The Hunt for Agmatine Receptors on Macrophages

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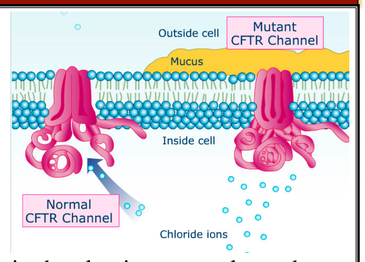
Abstract

Agmatine, a derivative of L-arginine, is known to act as a neurotransmitter, is associated with lung exacerbations in cystic fibrosis patients, and can augment biofilm formation in *Pseudomonas aeruginosa*. Most CF patients succumb to chronic airway infections from this opportunistic pathogen. Our lab is interested in the host-pathogen dynamic in the CF lung and has found that agmatine plays a pivotal role in this process. Known agmatine-binding receptors are being searched for on primary murine macrophages, a cell we have shown responds to agmatine. Candidates are 5HT-2C-serotonin receptors and α2-adrenoreceptors, whose existence has been putatively shown through adrenoreceptor blockade in the presence of agmatine. Western-blots were used to identify the presence of α2-adrenoreceptors and 5HT-2C-serotonin receptors and to quantify the level of expression following stimulus of macrophages with lipopolysaccharide or agmatine. Understanding the immunomodulatory effects of agmatine allows for future studies of host-pathogen interactions in CF patients.

Introduction

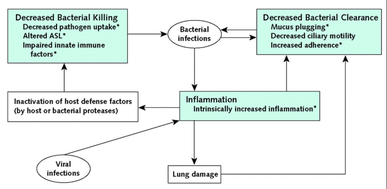
Cystic Fibrosis

Cystic fibrosis is an autosomal recessive disease due to a genetic mutation coding for the cystic fibrosis trans-membrane conductance regulator protein (CFTR), which is a critical chloride channel. The ionic current carried by chloride movement through individual CFTR channels is greatly reduced which results in the classic mucus clogged airways of a CF patient.

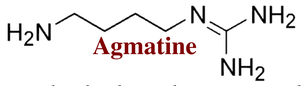


Chronic Infections by *Pseudomonas aeruginosa*

Mutations of CFTR impair the innate immunity of the pulmonary system, thus the lungs of a CF patient are predisposed to chronic bacterial infections. Infections of opportunistic pathogens such as *Pseudomonas aeruginosa* are linked to increased morbidity and mortality due to interconnected processes that lead to progressive lung damage[2]. Possible primary factors resulting from mutant CFTR are indicated by asterisks. ASL = airway surface liquid.



Agmatine: A Shared Metabolite



Agmatine is a pre-poly amine that is the result of decarboxylated L-arginine. It is formed via the arginine decarboxylase pathway by both prokaryotes and eukaryotes. Agmatine acts as an environmental trigger for biofilm formation of *P. aeruginosa* in the CF lung. Agmatine is also associated with and capable of causing inflammation in the host, and has been shown to act as a neurotransmitter through interaction with α2-adrenoreceptors and serotonin receptors, among others. Studying this shared recognition of agmatine could help elucidate novel host-pathogen interactions.

NF-kB and Inflammation

NF-kB proteins comprise a family of transcription factors that are involved in the control of immune and inflammatory responses. Our lab has demonstrated that agmatine is a direct immune modulator with effects on TNF-α production, likely through NF-kB. The agmatine receptors on the immune cells causing this response were unknown.

