Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Bijean Dorell Ford Date

Functional and transcriptional adaptations of blood monocytes recruited to the cystic fibrosis airway microenvironment *in vitro*

By

Bijean Dorell Ford

Doctor of Philosophy

In

Immunology and Molecular Pathogenesis

_____

Rabindra Tirouvanziam, Ph.D.
Advisor

_____

Jacob Kohlmeier, Ph.D.
Committee Member

_____

Larry Boise, Ph.D.
Committee Member

_____

Larry Anderson, M.D.
Committee Member

_____

Sean Stowell, M.D., Ph.D.
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____

Date

Functional and transcriptional adaptations of blood monocytes recruited to the cystic fibrosis airway microenvironment *in vitro*

By

Bijean Dorell Ford
Bachelor's in Biology

Advisor: Rabindra Tirouvanziam, Ph.D.

An abstract of
a dissertation submitted to the faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Immunology and Molecular Pathogenesis
2021

Abstract

Functional and transcriptional adaptations of blood monocytes recruited to the cystic fibrosis airway microenvironment *in vitro*

By Bijean Dorell Ford

Cystic Fibrosis (CF) is an autosomal recessive genetic disease, impacting an estimated 80,000 individuals worldwide, and still without cure. This disease disproportionately affects people of Caucasian descent with over 1,800 confirmed mutations divided into 6 classes impacting the structure and localization of the CF Transmembrane Conductance Regulator (CFTR) ion channel. The dysfunction of CFTR leads to a variety of systemic effects, with the manifestation of lung disease, derived from a pathological triad of obstruction, inflammation, and infection, representing the primary cause of death in patients with CF.

CF lung disease is dominated by the recruitment of myeloid cells (neutrophils and monocytes) from the blood which fail to clear the lung of colonizing microbes. In prior studies, our group showed that blood neutrophils migrated through the well-differentiated lung epithelium into CF airway fluid supernatant (ASN, purified from patient sputum) *in vitro* mimic the dysfunction of CF airway neutrophils *in vivo*, including decreased bactericidal activity despite an increased metabolism. In this study, we hypothesized that, in a similar manner to neutrophils, blood monocytes undergo significant adaptations upon recruitment into the lung lumen and exposure to CFASN. We tested this hypothesis by isolating primary human monocytes from blood and transmigrating them in our *in vitro* model into the ASN from healthy control (HC) or CF subjects to mimic *in vivo* recruitment to normal or CF airways, respectively. Surface phenotype, metabolic and bacterial killing activities, and transcriptomic profiling by RNA sequencing were quantified post-transmigration.

Unlike neutrophils, monocytes were not metabolically activated, nor did they show broad differences in activation and scavenger receptor expression upon recruitment to the CFASN compared to HCASN. However, monocytes recruited to CFASN showed decreased bactericidal activity. RNASeq analysis showed strong effects of transmigration on monocyte RNA profile, with differences between CFASN and HCASN conditions, notably in immune signaling, including lower expression in the former of the antimicrobial factor ISG15, defensin-like chemokine CXCL11, and nitric oxide-producing enzyme NOS3. While monocytes undergo qualitatively different adaptations from those seen in neutrophils upon recruitment to the CF airway microenvironment, their bactericidal activity is also dysregulated, which could explain why they, too, fail to protect CF airways from infection.

Functional and transcriptional adaptations of blood monocytes recruited to the cystic fibrosis airway microenvironment *in vitro*

By

Bijean Dorell Ford
Bachelor's in Biology

Advisor: Rabindra Tirouvanziam, Ph.D.

A dissertation submitted to the faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Immunology and Molecular Pathogenesis
2021

Acknowledgements

My experience at Emory University very likely would not have lasted long if it were not for my mentor, Dr. Rabin Tirouvanziam. After working through 4 rotations and being unable to find a laboratory home, Rabin selflessly helped picked up my baggage, brushed me off, and took me in. Although I may question what diversity really means to this institution, there certainly is no question that Rabin has fostered arguably one of the most diverse and inclusive lab environments I have run across on campus. Throughout my doctoral training, he exercised an enormous level of patience with me, allowing me to bump my head to figure things out in lab and in life, while being able to correctively intervene when the time was right. Up to, and possibly past, the moment of writing this acknowledgement, Rabin has not only pushed me scientifically to think and inquire in ways that I have never considered, but he has also inspired me with his brilliance. I seriously believe his intellect is super-human, and while he may humbly deny this claim, I proudly proclaim it to be true. For all this, I am indebted to him.

To the members of the lab who have either moved on or are still riding with me, I am grateful for each experiential aspect you all contributed to my time here:

- To Dr. Sarah Ingersoll, thanks for gracing me with your post-doctoral presence early on, albeit very temporarily, before moving on to your industrial career. You took me under your wing and acclimated me to the ways of the lab, and since your departure I have always wished you stayed longer. But such is life.

- To Dr. Milton Brown, you familiarized me with every nook and cranny of the lab, providing me with anecdotes for every item and process. In true fashion you served as the grandfather of the lab, and although you would always be self-critical of what you described as your "long-windedness", it was always a pleasure for me to soak up your knowledge and learn more than I asked. Besides, I have always been more of a listener than a talker. Thank you so much for your wisdom, assistance, and kind nature.

- To the CF patients, without you there is no lab, and without the lab my work does not exist. Your lives are precious, and we do all we can to contribute to their qualitative and quantitative extension. Thank you dearly for your extra time in the clinics to contribute samples for our research to continue. Another big thank you to all the clinicians, clinical coordinators, and scientists who all encompass the Center for Cystic Fibrosis and Airways Disease Research (CF-AIR). Your work is invaluable.

- To my past and present graduate student colleagues (Osric, Camilla, Brian, and Vincent), our most recent post-doctoral mentors (Alex and Diego), and the various undergrads who have come and gone in their respective capacities, you have all added to my laboratory experience immensely and never hesitated to aid me with my experiments or provide intellectual capital. If I may take a step back, I think it is prudent that I spotlight Diego to acknowledge his role in training me to use analytical tools for diving into big data, such as with my RNA sequencing experiments. I do not believe I have enjoyed working with anyone more than with him, and yet we accomplished so much while laughing between lines of code, and again during teleconference calls on Zoom or Microsoft Teams, during the pandemic.

- To the coronavirus pandemic, thank you for making life a little bit more difficult and the future a bit more uncertain. For over a year of my thesis, you helped create bottlenecks of frustration, and bouts of depression. But not even you could stop me. You, too, will not soon be forgotten; perhaps you have even changed our way of life. I will not miss you.

