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Review

The “missing link” in autoimmunity and autism: Extracellular mitochondrial components secreted from activated live mast cells

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ABSTRACT

Autoimmune diseases continue to increase, but the reason(s) remain obscure and infections have not proven to be major contributors. Mast cells are tissue immune cells responsible for allergies, but have been increasingly shown to be involved in innate and acquired immunity, as well as inflammation. This involvement is possible because of their ability to release multiple mediators in response to a great variety of triggers. We recently published that activation of mast cells is accompanied by mitochondrial fission and translocation to the cell surface from where they secrete at least ATP and DNA outside the cell *without* cell damage. These extracellular mitochondrial components are misconstrued by the body as “innate pathogens” leading to powerful autocrine and paracrine auto-immune/auto-inflammatory responses. We also showed that mitochondrial DNA is increased in the serum of young children with autism spectrum disorders (ASD), a condition that could involve “focal brain allergy/encephalitis”. Blocking the secretion of extracellular mitochondrial components could present unique possibilities for the therapy of ASD and other autoimmune diseases. Unique formulation of the flavonoid luteolin offers unique advantages.

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1. Introduction

Allergies [1], autoimmune diseases [2,3] and autism [4] have increased dramatically over the years. A possible common link may be the mast cells [5], which participate in innate [6], acquired immunity [7,8], autoimmunity [9] as well as in inflammation [5]. Mast cells are hematopoietically-derived tissue immune cells that secrete pre-stored mediators, such as histamine and tryptase, but also tumor necrosis factor (TNF) [10] through degranulation, as well as numerous newly synthesized chemokines and cytokines in response to allergic, environmental, hormonal, neuronal, stress and toxic triggers [8,11]. Interestingly, mast cells have the ability to release some of their mediators selectively without degranulation [12], and participate in autoimmunity through modulation by IL-32 [13] and IL-33 [14].

Mast cells are ubiquitous in the body, especially in tissues exposed to the outside, such as the skin, intestine, and lungs, including the brain, the richest place of which is the hypothalamus [15]. Mast cells interact with microglia, the innate brain immune cells that are increasingly implicated in a number of neuropsychiatric diseases [16], but also in neuroinflammatory diseases [17,18]. Mast cells also express toll-like receptors (TLRs) [19], including TLR9 [20] that can be activated by bacterial DNA sequences, leading to the release of different cytokines [21] that allow mast cells to participate in immunity against bacterial infections [22–24], especially by eliciting neutrophil infiltration [25]. Mast cells express a number of novel receptors that allow them to have important regulatory functions in health and disease [26]. Mast cells are also important for maturation of Th17 cells, important in autoimmune disorders [27]. In particular mast cells in the presence of IL-6 and transforming growth factor β (TGF β) are necessary for the production of Th17 cells [28], while TNF and vasoactive intestinal peptide (VIP) drive IL-6-independent Th17 cell maturation [28–30].

2. Extracellular mitochondrial DNA

2.1. Activated mast cells

We specifically showed that human mast cell degranulation and preformed TNF secretion in response to both allergic (IgE/anti-IgE) and non-allergic [substance P (SP)] triggers require mitochondrial fission and translocation to the cell surface [31]. We further showed that human mast cell degranulation is accompanied by secretion of mitochondrial DNA and ATP extracellularly without cell death [32]. These mitochondrial components then stimulated human mast cells, keratinocytes, and primary human microvascular endothelial cells (HMVEC) to release inflammatory cytokines [32] (Fig. 1). The

autocrine effect on mast cell was partially inhibited by a purinergic inhibitor [32]. However, most of the autocrine effect was due to mitochondrial protein (Fig. 2). The proteins involved are presently unknown. Human mast cell-derived mitochondrial DNA injected ip in rats was also detected in their serum within 4 h implying that extracellular mitochondrial components can reach distant sites [32]. These components outside the cell that are misconstrued by the body as “innate pathogens” and induce a strong auto-inflammatory response [32] (Fig. 3). This response could be due to the fact, as the late Lynn Margulis hypothesized, that mitochondria [33], were bacteria that became symbiotic with eukaryotic cells [34]. Mitochondria [35] typically undergo fission and fusion as a way of maintaining their health [36,37].

