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Extracellular Mitochondrial Components Secreted from Activated Live Mast Cells Act as "Innate Pathogens" and Contribute to Autism Pathogenesis

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Abstract— **Allergies, asthma and autism have reached epidemic proportions, but the reason(s) why remain elusive. Recent evidence indicztes that mitochondria, commonly known for their cellular energy production, undergo fission and translocation to the cell surface where they secrete some of their components extracellularly. These include DNA and ATP, which are misconstrued by the body as "innate pathogens" leading to an autoinflammatory response that may explain many sterile inflammatory disorders, especially autism.**

Index Terms Allergies, asthma, ATP, autism, autoimmunity, DNA, inflammation, innate pathogens, mast cells, mitochondria,

Allergies and asthma,[1], but also autism [2] have reached epidemic proportions over the last ten years. Autism is a neurodevelopmental disorder characterized by impaired social interactions, language loss, and stereotypic behaviors. Recent results from the Centers of Disease Control in the USA indicate that as many as 1/80 children have ASD [3]. Many such children regress at about age 3 years, often after a specific event such as reaction to vaccination, infection [4;5], trauma [6;7], or toxic exposures [8] or stress [9] implying the importance of some environmental triggers [10;11].

It is of interest that "allergic-like" reactions are common in autistic children [12;13] [14] implying activation of mast cells by non-allergic triggers [15]. The richest source of mast cells in the brain is the diencephalon [16] that regulates behavior. Mast cells are responsible for eliciting neutrophil infiltration that promotes inflammation [17]. Mast cell-microglial interactions are important in neuroinflammatory diseases [18;19]. Microglia are the innate brain immune cells that are increasingly implicated in a number of neuropsychiatric diseases [20]. In fact, abnormal microglial growth and activation was recently reported in the brain of ASD patients [21;22]. It is interesting that autistic patients exhibit neuro-immune aspects [23], and that children with mastocytosis have a higher risk of developing autism [24]. The cost of autism alone has been estimated to be $126 billion per year in the US [25].

All these conditions involve sterile inflammation and worsen with stress, but the pathogenesis is unknown, thus hampering the development of effective treatments. A possible common link appears to be the mast cells [26]. The effect of stress has been shown to be dependent on mast cells in the brain [27], lungs [28], and skin.

Mast cells are hematopoietic tissue immune cells that secrete pre-stored mediators, such as histamine and tryptase through degranulation, as well as numerous *de novo* synthesized chemokines and cytokines in response to allergic or non-immune triggers [30;31]. Interestingly, mast cells are the only cell type that stores pre-formed tumor necrosis factor (TNF) in secretory granules [32]. Moreover, mast cells have the ability to release their numerous mediators selectively not recognizable by routine histology [33], making diagnosis and the triggers involved .difficult to identify. Nevertheless, increasing evidence indicates that mast cells participate in innate and acquired immunity [34], as well as in inflammation [26].

We recently reported that during mast cell degranulation, mitochondria undergo fission and move to the cell surface [35] from where they release mitochondrial ATP, DNA and other components that are misconstrued by the body as "innate pathogens" and induce a strong auto-inflammatory response [36]. This finding is in line with the late Lynn Margulis' work (Fig. 1) indicating that mitochondria were bacteria that became symbiotic with eukaryotic cells [37]. Mast cells also express TLRs, including TLR9 that can be activated by bacterial DNA sequences, leading to release of different cytokines [38] that allow mast cells to participate in immunity against bacteria [39]. Given that mitochondria were bacteria that became symbiotic with eukaryotic cells [40], mitochondria health is regulated by autophagy that prevents mitochondria from being released outside the cell [41;42].

Mitochondria are the primary energy-generating organelles in eukaryotic cells [43], but they also participate in multiple intracellular processes and diseases [44], many of which require mitochondrial fission and translocation [45;46]. Mitochondrial shape and localization changes were shown to occur in T cell activation [47] and chemotaxis [48]. Moreover, damage-associated molecular patterns (DAMPs), released from damaged dead cells, can act as "alarmins" [49] and activate polymorphonuclear leukocytes (PMNs) through toll like receptors TLR9, leading to inflammatory responses in the absence of an active infection [50].

Instead, we hypothesized that stimulated live mast cells could secrete mitochondrial components extracellularly, that could further promote inflammation by acting as "innate

pathogens". We, therefore, investigated if mitochondria could be secreted from stimulated mast cells, in response to allergic and neuropeptide triggers. We specifically showed that human mast cell degranulation and preformed TNF secretion in response to both allergic and non-allergic triggers requires mitochondrial fission and translocation to the cell surface [35]. We further showed that human mast cell degranulation triggered by IgE/anti-IgE or substance P (SP) leads to secretion of mitochondrial DNA and ATP extracellularly without cell death. Extracellular ATP has been shown to trigger and maintain inflammation in asthmatic airways [51]. Moreover, extracellular ATP was recently considered a universal "alarm" signal released from cells under stress and affect neighboring cells [52].

Mitochondrial components then stimulated human mast cells, keratinocytes, and primary human microvascular endothelial cells (HMVEC) to release inflammatory cytokines. Human mast cell-derived mitochondrial DNA injected ip in rats was also detected in their serum within 4 hr implying that extracellular mitochondrial components can reach distant sites [36]. We also showed that such extracellular mitochondrial components could augment allergies and eczema [53], known to be increased in autistic children. Our results could provide a mechanism through which the increased serum mitochondrial DNA and ATP could contribute to autism (Fig. 2). In fact, we first reported increased serum mtDNA in young autistic children as compared to controls [54], and mitochondrial DNA has been reported to be neurotoxic in rat brain slices [55].