- To my family and true friends who have stuck with me throughout this training experience, your love, encouragement, and support are unmatched. To my childhood best friend David Gross, you are among 5 people in the entire world, living or deceased, who really know me for me; you truly are the brother I never had. Your support has been lifesaving; God bless you. To my wife, Alexandria, you are the foundation upon which I crumble and get rebuilt again. Through tears, through anger, through numbness, and through triumph you have been there to hold me steady while watching my evolution as a human being. Our quantum entanglement feeds my soul; I love you and God bless you. To my parents, without you there is no me. Without your love, support, and investment, I very likely don't make it this far. To my earthly father in heaven, I have missed you every year, for 13 years and incorporate aspects of you into my everyday life to always honor your name. To my mother, I write you every year on your birthday that I can never repay you for what you have done for me; I can only try. I will always have your back as much as you have had mine. I love you and I thank you both; God bless you. To my grandmother Evelyn, I love you so much. I wish you could see me become the doctor you always knew I could be, but I know your spirit is still with me. Sharing the responsibility of being your caretaker up to and through most of graduate school is an honor that I will carry with me for life. Every Chick-fil-A meal I could pick up and every cup of tea I could make was a privilege. Every wise word you uttered, and every loving gesture you expressed will stick with me. I will always be your 'Sonny Boy.'

  Finally, I would like to dedicate this dissertation and experience to the loved ones who have transitioned during my time in graduate school. This is for you:
  - Grandma Evelyn
  - Grandma Lena
  - Great Aunt Luscita
  - Uncle Walch
  - Uncle Lincoln
  - Uncle Loy
  - Aunt Gwyneth
  - Uncle Corlie
  - Uncle Donny

TABLE OF CONTENTS

Chapter 1: Introduction to monocyte development, phenotype, function, and relationship to cystic fibrosis airway disease

Overview of monocyte development and function

Monocytes originate from hematopoietic stem cells located in the bone marrow. Monocyte development proceeds through three differentiation events, progressing from stem cell to monoblast, monoblast to promonocyte, and promonocyte to differentiated monocytes **(Table 1.1)**. Differentiated monocytes express high levels of the CXC chemokine receptor 4 (CXCR4), leading to their retention in the bone marrow as they remain in close association with local stromal cells which express the CXCR4 ligand stromal cell derived factor-1 (SDF-1) **[1]**. Pulse-chase studies using the DNA labeling thymidine analog bromodeoxyuridine (BrdU) have shown that, on average, monocytes spend roughly 40 hours in the bone marrow during their maturation phase. Over the course of their development human monocytes increase their expression of CD14 (similar to Ly6C in mice). A gradual decrease in CXCR4 levels tracks with increasing expression of the other chemokine receptors CCR2 and CX3CR1. This change in expression licenses mature monocytes for release into the systemic circulation. Highlighting the importance of this process is the disease associated with monocytopenia termed WHIM syndrome (for warts, hypogammaglobulinemia, infections, and myelokathexis). In WHIM patients, persistent CXCR4 expression acts as an anchor for monocytes, preventing their release into blood **[2]**.

Table 1.1 Stages of monocyte development in the bone marrow of humans and mice. (originally featured in **[1, 3]**). HLA-DR: Human leukocyte antigen DR, HSC: hematopoietic stem cell.

Developmental stage	**Surface marker expression**	
	Humans	***Mice***
Multipotent HSC	Sca-1	Sca-1
Common myeloid progenitor	CD34, CD117	CD34
Myeloblast	CD16, CD32, CD34, CD117	CD16, CD32, CD34
Monoblast	CD117, CD135, CX3CR1	CD115, CXC3CR1, FLT-3
Promonocyte	CD13, CD115, CXC3CR1, HLA-DR	CD115, CXC3CR1, Ly6C
Differentiated monocyte	CCR2, CD13, CD14, $CD15^{Lo}$, HLA-DR	Classical: CD115, $CX3CR1^{Lo}$, CCR2-Hi, $CD62L^{Hi}$ Non-classical: CD115, $CX3CR1^{Hi}$, $CCR2^{Lo}$, $CD62L^{Lo}$

Acute modulation of CXCR4 in the final maturation step of monocytes in the bone marrow coincides with a transcriptional switch. Accordingly, certain genes are activated upon monocyte release into blood, while others that were on in the bone marrow stages are turned down **[4]**. Once in the systemic circulation, where they typically reside for 1 to 3 days, monocytes may be called upon to seed various tissues throughout the body. In the typical process of tissue inflammation, following a first wave of neutrophils, monocytes arrive 24-48 hours later to become a prominent cell type at the site. At that point, they may get activated and serve as a versatile effector population that can jumpstart immune responses and contribute to acute and chronic inflammation. Responses mounted by activated monocytes is crucial to displacing invading microorganisms and other foreign bodies. Alternatively, recruited monocytes may differentiate into tissue macrophages. The latter process serves to replenish the pre-existing, resident macrophage population that may be depleted by inflammation. The main cytokines involved in monocyte differentiation to macrophages include GM-CSF and M-CSF, signaling through the cognate receptors for GM-CSFR and M-CSFR, respectively **[5]**. As a third alternative, monocytes may differentiate into dendritic cells, which amplifies antigen presentation and kick-starts T- and B-cell responses, thus providing a bridge to adaptive immunity **[6]**.

Monocytes exhibit extensive plasticity of phenotype and function, yielding three main subsets **[7-11]**. The majority of human monocytes are identified as $CD14^{Hi}$ $CD16^{Lo}$ cells, with 80-90% of monocytes belonging to this 'classical' denominated subset **[7]**. Two other subsets manifest themselves in much smaller numbers, with 'non-classical', patrolling monocytes identified as $CD14^{Lo}$ $CD16^{Hi}$, and 'intermediate'/inflammatory monocytes as $CD14^{Hi}$ $CD16^{Hi}$ **[8]**. Typically, $CD14^{Hi}$ $CD16^{Lo}$ monocytes ($Ly6C^{Hi}$ in mice) are drawn early to sites of infection to combat microorganisms **[12]**, while $CD14^{Lo}$ $CD16^{Hi}$ monocytes ($Ly6C^{Lo}$ in mice) leave the circulation at sites to patrol the vasculature and aid in the removal of debris. Under other circumstances, $CD14^{Lo}$ $CD16^{Hi}$ may the first to the site of injury and recruit the next wave of leukocytes. In a positive feedback loop, the resulting release of proinflammatory cytokines like IL-6, GM-CSF, M-CSF can signal the bone marrow to release more monocytes **[5]**.

