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FELLOWSHIP FINAL REPORT

Targeting periostin reduces inflammation and respiratory barrier injury in lung diseases.

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REPORT INFO

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ABSTRACT

Periostin (POSTN) is a matricellular protein that plays a key role in development and repair within the biological matrix of the lung. POSTN is highly expressed in several cell types in lung such as epithelial or endothelial cells, fibroblasts, smooth muscle and mast cells, contributing to mucus secretion, alveolar epithelial repair, and lung fibrosis. However, the underlying mechanism how POSTN contributes to the development of lung inflammation remains unclear. In the current study, we attempted to determine whether treatment with a monoclonal anti-POSTN antibody induces a significant inhibition of asthmatic reactions in a mouse asthma model. Mice sensitized and challenged with papain evidenced an increased periostin expression in lung and typical asthmatic reactions, as follows: an increase in the number of eosinophils in bronchoalveolar lavage fluid; a marked influx of inflammatory cells into the lungaround blood vessels and airways, and Th2 cytokines including IL-4 and IL-5 and chemokines in the bronchoalveolar lavage (BAL) fluid; emphysema; the detection of thymic stromal lymphopoietin (TSLP) produced by epithelial cells. However, the administration of anti-POSTN prior to the final airwaypapain challenge resulted in a significant inhibition of all asthmatic reactions. We also demonstrated that anti-POSTN antibody treatment resulted in significant reductions on collagen expression and a reduction in the increased eosinophil. The treatment of animals with anti-POSTN resulted in a significant reduction in the concentrations of the chemokines (CCL-11 andCCL-17) in the airways, without any concomitant increase in the concentration of Th1 cytokines. This study identifies a novel therapeutic strategy for airway hyperresponsiveness, which uses antibodies reactive against POSTN via the inhibition of the Th2response. It also provides theoretical evidence for the control of allergic asthma and fibrosis by targeting POSTN.

1- Introduction

Lung diseases prevalence has significantly increased in the last 30 years, resulting in a severe human and economic burden for society affects 100-150 million people worldwide and results in over 180,000 deaths every year. Asthma and Idiopathic fibrosis (IPF) are examples of these diseases, been a major cause of morbidity and mortality worldwide. Risk factors associated with lung diseases are exposure to allergens in childhood and exposure to environmental factors, such as pollutants, allergens and chemicals.

Periostin (POSTN) is a matricellular protein belonging fasciclin family, which are involved not only in tissue developing and remodeling but also in the Th2-biased inflammation and eosinophilic infiltration of airway and skin allergic diseases, such as asthma 1. The gene encodes a 93 kD glycoprotein comprised of an N-terminal secretory signal sequence and 4 fasciclin domains [1]. Five isoforms have been described in man and 6 in mice (Uniprot data; [2]). The functional significance of the splice variants is not well understood. Recent studies have shown, POSTN in NPs is mainly produced by epithelial cells and regulated by several mediators including Th2 cytokines IL-4, IL-13 3. POSTN is able to stimulate epithelial cells to release TSLP, which may important in activating Th2 responses [1]. However, the underlying mechanism how POSTN contributes to the development of lung inflammation remains unclear.

POSTN functions as a matricellular protein in cell activation by binding to their receptors on cell surface, thereby exerting its biological activities. POSTN, a secreted homodimeric protein that binds integrins □v□3, □v□5, and □6□4, was originally found to be expressed in fetal tissues and in the adult upon injury particularly bone fractures due to its role in remodeling and repair. For example, eosinophils adhere to POSTN via the αMβ2 integrin CD11b and POSTN increases eosinophil adhesion to fibronectin [3]. POSTN also regulates TGF-β signaling [4].

Moreover, POSTN is a downstream molecule of interleukin (IL)-4 and IL-13, signature cytokines of type 2 immune responses, we showed that POSTN is a component of subepithelial fibrosis in bronchial asthma, the first formal proof that POSTN is involved in allergic inflammation. Recent evidence has accumulated demonstrating the significance of POSTN in allergic inflammation. It is of note that in skin tissues, POSTN is critical for amplification and persistence of allergic inflammation by communicating between fibroblasts and keratinocytes [5]. Furthermore, POSTN levels in serum may be a biomarker of severity of respiratory disease Ref. Serum POSTN can reflect local production of POSTN in inflamed lesions induced by Th2-type immune responses and also can predict the efficacy of Th2 antagonists against bronchial asthma [6]. Blocking the interaction between POSTN and its receptor, αv integrin, or down-regulating the POSTN expression shows improvement of POSTN-induced inflammation in mouse models or in in vitro systems [5].

