THE AUTODIGESTION HYPOTHESIS IN SHOCK AND MULTI-ORGAN FAILURE: DEGRADING PROTEASE ACTIVITY

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ABSTRACT

Shock and multi-organ failure have one of the highest levels of inflammatory markers, morbidities and mortality. The underlying mechanisms are currently unknown and no effective intervention exists. We present evidence for a previously untested mechanism due to *autodigestion* by the digestive enzymes synthesized in the pancreas and transported in the lumen of the intestine as normal part of food digestion. We summarize experimental evidence in support of the autodigestion hypothesis and a new approach for possible intervention against multi-organ failure that is currently entering clinical trials.

Key-words: Intestine; digestive pancreas; inflammation; trypsin; chymotrypsin; elastase; intestinal mucosa; hemorrhagic shock; sepsis.

INTRODUCTION

Multi-organ failure after shock, with its high mortality, is one of the most important clinical problems. No mechanism has yet been proposed that enjoys universal acceptance. We present here a new proposal for the rapid organ failure in shock that is linked to the digestive system.

To introduce the idea we consider the following question: What mechanisms allow digestion of a meal, even ingested intestine, while preventing digestion of one's own intestinal tissue? Why does our intestine not digest itself? Nature had to find a solution to this problem long before humans or other mammals walked the earth, and thus any protection mechanism against autodigestion is likely developmentally old and of course robust. That does not imply that the protection is limitless. We

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propose here that failure to protect against autodigestion by our own digestive enzymes may be a cause for multi-organ failure.

A Common Denominator in Human Diseases

In the past decades a large body of clinical evidence has brought to light evidence supporting the idea that the majority of human diseases are accompanied by significantly elevated levels of markers for inflammation (Ballantyne and Nambi, 2005; Blake and Ridker, 2001; Claus *et al.*, 2010). This evidence follows previous decades of experimental and smaller clinical studies analyzing many details of the inflammatory cascade (Zweifach *et al.*, 1974). The analysis brings to light that preclinical and clinical evidence is in agreement about a role of inflammation in human disease. Inflammation has moved into the center of research in many branches of medicine, from hypertension, diabetes, atherosclerosis, stroke, cardiac infarction, renal failure, chronic degenerative diseases, to cancer, to name just a few.

This development raises important questions about the significance of the inflammatory cascade in disease. Why would such a diversity of diseases in different organs utilize common biochemical and biophysical pathway? What mechanisms and pathways stimulate an inflammation?

Inflammation as requirement For tissue repair

The inflammatory cascade serves a lifetime for wound healing and tissue repair. The ability to repair is quite evident when we consider a small tissue injury, e.g. a cut into the skin. Following the initial injury, there is a stereotypic cascade of events that at the cellular level starts with:

- cell activation in the form of a transmembrane ion exchange and spontaneous degranulation by multiple cell types;
- breakdown of cell membrane adhesion mechanisms, e.g. elevated permeability of the vascular and lymphatic endothelium and other cell layers;
- expression of membrane adhesion molecules to facilitate binding of circulating cell to the endothelium including a specific sequence of steps for leukocyte adhesion that is highly cell-type specific;
- migration and differentiation of cells into the injured tissue;
- removal of injured cells and extracellular matrix fragments by apoptosis or necrotic phagocytosis;
- to the eventual generation of new tissues by local mitosis with growth factors and by infiltration and differentiation of stem cells.

At the end of this cascade is *resolution of inflammation* in the form of a new scar tissue that may or may not have the same structure and function as the injured tissue that it replaces (Carlo and Levy, 2010; Gonzalez--Periz and Claria, 2010; Gronert, 2010; Lawrence and Fong, 2010; Soehnlein and Lindbom, 2010). The repair process requires days and weeks and is repeated many times in life. According to our current understanding it is the *only repair* mecha-

nism in living tissues (Schmid-Schönbein, 2006).

Thus, if one looks at inflammatory makers as a sign of the repair mechanism in action, the immediate question arises for many diseases: What event caused injury to the tissue in the first place and triggered the inflammatory repair cascade? We have a keen interest to answer this important question, since it may serve as the key to preventive measures. In the following we will investigate these questions for the case of shock and multi-organ failure.

Tissue Injury in Shock

In physiological shock many injury mechanisms have been proposed, e.g. infections (viral, bacterial, fungal), trauma, exposure to elevated or reduced mechanical stress, extreme temperatures, and chemical exposures to name a few. Depletion of anti-inflammatory pathways may also trigger an inflammation.