Methods and Results

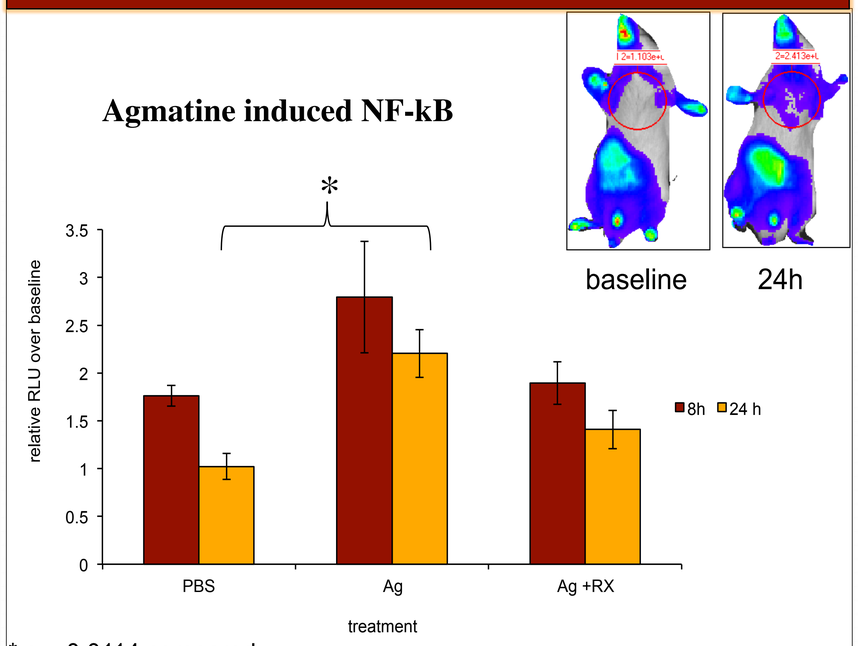


Figure 1: Agmatine induces lung inflammation through α2-adrenoreceptors
Agmatine or PBS was injected intratracheally to NF-kB reporter mice and the relative luminescence over the chest was measured after IV luciferin injection at 8 and 24 hours. Another group received identical intratracheal treatment but also received ip RX821002, which is an α2-adrenoreceptor antagonist. PBS group = 3 mice, Ag and Ag+Rx groups had 6 mice each. Agmatine dose was 100 µg in 50 µl and RX821002 was dosed at 10mg/kg.

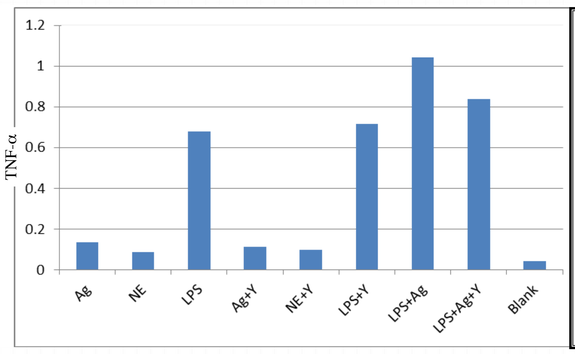


Figure 2: Role for α2-adrenoreceptors in agmatine response
Macrophages were incubated in agents at bottom of graph for 24 h and an ELISA for TNF-α was done on supernatants. Agmatine (Ag) and norepinephrine (NE) act through adrenoreceptors. Y is yohimbine which blocks adrenoreceptors.

Figure 3: Expression of α2-adrenoreceptors on macrophages
A western blot probing for α2-adrenoreceptors was performed on peritoneal murine macrophages stimulated with agents listed above blot.

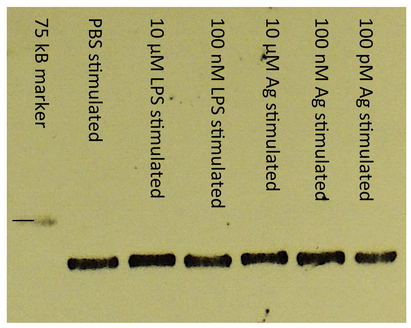
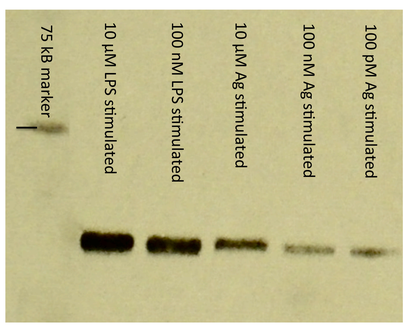


Figure 4: Expression of 5HT-2C serotonin receptors on macrophages
A western blot probing for 5HT-2C serotonin receptors was performed on peritoneal murine macrophages stimulated with agents listed above blot.



Discussion

Our lab has discovered an immunomodulatory role for agmatine in the lung. The preliminary data suggest its source is either from bacterial infections or through hemorrhage during pneumonia, as it does not appear to be created by inflammatory cells. Depending on the situation, agmatine can either augment or suppress inflammation as monitored by TNF-α (data not shown). Agmatine has a number of known receptors including α2-adrenoreceptors, serotonin receptors and imadazoline receptors, but it is still unknown which of these are key players in the aforementioned systems. Our data in this presentation clearly suggest both 5HT-2C receptors and α2-adrenoreceptors are present in our cell of interest. α2-adrenoreceptors have recently been implicated in catecholamine-induced inflammation in the lung[1]. The role of serotonin receptors on macrophages is poorly understood but they appear to be under the influence of both LPS and agmatine exposure. These preliminary studies provide a starting point to unravel the mechanism by which agmatine imparts its immunomodulatory effects on inflammatory cells.

Conclusion

- Agmatine has a direct impact on inflammation with NF-kB induction in mouse lungs, and its effects are blocked by α2-adrenoreceptor blockers (Figure 1).
- Agmatine induces a TNF-α response in macrophages that is also blocked by α2-adrenoreceptor blockers (Figure 2).
- α2-adrenoreceptors are present on primary murine macrophages but expression levels seemed to be unaffected by stimulus with different levels of LPS or agmatine (Figure 3).
- Macrophages were found to express 5HT-2C serotonin receptors also, and expression level was affected in a dose-dependent manner for LPS or agmatine stimulation (Figure 4).

Acknowledgements and References

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References

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