Several studies have been described the correlation between POSTN levels with diseases severity. For example, treatment with the anti-IL-13 antibody lebrikizumab improves lung function in patients with asthma, and patients with high pretreatment levels of serum POSTN had greater improvement in lung function than patients with low POSTN levels [7]. It has been described in patients with persistent symptoms despite inhaled corticosteroids, serum POSTN is the single best predictor of airway eosinophilia [8]. Moreover, POSTN is a marker of omalizumab responsiveness in patients with uncontrolled severe persistent allergic asthma [9]. Among several biomarkers of asthma, increased levels of POSTN were identified in asthmatic and COPD patients [10].

POSTN is also involved in modulating cell functions, plays a unique role as an inducer of chemokines to recruit neutrophils and macrophages important for the process of pulmonary fibrosis in bleomycin-administered model mice. This suggests a therapeutic potential for POSTN in idiopathic pulmonary fibrosis and drug-induced interstitial lung disease [11].

It has proven very difficult to obtain monoclonal antibodies (mAbs) against this highly conserved protein. However, Field et al. reported that combining infection of mice with lactate dehydrogenase elevating virus (LDV), a B cell activating arterivirus, with conjugation of human POSTN to ovalbumin as an immunogenic carrier, enabled to develop six mAbs recognizing both human and mouse POSTN and inhibiting its binding to avb3 integrin. Two of the mAbs, MPB4B1 (IgM) and MPC5B4 (IgG1), were tested and found to inhibit POSTN-induced migration of human endothelial colony forming cells Fields. Here we focused on anti PSTN MPC5B4, which recognizes the amino acid sequence 136-151, located within the fascilin (FAS)1-1 domain of the human POSTN isoforms, and conserved in murine isoforms, and binds similarly human and murine POSTN.

In order to better understand the role of POSTN in lung inflammation, we investigated the expression of POSTN in mouse models of experimental allergic asthma or IPF. In the course of this studies, we observed an upregulated expression of POSTN after papain or bleomicin stimulation. Administration of anti-POSTN neutralizing antibody significantly decreased the pathology associated with airway inflammation in a pre-clinical model of airway allergy using the allergen papain.

2- Experimental details

Mice: Female eight-weeks old C5BL/6 mice were purchased from Janvier Laboratories (Saint Berthevin, France). Mice were maintained under a 12-hours light-dark cycle and they were fed with a standard laboratory diet. The inhalational anesthetic isoflurane was administered during intratracheal administration. All studies were approved by Institutional Animal Care and Use Committee of CNRS.

Papain-induced lung inflammation model in mice. Mice were anesthetized by 2-3% isofluorane and 2% O_2 follow asthma was induced by the administration of papain (Calbiochem, Darmstadt, Germany). Papain was dissolved in sterile NaCl solution. Animal were sensitized 3 times with 40 ul solution containing 25 ug papain administrated intranasally [12]. Mice were sacrificed at day 4 following asthma induction. Control animals were either untreated.

Mice were euthanized by CO_2 inhalation 4 days after papain administration and BALF was collected. After a hearth perfusion with ISOTON II (acid-free balanced electrolyte solution Beckman Coulter, Krefeld, Germany), lungs were collected and sampled for analyses.

Bleomycin-induced lung fibrosis model in mice. Mice were anesthetized by 2-3% isofluorane and 2% O_2 follow pulmonary fibrosis was induced by the administration of bleomycin (Merck KGaA, Darmstadt, Germany). Bleomycin was dissolved in sterile NaCl solution. Animal were sensitized 3 times with 40 ul solution containing 3 mg/kg administrated intranasally. Mice were sacrificed at day 14 following emphysema induction. Control animals were either untreated. BALF and lungs were collected and sampled for analyses.

Periostin neutralization: MPC5B4 blocks periostin interaction with the □v□3 and □v□5 integrins [13]. Anti-periostin (MPC5B4) or isotype control (IgG1) antibody were given at indicated time points. Different doses of monoclonal antibody (12.5, 5, 2.5 mg/kg; i.p. or 10, 1 mg/kg; it.) were used for dose response study.

Analysis of airway inflammation and collagen deposition: The left lobe of lung was fixed in 4% buffered formaldehyde and paraffin embedded under standard conditions. Tissue sections (3 μm) were stained with PAS. Histological changes such as inflammation and emphysema were assessed by a semi-quantitative score from 0 to 5 for cell infiltration (with increasing severity) as described before [14].

Sample size and statistical analysis: The statistical analysis was performed using Prism 5 (GraghPad software, San Diego, CA). Results were analyzed using non-parametric test (Mann Whitney tests), T test expressed in terms of probability (P). Differences were considered significant when $p<0.05$. All data were expressed as mean± SEM.

3- Results

Papain increase POSTN production.

