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Role of Fetuin-A in Injury and Infection

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1. Introduction

Injury and infection, seemingly unrelated conditions, converge on a common process - inflammation, which is mediated partly by innate immune cells including macrophages and monocytes. These innate immune cells are equipped with pattern recognition receptors (such as TLR2, TLR4, and TLR9) (Brightbill *et al.* 1999; Poltorak *et al.* 1998; Hemmi *et al.* 2000), and can recognize both damage- and pathogen-associated molecular patterns (DAMPs, such as HMGB1; and PAMPs, such as endotoxin) (Andersson *et al.* 2000; Chen *et al.* 2009;Krieg 2002;Wang *et al.* 1999;Ivanov *et al.* 2007). In response to various PAMPs or DAMPs, innate immune cells release proinflammatory cytokines (such as TNF, IL-1, IFN-γ or HMGB1) to mount inflammatory responses. If dysregulated, an uncontrolled inflammation may adversely lead to detrimental consequences. To orchestrate the inflammatory response to infection and injury, the liver strategically re-prioritizes the synthesis and systemic release of a group of proteins collectively termed "acute phase proteins" (APPs). For instance, fetuin-A, also called the alpha-2-HS-glycoprotein for the human homologue (Christie *et al.* 1987), has been implicated as an anti-inflammatory protein during injury or infections. In this book chapter, we summarize emerging evidence to support fetuin-A as an acute phase protein capable of attenuating injury- or infection-elicited inflammatory responses.

2. Fetuin-A as a negative or positive APP

Fetuin-A was first isolated by Pederson more than sixty years ago as a major plasma protein in the fetus (Pedersen 1944). During fetal development, it is expressed in most organs including the liver, kidney, gastrointestinal tract, skin and brain (Terkelsen *et al.* 1998; Kitchener *et al.* 1997; Dziegielewska *et al.* 2000; Kitchener *et al.* 1999). In adults however, fetuin-A is produced primarily by the liver, and its synthesis is divergently regulated during injury or infection, classifying it as a negative or positive APP.

2.1 Regulators of hepatic Fetuin-A expression

Although fetuin-A is constitutively expressed in hepatocytes, its expression is negatively regulated by several proinflammatory cytokines. For instance, the fetuin-A expression levels in human HepG2 hepatoma cells were reduced by proinflammatory cytokines such as TNF, IL-1, IL-6, and IFN-γ (Daveau *et al.* 1988;Li *et al.* 2011a). IFN-γ, at concentrations as low as 10-50

ng/ml, reduced fetuin-A expression levels by as much as 50-70% (Li *et al.* 2011a). In contrast, HMGB1 (1 μg/ml), a late proinflammatory mediator of lethal systemic inflammation (Wang *et al.* 1999; Yang *et al.* 2004; Wang *et al.* 2008), elevated fetuin-A expression levels by 2-3 folds in HepG2 cells, suggesting that different cytokines divergently regulate hepatic fetuin-A expression.

2.2 Elevation of Fetuin-A Levels during ischemia

In patients with cerebral ischemic injury (stroke), plasma fetuin-A levels were paradoxically elevated (Weikert *et al.* 2008;Tuttolomondo *et al.* 2010). The elevation of circulating fetuin-A levels correlated with an increase not only in LDL-cholesterol levels (Tuttolomondo *et al.* 2010) but also in risk of cardiovascular disorders (Weikert *et al.* 2008). Similarly, serum fetuin-A levels were increased up to 10-fold in cattle following traumatic injury (Dziegielewska *et al.* 1992), suggesting fetuin-A as a positive APP during ischemic or traumatic injury. Notably, HMGB1 can be passively leaked from injured cells (Peltz *et al.* 2009), and functions as an early mediator of traumatic or ischemic injury (Zhu *et al.* 2010; Wu *et al.* 2007; Liu *et al.* 2007b; Tsung *et al.* 2005; Tsung *et al.* 2007; Watanabe *et al.* 2005). It is thus plausible that HMGB1 participates in the up-regulation of hepatic fetuin-A expression during injury.

In an animal model of focal cerebral ischemia (i.e., permanent middle cerebral artery occlusion, MCAo), fetuin-A levels in the ischemic brain tissue were also elevated in a time-dependent manner, starting between 2-6 h, peaking around 24-48 h, and returning towards base-line at 72 h post MCAo (Wang *et al.* 2010). This time-dependent increase in cerebral fetuin-A levels parallels with the transient elevation of the blood-brain barrier (BBB) permeability (Belayev *et al.* 1996), suggesting that circulating fetuin-A can gain entry across the BBB into the ischemic brain tissue. This possibility was supported by the observation that peripherally (intravenously) administered FITC-labeled fetuin-A was found in the ischemic brain region at 24 h after MCAo (Wang *et al.* 2010).