Mitochondrial translocation was shown at the “immune synapse” of activated T cells [38]. Our results indicate that most of the mast cell-derived mtDNA is free as it is destroyed by DNAase I; some of this DNA was inside exosomes [32]. Mast cells can secrete exosomes [39] and mtDNA was reported inside exosomes secreted from glioblastoma cells [40]. Mitochondria health is regulated by autophagy that prevents them from leaving the cell [41,42]. Extracellular mitochondrial DNA was detected in the synovial fluid of patients (70%) with rheumatoid arthritis but not in any controls, as well as in the plasma of such patients (56%) as compared to healthy volunteers (27%) [43].

2.2. Damage-associated molecular patterns (DAMPs)

Damage-associated molecular patterns (DAMPs) can act as “alarmins” [44]. DAMPs released from damaged dead cells following major trauma in humans can activate polymorphonuclear leukocytes (PMNs) through TLR-9 [45]. Similar findings were also reported in shock-injured rat tissues [46]. In both these cases, the DAMPs that came from damaged cells and extracellular nucleic acids (not necessarily from mitochondria) are considered as sensors of cell damage and are involved in autoimmunity [47]. Antibodies recognizing mitochondrial antigens have been identified and considered a possible link with autoimmunity [48], but had not been shown to be associated with any disease until now except for primary biliary cirrhosis [49].

3. Brain inflammation

Inflammation has been increasingly implicated in damage to the brain [50] and in neuropsychiatric diseases [51,52].

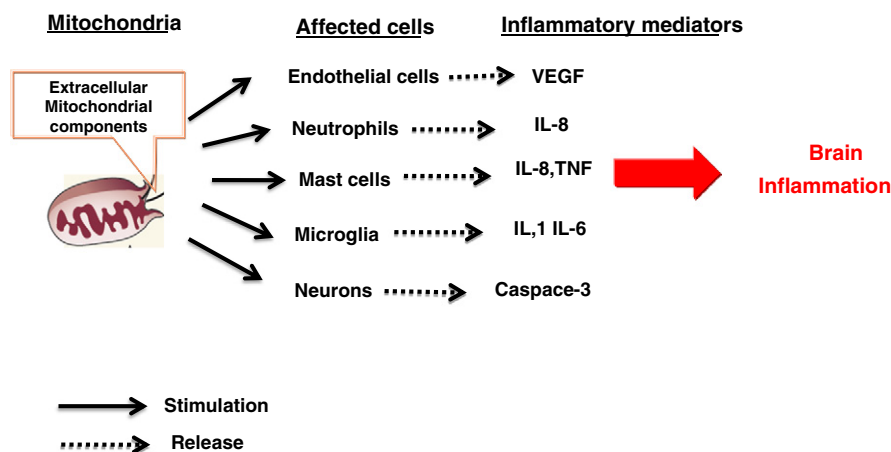


Fig. 1. Diagrammatic representation of the effect of extracellular mitochondrial component on stimulation of a variety of cells to release pro-inflammatory molecules leading to brain inflammation.