To assess the capacity of papain to induce POSTN production, C57BL/6 mice were treated or not with 25 ug papain intratracheal. Mice received one dose of papain. POSTN levels were measure 48h after papain administration. Papain-treated mice shown significant increased levels of POSTN in bronchoalveolar lavage (BAL) fluid compared to control mice.

Anti-POSTN antibody prevent lung inflammation in papain-induced asthma.

Monoclonal mouse anti-POSTN antibody (MPC5B4, IgG1 isotype), which blocks POSTN binding site interaction (aa140-150) with integrin □v□3 [13]. The treatment with MPC5B4 was included to assess the impact of POSTN signaling modulation in lung inflammation. C57BL/6 mice were injected with anti-POSTN or Isotype control antibody (12.5 µg/mouse; i.p.). 1h after injection, mice were treated or not with 25 ug papain to induce allergic asthma. Mice received 3 doses of papain on 3 consecutive days. Cell counts were monitored in bronchoalveolar lavage fluid (BALF) and lung 4 days after first administration. Papain treated mice as control group shown increase total cells count, eosinophils and lymphocyte number. Moreover, papain treated mice shown that anti-POSTN therapy protected the recipient mice against lung inflammation reducing significantly eosinophils and lymphocytes counts. However, control isotype antibody therapy failed to protect the recipient mice against inflammation.

Lung CCL11 was increased significantly after papain exposure, and this was reversed by anti POSTN antibody. Thymus- and activation-regulated chemokine (TARC/CCL17) induces a Th2-dominated inflammatory reaction in mice. Papain treated mice showed a trend increased CCL17 levels in lung, however anti-POSTN antibody treatment reduced the CCL17 production. Papain treated-mice significantly increased the tissue damage in lung measured by histological score emphysema after the 4 days. Anti-POSTN antibody treatment significantly reduced the lung tissue damage.

Local administration of anti-POSTN antibody prevent lung inflammation.

The treatment local with MPC5B4 by intratracheal route was included also to assess the impact of POSTN in lung inflammation. C57BL/6 mice were injected with anti-POSTN antibody (10, 1 mg/kg; i.t.). 1h after injection, mice were treated or not with 25 ug papain to induce asthma allergic. Mice received 3 doses of papain. Cells counts was monitored in BALF 4 days after first administration. Papain treated mice as control group shown increase total cells count, eosinophils, lymphocyte, neutrophils and macrophages number. Moreover, papain treated mice shown that anti-POSTN therapy protected the recipient mice against lung inflammation reducing significantly eosinophils, lymphocytes and neutrophils counts. POSTN production quantified in BALF is also reduce but not MPO.

Papain treated-mice significantly increased the tissue damage in lung measured by histological score emphysema after the 4 days. Anti-POSTN antibody treatment significantly reduced the lung tissue damage and collagen production.

Therapeutic administration of anti-POSTN antibody blocks chronic severe papaininduced asthma

To assess the capacity of therapeutic treatment with MPC5B4 anti-POSTN antibody to treat chronic severe asthma, mice received saline vehicle or 4 administrations of papain (25 µg i.n.) on day 1, 2, and further on days 14 and 21. Anti-POSTN antibody MPC5B4 (12.5 mg/kg; i.p.) administered on day 14, 18 and 21 reduced lung inflammation and collagen deposition in this severe asthma model. Cells counts wasmonitored in the BAL 22 days after the first administration. Papain treated-mice as control group showed increase in total cells count, eosinophils, lymphocytes, neutrophils and macrophages numbers. Moreover, anti-POSTN therapy protected the papain treated mice against lung inflammation reducing significantly eosinophils, lymphocytes and neutrophils counts.

Papain treated-mice significantly increased the tissue damage in lung measured by histological score emphysema after the 22 days. Anti-POSTN

antibody treatment significantly reduced the lung tissue damage and collagen production.

Total cells, Eosinophil, Lymphocyte, Macrophage and Neutrophil infiltration were reduced in BALF on day 22, as were lung tissue damage, soluble collagen protein and collagen message transcription after anti-POSTN MPC5B4 antibody as compared to isotypecontrol treated-mice.

Anti-POSTN antibody reduces pulmonary fibrosis.

The treatment with monoclonal anti-POSTN antibody (MPC5B4), was also included to assess the impact of POSTN signaling modulation in pulmonary fibrosis. C57BL/6 mice were injected with anti-POSTN or Isotype control antibody (12.5 mg/kg; i.p.). Mice received three doses of monoclonal antibody as preventive treatment. 1h after injection, mice were treated or not with 3 mg/kg bleomycin to induce lung fibrosis. Cells counts was monitored in BAL and lung 14 days after first administration. Bleomycin-treated mice as control group shown increase total cells, neutrophils and lymphocyte numbers. Moreover, bleomycin-treated mice shown that preventive anti-POSTN therapy protected the recipient mice against lung fibrosis reducing significantly neutrophils and lymphocytes number. However, control isotype antibody therapy failed to protect the recipient mice against fibrosis.