However, it is also evident that other mechanisms exist. For example in hemorrhagic and in several forms of septic shock, severe inflammation can be rapidly generated in the absence of any of the tissue injury mechanisms listed above. Anti-biotic treatment has so far been largely ineffective in clinical trials of septic patients and so have been interventions against mediators derived from infection (e.g. endotoxin) or against some inflammatory mediators/markers (e.g. TNFα, Il-1, complement) (Brierre *et al.*, 2004; Derkx *et al.*, 1999; Harlan and Winn, 2002; Kalil and Sun, 2011; Kumar *et al.*, 2010; Solomkin, 1994; Zanotti and Kumar, 2002; Ziegler, 1988). The lack of positive outcomes of clinical trials suggests that even though these markers of inflammation may be present in shock plasma, they themselves may not be the cause for the tissue injury in shock.

Shock and Multi-Organ Failure: Plasma-Derived Mediators

It is useful to look more in detail into the properties of the plasma that one can collect in shock. Shock plasma (and also lymphatic fluid derived from the intestine) contains numerous inflammatory mediators. In hemorrhagic shock for example, the earliest signs of inflammation in the form of enhanced circulating leukocyte activation can already be detected within minutes after reduction of central blood pressure (Barroso-Aranda *et al.*, 1995). It is apparent that there is early proinflammatory signal generation that does not require de novo gene expression. Within one hour this cell activation reaches levels that can help to discriminate between survivors and non-survivors (Barroso-Aranda and Schmid-Schönbein, 1989).

The plasma of animals in shock exhibits a diversity of activities, e.g. it activates naive donor leukocytes and at the same time depresses many normal cell functions. In the case of the heart muscle this property has been designated as *myocardial depressing factor* (Lefer, 1974).

A number of candidate mediators have been proposed to explain the proinflammatory activity in the plasma, e.g. endotoxin, complement cascade products, cytokines, arachado-

nic acid products, to name a few. Depletion of anti-inflammatory mediators has also been suggested (e.g. IL-10, glucocorticoids).

Decades of research have not led to identification of a unique chemical entity responsible for the proinflammatory activity in plasma of shock animals or humans. Instead we propose here a new approach to this important problem.

The Pancreatic Enzymes in Inflammation

The idea is as follows: If early during hemorrhagic shock (when only blood volume is reduced and no agents or drugs were administered) inflammatory mediators are detected then the implication is that these mediators must be pre-formed or otherwise rapidly produced, rather than synthesized de novo. Analysis of homogenates from different tissues in the rat shows that the *pancreas* – but less so other organs – is able to generate in a short period of time (minutes) powerful proinflammatory (in form of leukocyte activation) and even cytotoxic mediators (Kistler *et al.*, 2000a). The intestine is also able to do so, but only if pancreatic digestive enzymes are present (Penn *et al.*, 2007). In the absence of pancreatic digestive enzymes in the lumen of the intestine, intestinal homogenates produce low levels of inflammation. Similarly, other organ homogenates (heart, brain, liver, kidney and others) induce low levels of inflammation, but they are equally inflammatory or cytotoxic if mixed with pancreatic digestive enzymes (Penn *et al.*, 2007; Waldo *et al.*, 2003). This evidence implicates *pancreatic digestive enzymes* as key players in the formation of a pro-inflammatory mediator in plasma already during early periods of hemorrhagic shock.

Pancreatic enzymes are also implicated in the production of myocardial depressant factor, which is thought to be a proteolytically derived peptide of pancreatic origin (Lefer and Glenn, 1971). Direct test of the pancreatic homogenates shows that they activate neutrophils and simultaneously depress myocardial contraction (Kistler *et al.*, 2000b). Inflammation generated by pancreatic homogenates can largely be recreated by incubating previously non-inflammatory tissues with pancreatic enzymes such as trypsin and chymotrypsin.

The activity generated by the pancreas is largely derived from lower molecular weight constituent (<10 kD), implicating cleaved pancreatic peptides as inflammatory mediators (Kistler *et al.*, 2000b). In addition, free fatty acids formed in the autodigestion process have been implicated in inflammation generated by pancreatic homogenates. Systemic circulatory effects of the homogenates appear to be largely peptide related, while fatty acid production may account for a large proportion of white blood cell activation and direct cytotoxicity (Kramp *et al.*, 2003; Penn and Schmid-Schönbein, 2008; Waldo *et al.*, 2003).

Digestive Enzymes in the Intestine: What prevents Autodigestion?

