The post-viral GPNMB^{+} immune niche persists in long-term Covid, asthma, and COPD

Kangyun Wu[1], Yong Zhang[1], Huiqing Yin-DeClue[1], Kelly Sun[1], Dailing Mao[1], Erika C. Crouch[2], Derek E. Byers[1], and Michael J. Holtzman[1,3,4]

[1]Pulmonary and Critical Care Medicine, Department of Medicine, [2]Department of Pathology and Immunology, [3]Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO, USA 63110 and [4]NuPeak Therapeutics Inc., St. Louis, MO 63105

Running title: Immune niche persistence in long-term lung disease

Address correspondence to: Michael J. Holtzman, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, mjholtzman@wustl.edu.

Abbreviations used in this article: basal-ESC, basal-epithelial stem cell; COPD, chronic obstructive pulmonary disease; Covid-19, coronavirus disease of 2019; GPNMB, glycoprotein nometastatic melanoma B; monocyte-derived dendritic cell (moDC), PVLD, post-viral lung disease.

Keywords: asthma, disease biomarker, chronic obstructive pulmonary disease (COPD), epithelial stem cell, GPNMB, glycoprotein nometastatic melanoma B (GPNMB), inflammatory memory, long-term Covid, macrophage, monocyte-derived dendritic cell (moDC), post-viral lung disease, respiratory viral infection.

Abstract

Epithelial injury calls for a regenerative response from a coordinated network of epithelial stem cells and immune cells. Defining this network is key to preserving the repair process for acute resolution, but also for preventing a remodeling process with chronic dysfunction. We recently identified an immune niche for basal-epithelial stem cells using mouse models of injury after respiratory viral infection. Niche function depended on an early sentinel population of monocyte-derived dendritic cells (moDCs) that provided ligand GPNMB to basal-ESC receptor CD44 for reprogramming towards chronic lung disease. These same cell and molecular control points worked directly in mouse and human basal-ESC organoids, but the findings were not yet validated in vivo in human disease. Further, persistence of GPNMB expression in moDCs and M2-macrophages in mouse models suggested utility as a long-term disease biomarker in humans. Here we show increased expression of GPNMB localized to moDC-macrophage populations in lung tissue samples from long-term Covid, asthma, and COPD. The findings thereby provide initial evidence of a persistent and correctable pathway from acute injury to chronic disease with implications for cellular reprogramming and inflammatory memory.

New and noteworthy

Recent work indicates that a sentinel immune niche provides GPNMB to epithelial stem cells to drive structural remodeling and disease as exemplified by the response to respiratory viral injury. The present study provides initial evidence that this niche can be detected in humans in the context of comparable diseases (long-term Covid, asthma, and COPD) also linked to viral infection. The results support a persistent mechanism for inflammatory disease that might be correctable with GPNMB blockade directly or indirectly through related signaling pathways.

Introduction

Epithelial barriers are carefully programmed for primordial defense and repair in response to injuries from infectious and other toxic agents. In that context, one of the most common forms of epithelial injury derives from respiratory viral infection, including childhood outbreaks of RSV and HEV-D68 and pandemics of influenza virus and coronavirus (1, 2). In each case, the host goal is to wall off infection and restore integrity at the barrier site. A key step in this repair process is the growth and differentiation of epithelial stem cells and the coordinated actions of immune cells designed to clear infectious agents. However, based on viral and host factors, the normal program for recovery can be skewed to an ongoing response that results in structural remodeling and long-term post-viral lung disease (PVLD) that can manifest as long-Covid, post-influenza sequelae and related virus-triggered diseases such as asthma and COPD (3-6). In fact, basal-epithelial stem cells (basal-ESCs) represent a stereotyped feature of the epithelial barrier program. In experimental models, this cell population can be reprogrammed for hyperplasia and metaplasia that disrupts lung function after native Sendai virus (SeV) or adapted influenza A virus (IAV) infections (7-9). Similar activation of basal cell growth is found in Covid-19 (6, 10) that might be linked to asthma exacerbation (11). Defining and correcting a renewable stem cell component with the capacity for inflammatory memory is entry point for a disease-modifying therapy. However, the molecular basis for excess growth and immune activation still needed to be defined as a basis for precisely targeted correction.