Monoclonal anti-POSTN antibody was administrated twice to show its therapeutic efficacy. Bleomycin-treated mice show that therapeutic monoclonal anti-POSTN treatment significantly reduced the lung inflammation measured by cells counting after the 14 days. Collagen production shown be reduced after anti-POSTN antibody treatment by histology.

A therapeutic model to analyze the efficacy of anti-POSTN antibody therapy to control lung inflammation and collagen production in lung fibrosis show to be efface.

Conclusion

In conclusion, we show that periostin, a matricellular protein, influences the progression of pulmonary inflammation and fibrosis by inducing chemokines and proinflammatory cytokines and collagen deposition in papain or BLM-administered mice. This suggests a therapeutic potential for targeting periostin in inflammatory lung diseases.

4- Perspectives of future collaborations with the host laboratory

Increasing evidence has shown the significance of periostin as a novel mediator in allergic inflammation. Periostin has a function as an inflammatory mediator linking immune cells and resident cells in inflamed lesions. However more studies are needed to continue understanding the role of POSTN in lung inflammation and fibrosis. Both the concept of a molecular mechanism in which periostin is involved and the clinical application targeting periostin in allergic inflammation can be extended to other inflammatory diseases, malignancies, and developmental biology because periostin acts as a remodeling molecule in a wide variety of healthy and pathologic states.

As this project progresses, we applied for a C-Valo project, a regional program may be the necessary support for the maturation of the current project and create a new Start-up. The intellectual property that protects the use of the anti-periostin antibody as a new therapy to treat respiratory diseases will be transferred to the Start-up in order to fully explore the translation of our findings into clinical studies for treatment of asthma.

5- Articles published in the framework of the fellowship

The relevance of results obtained during the fellowship give us the opportunity to file a patent in mars 2021. We write two manuscript and they are in publication process. Moreover, a seminary on INEM-CNRS was done:

- **Patent:** Use of a periostin antibody for treating inflammation, fibrosis and lung diseases. Europe, 2021.
- **Review:** Periostin in lung inflammation. Frontiers in Immunology. 2021 (In preparation).

- **Article:** Targeting periostin reduces inflammation and respiratory barrier injury in lung diseases (In preparation).

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7- References

1. Masuoka, M. *et al.* Periostin promotes chronic allergic inflammation in response to Th2 cytokines. *J Clin Invest* **122**, 2590-2600 (2012).

2. Morra, L. *et al.* Characterization of periostin isoform pattern in non-small cell lung cancer. *Lung Cancer* **76**, 183-190 (2012).

3. Blanchard, C. *et al.* Periostin facilitates eosinophil tissue infiltration in allergic lung and esophageal responses. *Mucosal Immunol* **1**, 289-296 (2008).

4. Snider, P. *et al.* Periostin is required for maturation and extracellular matrix stabilization of noncardiomyocyte lineages of the heart. *Circ Res* **102**, 752-760 (2008).

5. Izuhara, K. *et al.* Periostin in allergic inflammation. *Allergol Int* **63**, 143-151 (2014).

6. Izuhara, K. *et al.* Periostin: An emerging biomarker for allergic diseases. *Allergy* **74**, 2116-2128 (2019).

7. Corren, J. *et al.* Lebrikizumab treatment in adults with asthma. *N Engl J Med* **365**, 1088-1098 (2011).

8. Jia, G. *et al.* Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* **130**, 647-654 e610 (2012).

9. Hanania, N.A. *et al.* Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* **187**, 804-811 (2013).

10. Carpaij, O.A. *et al.* Serum periostin does not reflect type 2-driven inflammation in COPD. *Respir Res* **19**, 112 (2018).

11. Uchida, M. *et al.* Periostin, a matricellular protein, plays a role in the induction of chemokines in pulmonary fibrosis. *Am J Respir Cell Mol Biol* **46**, 677-686 (2012).

12. Agoro, R. *et al.* IL-1R1-MyD88 axis elicits papain-induced lung inflammation. *European journal of immunology* **46**, 2531-2541 (2016).

13. Field, S. *et al.* Novel highly specific anti-periostin antibodies uncover the functional importance of the fascilin 1-1 domain and highlight preferential expression of periostin in aggressive breast cancer. *Int J Cancer* **138**, 1959-1970 (2016).

14. Yu, H.S., Angkasekwinai, P., Chang, S.H., Chung, Y. & Dong, C. Protease allergens induce the expression of IL-25 via Erk and p38 MAPK pathway. *J Korean Med Sci* **25**, 829-834 (2010).