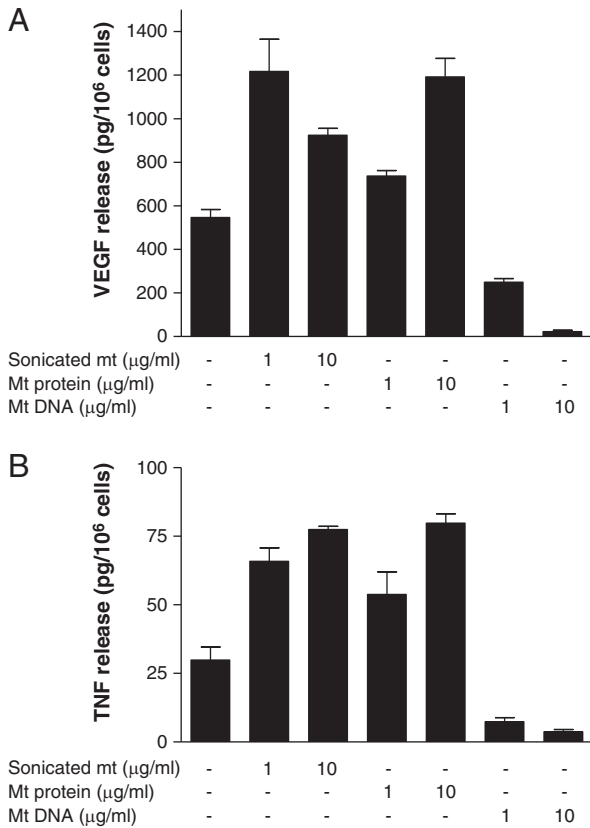


Fig. 2. Mitochondrial components stimulate human mast cell pro-inflammatory mediator release. LAD2 cells were incubated with sonicated mitochondria, mitochondrial protein, or mitochondrial DNA isolated from other LAD2 mast cells for 48 h. Supernatant fluids from different conditions were collected. (A) VEGF, (B) TNF were assayed at 48 h using commercial ELISA from R&D Systems, Minneapolis, MN (n = 3; *p < 0.05, compared to control). Mt = Mitochondria.

3.1. Autism Spectrum Disorders

Autism Spectrum Disorders (ASD) are neurodevelopmental disorders characterized by impaired social interactions and communication, as well as stereotypic behaviors [53–56]. Recent results from the Centers of Disease Control in the USA indicate that as many as 1/80 children have ASD [57]. Many such children regress at about age 3 years, often after a specific event such as reaction to vaccination, infection [58,59], trauma [60,61], toxic exposures [62] or stress [63] implying the importance of some environmental triggers [64,65]. A number of pieces of evidence suggest that ASD may have some aspects of autoimmunity [66], defective immune responses [67,68], as well as some neuroimmune component [69]. In addition, recent papers have suggested that a number of ASD patients have either mitochondrial diseases or mitochondrial dysfunction [70].

3.2. Allergic symptomatology

It is of interest that “allergic-like” reactions, especially chronic urticaria and eczema, are common in autistic children [71,72]. Such skin diseases are considered to have a strong autoimmune component [73]. A recent epidemiological study of 92,642 noninstitutionalized children (0–17 years old) showed that eczema is strongly associated with ADHD and ASD [74]. Moreover, children with mastocytosis have a higher risk of developing autism [75], implying activation of mast cells [76].

Abnormal microglial growth and activation was recently reported in the brain of ASD patients [77,78]. It is of interest that mast cell tryptase induced microglia activation [79]. We further reported that mitochondrial DNA could augment allergies and eczema [80], known to be increased in autistic children. Our results could provide a mechanism (Fig. 4) through which extracellular mitochondrial components, such as DNA and ATP, could contribute to ASD. We also reported increased serum mitochondrial DNA in young autistic children as compared to controls [81]. This is uniquely important since mitochondrial DNA is neurotoxic in rat brain slices [82]. Extracellular ATP has been shown to trigger and maintain inflammation