To address these issues, we recently identified a sentinel immune niche for basal-ESC reprogramming in mouse models of epithelial injury after respiratory viral infection. Niche function depended on monocyte-derived dendritic cell (moDC) recruitment and then production of ligand glycoprotein nometastatic melanoma B (GPNMB) for delivery to receptor CD44 on basal-ESCs. This ligand-receptor interaction could be antibody-blocked early after infection (5-12 d) to prevent the subsequent reprogramming and PVLD that developed later after infection (49 d). These same moDC and GPNMB-CD44 control points worked directly in mouse and human basal-ESC organoids, but the findings were not yet extended to studies of human disease conditions. Further, persistence of GPNMB expression in moDCs and then M2-macrophages after clearance of infection suggested utility as a long-term biomarker for chronic lung disease. In line with these concepts, we show here that expression of GPNMB can also be localized to lung moDCs and macrophages in situ in humans using post-mortem tissues from long-term Covid, asthma, and COPD. The results thereby provide initial evidence of methods to stratify and modify post-injury disease in the lung and perhaps other sites of epithelial injury.

Results and Discussion

To determine whether findings in experimental animal and human models translate to similar characteristics in human lung disease, we engaged a tissue registry of human lung samples constructed and validated as

described previously (3, 6, 12, 13). For long-term Covid samples, human lung tissue was obtained from a series of consecutive autopsies performed from April-August 2020 at 27-51 d after onset of infectious illness (6). For asthma, COPD, and non-disease control samples, lung tissue was obtained from a Tissue Registry for Advanced Lung Disease that contains whole lung explants harvested but not used for lung transplantation and from a tissue procurement service (IIAM, Edison, NJ) (3, 12, 13). For the present experiments, the clinical characteristics of lung tissue donors is summarized in **Table 1,** recognizing that full characteristics were unavailable for some donors that provided tissues collected post-mortem.

The primary endpoints for study were the site and level of expression for GPNMB in disease versus non-disease control conditions. Detection methods were the same those applied to studies of GPNMB expression in mouse models of PVLD (14) to allow for comparison across experimental and clinical conditions. Using this approach, immunostaining of lung tissue sections showed expression of GPNMB localized to $CD11c^+$ and $CD68^+$ cells with moDC and macrophage morphology in long-term Covid, asthma, and COPD (**Figure 1A**). Quantitative morphology demonstrated that levels of GPNMB expression were significantly increased in each disease condition compared to non-disease control (**Figure 1B**). In concert with GPNMB expression, immunostaining for CD44 was also localized (although not exclusively) to basal epithelial cells under disease and control conditions (**Figure 1A**).

Together, the present findings provide initial validation for comparable cell and molecular components in experimental models and clinical samples of chronic lung disease. In the experimental setting, GPNMB expression is significantly increased and localized to moDCs and macrophages in concert with basal-ESC hyperplasia/metaplasia and immune activation that feed-forward to promote additional immune cell infiltration. The relatively prolonged time course predicted a comparable $GPNMB^+$ moDC and macrophage signature in chronic lung disease even long after any previous injury. Indeed, that appears to be the case given the prominence of $GPNMB^+CD68^+$ macrophages found in clinical samples of lung tissue in long-term Covid-19, asthma, and COPD. Thus, the present findings are comparable to the later phase (21-49 d after infection) of the viral mouse model wherein similar GPNMB expression can be localized to M2 macrophages (14).