2.3 Reduction of circulating Fetuin-A levels during infection

In animal models of endotoxemia and sepsis (induced by cecal ligation and puncture, CLP), circulating fetuin-A levels were decreased in a time-dependent fashion, starting between 2-6 h, reaching a nadir (with maximal reduction by 50-60%) around 24-48 h. Afterwards, fetuin-A levels started to increase, returning towards basal levels approximately 72 h post endotoxemia or sepsis, supporting fetuin-A as a negative APP in animal models of lethal endotoxemia and sepsis (Li *et al.* 2011a). Interestingly, disruption of expression of early proinflammatory cytokines (such as IFN-γ) impaired bacterial endotoxin-mediated down-regulation of fetuin-A expression (Li *et al.* 2011a). It thus appears that early proinflammatory cytokines (such as TNF and IFN-γ) function as negative regulators to reduce circulating fetuin-A levels during an early stage of endotoxemia or sepsis; whereas late-acting proinflammatory mediators (e.g., HMGB1) stimulate fetuin-A expression to restore its circulating levels at a late stage.

In patients with other inflammatory diseases such as pancreatitis (Kusnierz-Cabala *et al.* 2010), chronic kidney diseases (Metry *et al.* 2008), and rheumatoid arthritis (Sato *et al.* 2007), serum fetuin A levels were also decreased by 20-30%. In these patients, circulating fetuin-A levels were not only inversely correlated with levels of inflammatory cytokines (such as IL-6) (Kusnierz-Cabala *et al.* 2010), but also associated with increased mortality rates (Metry *et al.* 2008). Collectively, these observations classify fetuin-A as a negative APP during infection or other inflammatory illness.

3. Biological functions of Fetuin-A

Despite its abundance, the functions of fetuin-A remain poorly understood. A wide range of biological functions have been proposed for fetuin-A based on its structural similarities to other proteins or physical interactions with biogenic molecules.

3.1 Inhibitor of insulin or TGF-β Signalling

Fetuin-A shares sequence similarity to type II TGF-β receptors (Demetriou *et al.* 1996) and insulin receptor tyrosine kinases (Mathews *et al.* 1997;Haasemann *et al.* 1991), and has thus been proposed as an inhibitor of the TGF-β or insulin signaling pathways. After binding to to TGF-β1, fetuin-A prevents TGF-β1 from binding to its receptors, thereby antagonizing TGF-β1-mediated antiproliferative effects (Demetriou *et al.* 1996). Similarly, fetuin-A can also bind to the insulin receptor, and consequently inactivate (rather than activate, as in the case for insulin) the receptor tyrosine kinase (Goustin & Abou-Samra 2010). This may partly explain why higher fetuin-A levels were associated with insulin resistance in some patients with type 2 diabetes (Ix *et al.* 2008).

3.2 Inhibition of pathological calcification

As a glycoprotein, fetuin-A carries two N-linked and three O-linked oligosaccharide chains that terminate with sialic acid residues, and can bind cationic Ca^{2+} ions. Accordingly, fetuin-A has been proposed as an endogenous inhibitor of pathological mineralization or calcification in soft tissues (Jahnen-Dechent *et al.* 2001;Schinke *et al.* 1996;Szweras *et al.* 2002;Schafer *et al.* 2003;Ketteler *et al.* 2003). Specifically, fetuin-A forms protein-mineral colloids with calcium and phosphate (Heiss *et al.* 2003;Wu *et al.* 2009), thereby preventing uncontrolled mineralization that may otherwise occur under pathological conditions (Rochette *et al.* 2009).

3.3 Inhibition of inflammation

While investigating the mechanism underlying a cationic molecule spermine-mediated anti-inflammatory actions, we serendipitously discovered that macrophages lost their responsiveness to spermine when cultured under low serum conditions (Wang *et al.* 1997). That is, despite the addition of cytokine-suppressing concentrations of spermine, the bacterial lipopolysaccharide (LPS)-induced production of TNF by these serum-starved macrophages was uninhibited. Subsequently, we discovered that these serum-starved macrophages became deprived of fetuin-A that was required for spermine to inhibit TNF production (Wang *et al.* 1997). The involvement of fetuin-A in spermine-mediated immunosuppression was confirmed by adding highly purified fetuin-A or fetuin-specific antibodies, which respectively restored or impaired spermine-mediated TNF inhibition (Wang *et al.* 1997).