The role of pancreatic enzymes within the lumen of the intestine is in agreement with many studies that

have recognized the special role of the intestine during shock (Chang, 1997). As part of its fundamental role in digestion, the intestine is the only organ that normally receives pancreatic digestive enzymes into its lumen. Under normal physiologic conditions, pancreatic digestive enzymes are activated by enterokinases in the intestinal lumen and digest most biological polymers into their monomeric constituents, thus facilitating transport across the mucosal barrier.

Thus we return to the question: What mechanisms prevent digestion of one's own intestine when such powerful digestive enzymes are present and activated? Current evidence points to the mucosal epithelial barrier as the predominant mechanism that serves to compartmentalize the digestive enzymes in the lumen of the intestine. Under normal physiological conditions intestinal permeability is low enough to prevent escape of digestive enzymes from the bowel lumen into the intestinal wall (including smaller proteases, such as trypsin (~19K Da). Circulating plasma protease inhibitors (e.g. serpins) act as a second buffer against intestinal protease leakage and deleterious systemic proteolytic activation (e.g. leukocyte elastase).

Entry of Digestive Enzymes into the Ischemic Intestinal Wall

The selective barrier properties of the mucosal epithelium depend on mucin secretion (Qin et al., 2010) and on the tight junctions between epithelial cells covering the villi (Perry et al., 1999). This barrier is essential to prevent entry of digestive enzymes into the intestinal wall. As a biological barrier it is, however, sensitive to many influences, including oxygen depletion, the presence of inflammatory mediators, the intestinal bacteria, and passage of partially digested food items.

In hemorrhagic shock the intestinal perfusion and oxygen levels are reduced. This process is sufficient to enhance the permeability of the mucosal barrier by opening the tight junctions between epithelial cells (Rollwagen et al., 2000) and allowing pancreatic digestive enzymes, like trypsin, access into the wall of the intestine. The digestive enzyme are transported into the villi and the smooth muscle layer, and even across the outermost collagen sheet of the intestine (serosa) into the peritoneum (Ishimaru *et al.*, 2004; Rosario *et al.*, 2004).

Pancreatic enzyme leakage into the intestinal wall is a truly catastrophic event for the structure and function of the intestine since there is little inhibition of digestive protease once inside the wall of the intestine. The intestinal villi are subject to enzymatic digestion and rapidly lose their morphological structure, the digested tissue detaches from the intestine to the point of complete cleavage of the villi down to their bases. This mucosal barrier and tissue destruction provides the digestive enzymes unimpeded access into the intestinal wall (Fig. 1) (Fitzal *et al.*, 2002; Mitsuoka *et al.*, 2000).

With the ensuing injury, the intestinal tissue and Peyer's patches swell and exhibit hemorrhage into the interstitial tissue, a sign of blood vessel wall destruction in the intestinal microvasculature. All interstitial struc-