The present data suggests the presence of a persistent GPNMB signal in disease, raising the question of mechanism for how this signal remains active. Certainly having the signal in long-lived cells (in this case moDCs and macrophages but in other cases basal-ESCs) is a contributing factor, but this might not be sufficient to explain long-term persistence over years. In that regard, beneficial instructions for host defense, repair, and inflammatory memory after viral infection (15) or other injuries might instead manifest as long-term, epigenetic reprogramming for inflammatory disease. Passing these instructions in renewable cell populations would provide even longer reprogramming towards disease. In this case, GPNMB and related

biomarkers could provide guidance for detecting and correcting this type of disease. Thus, correlation with GPNMB signaling partners as recently identified (14, 16, 17) will also be instructive. Extending the present studies of post-mortem tissues to the clinical precision of a planned patient enrollment and comprehensive survey study will also better define the differences between non-disease and disease conditions. The present and pending information should provide significant practical value given the potential for correcting GPNMB and related signaling activities as a mechanism to modify post-injury disease.

Materials and Methods

Human clinical samples

For Covid-19 samples, human lung tissue was obtained from a series of consecutive autopsies performed from April-August 2020 at Barnes-Jewish Hospital as described previously (6). For asthma, COPD, and non-disease control samples, lung tissue was obtained from a Tissue Registry for Advanced Lung Disease that contains whole lung explants harvested but not used for lung transplantation and from a tissue procurement service (IIAM, Edison, NJ) as described previously (3, 6, 12, 13). Human studies were conducted with protocols approved by the Washington University (St. Louis, MO) Institutional Review Board and U.S. Army Medical Research and Development Command (USAMRDC) Office of Research Protections.

Histology and immunostaining

Lung tissue was fixed with 10% formalin, embedded in paraffin, cut into 5-µm sections and adhered to charged slides. Sections were stained with PAS and hematoxylin as described previously (9, 18). For immunostaining, sections were deparaffinized in Fisherbrand® CitriSolv® (Fisher), hydrated, and heat-treated with antigen unmasking solution (Vector Laboratories, Inc). Immunostaining was performed with the commercially available primary antibodies as listed in **Table 2**. Primary Abs were detected with secondary Abs labeled with Alexa Fluor 488 (Thermo Fisher Scientific) or Alexa Fluor 594 (Thermo Fisher Scientific) followed by DAPI counterstaining. Slides were imaged by light microscopy using a Leica DM5000 B and by immunofluorescent microscopy using an Olympus BX51, and staining was quantified in whole lung sections using a NanoZoomer S60 slide scanner (Hamamatsu) and ImageJ software as described previously (9, 18).

Statistical analysis

All data presented in bar-graph formats were expressed as mean ± SEM or SD as indicated. For this data, statistical differences between means for sample conditions were assessed using one-way analysis of variance (ANOVA) with Tukey correction for multiple comparisons. For all data, significance threshold was set at $P<0.05$. The number of human subjects for each condition is defined in the legend for **Figure 1**. These subjects were selected at random from the total group of subjects shown in **Table 1**.

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Disclosures

MJH is the Founder of NuPeak Therapeutics, Inc. KW, YZ, AGR, and MJH are inventors on a patent for MAPK inhibitors and methods of use thereof. MJH, KW, and YZ are inventors on a provisional patent for Methods of use for GPNMB-CD44 blockade in chronic respiratory disease.

Author contributions

K.W. organized and performed experiments, Y.Z. organized experiments, H.Y-D. performed immunostaining; D.M. performed experiments, K.S. performed experiments; E.C.C. identified and analyzed autopsy samples; D.E.B. obtained and libraried donor samples; and M. J. H. directed the project and wrote the manuscript.