It is plausible that fetuin-A functions as an opsonin for cationic spermine, and its availability to immune cells may be critical in regulating the innate immune response (Wang & Tracey 1999). Indeed, levels of fetuin-A in macrophage cultures could be altered by LPS stimulation or fetuin-A supplementation (**Figure 1A**). Intriguingly, the exogenously administered fetuin-A was predominantly localized in cytoplasmic punctate structures (**Figure 1B**), which co-localized with vesicles containing an autophagy marker (LC3) - possibly autophagosomes or amphisomes - in LPS-stimulated macrophages.

When given at higher concentrations (e.g., 3.5 mg/ml), crude fetuin-A (> 98%, Sigma-Aldrich) abrogated endotoxin-induced release of IL-1 and nitric oxide (Dziegielewska *et al.* 1998). Upon purification by gel filtration and ion-exchange chromatography, the highly purified intact fetuin-A could effectively inhibit IFN-γ- or LPS-induced release of HMGB1 (Li *et al.* 2011a), a newly identified late mediator of lethal endotoxemia and sepsis (Wang *et al.* 2008;Wang *et al.* 2009). However, even at the concentrations (e.g., 100 μg/ml) that abrogated LPS-induced HMGB1 release, fetuin-A only partly inhibited LPS-induced TNF secretion, suggesting fetuin-A as an effective inhibitor of HMGB1 release.

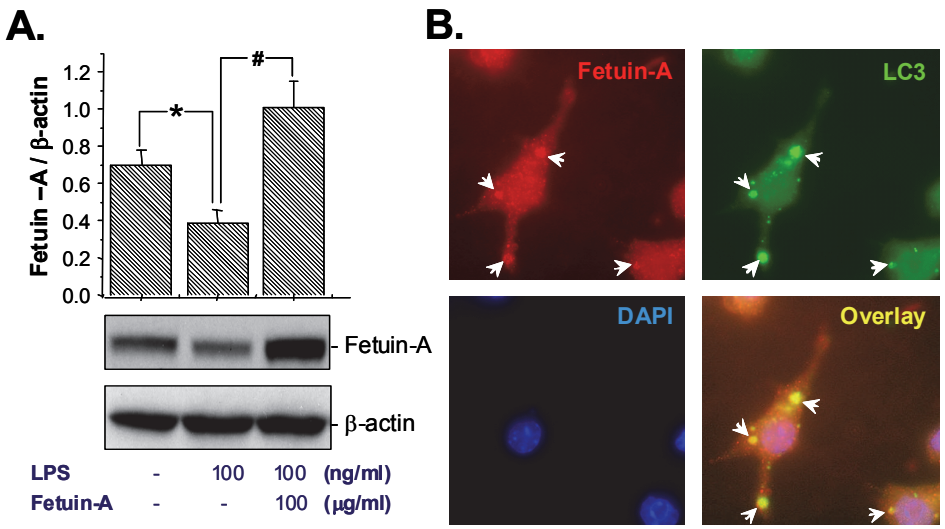

Fig. 1. **Exogenous fetuin-A was internalized into cytoplasmic vesicles in macrophage cultures**. A). Supplementation of exogenous fetuin-A prevented endotoxin-induced fetuin-A depletion**.** Murine macrophage-like RAW 264.7 cells were stimulated with LPS (100 ng/ml) in the absence or presence of fetuin-A (100 μg/ml) for 2 h, and cellular fetuin-A levels were determined by Western blotting. The relative fetuin-A levels, as a ratio to β-actin, were expressed as the mean ± SD of three independent experiments. *, $p < 0.05$ versus control ("+LPS"); #, $p < 0.01$ vs control ("+LPS"). B) Exogenous fetuin-A was internalized into LC3-containing cytoplasmic vesicles. GFP-LC3-transfected RAW 264.7 cells were stimulated with LPS (200 ng/ml) in the presence of fetuin-A (100 μg/ml) overnight, and immunostained with fetuin-A-specific antibodies.