Debate still exists over the role of the intermediate monocytes as their function and proportion can vary depending on the context. For instance, classical monocytes exercise phagocytic capabilities at the site of inflammation. Under the same conditions, patrolling (non-classical) monocytes express high levels of proinflammatory cytokines, with this subset secreting the most IL-1β of all three subsets. Intermediate monocytes share attributes of both classical and non-classical subsets as they seem to represent a transitional state; in some conditions they secrete the anti-inflammatory cytokine IL-10 **[13]**, while in others they may upregulate expression of co-stimulatory CD80 and CD86 receptors and thereby provide necessary signals to trigger T-cell activation upon antigen presentation **[14]**.

In the context of microbial infection, it is crucial for myeloid cells, notably monocytes, to effectively exercise their functions as first-responders by migrating into the site of infection as quickly as possible. For instance, CD16+ monocytes can quickly transition from patrolling activities to a first responder profile that may then signal for additional backup. Indeed, such non-classical monocytes, in coordination with the damaged tissue, can release damage signals (alarmins) and pro-inflammatory mediators that usher in subsequent waves of neutrophils and CD14+ classical and intermediate monocytes from blood.

Following this early response, monocytes utilize their antigen presentation capabilities, as inflammatory cytokines induce them to upregulate major histocompatibility complex (MHC)-class II molecules such as HLA-DP, HLA-DQ and HLA-DR **[15]** to initiate adaptive immune responses. In contrast to this pro-inflammatory scenario, *in vitro* studies assessing the response of monocytes exposed to the anti-inflammatory cytokine IL-10*,* have shown the down-regulation of MHC-class II antigen presentation machinery by monocytes from all three subsets, with the expression of the Class II-associated invariant chain peptide (CLIP) inversely related to HLA-DR expression **[16, 17]**. This finding supports the general premise of classical and intermediate monocytes performing the majority of phagocytic functions during an immune response.

Tissue migration and differentiation

To migrate into tissues, monocytes use an array of chemokine receptors and adhesion molecules such as CCR2 (receptor for CCL2), CX3CR1 (receptor for CX3CL1), MAC-1, LFA-1, and L-selectin, which allow them to adhere to the endothelium and extravasate [18]. In the case of interstitial lung disease, a gradient of CX3CL1 (fractalkine) licenses non-classical monocytes which express CX3CR1 to migrate into the tissue, which in turn increases the relative frequency of classical monocytes in blood [19]. In the context of alveolar infection, CCL2 secretion by epithelial cells contributes to developing a pro-inflammatory environment by recruiting CCR2-expressing monocytes **[20]**. Blood monocytes entering tissues may exit by way of the lymphatic system and carrying out other duties, while retaining their identity as monocytes **[21]**. Tissue-recruited monocytes may also opt to differentiate into monocyte-derived dendritic cells and monocyte-derived macrophages (MDMs), both of which are professional antigen-presenting cells (APCs) **[22]**. GM-CSF signaling **[23]** for example aids in guiding monocytes toward a dendritic cell phenotype by inhibiting the autophagy pathway and further cytokine production and differentiation. When monocytes differentiate into macrophages, they can then adopt multiple functional paths upon differentiation (typically M0, M1, M17), which impacts their function **(Figure 1.1)**.

Although macrophage differentiation from monocytes is common, not all tissue macrophages are derived from monocytes. Developmentally, tissue-resident macrophages migrate from the fetal yolk sac **[24, 25]** into local environments such as the brain, lungs, kidneys, spleen, and liver, maintaining homeostasis at these sites [26]. Under homeostatic conditions, tissue-resident macrophages are replenished through self-renewal **[27]**. However, upon local injury/inflammation, as in the case of an ischemic event following myocardial infarction, tissue-resident macrophages can be replaced by blood monocytes that differentiate into myocardial macrophages **[21, 28]**. However, monocyte-derived macrophages replacing depleted tissue-resident macrophages may not be equipped with functional capabilities; this has been documented within the liver, brain, heart, bladder, and lungs **[29-31].**

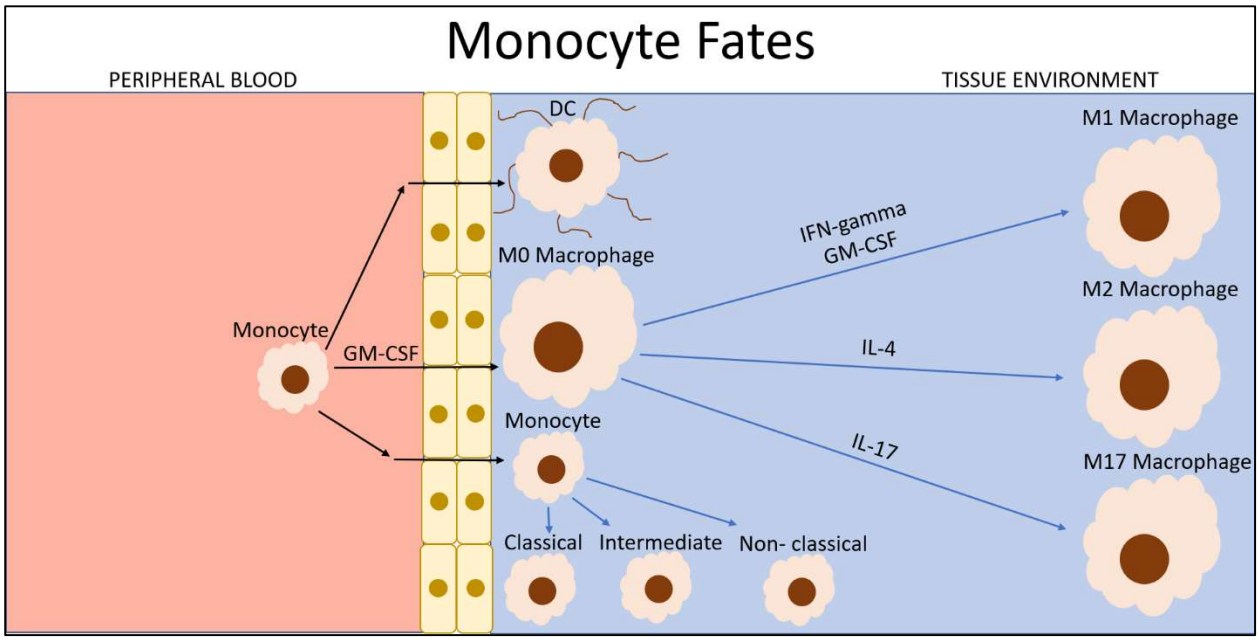

Figure 1.1. Monocyte differentiation into macrophage and subsequent polarization following exposure to microenvironment-specific factors. Tissues naturally possess resident macrophage populations derived from the fetal yolk sac. During stress-induced tissue trauma, apoptosis of resident macrophages may occur, inducing blood monocyte recruitment and differentiation into macrophages.

