protein kinase ribonucleic acid (PKR) and inflam-masomes with release of *interleukin* (IL) -1 and IL-18 ***(lamkanfi et al., 2010).***

- **NETosis:**

NETosis is another form of regulated cell death that occurs primarily with neutrophils. Following the death of neutrophils by apoptosis, macrophages can clear their remains by a process called efferocytosis. In addition to apoptosis, neutrophils can display another, more dramatic response to stimuli such as bacteria, LPS, and cytokines, undergoing of a process called NETosis. This process culminates in cell death, releasing structures called neutrophil extracellular traps (NETs) ***(Brinkmann and Zychlinsky, 2012).***

HMGB1 is a component of NETs**.** Nevertheless, the process of NET release, with or without cell death, can be a source of extracellular HMGB1 that appears in tissue or can serve as a biomarker. This material can also be a source of autoantigen to simulate antibody production or form immune complexes in autoimmune diseases ***(Mitroulis et al., 2011; Yipp et al., 2012).***

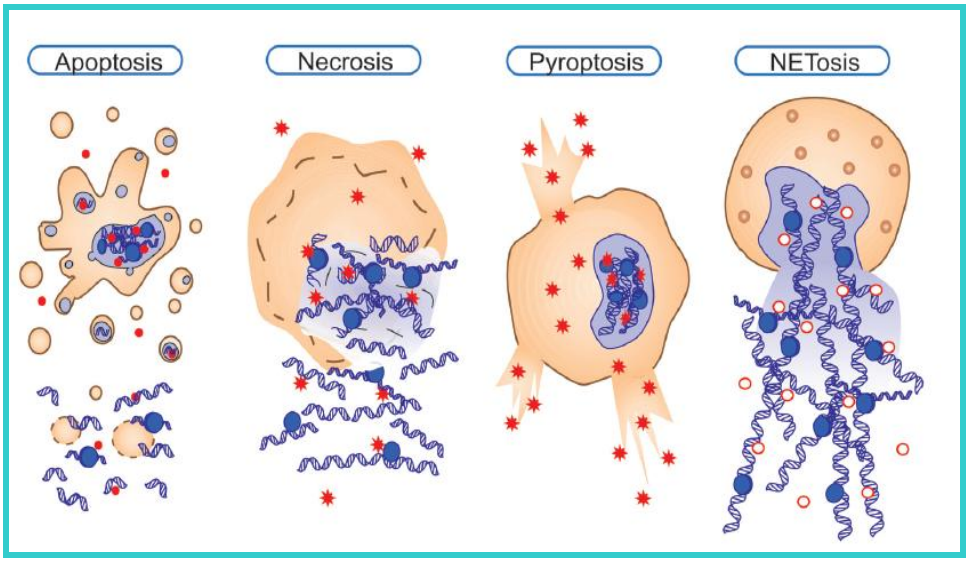

Figure (3): The pattern of HMGB1 translocation during cell death. Apoptotic cells can retain HMGB1 tightly bound to chromatin in the nucleus, but, during late apoptosis or secondary necrosis, can release this protein; this isoform is oxidized and lacks immunological activity (depicted by red dots). During **necrosis**, the plasma membrane and nuclear membrane lose integrity, releasing a proinflammatory form of HMGB1 (red stars). During **pyroptosis**, following inflammasome activation, the plasma membrane opens and HMGB1 release occur (red stars). If, with pyroptosis, TLR activation occurs, a cytokine-inducing form can also be released (not shown). While **NETosis** also induces inflammation and releases nuclear material, the redox state of the released HMGB1 in this type of cell death is yet unknown (depicted by red circles). The helical symbols indicate DNA. In **NETosis**, DNA is released in the form of strands or meshes; while the DNA has a high molecular weight with necrosis, it is not organized or associated with cytoplasmic proteins. The blue dots indicate a nucleosome structure. In apoptosis and pyroptosis, the DNA is cleaved (not shown); laddering occurs with apoptosis but not pyroptosis ***(Yipp et al., 2012).***

Mode of action and HMGB1 receptors:

It has been reported that HMGB1 can interact with multiple immune sensors and receptors. These receptors included receptor for advanced glycation end products (RAGE) as well as toll like receptors (TLRs) 2, 4 and 9 and chemokine receptors C-X-C motif receptor 4 (CXCR4) **(Figure 4**) ***(Schiraldi et al., 2012).***

