Assessment of Serum Zonulin as a Marker of Altered Intestinal Permeability in Patients with Multiple Sclerosis

Thesis

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By

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List of Abbreviations

Abb.	Full term
APCs	*Antigen presenting cells*
CD	*Cluster of differentiation*
CNS	*Central nervous system*
CO	*Carbon monoxide*
DCs	*Dendritic cells*
EAE	*Experimental autoimmune encephalomyelitis*
EBV	*Epstein Barr virus*
FABP	*Fatty acid binding proteins*
FasL	*Fas ligand*
GABA	*Gamma amino buteric acid*
GALT	*Gut associated lymphoid tissue*
GSTs	*Glutathione S-transferases*
HLA	*Human leukocyte antigen*
IBD	*Inflammatory bowel disease*
IL	*Interleukin*
INF	*Interferon*
LPS	*Lipopolysaccharide*
MAGKH	*Membrane-associated guanylate kinase homologs*
MBP	*Myelin basic protein*
MOG	*Myelin oligodendrocyte glycoprotein*
MS	*Multiple sclerosis*
NO	*Nitric oxide*

List of Abbreviations (cont...)

Abb.	Full term
PD	*Parkinson's disease*
PPMS	*Primary progressive multiple sclerosis*
PSA	*Polysaccharide A*
RA	*Rheumatoid arthritis*
RRMS	*Relapsing remittent multiple sclerosis*
SCFA	*Short chain fatty acids*
SIRS	*Systemic inflammatory response syndrome*
SPMS	*Secondary progressive multiple sclerosis*
T reg	*Regulatory T cells*
TGF	*Transforming growth factor*
TJ	*Tight junctions*
TNF	*Tumor necrosis factor*
ZO	*Zonula occludens*

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INTRODUCTION

The pathogenic process of multiple sclerosis is immune-mediated. A change in intestinal permeability may be considered biomarker of local or even distant immune-mediated disorders. Increased gut permeability allows the passage, through the intestinal epithelial layer, of macromolecules, toxins, and bacterial species (both pathogenic and commensal) that may trigger immune-mediated diseases in different systems even distant from the gastrointestinal tract, such as the central nervous system.

Multiple sclerosis (MS) is an autoimmune-mediated disorder that affects the central nervous system (CNS) and often leads to severe physical or cognitive incapacitation as well as neurological problems in young adults ***(Compston and Coles, 2008).***

Multifocal zones of inflammation due to focal T-lymphocytic and macrophage infiltrations, and oligodendrocyte death are the primary causes of myelin sheath destruction that result in the formation of CNS plaques composed of inflammatory cells and their products, demyelinated and transected axons, and astrogliosis in both white and gray matter. These lesions interrupt with the correct transmission of nerve impulses and lead to neuronal dysfunction such as autonomic and sensorimotor defects, visual disturbances,

ataxia, fatigue, cognitive and emotional problem ***(Loma and Heyman, 2011).***

The etiology of MS remains unclear, however it can be considered a multifactorial disease and include a genetic predisposition combined with environmental influences ***(Hatch et al., 2009).***

Environmental factors, including exposure to viral and bacterial agents such as Epstein Barr virus (EBV), human herpes virus type 6, and mycoplasma pneumonia are associated with the onset of MS. The foreign agents may have a nuclear antigen that is structurally homologous with myelin sheet components such as proteolipid protein, myelin basic protein, and myelin-associated glycoprotein. Thus, when immune cells are activated by these pathogens, myelin sheath lesions will form ***(Fujinami et al., 2006; Sloka et al., 2011).***

Among this multiplicity of factors, microbiota and gut function are increasingly recognized as relevant in autoimmune disorders. An altered physiology of gut mucosa and/or of the gut-associated lymphoid tissue may impact intestinal permeability (IP), leading to an increase in epithelial paracellular space. A change in IP may be considered a biomarker of local or even distant immune-mediated disorders. In fact, increased gut permeability allows the passage, through the intestinal epithelial layer, of macromolecules, toxins, and bacterial species, both pathogenic and commensal that may

trigger immune-mediated diseases in different systems even distant from the gastrointestinal tract, such as the CNS. Conversely, CNS inflammation can increase gut permeability and alter the mucosal structure in the small intestine ***(Nouri et al., 2014; Haghikia et al., 2015).***