Monocytes: "of mice and men"

When characterizing monocytes in mice, common cell surface markers are Ly6C, CCR2 and CX3CR1, mirroring human monocyte subset expression of CD14 (Ly6CHi, CCR2Hi) and CD16 (Ly6CLo, CX3CR1Hi), respectively **[32] (Table 1.2)**. Additional markers such as CD86, HLA-DR and CD45 aid in discerning monocytes from other leukocytes with significant expression of Ly6C, CCR2 and CX3CR1 **[14]**. Mouse monocytes are generally segregated into classical inflammatory monocytes (Ly6CHi, CCR2Hi) and non-classical patrolling monocytes (Ly6CLo, CX3CR1Hi). The intermediate monocyte subset seen in humans may be identified in mice by way of TREML4, which appears upregulated in a putative intermediate monocyte subset differing from Ly6CHi and Ly6CLo subsets **[33]**. Hence, one of the limitations of mouse studies is the relative inability to adequately dissect the role of intermediate monocyte, whose impact on immune responses is still debated, notably in acute bacterial / viral infections, chronic immunoinflammatory diseases such as rheumatoid arthritis and lupus erythematosus and CF **[8, 10, 34-38]**. Additional methods for human monocyte subsetting based on transcriptomics have led to further segregation of the intermediate subset into Mono3 and Mono4. Mono3 cells express cell cycle, differentiation, and trafficking signatures, while Mono4 cells express cytotoxic signatures **[39]**. More work is needed to better substantiate these subsets in humans and in mice.

Table 1.2. Monocyte subsets in humans and mice. (originally featured in **[7, 39]**).

Monocyte Subset	Human Expression Markers	Mouse Expression Markers	Percent of Monocytes in Blood	Hallmark Functional Role(s)	Gene Expression
Classical Monocyte	CD14++ CD16-	Ly6C^{hi}CX3CR1^{lo}	85%	Phagocytosis	*CD14, CD163, TREM1, CD36*
Intermediate (I) Monocyte	CD14++CD16+	Ly6C^{int}CX3CR1^{hi}	5%	Cytokine Signaling	*MXD1, CXCR1, CXCR2, VNN2*
Intermediate (II) Monocyte	CD14+CD16+				*PRF1*, *GNLY*, *CTSW*
Non-classical Monocyte	CD14dimCD16+	Ly6C^{lo}CX3CR1^{hi}	10%	Cytokine Signaling/Antigen Presentation	SIGLEC10, ATSS, IFITM2, NFAT1

Monocyte transcriptional profile

In the bone marrow, the transcription factor GATA2 assists in myeloid progenitor differentiation and self-renewal. The transcription factor Fli-1 serves an opposite role by negatively regulating myeloid progenitor development **[1]**. The runt-related transcription factor 1 (RUNX1) directly upregulates the transcription of PU.1, leading to its stable expression. The transcription factor PU.1 plays an essential role in early monocyte development **[1, 40]**. Studies in knockout mice have shown that lack of PU.1 expression leads to severely depleted levels of monocyte and DC subsets, resulting in early death. Additionally, low-level expression of PU.1 favors differentiation of cells into the granulocytic lineage, resulting in systemic neutrophilia **[1]**. In subsequent steps, other transcription factors take over, such as IRF8 and KLF4; In mice deficient in these two factors, Ly6CHi monocyte development is stunted. Meanwhile, the transcription factors NR4A1 and Nur77 play crucial roles in the specification and development of Ly6CLo monocytes.

Consistently, transcriptional profiles differ among monocyte subsets. Classical monocytes express higher levels of genes associated with the regulation of cell cycle, cytoskeletal organization, cellular

protein catabolism, cell death, and phagocytosis. Among the latter, highly expressed genes encode CD93, CD64, CD32, CD36, ficolin 1 (FCN1) and signal regulatory protein-alpha (CD172a/SIRPα). This is consistent with clearance of pathogens being the primary function of classical monocytes. Typical gene signatures of classical monocytes include VCAN, S100A12, CD163, SLC2A3, RNASE6, CCR2, VEGF, EMB, S100A8, and ASGR2 **[41]**. The transcriptional profile of non-classical monocytes reveals expression of genes regulating cell cycle, macromolecule catabolism, transcription, translation, and apoptosis. Typical gene signatures of non-classical monocyte include IFITM1, CKB, RHOC, CDKN1C, CD79B, MTSS1, ADA, IL21R, IFITM2, NEURL, and HIST1H2BF **[41]**. Lastly, intermediate monocytes express genes involved in the regulation of RNA processing, cellular protein metabolism, responses to DNA damage, transcription, and lipid transport. Typical gene signatures of intermediate monocytes include NKG7, CD1C, HLA-DMA, ICAM-1, MGLL, PTX3, WARS, and CDKN1A **[41]**.

Transcripts involved in monocyte-to-macrophage differentiation

Despite the unique gene signatures of each of the three distinct monocyte subsets, these may overlap in their expression of certain genes. Indeed, classical monocytes and intermediate monocytes have more in common than with non-classical monocytes, which are considered more mature than their classical and intermediate counterparts **[1]**. CD16+ monocytes (non-classical) express macrophage-like and DC-like transcripts for CD16, CD83, SPN, ITGAL and BTLA. By contrast, CD14+(classical) and CD16+CD14+ (intermediate) monocytes express signatures of immaturity such as CD14, TREM1, CD93, CD114, and CD116 **[41]**. This aligns with the premise that monocytes lose CD14 expression as circulation time increases and monocytes further their maturation. In further support of this notion, proliferation-associated genes are upregulated in classical monocytes while genes associated with the inhibition of the cell cycle are present in non-classical monocytes. Interestingly, monocyte-derived macrophages which have the ability to replenish themselves upregulate *Myc, Klf2,* and *Klf4* while simultaneously

downregulating MafB and c-Maf. Collectively, these changes effectively release the brake on self-renewal process. This is aided by M-CSF, which helps establish a MycHi Maf-BLo phenotype **[1]**.

Aging also impacts monocyte gene expression. Stimulation of monocytes with the Toll-like receptor 4 (TLR4) agonist lipopolysaccharide (LPS), non-classical monocytes in middle-aged adults upregulate genes for tRNA metabolic processes, extrinsic pathways for apoptosis, and mitosis. In contrast, similar treatment in older adults upregulates genes supporting antigen presentation (MHC Class II), programmed death -PD-1 signaling, bacterial and viral recognition via pattern recognition receptors (PRRs), and superoxide production **[42]**. In classical monocytes stimulated with single stranded RNA (TLR7/8 agonist), monocytes from middle-aged adults upregulate genes for TNFR2, co-stimulation signaling, interferon, antiviral, and proinflammatory signaling. Meanwhile, classical monocytes from older adults under the same stimulus upregulate genes supportive of ROS and superoxide production; basically, anaerobic metabolic processes are upregulated in the elderly.