4. Therapeutic potential of Fetuin-A in infection or injury

4.1 Carrageenan-induced paw edema

In an animal model of carrageenan-induced inflammation, intraperitoneal administration of fetuin-A (5 to 500 mg/kg) dose-dependently attenuated the development of paw edema (Ombrellino *et al.* 2001). The sialic acid moieties of fetuin-A might be required for its anti-inflammatory activities. When these sialic acid residues were removed by neuraminidase, the resultant asialofetuin-A failed to potentiate the anti-inflammatory activities of spermine (Wang *et al.* 1997) and failed to attenuate carrageenan-induced TNF production *in vivo*

(Ombrellino *et al.* 2001). In contrast, administration of anti-fetuin-A neutralizing antibodies in combination with carrageenan led to significantly increased paw edema, indicating that fetuin-A plays an important role in counter-regulating inflammatory responses.

4.2 Cerebral ischemic injury

Cerebral ischemia is frequently caused by an obstruction of a cerebral artery. Despite advances in acute and prophylactic therapies, stroke represents the leading cause of long-term disability (500,000-700,000 cases per year), and the third most common cause of death (with a mortality rate of 20-25%) in the United States.

4.2.1 Pathogenesis of cerebral ischemic injury

Cerebral ischemic injury consists of two stages: primary tissue damage in the ischemic core and secondary tissue injury in the surrounding penumbra. The primary injury in the ischemic core is primarily mediated by tissue ion (Ca^{2+} and Na^{+}) overload (Taylor & Meldrum 1995) and excitotoxicity (Lee *et al.* 1999); whereas the secondary injury in the surrounding penumbra is partly mediated by proinflammatory cytokines (**Figure 2**, Feuerstein *et al.* 1998).

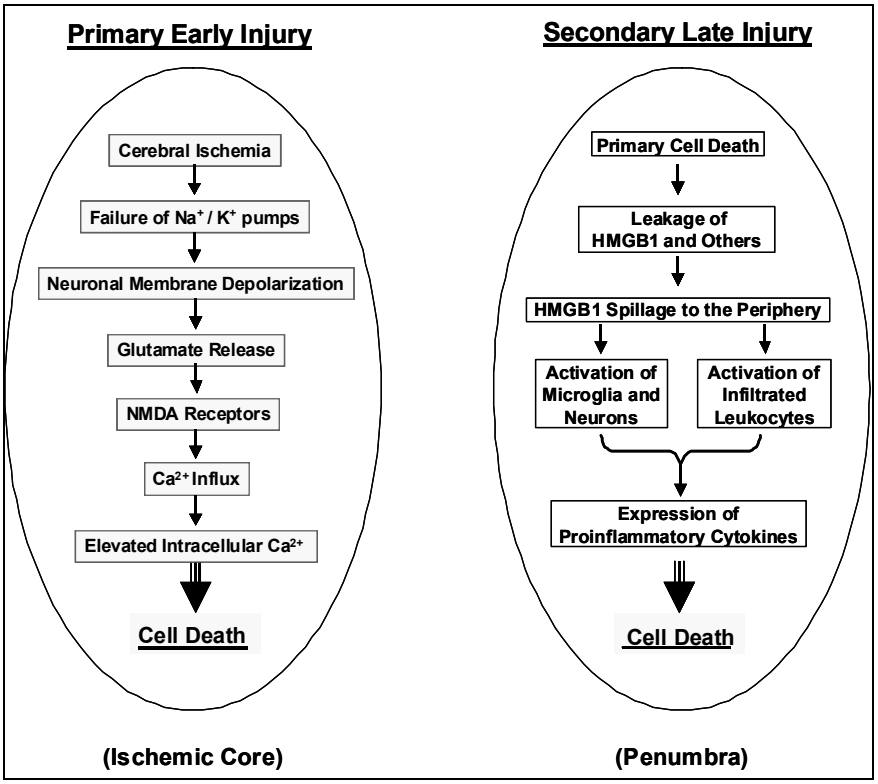

Fig. 2. **Cascade of events leading to primary injury in the ischemic core and secondary injury in the surrounding penumbra.**

4.2.1.1 Primary early injury in the core

Within seconds to minutes after cerebral ischemia, decreased ATP production leads to failure of the Na^+/K^+-ATPase pump, disruption of membrane potentials, influx of sodium and calcium, and subsequent release of excitatory amino acids (such as glutamate, **Figure 2**). Engagement of glutamate with the ionotropic N-methyl-D-aspartate receptor (NMDA) leads to Ca^{2+} influx and activation of damaging proteases (e.g., phospholipase A_2, nitric oxide synthase, endonucleases, and calpain) that compromise the functional and structural integrity of neuronal cells within 20-60 minutes (**Figure 2**). Early-stage therapeutics that block ion (Na^+ and Ca^{2+}) channels (Taylor & Meldrum 1995) and glutamate receptors (Meldrum 1990) fail in clinical trials, partly because of the impracticalities of administering such drugs at a time when those mechanisms are already activated. These failures have prompted the search for downstream targets that also mediate ischemic injury.