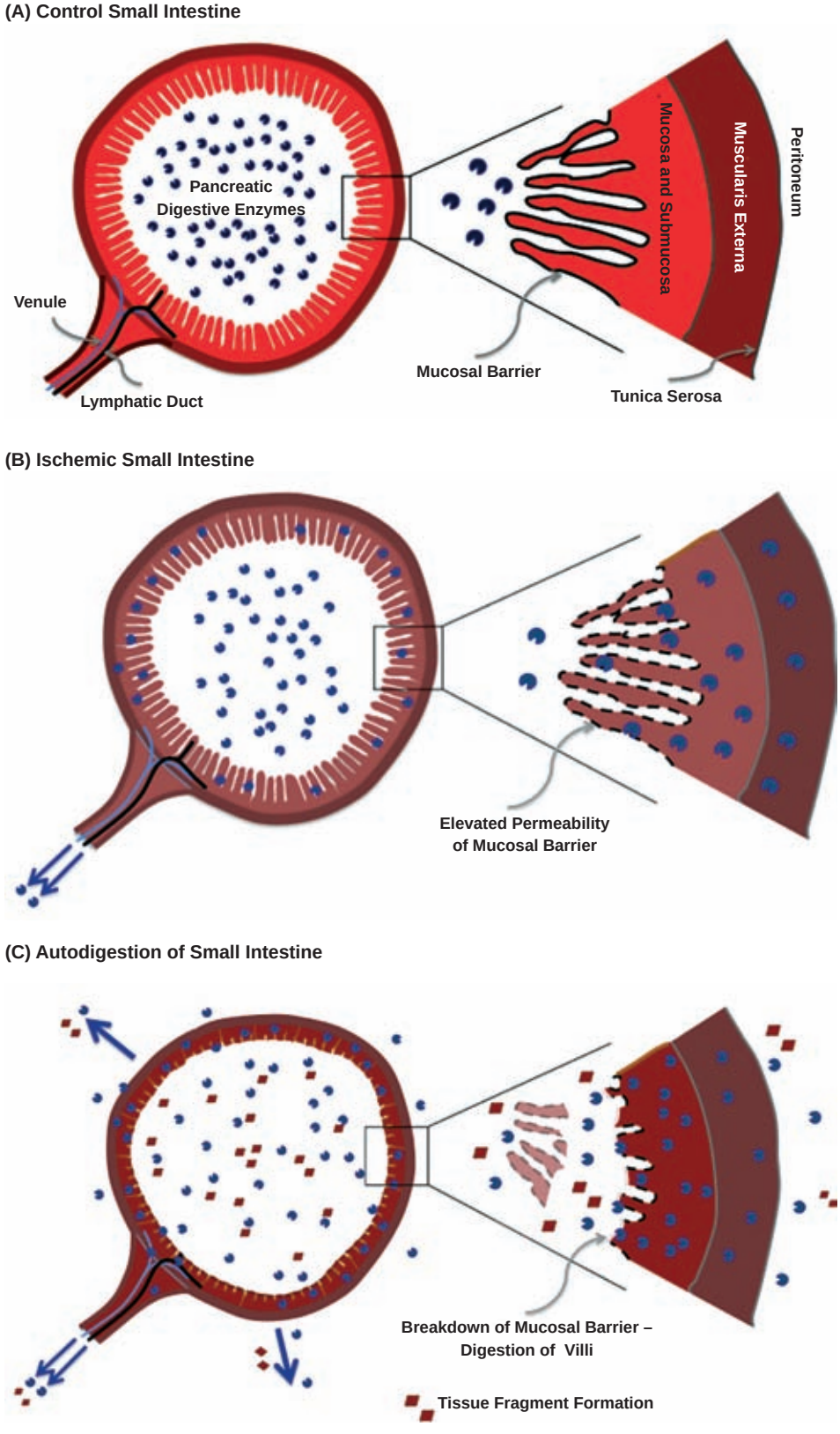

Fig. 1 – Schematic diagram of pancreatic digestive enzyme transport in the small intestine. (Panel A) Compartmentalization of digestive enzymes inside the lumen of a normal intestine by the mucosal barrier with minimal transport into the intestinal wall; (B) escape of digestive enzymes into the wall of an ischemic intestine after elevation of the mucosal barrier permeability, (C) autodigestion of the wall structures by the pancreatic digestive enzymes with loss of intestinal villi and loss of mucosal barrier function. During autodigestion (panel C), the digestive enzymes enter into venules and lymphatics draining the intestine (as long as they are not themselves enzymatically digested), and they pass across the serosa into the peritoneal space. In the intestinal wall the digestive enzymes generate a variety of tissue fragments (e.g. peptide and lipid fragments) that are transported in venules and lymphatics into the portal venous and the central circulation where they act as inflammatory mediators.

tures are destroyed. Molecular absorption by the mucosal barrier and intestinal peristalsis may be severely compromised since none of the cell and membrane structures responsible for these functions remain intact. In addition, attempts to interfere with this mucosal barrier breakdown by intervening with cell signaling pathways are likely to fail, since the cells required to signal may no longer be viable or even present.

Inflammatory Mediators Generated by Digestive Enzymes in the Wall of the Intestine

Besides destruction of tissue structure, entry of digestive enzymes into the wall of the intestine generates a second problem that arises during intestinal autodigestion; that of lipolytic and proteolytic degradation of intestinal tissue with subsequent generation of proinflammatory cytotoxic mediators.

Lipases in the lumen of the intestine as part of normal digestion break dietary triglycerides into non-esterified ("free") fatty acids and glycerol. At the concentrations at which they are present these free fatty acids are cytotoxic (Penn *et al.*, 2007). When the mucosal barrier fails, these fatty acids are able to enter the intestinal wall. Moreover, the lipases that enter the intestinal wall can generate even more free fatty acids from the intestinal tissue. The body's normal defense against necrosis from free fatty acids is to bind them to proteins such as the Fatty Acid Binding Proteins and albumin, which is ubiquitous in the plasma, lymph, and interstitial spaces. However, these binding proteins in the intestine can be destroyed by pancreatic proteases that cross the mucosal barrier, preventing them from binding the free fatty acids and further liberating any free fatty acids already bound (Penn and Schmid-Schönbein, 2008). Thus, digestive enzymes may result in intestinal tissue necrosis via creation and release of free fatty acids.