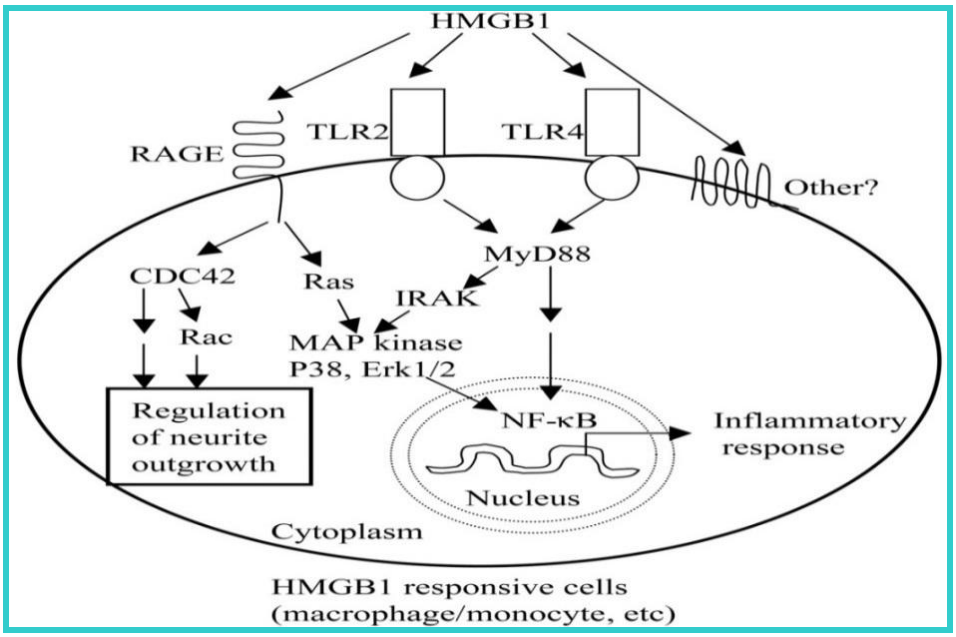

Figure (4): Receptors for HMGB1. Interaction of HMGB1 with RAGE can activate CDC42 pathway and MAPKs pathway leads to cytoskeletal changes and NF-κB activation. The binding of HMGB1 to TLR4 in particular can lead to activation of NF-κB and production of inflammatory cytokines such as IL-6 and TNF-αo by macrophages***. CDC*** *(cell devision control protein)**, Erk*** *(extracellular signal-regulated kinase)**, IRAK*** *(interleukin receptor associated kinase),* ***RAGE*** *(receptor for advanced glycation end products),* ***NF-kB*** *(nuclear factor κB),* ***TLR*** *(toll-like receptors)* ***(Stern et al., 2002).***

RAGE was the first identified receptor for HMGB1, it is a transmembrane protein and a member of the immunoglobulin superfamily. RAGE is expressed in endothelial cells, vascular

smooth muscle cells, neurons, and macrophage/ monocyte ***(Kokkola et al., 2005)***.

Interaction of HMGB1 with RAGE can activate two major signaling pathways, one encompassing cell division control protein 42 (CDC42) and the other involving diverse mitogen-activated protein kinases (MAPKs) that finally leads to cytoskeletal changes and nuclear factor-kappa B (NF-êB) activation, respectively ***(Taguchi et al., 2000)***. Binding of HMGB1 to RAGE initiates cell migration–dependent pathways via NF-êB activation ***(Palumbo et al., 2009).***

In addition to RAGE, the importance of TLRs has been demonstrated in HMGB1 signaling pathways. TLRs are highly conserved proteins and important pathogen-recognized patterns both in innate and adaptive immunity. Signaling by activation of TLRs-2,4,9 culminates in NF-êB and MAPKs that regulate gene expression of various immune and inflammatory mediators ***(Park et al., 2006).***

The binding of HMGB1 to TLR4 in particular can lead to activation of NF-κB and production of cytokines such as IL-6 and TNF-αo by macrophages ***(Park et al., 2006)***. Also HMGB1 can induce chemotaxis, a process which involves a distinct receptor set ***(Yang et al., 2013).***

HMGB1 also promotes recruitment of inflammatory cells to damaged tissue by forming a complex with the chemokine CXCL12 and signaling via CXCR4 independent of RAGE and

TLR4. Moreover, cell migration can also occur when HMGB1 is complexed to chemokine CXCL12, and then it acts synergistically through CXCR4 ***(Schiraldi et al., 2012)***.