4.2.1.2 Secondary late injury in the penumbral zone

Outside of the ischemic core where cells are destined to die lies a penumbral zone where brain cell death continues slowly for hours and even days after the onset of ischemia (**Figure 2**). This progressive expansion of cell death in the penumbra (i.e., secondary injury) is mediated by ischemia-elicited inflammatory responses. Within a few hours, microglia and neurons become activated to produce TNF and other cytokines (Kato *et al.* 1996;Botchkina *et al.* 1997). Subsequently, polymorphonuclear cells infiltrate into the ischemic brain tissue within 12-48 hours (Akopov *et al.* 1996), followed by an influx of monocytes and macrophages over a period of one to several days. Together, these centrally- and peripherally-derived cells orchestrate a potentially injurious inflammatory response by overproducing various proinflammatory cytokines (**Figure 2**).

Many pro-inflammatory cytokines (e.g., TNF and IL-1) contribute to cerebral ischemic injury (Buttini *et al.* 1996;Zaremba & Losy 2001), because inhibition of their production (Meistrell *et al.* 1997;Bertorelli *et al.* 1998) or activity (Barone *et al.* 1997;Yang *et al.* 1999) confers protective effects. In addition, an ubiquitous nuclear protein, HMGB1, can be passively released from the ischemic core, and spilled into the surrounding periphery (Qiu *et al.* 2008). In the penumbra, it amplifies a potentially injurious inflammatory response by inducing various cytokines, chemokines, tissue factor and adhesion molecules (Andersson *et al.* 2000;Lv *et al.* 2009;Fiuza *et al.* 2003;Treutiger *et al.* 2003) (**Figure 2**). Indeed, HMGB1-specific neutralizing antibodies and antagonists (e.g., the A box) have been proven protective (Liu *et al.* 2007a;Muhammad *et al.* 2008), supporting a pathogenic role for HMGB1 in ischemic injury.

4.2.2 Divergent roles of spermine in cerebral ischemic injury

Another abundant molecule, spermine, can also be passively released by injured cells (Paschen 1992). At higher (millimolar) concentrations, spermine could be neuroprotective by binding and blocking the NMDA receptor (Araneda *et al.* 1999;Ferchmin *et al.* 2000). In addition, it counter-regulates expression of inflammatory cytokines (Zhang *et al.* 2000;Zhang *et al.* 1997;Zhang *et al.* 1999;Zhu *et al.* 2009) and scavenges free radicals (Ha *et al.* 1998;Adibhatla *et al.* 2002). However, spermine can be enzymatically converted by polyamine oxidases into cytotoxic metabolites (e.g., 3-aminopropanal) (Ivanova *et al.* 1998), which readily spreads and mediates direct cytotoxicities (Ivanova *et al.* 1998). At low (micromolar) concentrations, spermine activates the NMDA receptor (Zubrow *et al.* 2000;Ferchmin *et al.* 2000;Williams 1997), thereby augmenting glutamate-mediated neurotoxicity by overactivating Ca^{2+} fluxes and disturbances of the calcium homeostasis.

During cerebral ischemia, brain spermine levels are decreased (Paschen *et al.* 1992), owing largely to an accompanying increase in the enzymatic activity of brain polyamine oxidase (Ivanova *et al.* 1998). The loss of spermine consequently tilts the balance towards neurotoxicity through activating the NMDA receptor, and increasing susceptibility to oxidative stress as well as excessive inflammatory response.