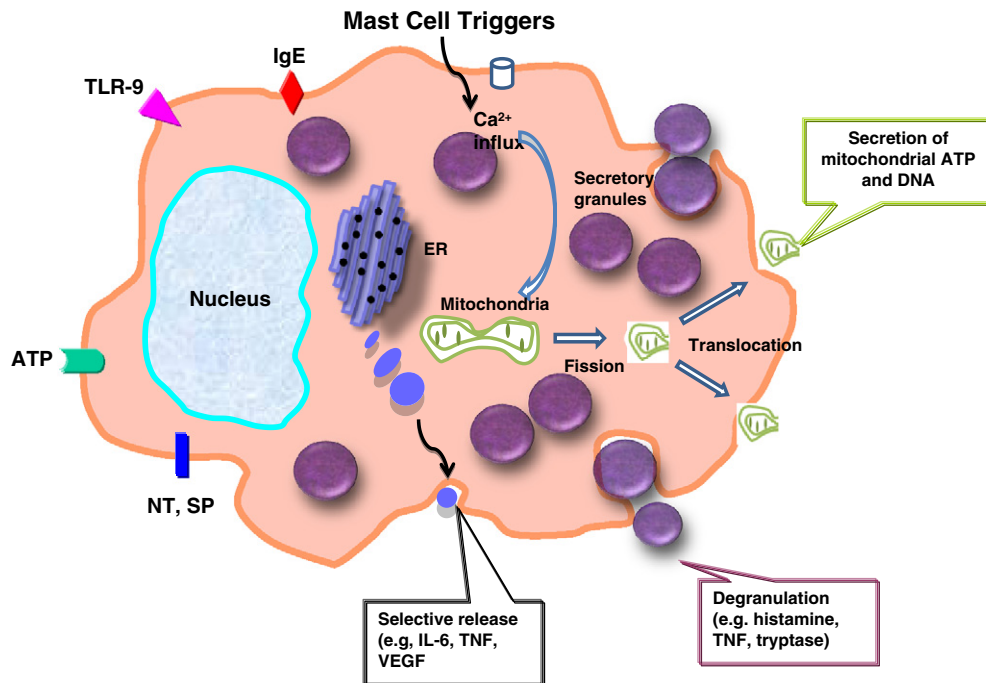


Fig. 3. Diagrammatic representation of mast cell activation through a variety of different receptors leading to mitochondrial fission, translocation to the cell surface and secretion of mitochondrial ATP and DNA, which are misconstrued by the body as “innate pathogens” and trigger auto-inflammatory responses.