Fatty acids generated in the intestine may enter the circulation, stimulating apoptosis (Dersch et al., 2005) and inflammation elsewhere. Furthermore, the intestinal necrosis itself may release many inflammatory mediators into the circulation (HMGB1, mitochondrial DNA, etc.).

Proinflammatory Signals in the System Circulation

The mixture of degrading enzymes and small molecular weight fragments generates many proinflammatory signals that are detected in the portal venous blood and in intestinal lymphatics in shock. In the early phase of shock, the liver helps absorb these inflammatory products and systemic levels the mediators remain low in spite of an ischemic intestine. However, by the time proinflammatory signals appear in the systemic circulation (e.g. in the early period of blood volume restoration in hemorrhagic shock) the first signs of multi-organ dysfunction and failure become visible (Mitsuoka *et al.*, 2000). At this point there is enhanced pulmonary permeability and interstitial lung fluid accumulation, morphological damage with microhemorrhage in the pulmonary and cardiac circulations and typical signs of in-

flammation in peripheral organs, e.g. enhanced leukocyte adhesion to the endothelium with membrane adhesion molecule expression, cytokine production, mast cell degranulation, coagulation, and eventual apoptosis (Fitzal *et al.*, 2002), to name a few of the proinflammatory events.

Evidence derived from in-vivo experimental observations is in line with the clinical evidence for proinflammatory activity in the plasma of shock victims. However, patient plasma mediators and those found in tissue may not be the same, an issue that limits the utility of biomarkers in clinical samples.

Blockade of Digestive Enzymes in the Lumen of the Intestine

It is evident from the discussion above that to prevent entry of digestive enzymes into the wall of the intestine any intervention against autodigestion requires an action against the digestive enzyme activity in the intestinal lumen; in some medical situations it may also require action against the enzyme activity in the pancreas per se. What are the possibilities in this respect?

The *first line of defense* against autodigestion is to prevent elevation of mucosal permeability in the first place or to restore its functionality as soon as possible. If the mucosal barrier has already been compromised, a second line of defense is to block the activity of digestive enzymes and minimize their ability to autodigest host tissue. The first line of defense is less of an option in many trauma situations, but may be so in elective surgery.

Instead we will focus in the following on the *second line of defense*. This approach is to block the digestive enzymes directly in the lumen of the intestine where they are in high concentrations and can be reached by direct (enteral) administration of inhibitors to these enzymes into the lumen of the intestine. In a splanchnic artery occlusion model of shock enteral blockade of digestive protease leads to a significantly reduced autodigestion of the intestinal wall, it reduces the morphological damage and the inflammatory response (Mitsuoka *et al.*, 2000; Mitsuoka *et al.*, 2002) and attenuates multiorgan failure (Fitzal *et al.*, 2002) even when administered with some delay (Fitzal *et al.*, 2004) but before major tissue damage has occurred. There is significantly less swelling of the tissues, including the lung, and reduced signs for inflammation and organ failure in peripheral tissues (Fitzal *et al.*, 2002). This protection against autodigestion and its consequences can be achieved with different protease inhibitors but is not significantly improved by the addition of an oxygen free radical inhibitor to the protease blocker or by phospholipase inhibitors (Mitsuoka and Schmid-Schönbein, 2000).

A similar protection by enteral blockade of digestive proteases against autodigestion is observed also in *endotoxic shock* (Fitzal *et al.*, 2003). If the digestive enzymes in the lumen of intestine are blocked before endotoxin administration there is transient inflammation by the endotoxin but no progression into multi-organ failure. Even though a bolus administration of endotoxin into the circulation has the ability to stimulate an inflammation response, it is tran-

sient and not *directly* responsible for the lethal course into multiorgan failure. Instead, endotoxin may elevate the mucosal permeability in the intestine, so that digestive enzymes can enter the wall of the intestine and start an autodigestion process with eventual multi-organ failure.

Enteral blockade of digestive also reduces also the need for resuscitation fluid (Doucet *et al*., 2004) and it improves morbidity after shock (Kim *et al.*, 2010). Its utility in shock or septic patients remains to be tested.

SYNOPSIS

Multiple and independent pieces of preclinical evidence support the hypothesis that the pancreatic digestive enzymes in the lumen of the intestine, an integral part of normal food digestion, can be major mediators for cell and organ dysfunction in shock. If not compartmentalized in the lumen of intestine, pancreatic serine proteases will autodigest intestinal wall structures and promote inflammation and cell and organ dysfunction (Fig. 1). There exists a possibility to block these enzymes pharmacologically in the lumen of the intestine, which in preclinical studies has led to a significant reduction of markers for multi-organ failure.

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CONFLICT

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