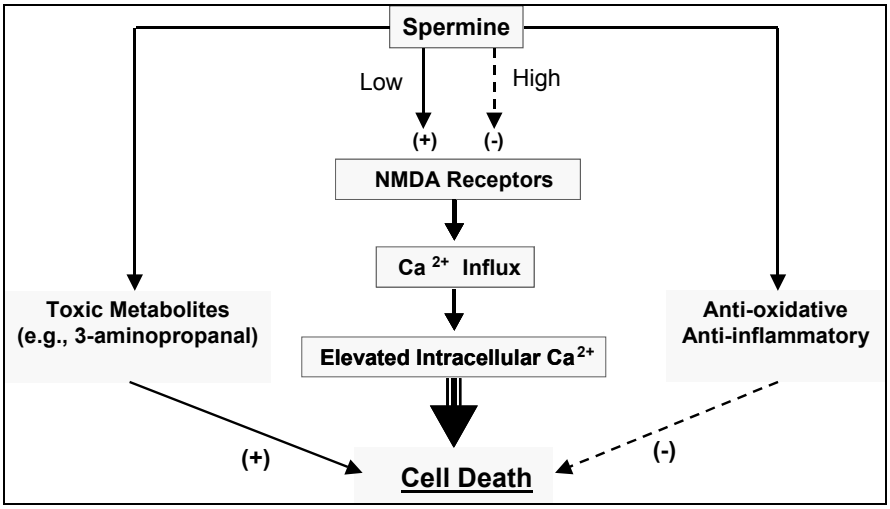

Fig. 3. **Divergent roles of spermine in cerebral ischemic injury.**

4.2.3 Peripheral administration of fetuin-A reduced cerebral ischemic injury

As mentioned earlier, when given peripherally, exogenous fetuin-A gains entry across the BBB into the ischemic brain tissue (**Figure 4**). The time-course of fetuin-A extravasation in the ischemic brain tissue parallels with the time-dependent alteration of the BBB permeability (Belayev *et al.* 1996), which was transiently elevated (5 -25 h post MCAo) followed by a return towards baseline at 72 h post MCAo (Belayev *et al.* 1996). It is possible that the temporal breakdown of the BBB is required for circulating fetuin-A to transiently gain entry into the brain. Consistently, peripheral administration of fetuin-A (50 mg/kg) promoted a dose-dependent protection against cerebral ischemic injury during an early stage of cerebral ischemia (i.e., 24 h post MCAo) (Wang *et al.* 2010). However, the fetuin-A-mediated protection was not long-lasting, and gradually diminished at a later stage (e.g., 7 days post MCAo). It is possible that the restore of BBB function at a late stage (3 days after MCAo) limits subsequent fetuin-A extravasation, thereby diminishing fetuin-A-mediated long-lasting protective effects.

Given the aforementioned pathogenic roles of Ca^{2+} and spermine in cerebral ischemia (in **section 4.2.1 and 4.2.2**), as well as the capacity of fetuin-A in binding Ca^{2+} and spermine (in **secton 3.2 and 3.3**) (Suzuki *et al.* 1994;Wang *et al.* 1997), it is plausible that fetuin-A confers protection by caging these toxic cationic molecules (Lee *et al.* 1999;Ivanova *et al.* 1998), thereby depriving them from damaging enzymes (such as Ca^{2+} -dependent proteases and polyamine oxidase). Furthermore, the fetuin-A-mediated protection is associated with a reduction of ischemia-elicited HMGB1 leakage from the ischemic core, and an inhibition of

expression of proinflammatory cytokines (e.g., TNF) in the penumbra (Wang *et al.* 2010) (**Figure 4**), suggesting that fetuin-A confers protection partly by attenuating early inflammatory responses.

