Inverse Expression of Fas Ligand and Asthma-Related Cytokines in an Aspergillus Challenge Murine Model of Asthma

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Abstract

Rationale: Resolution of inflammation in asthma is typically thought of as a passive phenomenon in which proinflammatory Th2-type cytokines wane after the initial trigger. We hypothesized that active mechanisms, including anti-inflammatory cytokines and pro-apoptotic factors, contribute to this process. Fas Ligand (FasL) expression was particularly interesting since important effector cells in asthma (e.g. eosinophils and T helper cells) are Fas-sensitive.
Methods: BALB/c mice were sensitized and challenged with an Aspergillus fumigatus extract, and sacrificed 1, 7 and 10 days later to capture events during initiation of the inflammatory response and its resolution. Endpoints included bronchoalveolar lavage (BAL) cell counts, protein array analysis of BAL fluid, and cytokine gene array of total lung RNA.
Results: BAL eosinophilia peaked on day 1 and was associated with a marked increase in both Th1 and Th2 type cytokines including IL-12, IFN, IL-4, IL-5, IL-6, and IL-10 and eosinophil-active chemotactic factors. In contrast, FasL protein and gene expression was suppressed at this time point compared to baseline. Resolving eosinophilia was coincident with a marked increase in FasL expression and the return of most cytokines towards baseline although residual chemokine levels were noted 10 days after allergen challenge.
Conclusions: We observed an inverse relationship between expression of FasL and asthma-related cytokines in an experimental asthma model. These data are consistent with a regulatory role for FasL during resolution. While Th2 cytokines are thought to orchestrate the initial response to antigen, multiple classically anti-inflammatory cytokines appear to be involved as well.
Funded By: NIH HL076646, AI055593

Background

Asthma is a disease of chronic inflammation as well as hyperresponsiveness of the airways. Airway inflammation in asthma is characterized by initiation and maintenance of inflammatory cells followed by resolution. Fas ligand may act as an endogenous anti-inflammatory mediator in asthma by promoting the apoptosis of inflammatory effector cells such as eosinophils and lymphocytes. In previous experimentation, anti-FasL given systemically to mice appeared to potentiate eosinophilia locally in a murine model (Almeida et al, Am J Respir Crit Care Med 2006; 3:A340). This would seem to implicate locally generated FasL in the clearance of pro-inflammatory cells. In this study we investigated the expression of asthma-related cytokines in the context of FasL protein and gene expression in an established Aspergillus mouse model of asthma.

Questions

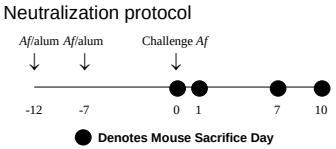
- What is the relationship between local lung cytokine expression and resolution of inflammation?
- What is the relationship between local lung cytokine expression and FasL protein and gene expression?

Hypothesis: We hypothesize that active mechanisms, including anti-inflammatory cytokines and pro-apoptotic factors such as FasL are involved in the resolution of inflammation in asthma.

Methods

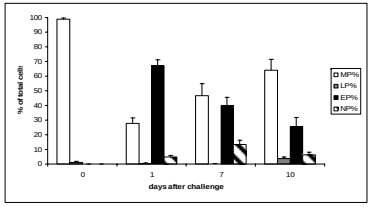
- Balb/c mice were sensitized with *Af*/alum (20 ug/mouse) on days -12 and -7, and nasally challenged with *Af* on day 0
- Mice were sacrificed at baseline (d0) and 1, 7 and 10 days post challenge.
- Differential BAL cell counts were determined by Wright's stain
- Tissue eosinophils were shown by staining with major basic protein
- Soluble FasL levels in BALF were analyzed using ELISA (R&D systems).
- Quantification of the FasL target sequences in the whole lung RNA was performed by a gene expression array (Superarray Bioscience Corp) and confirmed by real-time PCR (Taqman; Applied Biosystems).
- BAL Fluid was analyzed using a Quansys Q-Plex™ Mouse Cytokine array, a fully quantitative ELISA able to detect 16 different cytokines on multiple samples

Neutralization protocol

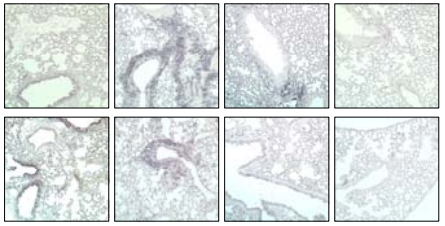


Results

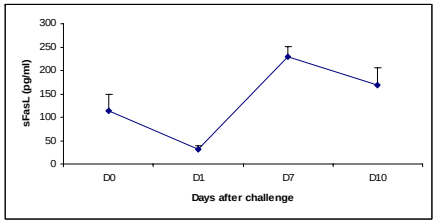
Kinetics of inflammation in mouse lung after *Af* challenge



Tissue sections stained with eosinophil MBP antibody

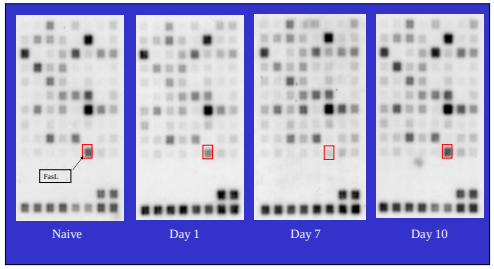


Appearance of soluble FasL in the BAL fluid

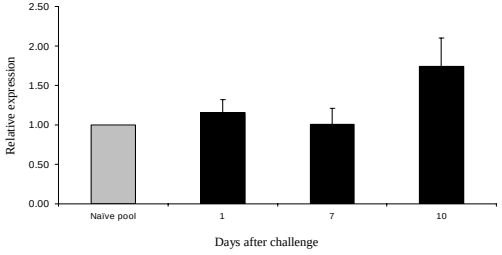


FasL gene expression over time: whole lung RNA

Murine Asthma Gene Array

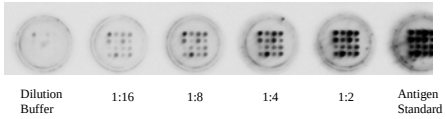


Real Time PCR: FasL expression relative to baseline

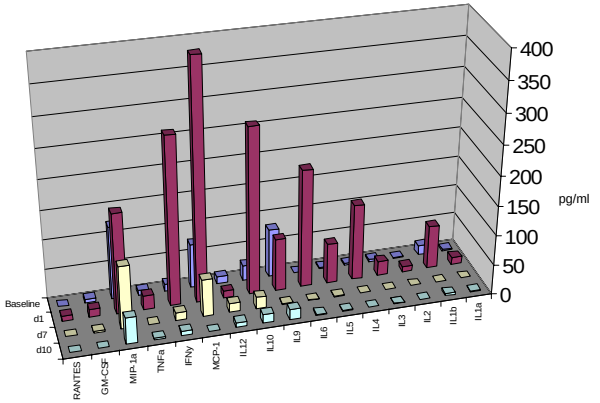


Mouse cytokine array

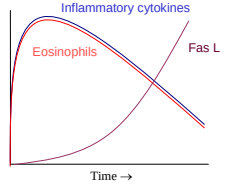
Standard curve



Cytokine array at Days 0, 1, 7, and 10



Summary/Conclusion



- Antigen challenge was associated with airway eosinophilia and a marked initial increase in both Th1 and Th2 type cytokines that waned over time.
- Resolving eosinophilia was coincident with the return of most cytokines toward baseline.
- There was an inverse relationship between the expression of FasL and asthma related cytokines.
- These results support the concept that endogenous FasL is involved in the resolution phase of allergic airway inflammation.