These findings are unlike DAMPs, which are released following major trauma in humans [50] or shock-injured rat tissues [56] that can activate TLR9 receptors on human PMNs leading to inflammation [50]. In both these cases, the DAMPs came from damaged cells. Extracellular nucleic acids are now considered as sensors of cell damage and are involved in autoimmunity [57].

Given our results, it would be reasonable to block mast cell activation. However, there are no clinically available drugs that can block mast cell secretion. The so called "mast cell stabilizer" disodium cromoglycate (cromolyn) is quite effective in rats [58], but has been recently shown *not* to inhibit human mast cells [59-61]. Instead, the natural flavonoids luteolin and quercetin are generally safe [62-65], and can even protect against chemically-induced liver toxicity, a common consequence of many drugs [66]. Quercetin and luteoilin have potent anti-inflammatory and mast cell inhibitory actions [67;68]. Luteolin inhibits: oxidative stress [68], inflammation [68], mast cell degranulation [69], mast cell cytokine release [53], thimerosal-induced inflammatory mediator release [70], microglial activation and proliferation [71-73], and auto-immune T cell activation [67;74]. Luteolin is also protective against methylmercury-induced mitochondrial damage [75], is neuroprotective [76] and mimics brain-derived neurotrophic factor (BDNF) [77], reduction of which was recently associated with autistic-like-behavior in mice [78]. Finally, luteolin could reverse ASD-like behavior in mice [13], and was shown to have significant benefit in ASD children [79].

Recently, Autism Speaks issued a press release ahead of any specific peer-reviewed scientific publication (http://www.autismspeaks.org/about-us/press-releases/research-blocking-cell-distress-signals-can-ease-autism-symptoms) confirming our findings in a "mouse model" of autism. Interestingly, they indicated that this was the first mention of the inflammatory action of extracellular ATP. The press release went on to state that suramin, a drug used for the rare disease trypanosomiasis, reversed the findings in mice. This created an ethical and social problem as numerous parents are now considering using suramin for their autistic children. [80]. Parents should be aware of the fact that suramin *has not* been used in autistic children, that it has numerous adverse effects. Suramin is contraindicated in hepatic function impairment, causes hypersensitivity, can impair renal function, and has many serious adverse effects including kidney damage, blood dyscrasias, optic atrophy, vomiting, urticaria, paresthesias, peripheral neuropathy, and can even lead to shock. Moreover, there may also be additional interactions with other drugs [81], or with supplements commonly given to autistic children [82].

In conclusion, there is a unique and heretofore unrecognized function of mitochondrial components secreted from live activated mast cells with autocrine and paracrine pro-inflammatory effects. Extracellular mitochondrial components could act as "innate pathogens" and be the "missing trigger" in certain auto-immune and auto-inflammatory diseases, especially autism. Detecting circulating mitochondrial DNA and/or ATP could be used for diagnosis, while preventing secretion and/or neutralizing extracellular mitochondrial components may be used as novel therapeutic approaches. Recent efforts to compare the efficacy of non-steroidal anti-inflammatory drugs (NSAIDs) to that of flavonoids [83] is a necessary exercise to clarify the usefulness of various molecules in inflammatory diseases, such as autism.

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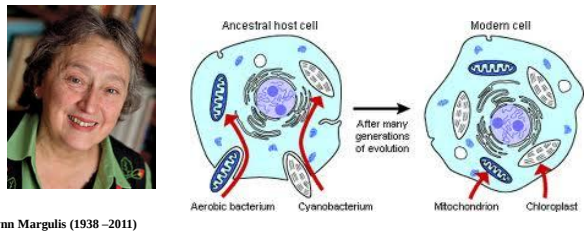

Lynn Margulis (1938 –2011)

Margulis L. Symbiotic theory of the origin of eukaryotic organelles: criteria for proof. *Symp Soc Exp Biol* 29:21-38, 1975

Figure 1

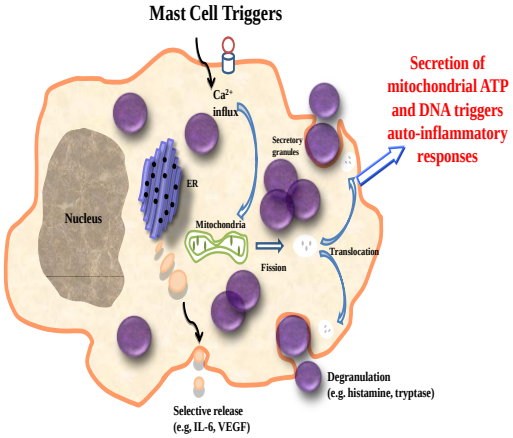

Figure 2

Legends

Figure 1. Photogrtaph of the late Dr. Margulis and representation of one of her drawings showing the bacterial origin of mitochondria.

Figure 2. Diagrammatic representation of mast cell activation leading to mitochondrial fission, translocation to the cell surface and secretion of mitochondrial ATP and DNA, which are misconstrued by the body as "innate pathogens" and trigger auto-inflammatory responses.