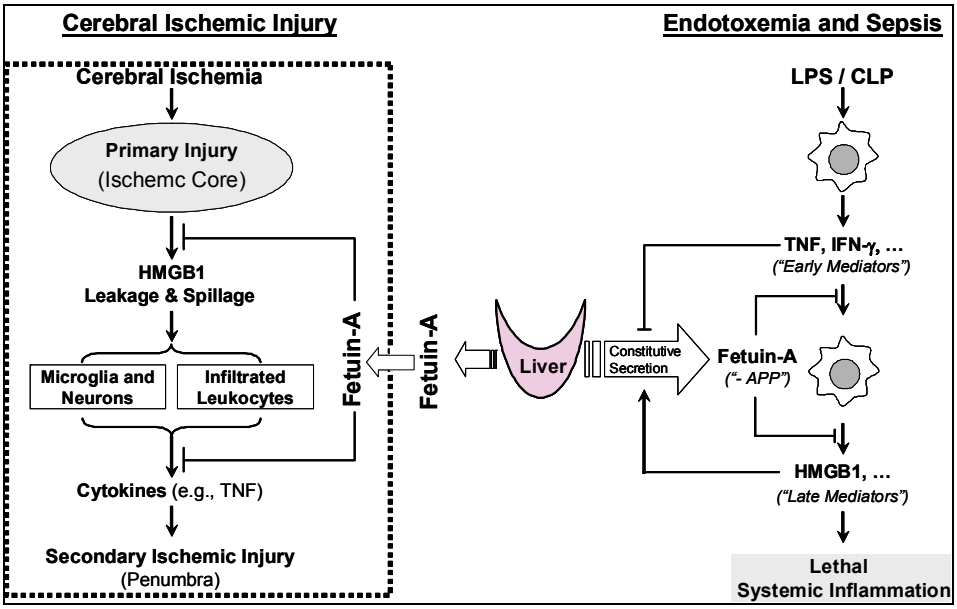

Fig. 4. **Protective roles of fetuin-A in cerebral ischemic injury and sepsis.**

4.3 Experimental sepsis

Sepsis is the most common cause of death in intensive care units, claiming approximately 225,000 victims annually in the U.S. alone. The high mortality of sepsis is in part mediated by bacterial endotoxin , which activates macrophages and monocytes to sequentially release early (e.g., TNF and IL-1) (Dinarello 1996) and late (e.g., HMGB1) proinflammatory cytokines.

4.3.1 Pathogenesis of sepsis

The pathogenesis of sepsis is partly attributable to dysregulated systemic inflammatory responses that are initiated by early proinflammatory cytokines and sustained by late-acting proinflammatory mediators. For instance, excessive accumulation of early proinflammatory cytokines, including TNF (Tracey *et al.* 1987), interleukin (IL)-1 (Dinarello & Thompson 1991), interferon (IFN)-γ (Heinzel 1990), individually or in combination, contribute to the pathogenesis of lethal systemic inflammation. Because these early cytokines are difficult to target in clinical settings, we searched for other late proinflammatory mediators that may offer a wider therapeutic window.

As aforementioned, HMGB1 is released from activated innate immune cells in response to microbial products (such as endotoxin or CpG-DNA) (Wang *et al.* 1999;Ivanov *et al.* 2007), or host cytokines (e.g., TNF or IFN-γ) (Wang *et al.* 1999;Rendon-Mitchell *et al.* 2003), and

functions as a late mediator of endotoxemia and sepsis (Wang *et al.* 1999;Yang *et al.* 2004;Wang *et al.* 2008;Wang *et al.* 2009). In murine models of endotoxemia and sepsis, HMGB1 is first detectable in the circulation eight hours after the onset of diseases, subsequently increasing to plateau levels from 16 to 32 hours (Wang *et al.* 1999;Yang *et al.* 2004) (**Figure 4**). This late appearance of circulating HMGB1 parallels with the onset of animal lethality from endotoxemia or sepsis, and distinguishes itself from TNF and other early proinflammatory cytokines (Wang *et al.* 2001). Therefore, agents capable of selectively attenuating systemic HMGB1 accumulation at a late stage may hold potential in the treatment of lethal sepsis.

4.3.2 Dual roles of spermine in experimental sepsis

In light of the anti-inflammatory activities of spermine *in vitro* (Zhang *et al.* 1997;Zhu *et al.* 2009), we evaluated the effects of spermine on animal survival in animal models of sepsis. Intraperitoneal administration of spermine (1.0 -10 mg/kg, twice daily, for three days) did not protect mice against lethal endotoxemia, but confers a dose-dependent protection against lethal sepsis. This protection was associated with a significant attenuation of systemic accumulation of HMGB1 and other cytokines (e.g., IL-6, KC, MCP-1, MIP-2, TIMP-1, sTNFRI and sTNFRII) (Zhu *et al.* 2009). At a higher dose (100 mg/kg), however, spermine decreased animal survival rate from 58% to 38% at 48 h post CLP, and further decreasing it to 0% at 72 h post CLP. It is possible that spermine is enzymatically converted by polyamine oxidases into cytotoxic metabolites (e.g., 3-aminopropanal), thereby exerting these potentially toxic effects when given at higher doses.

4.3.3 Protective role of Fetuin-A in sepsis

To understand the role of fetuin-A in systemic inflammatory diseases, we determined the influence of fetuin-A disruption on endotoxemic and septic lethality. Although fetuin-A-deficient C57BL/6J mice were not more susceptible to cerebral ischemic insult than sex- and body-matched (male, 27-29 g) wild-type C57BL/6J mice (Wang *et al.* 2010), they were more susceptible to lethal endotoxemic or septic insult (Li *et al.* 2011a). It suggests that endogenous fetuin-A occupies an integral role in host defense against lethal systemic inflammation.