Monocyte metabolism and reactive oxygen species (ROS) production

Metabolism is essential to support cell function, and the extent of it mirrors the cell's level of activation. During inflammation, monocyte effector functions and proliferation are particularly energy-demanding **[43, 44]**. Various pathways support the metabolic needs of monocytes, including glycolysis and oxidative phosphorylation (OxPhos). Glycolysis uses a set of cytosolic enzymes to catabolize glucose for partial ATP production (2 ATP per glucose molecule), generating lactate as a by-product, and is an option during rapid activation phases. In certain activation conditions necessitating reactive oxygen species (ROS) production, glycolysis can be shunted into the pentose phosphate pathway (PPP). Glucose catabolism through the PPP leads to the production of NADPH (instead of ATP), which is then oxidized by nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase or NOX) enzymes to form superoxide ions, the precursor of all ROS **[45]**.

Superoxide dismutates spontaneously or enzymatically to hydrogen peroxide **[46, 47]**. In turn, hydrogen peroxide associates with chloride ions to form hypochlorous acid (the active component of bleach) under enzymatic activity of myeloperoxidase (MPO) **[48, 49]**. While monocyte precursors actively synthesize MPO during bone marrow differentiation, circulating monocytes do not. Highlighting the importance of this bleach-producing enzyme, studies have shown that higher plasma MPO levels associate with a greater risk and incidence of cardiovascular disease, including atherosclerosis **[50]**. Indeed, excessive ROS production can make the tissue microenvironment highly oxidative and potentially toxic not just to microbes, but also to host cells **[49]**. Conversely, suboptimal ROS production can cause resistant pathogens to escape killing by myeloid cells. Traumatic events such as stroke may negatively impact ROS production by monocytes, making patients susceptible to secondary bacterial infection **[51]**.

In contract with glycolysis and PPP, OxPhos requires mitochondrial involvement and can use glucose, fatty acids, or glutamine as substrates, leading to slower but complete catabolism and maximal ATP production (36 ATP per glucose molecule). Regulatory functions of monocytes that do not require quick bursts of energy often leverage fatty acid catabolism via OxPhos **[52]**. Amino acids can also serve as substrates for OxPhos. In patients with rheumatoid arthritis (RA), monocytes modulate the expression of the amino acid transporter SLC7A5 **[53]**, which in turn leads to the activation of mechanistic target of rapamycin complex 1 (mTORC1) a key anabolic switch in cells **[54-56]**. Stimulation of monocytes with LPS upregulates SLC7A5 to higher levels in monocytes from RA patients compared to healthy controls, whereas blockade of SLC7A5 and mTORC1 lowers inflammatory cytokine secretion and glycolysis.

Both NOX activity and OxPhos consume oxygen. Therefore, rapid activation of monocytes (and of neutrophils and macrophages also endowed with high glycolytic capacity and NOX activity) during inflammation can decrease the local pH (due to lactate release) and oxygen supply, steering metabolism toward anaerobic pathways **[45]**. In turn, expression of hypoxia inducible factor-1 (HIF-1) dependent signaling in monocytes signals for a switch in gene expression to adapt to the lack of oxygen **[8, 57]**.

Monocyte phagocytosis and clearance of pathogens

Recognition of pathogens by monocytes is conducted via pattern recognition receptors (PRRs), such as TLRs, which are located at the cell surface and within endosomes **[58]**. For instance, different types of bacteria can be recognized by TLRs 2, 4, and 6 **[59]**. Binding of antibodies to pathogen-expressed epitopes may also enable their uptake via common Fcγ receptors I-III (CD16, CD32, CD64). Whether via PRRs of Fc receptors, pathogen uptake results in the formation of a "phagosome" **(Figure 1.2)**.

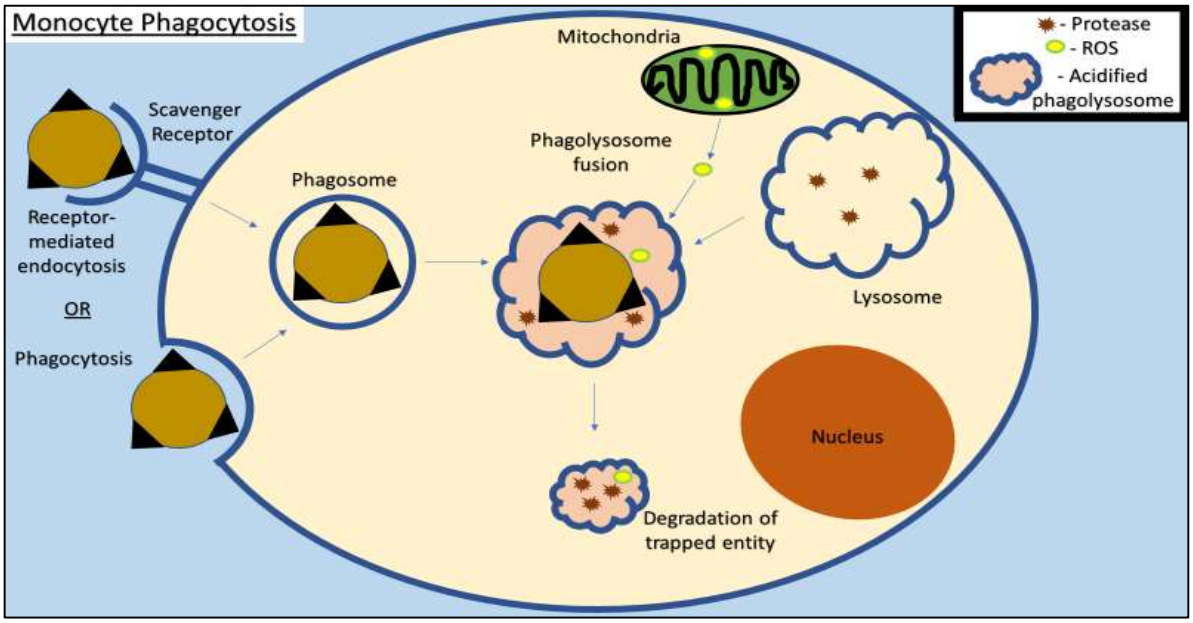

Figure 1.2 Monocyte phagocytosis and pathogen killing. Monocytes can kill pathogens via sequential uptake and fusion of the resulting phagosome with other compartments, such as the lysosome.