Furthermore, previous studies indicated that HMGB1 can bind to other molecules, such as PAMPs such as LPS, double-stranded RNA or DNA, or a cytokine such as IL-1β, and act through the partner's receptor. This synergism could induce expression of mediators like the prostaglandins to promote pain and inflammation in arthritis ***(Wähämaa et al., 2011; Leclerc et al., 2013).***

Pathophysiological effects of HMGB1:

- ***Pro-inflammatory effects of HMGB1****:*

1- Intranuclear effect

The intranuclear functions of HMGB1are regulation of gene transcription and maintenance of nucleosome structure ***(Stros et al., 2007)***. HMGB1 binds the minor groove of DNA facilitating the assembly of site-specific DNA-binding proteins, including nuclear hormone/nuclear hormone receptor complexes and p53 or p73 transcriptional complexes ***(Brezniceanu et al., 2003).***

HMGB1 has been found to increase the binding affinity of many sequence-specific transcription factors to their cognate DNA, such as p53, p73, Rb protein, NF-κB, and estrogen receptors (ER). HMGB1 directly binds to a variety of bulky DNA lesions which allows it to participate in DNA repair

pathways including nucleotide excision repair, base excision repair, mismatch repair, and double strand break repair via non homologous end-joining ***(Kanget al., 2013)***.

2- Cytosolic effect

HMGB1 has been found to have a cytosolic function in cells of the innate immune system ***(Yanai et al., 2009)***. It binds foreign immunogenic nucleic acids and activates innate immune responses via endosome-based cytosolic receptors like TLR3, TLR7 and TLR9. RNA- and DNA- sensing receptors in the cytosol like *Rig-I like receptors* (RLRs), *melanoma differentiation-associated protein 5* (MDA5), *DNA-dependent activator of interferon regulatory factors* (DAI) and *absent in melanoma 2* (AIM2) will trigger the innate immune system and induce production of type I IFN, proinflammatory cytokines and chemokines ***(Harris et al., 2012)***. It has been shown that absence of cytosolic HMGB1 protein will reduce induction of type I IFN and cytokines by foreign DNA and RNA ***(Andersson and Rauvala, 2011; Yanai et al., 2011)***. HMGB1 is involved in inflammasome activation and autophagy by binding to Beclin 1 receptors ***(Tang et al., 2010)***.

3- Extracellular effect

Upon release from cells, HMGB1 will bind to cell-surface receptors on monocytes, macrophages and DCs, such RAGE, TLR2 and TLR4 ***(Park et al., 2004; Lotze and Tracey, 2005)***. Receptor binding leads to activation of the transcription factor NF-êB which induces production of multiple

proinflammatory molecules such as TNF-á, IL-1â, IL-6 and IL-8, etc ***(Andersson et al., 2000)***.

Signaling through cell surface receptors cannot alone explain all the downstream effects of HMGB1. Different biological effects may be related to whether it is complexed or free. HMGB1 will easily form complexes with IL-1â, nucleosomes, LPS and DNA which interacts with receptors like IL-1R, TLR2, TLR4, and TLR9 respectively **(Figure 5).** HMGB1- complexing has an enhancing effect on the activation of the receptors and the immune response ***(Sha et al., 2008; Hreggvidsdottir et al., 2012)*.** This complex-binding ability is probably the source of analytical difficulties in various immunological assays ***(Hreggvidsdottir et al., 2009)***.

As HMGB1 has multiple downstream signaling responses due to activation of different receptors, it also induces cell specific responses when it stimulates cells of the immune system ***(Jiang et al., 2007)***. Extra cellular HMGB1 has become a focus in the field, as it is involved in a variety of immune responses, including neurite outgrowth, platelet activation, and cytokine- and chemokine-like activity ***(Rouhiainen et al., 2000)***.