The protective role of fetuin-A was further supported by the observations that supplementation with exogenous fetuin-A (20-100 mg/kg) provided a dose-dependent protection against lethal endotoxemia (Li *et al.* 2011a). In an animal model of sepsis, delayed administration of fetuin-A (20 - 100 mg/kg), beginning 24 h *after* the onset of sepsis and followed by an additional dose at 48 h post CLP, dose-dependently and significantly increased long-term animal survival rates from 45% to 90% (Li *et al.* 2011a).

4.3.4 Protective mechanisms

Supplementation of fetuin-A was associated with significant reduction of circulating HMGB1 levels, suggesting that fetuin-A confers protection by inhibiting late-acting proinflammatory mediators (Li *et al.* 2011a). The mechanisms underlying fetuin-A-mediated suppression of HMGB1 release may be complex. At the concentrations (100 μg/ml) that fetuin-A attenuated LPS-induced HMGB1 release in macrophage cultures, fetuin-A stimulated autophagy and impaired LPS-induced elevation of cytoplasmic and nuclear HMGB1 levels (Li et al. 2011a). It is presently unknown whether fetuin-A reduces

cytoplasmic HMGB1 levels by stimulating its degradation in an autophagy-dependent fashion, as what has been shown for other HMGB1 inhibitors such as EGCG, the major catechin of Green tea (*Camellia sinensis*) (Li *et al*. 2011b).

Accumulating evidence has suggested the possibility that fetuin-A functions as a negative regulator of HMGB1 release during lethal systemic inflammation (**Figure 4**). First, the time-dependent decrease of circulating fetuin-A levels was accompanied by parallel but opposite changes – a time-dependent increase - of circulating HMGB1 levels in animal models of endotoxemia (Wang *et al.* 1999) or sepsis (Yang *et al.* 2004). Second, disruption of fetuin-A expression led to elevation of serum HMGB1 levels in endotoxemia and sepsis *(Li et al. 2011a)*. Lastly, supplementation of fetuin-A resulted in significant reduction of circulating HMGB1 levels during endotoxemia and sepsis *(Li et al. 2011a)*.

Nevertheless, the current study can not exclude other alternative mechanisms by which fetuin-A confers these protective effects. For instance, fetuin-A may be capable of binding bacteria (Chmiela *et al.* 1997;Dubreuil *et al.* 2002), thereby affecting macrophage-mediated pathogen elimination. Furthermore, fetuin-A may facilitate macrophages-mediated ingestion and elimination of apoptotic neutrophils (Lord 2003;Jersmann *et al.* 2003), thereby preventing secondary necrosis and passive leakage of injurious molecules (e.g., proteases, reactive oxygen species, and HMGB1) (Bell *et al*. 2006).

5. Conclusions

A liver-derived acute phase protein, fetuin-A, appears to be distinctly regulated by different proinflammatory mediators. A previously under-appreciated protective role for fetuin-A in injury and infection has been suggested by recent studies. Fetuin-A is capable of crossing the blood-brain barrier, inhibiting early inflammatory response in animal models of cerebral ischemia, thereby conferring a short-term neuroprotection against ischemic injury. Disruption of fetuin-A expression renders mice significantly more susceptible to lethal endotoxemia or sepsis; whereas repetitive administration of fetuin-A confers a dose-dependent and long-lasting protection in animal models of lethal endotoxemia and sepsis. Thus, fetuin-A occupies protective roles against injury- or infection-elicited inflammation.

6. Acknowledgment

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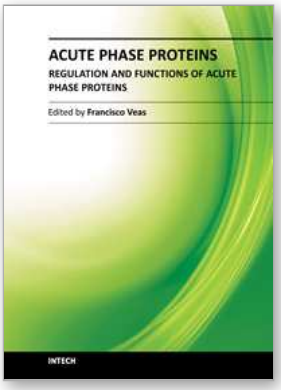

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The two volumes of Acute Phase Proteins book consist of chapters that give a large panel of fundamental and applied knowledge on one of the major elements of the inflammatory process during the acute phase response, i.e., the acute phase proteins expression and functions that regulate homeostasis. We have organized this book in two volumes - the first volume, mainly containing chapters on structure, biology and functions of APP, the second volume discussing different uses of APP as diagnostic tools in human and veterinary medicine.

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