Killing of the target pathogen is then conducted by fusion of the phagosome with the lysosome, thus forming the phagolysosome **[60]** which is enriched with ROS and other toxic effectors (proteases, antimicrobial peptides). This process is not always effective, as various pathogens have evolved escape mechanisms to leave the phagosome or to prevent its fusion with the lysosome **[61]**. This may be countered by phagosome fusion first with autophagy vesicles, resulting in an autophagosome, and then with lysosomes, resulting in an autophagolysosome **[62],** which can precipitate the demise of pathogens.

Monocyte scavenging

Scavenging of endogenous debris is another critical function of monocytes, which they share with macrophages and neutrophils. Scavenging may be facilitated by tagging of material with antibodies. Thus, Fc receptors play a key role not only in phagocytosis, as described above, but also in uptake of debris, antibody-dependent cellular cytotoxicity, and cellular inhibition **[63]** of other host cells by monocytes. Beyond Fc receptors, a broad set of scavenger receptors with affinity for proteinaceous ligands, lipids, nucleic acids, or apoptotic bodies mediate monocyte uptake of various kinds of material **(Table 1.3)**. These scavenger receptors categorized by class, from A to L that are expressed monocytes and macrophages at varying degrees and with differences between organs, physiological conditions, and animal models. With the exception of class C, all classes are expressed on human cells **[64]**.

Table 1.3 Human-expressed scavenger receptors. (originally featured in **[64]**).

Scavenger Receptor Protein	Class A Scavenger Receptor	Class B Scavenger Receptor	Class C Scavenger Receptor	Class D Scavenger Receptor	Class E Scavenger Receptor	Class F Scavenger Receptor	Class G Scavenger Receptor	Class H Scavenger Receptor	Class I Scavenger Receptor	Class J Scavenger Receptor	Class K Scavenger Receptor	Class L Scavenger Receptor
1.	SR-A1/ MSR-1	SR-B1/ CD36L1	N/A	CD68	SR-E1	SR-F1	SR-G1/ CXCL16	SR-H1/ STABILIN-1	SR-I1/ CD163	SR-J1/ AGER	SR-K1/ CD44	SR-L1/ CD91
2.	SR-A1.1	SR-B1.1	N/A		SR-E1.1	SR-F2		SR-H2/ STABILIN-2		SR-J1.1		SR-L2/ GP330
3.	SR-A3/ MSRL1/ APC7	SR-B2/ CD36	N/A		SR-E2/ DECTIN-1							
4.	SR-A4/ SR-CL		N/A		SR-E3/ CD206							
5.	SR-A5/ TESR		N/A		SR-E4/ CLEC4H1							
6.	SR-A6/ SCARA2		N/A									
Associated Scavenger Receptor Gene	Class A Scavenger Receptor	Class B Scavenger Receptor	Class C Scavenger Receptor	Class D Scavenger Receptor	Class E Scavenger Receptor	Class F Scavenger Receptor	Class G Scavenger Receptor	Class H Scavenger Receptor	Class I Scavenger Receptor	Class J Scavenger Receptor	Class K Scavenger Receptor	Class L Scavenger Receptor
1.	*SCARA1*	*SCARB1*	*N/A*	*CD68*	*OLR1*	*SCARF1*	*CXCL16*	*STAB1*	*CD163*	*RAGE*	*CD44*	*LRP1*
2.	*SCARA1*	*SR-BII*	*N/A*		*OLR1*	*MEGF10*		*STAB2*		*RAGE*		*LRP2*
3.	*SCARA3*	*CD36*	*N/A*		*CLEC7A*							
4.	*COLEC12*		*N/A*		*MRC1*							
5.	*SCARA5*		*N/A*		*ASGR1*							
6.	*MARCO*		*N/A*									

For instance, monocytes and macrophages are known to be avid scavengers of lipids, and an elevated number of monocytes in blood is a correlate of atherosclerotic disease severity **[9]**. CD36 (monocytes and macrophages), as well as LDLR and SR-A1 (macrophages) mediate uptake of cholesterol, lipoproteins, lysophosphatidylcholine, and oxidized lipids **[65-67]**. Lipid-laden monocytes predominately carrying LDLs and VLDLs are inhibited in their migratory capacity as their cytoskeletal structure modifications are reduced to inhibit their movement **[68]**. Over time, these scavenger cells stiffen the vascular wall, adding to the risk of obstruction and rupture. Other prominent scavenger receptors include CD91, CD172a, CD163, and Tyro3/Ax/Mer (TAMs), as detailed below.

CD91 (LRP1) is a key efferocytosis receptor which facilitates the update of apoptotic bodies bound to particular proteins, such as complement, collectins **[69]**, and surfactant proteins **[70]**. There are over 30 binding partners to CD91 and its expression on monocytes and macrophages signals their activation **[66, 69]**. Additionally, this marker has been more recently utilized as an alternative method of categorizing monocytes into classical and non-classical subsets, based on surface CD91 expression **[71]**. CD172a (SIRPα) serves as another important receptor via binds to its ligand CD47 on target cells. CD47 is a "don't eat me" signal that serves as a means by which monocytes and macrophages verify that the cell in direct proximity is not ready for clearance. Accordingly, apoptotic cells that lose their CD47 clustering ability at the surface become amenable to scavenging by monocytes and macrophages **[72]**. Studies have also targeted the CD172a/CD47 axis in cancer, by way of antibody blockade, to restore tumor removal of monocytes and macrophages **[73]**. CD172a is an inhibitory receptor, and loss of its surface expression increases the inflammatory poise of monocytes **[74]**. CD172a also has been shown to play an important role in monocyte transmigration across the CD47-expressing cerebral endothelium, as inhibition of this interaction delayed transmigration time **[75]**. Interestingly, collectins can elicit opposite responses in monocytes/macrophages via binding to CD172a (inhibition) or CD91 (activation) following engulfment of extracellular material by these scavenger cells **[69, 76]**.

CD163 is a surface receptor with a role in the resolution of inflammation. Indeed, increasing numbers of monocytes / macrophages expressing CD163 correlates with survival and positive prognosis in chronic disease **[77]**. On the flipside, increased levels of the decoy receptor CD163 in the plasma is an indicator of deteriorating health status, as illustrated in patients with liver failure **[78]**. TAM receptors, including Tyro3, Axl, and Mer are also active in the resolution phase of inflammatory responses **[79, 80]**. TAM receptors enable macrophages to endocytose apoptotic bodies via binding to phosphatidylserine leaflets on cell membranes and rid the microenvironment of potentially inflammatory stimuli, thus avoiding pro-inflammatory signaling. However, in some pathological environments, proteases from host cells such as neutrophil elastase (NE) **[81]** and matrix metalloproteinase-9 (MMP-9) **[82]** may cleave TAM receptors. Similarly, microbial proteases may also cleave these receptors **[83, 84]**, rendering them ineffective. The net effect of such protease-mediated cleavage of TAM receptors would be to enable the accumulation of cellular debris as disease progresses and inhibit the resolution of inflammation and subsequent repair.