Tight junctions are the most apical junctional complex connecting both neighboring epithelial and endothelial cells and are comprised of transmembrane proteins. These transmembrane proteins interact between themselves and with intracellular scaf folding proteins, including zonulaoccludins (ZOs), which are anchored to the actin cytoskeleton. The interaction of transmembrane proteins between cells and with ZOs maintains the integrity of the tight junction and controls the passage of molecules through the paracellular space. Zonulin is the only physiological mediator that is known to influence gut barrier permeability by deranging intestinal tight junctions ***(Higashi et al., 2013).***

Zonulaoccludens toxin (Zot) is an enterotoxin which is able to reversibly open intracellular tight junctions. Zot is able to interact with epithelial cells along the gastrointestinal tract with the highest binding in the jejunum and distal ileum. Given the complexity of the intracellular signaling activated by Zot leading to tight junction modulation, it was hypothesized that the toxin may mimic an endogenous protein which is able to regulate the epithelial tight junctions. Zonulin is a human analog to Zot. Ex vivo studies show endogenous human zonulin

is able to increase permeability in both the jejunum and ileum ***(Fasano et al., 1991; Fasano et al., 2000).***

The inappropriate production of increased amount of Zonulin causes a functional loss of barrier function, with subsequent inappropriate and uncontrolled antigen trafficking instigating an innate immune response by the submucosal immune compartment. If this process continues, an adaptive immune response is mounted causing production of pro-inflammatory cytokines, including IFN-ɣ and TNF-α that cause further opening of the paracellular pathway to the passage of antigens, creating a vicious cycle. Ultimately, these processes lead to break of tolerance with subsequent onset of chronic inflammatory disease.

A study focused on the experimental autoimmune encephalomyelitis (EAE) mouse model of MS has further described how Zonulin is involved in MS. Intestinal permeability and intestinal Zonulin are increased during the pre-clinical phase of neurological symptoms, suggesting a role for Zonulin in disease development ***(Nouri et al., 2014).***

Interestingly, patients with progressive MS showed increased levels of serum Zonulin, while those with relapsing-remitting MS who were in remission showed serum Zonulin levels similar to controls ***(Fasano, 2011).***

Aim of the Work

To investigate the possible association between IP changes and MS, through measurement of serum Zonulin in different population of MS, this may affect treatment of MS next years.

Chapter 1

PATHOGENESIS OF MS

Multiple sclerosis (MS) is an autoimmune-mediated disorder that affects the central nervous system (CNS) and often leads to severe physical or cognitive incapacitation as well as neurological problems in young adults. Multifocal zones of inflammation due to focal T-lymphocytic and macrophage infiltrations, and oligo dendrocyte death are the primary causes of myelin sheath destruction that result in the formation of CNS plaques composed of inflammatory cells and their products, demyelinated and transected axons, and astrogliosis in both white and gray matter. These lesions can cross-talk with the correct transmission of nerve impulses and lead to neuronal dysfunction such as autonomic and sensorimotor defects, visual disturbances, ataxia, fatigue, difficulties in thinking, and emotional problems ***(Weiner, 2008; Goldberg et al., 2009).***

Demyelinated MS lesions are classified on the basis of myelin ladden macrophages. In acute and chronic active lesions the axons are commonly preserved while many macrophages are present in these lesions that have taken up myelin. In contrast, in inactive plaques there is loss of axons and oligodendrocytes and only few macrophages or/and microglia cells are present. There are also smoldering plaques that represent an intermediate stage of chronic active and inactive

lesions with a hypocellular center and the presence of macrophages at the sharp borders. Shadow plaques represent remyelinated lesions. In relapsing remittent multiple sclerosis (RR-MS) and secondary progressive multiple sclerosis (SP-MS) with relapses (rSP-MS), acute and chronic active plaques are quite typically found. In SP-MS without relapses (nrSP-MS) and primary progressive multiple sclerosis (PP-MS) most lesions are inactive lesions ***(Popescu and Lucchinetti, 2012).***

Cortical plaques are found in early and in late MS. For long time they had been ignored, even though they had been described in the literature. They are divided in type I lesions that have leucocortical location and involve the grey and adjacent white matter. Type II lesions are located intracortical and purely lie within the cortex and type III lesions extend from and run parallel to pial surface. The mechanisms that lead to this kind of pathology are not well known so far, but most probably there is an immune response against the sparse myelin in this region and also against neuronal antigens ***(Peterson et al., 2001; Trapp and Nave, 2008).***

In figure 1: Auto reactive T cells that migrate across the blood–brain barrier (BBB) are activated locally by immune cells in the CNS (microglia and astrocytes), leading to CNS inflammation ***(Constantinescu and Gran, 2014).***

Cortical plaques are associated with affection of cognition and cognitive decline and disease progression. While