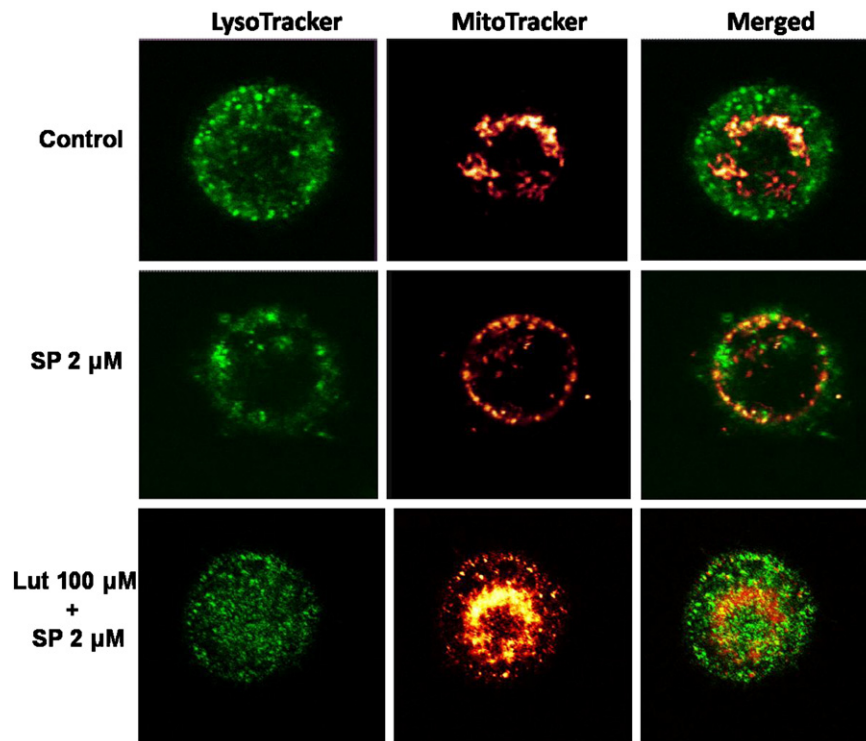


Fig. 4. Photomicrographs of human cultured LAD2 cells. Cells were incubated with 20 nM MitoTracker deep red for 20 min and 100 nM LysoTracker DND for 30 min. Cells were washed, moved to glass-bottom Confocal dishes and incubated with luteolin (10, 50 or 100 μM , 30 min) before SP stimulation (2 μM , 30 min). Cells were then observed under Confocal microscopy. Green color indicates mast cell granules and red color indicates mitochondria. The top horizontal row represents control cells, the middle horizontal row represents cell stimulated with SP and the lower horizontal row represents cells preincubated with luteolin (100 μM) before stimulation with SP (2 μM). The left vertical panel shows cells stained with MitoTracker, the middle vertical panel shows cells stained with LysoTracker and the right handside vertical panel shows cells of merged images from the previous two panels. These are representative images from 100 randomly selected cells (magnification = $\times 1000$). Lut = Luteolin; SP = Substance P.

in asthmatic airways [83]. Moreover, extracellular ATP is considered a universal “alarm” signal released from cells under stress and capable of affecting neighboring cells [84], including mast cells [85]. It was recently reported that DNA released from dying cells has synergistic effect with aluminum increasing production of IgE and IgG1 leading to increasing adjuvant activity [86]. It is interesting that in addition to the possible association between mercury and ASD [87] and mercury’s ability to stimulate mast cells [88], aluminum has also been shown to be associated with symptom severity in ASD children [89,90].

3.3. Purinergic effects

Recently, a paper using a mouse “model” of “ASD” like behavior reported that the nonspecific anti-purinergic drug Suramin, used for the rare disease trypanosomiasis, reversed the findings in mice [91]. Suramin *has not* been used in any autoimmune disease, let alone ASD, and has numerous adverse effects, especially severe urticarial in over 90% of patients and adrenal damage in about 50% of patients [32]. Suramin is also contraindicated in hepatic function impairment [92], can impair renal function, induce blood dyscrasias, optic atrophy, and peripheral neuropathy, even shock [93]. Additional interactions with other drugs [94], or with supplements commonly given to autistic children [95] may also occur. In fact, Suramin can also block G-coupled receptors, ATPases [96] and mast cells [97,98]. Given the fact that poly(I:C) maternal infection model does not occur in IL-6 knockout mice [99,100], and that most of the IL-6 depends on mast cells [101], and wonders of the actions of Suramin reported are not due to anti-purinergic actions as reported, but more due to effects on mast cells [102].

4. Possible treatments

4.1. Mast cell inhibitors

Given the above, it would be advantageous to prevent secretion of extracellular mitochondrial components from mast cells or other immune cells. Unfortunately, there are no clinically available mast cell blockers. The so called “mast cell stabilizer” disodium cromoglycate (cromolyn) is quite effective in rats [103], but has been recently shown *not* to inhibit human mast cells [104–106]. Instead, the natural flavonoids quercetin and luteolin have potent anti-inflammatory and mast cell inhibitory actions [107,108].

4.2. Luteolin

Luteolin inhibits oxidative stress [108], inflammation [108], mast cell degranulation [109], mast cell cytokine release [80], thimerosal-induced inflammatory mediator release [110], microglial activation and proliferation [111–113], as well as auto-immune T cell activation [107,114]. Luteolin is also protective against methylmercury-induced mitochondrial damage [115], and is neuroprotective [116]. Luteolin (5,7,3',4'-tetrahydroxyflavone) is structurally closely related to 7,8-dihydroflavone [117], which was shown to have brain-derived neurotrophic factor (BDNF) activity. In fact, the absence of BDNF was associated with autistic-like-behavior in mice [118], while 7,8-dihydroflavone was recently shown to reduce symptoms in a mouse model of Rett syndrome [119]. Our preliminary evidence indicates that luteolin also inhibits mitochondrial translocation during mast cell secretion (Fig. 3) and mitochondrial-induced histamine and PGD_2 release (Fig. 5). Moreover, a luteolin analog,