An inherent difficulty to studies of scavenger receptors and their relative role in pathology is their partial overlap in expression, and ligand promiscuity, leading to functional redundancy. Thus, it is unclear which scavenger receptors are critical because of the cell's absolute dependency on them for specific tasks, while others may be optional. Additionally, as illustrated by protease-dependent cleavage discussed above, there are post-translationally modified isoforms (as well as splice variants) of some of these scavenger receptors that may confer different functions, and also need to be taken into account in complex inflammatory conditions **[64]**.

Overview of cystic fibrosis

Cystic fibrosis (CF) is a monogenic disease resulting from recessive mutations of the *cftr* gene, leading to partial or complete loss of CFTR anion channel and abnormal function of exocrine epithelial in the genital tract, sweat glands, salivary glands, pancreas, gut and lung **[85]**. There are 2,000 known

mutations of the *cftr* gene, affecting about 80,000 patients worldwide. Caucasians are disproportionately impacted by this disease, with a carrier frequency of ~1:25 and an incidence of ~ 1:2,500 **[86, 87]**. Due to the partial penetrance of *cftr* mutations, not all organs are affected equally, even in patients carrying the same mutations. While CF remains intractable to this day, maintenance therapies include: (i) pancreatic enzyme replacement therapy and high-calorie diet to enable patients to thrive nutritionally; (ii) high-frequency chest wall oscillation vests **[88, 89]**, alone or combined with nebulized hypertonic saline (3-5% salt water) to help mobilize the secretion of water from the airway epithelium into the lumen and subsequently induce effective coughing for mucus clearance **[90]**. Thanks to these measures, CF is no longer a diagnosis leading to short-term death after birth, and patients live long enough to allow the manifestation of comorbidities, such as CF-related diabetes (CFRD) and osteoporosis **[91, 92]**.

CF airway disease pathogenesis

The most frequent cause of morbidity and mortality in patients with CF is lung disease **[93]**. Symptoms start in the small airways, featuring (i) obstruction, (ii) chronic infection, and (iii) early and sustained inflammation. This pathological triad induced cycles of acute pulmonary exacerbations (APEs) leading to frequent hospitalizations **[94, 95]**. With each APE, lung function drops and even upon treatment, often fails to return to the prior baseline **[94, 96-98]**. While CF airway disease has long been thought to manifest initially as an obstructive process., recent studies showed that inflammation with neutrophils from blood may precede obstruction **[99]**. Multiple *in vivo* studies conducted by the our group in infants, children and adults with CF further demonstrated that naïve blood neutrophils recruited to the CF airway microenvironment *in vivo* undergo profound functional and signaling adaptations **[100]**.

Functionally, CF airway neutrophils adopt a new fate which we dubbed GRIM for granule-releasing (active exocytosis of NE and MPO containing primary granules), immunomodulatory (inhibition of T-cells via the release of the metabolic enzyme arginase-1), and metabolically active (high glycolytic rate, anabolic/mTOR/pinocytic poise and catabolic/lysosome capacity), and an active inhibition of bacterial

killing **[101-107].** These adaptations are driven by a rapid and broad derepression of their chromatin and *de novo* transcription **[106]**. GRIM neutrophils are present in the lumen of CF airways shortly after birth, and their by-products including NE and MPO correlate positively with structural lung damage in children **[108, 109]**, and negatively with lung function in adults **[107]**. As the number of neutrophils entering CF airways increases, more neutrophil by-products (NE, MPO, arginase-1, etc.) are released actively into the lumen. The milieu that is created by this admixture of molecules **(Figure 1.3)**, fuels the recruitment of more neutrophils, epithelial dysfunction and chronic bacterial infection **[110].**

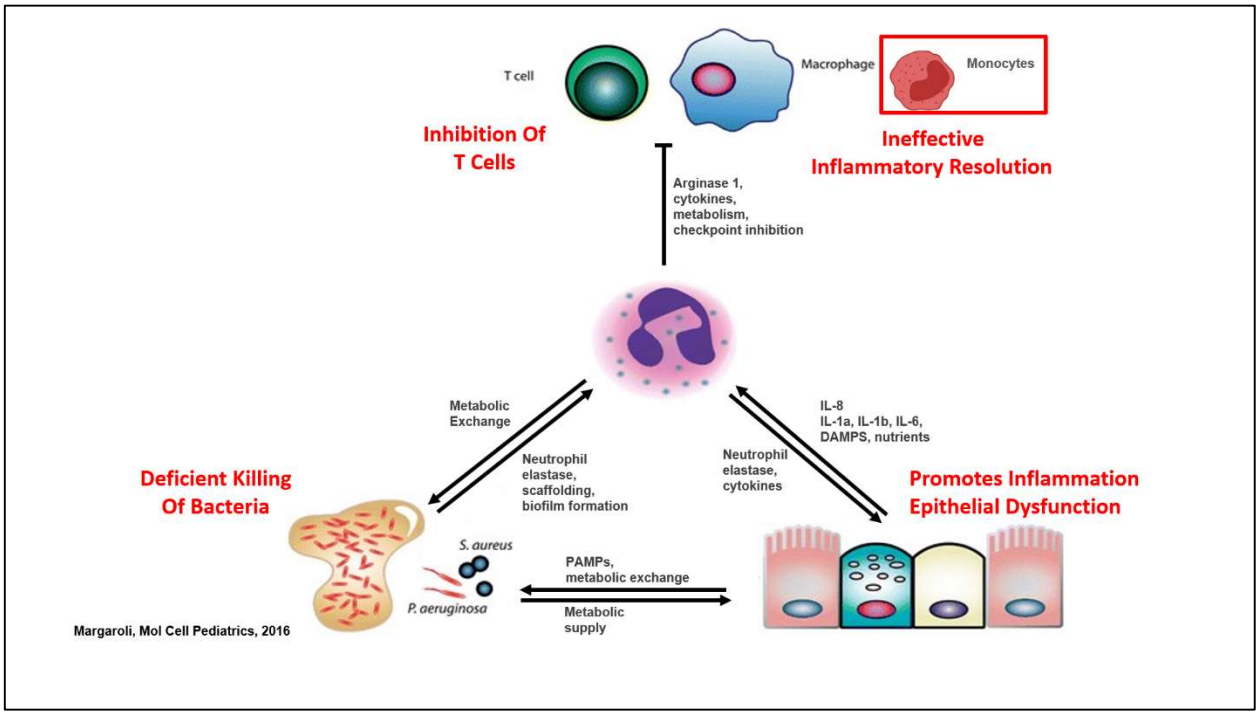

Figure 1.3. Cellular interplay within the CF airway microenvironment. The schematic highlights how each component of the CF airway milieu contribute to perpetuating disease. (originally featured in **[100]**).