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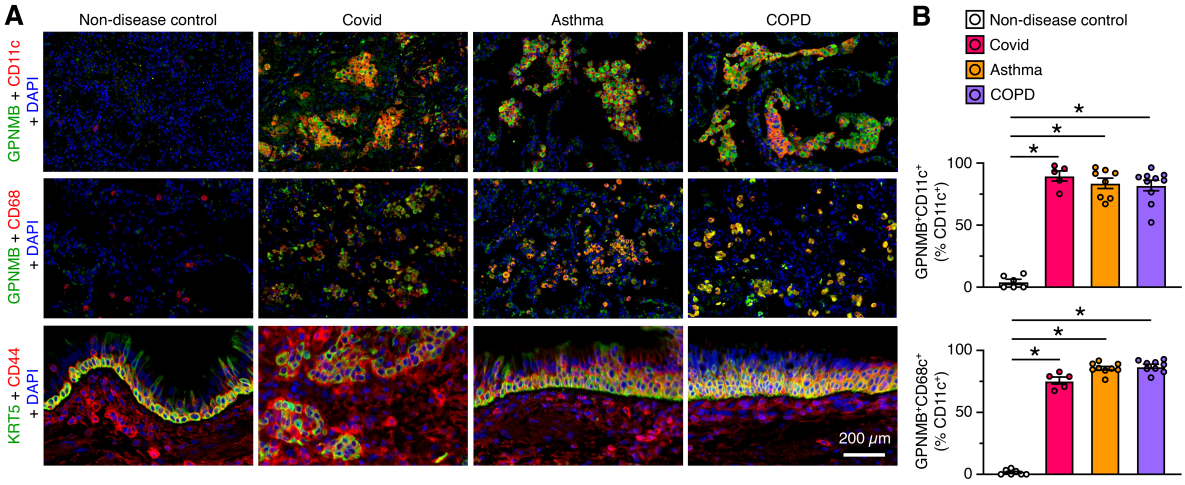

Figure 1

Figure 1. GPNMB and CD44 expression in chronic lung disease. A, Representative immunostaining for GPNMB plus CD11c or CD68 and KRT5 plus CD44 with DAPI counterstaining in lung sections from non-disease control (n=5-6), Covid (n=5), asthma (n=8-9), and COPD (n=9-10) subjects. **B**, Quantitation of immunostaining from (A). Values represent mean ± SEM. *P <0.05 by ANOVA and Tukey correction for multiple comparisons. No significant differences were found among disease conditions.

Table 1: Clinical characteristics of tissue sample groups.

Characteristic	Non-disease control (n=7)	Covid-19 (n=5)	Asthma (n=9)	COPD (n=14)
Age	41.7 ± 19.8	52.0 ± 6.5	47.0 ± 15.2	59.9 ± 6.2
Sex (M/F)	5/2	3/2	4/5	10/4
Race (W/B/H)	3/0/0[2]	1/4/0	4/3/2	13/1/0
Pack-year	ND[3]	ND	None[4]	57.0 ± 27.2
Years quit	ND	ND	ND	4.4 ± 3.4
FVC % pred	ND	ND	ND	62.7 ± 15.2
FEV1 % pred	ND	ND	ND	17.7 ± 3.5
FEV1/FVC ratio	ND	ND	ND	0.22 ± 0.04

[1]Data provided for the entire group of deceased subjects. The number of subjects used in analyses are included in the figures. Values represent mean ± SD.

[2]Race data missing for some non-disease control subjects.

[3]Two deceased non-disease control subjects had a history of tobacco smoking 10 pack-years and 15 pack-years, but smoking data was not available for all subjects.

[4]Two deceased asthmatics had less than a 5 pack-year history of tobacco smoking.

Abbreviations: ND, not determined.

Table 2. Antibodies for human tissue immunostaining.

Target Protein	Antibody Type	Vendor	Catalogue #
CD11c	Rabbit mAb	Cell Signaling	97585/45581
CD44	Rabbit mAb	Abcam	Ab189524
CD68	Rabbit mAb	Cell Signaling	76437
GPNMB	Mouse mAb	Proteintech	66926
KRT5	Rabbit pAb	Abcam	Ab53121