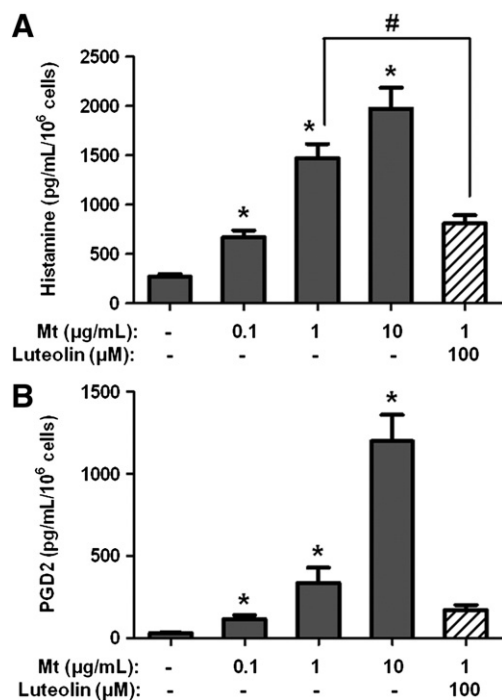


Fig. 5. Mitochondrial components stimulate histamine and PGD2 release from human mast cells, which is decreased by luteolin. Human cord blood-derived cultured mast cells (hCBMCs) were incubated with mitochondria (Mt) isolated from LAD2 mast cells for 30 min. In some experiments, hCBMCs were pretreated with luteolin (100 µM, 30 min). Supernatant fluids were collected and assayed for (A) histamine and (B) PGD2 release ($n = 3$; * $p < 0.05$ compared to control, # $p < 0.05$ compared to Mt 1). Mt = Mitochondria; PGD2 = Prostaglandin 2.

tetramethoxyluteolin, is more potent than luteolin as an inhibitor of inflammatory cytokine release [120]. Finally, luteolin could reverse ASD-like behavior in mice [72], and has significant benefit in ASD children [121,122]. Moreover, luteolin and quercetin are generally safe [123–126].

5. Conclusion

We hypothesized that ASD patients exhibit neuro-immune aspects [69], leading to “allergies in the brain” [127] and “focal encephalitis” [102]. Extracellular mitochondrial components, acting as “innate pathogens”, could be the “missing link” in certain auto-immune and auto-inflammatory diseases especially ASD. This problem could be worse in the subgroup of ASD patients with mitochondrial dysfunction. Preventing secretion of extracellular mitochondrial components may be used as a novel therapeutic approach.

Authors' contributions

All authors have read and approved the final manuscript. TCT designed and wrote most of the paper, SA researched the literature, ZW provided the confocal picture of the mitochondria and SP drew the graphics and prepared the manuscript.

Take-home messages

- Brain inflammation is involved in neuropsychiatric diseases.
- Mast cells secrete extracellular mitochondrial components that induce autoimmune responses.
- Damage associated molecular patterns are released from damaged cells.
- Autism spectrum diseases (ASD) involve brain inflammation.
- Mitochondrial DNA is increased in serum of ASD patients.

- Extracellular mitochondria components could be target of treatment of ASD.
- The flavonoid luteolin can block mast cell secretion of mitochondrial components.

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Disclosures

TCT has been awarded US Patent No 8,268,365, entitled “Anti-inflammatory compositions for treating brain inflammation” and PCT No. 13/722, 397. Tufts University has filed (10/21/10) on behalf of Dr. Theoharides PCT Application No. 61/405,414 entitled, “Extracellular mitochondria-based screening and treatment”.

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