Consistent with a key "feed-forward" role of the CF airway milieu in perpetuating disease **(Figure 1.4)**, our group was able to recapitulate the profound adaptations observed in CF lung neutrophils *in vivo* (transcriptional reprogramming and GRIM fate, including active inhibition of bacterial killing) by transmigrating naïve human blood neutrophils through a differentiated human epithelium topped with CF

sputum **[101, 106]**. This novel transmigration model has since then been applied to other lung diseases (e.g., asthma, acute lung injury **[111])** and opens avenues for further investigations into the mechanisms of leukocyte transmigration and adaptation/activation post-transmigration into human airways.

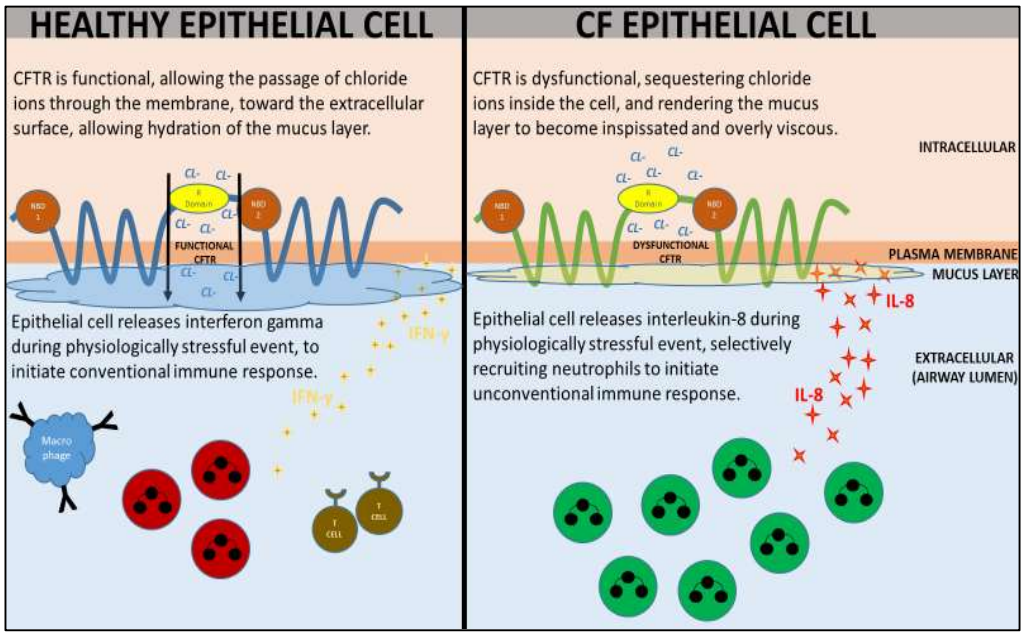

Figure 1.4. Comparison of CF and healthy airway microenvironments. CFTR dysfunction affects the properties of the airway fluid (ionic content, pH, hydration, inflammatory content, viscosity), which is concomitant with an early and sustained recruitment of neutrophils from blood. These neutrophils adopt the pathological GRIM phenotype, which is responsible for perpetuating disease (see also **Figure 1.3**).

Although neutrophils dominate the CF airway microenvironment the role of other immune subsets such as resident macrophages and T-cells. While T-cells are primarily accumulating in the lamina propria of CF airways and are quickly inhibited upon migration into the lumen **[102],** macrophages are present in the lumen, but their relative frequency and activity appear to diminish as those of neutrophils become predominant **[112]**. However, the role of blood monocytes in CF airways remains unclear, as does the ability of the CF airway microenvironment to impact their function. This is the focus of this work, which leverages our transmigration model to study monocyte-dependent mechanisms in CF inflammation.

CFTR effects on monocyte/macrophage function

While the *cftr* gene is controlled by a housekeeping promoter, its dysfunction resulting from mutations seems to manifest primarily in exocrine epithelial cells **[113]**. However, there is contention over the impact that CFTR may have on the bacterial killing capacity of monocytes / macrophages **[114-117]**. In addition, it has been suggested that human monocyte-derived macrophages from non-CF donors (expressing normal CFTR function) are less prone to apoptosis, and show greater phagocytic ability, than those from CF donors **[101, 116, 118, 119]**. In mice, alveolar macrophages from CFTR-deficient mice, exhibit defective bacterial killing which may relate to its bicarbonate ion transport function as a regulator of phagosomal acidification**[61]**. In human blood monocytes, depolarization triggered by a CFTR agonist was higher in non-CF than in CF carrier (heterozygote) donors while CF donors were unresponsive **[120]**.

Drugs targeted at the CFTR channel defect (highly effective modulator therapy – HEMT) have been introduced in the past five years and offer an opportunity to study the effect of CFTR correction on the properties of monocytes *in vivo*. In a pilot study of ivacaftor (CFTR corrector enabling its escape from the ER/Golgi) , the effect on monocytes was assessed by observing their CFTR-dependent chloride efflux, at baseline, and 1, 3 and 6 months post treatment, revealing an increase over time, with a peak at the 6-month timepoint **[121]**. In a larger trial of combination therapy with ivacaftor and lumacaftor (CFTR modulator leading to increased open channel probability) which is known to positively impact lung function and reduce the frequency of APEs **[94, 122]**, greater normalization of macrophage function was achieved than with ivacaftor alone **[123]**. Further studies featuring the novel HEMT combination of ivacaftor, elexacaftor, and tezacaftor with improved positive effects on lung function and APEs is under study for its impact on monocytes/macrophages **[124, 125]**.

Models of CF disease

Although CF is a human disease, since the discovery of the *cftr* gene in 1989 **[85]**, various animal models in mice, rats, rabbits, sheep, ferrets, and pigs **(Table 1.4)** have been established to conduct in-depth pathophysiological and therapeutic investigations **[126, 127]**. While CF mice fail to spontaneously develop lung disease, the beta-ENaC mouse model features increased baseline sodium absorption resulting in the depletion of the airway surface liquid and defective mucus clearance **[128]**, mimicking some aspects of CF lung disease in humans. Lung disease is more apparent in rats, pigs, and ferrets, although its mechanisms do not fully seem to recapitulate those seen in human infants with CF.

Table 1.4. Current animal models of CF and respective disease manifestations.

CF animal model	Spontaneous lung infection	Pancreatic insufficiency	Impaired growth	Intestinal blockage	Reproductive dysfunctional	Hepatic/gallbladder disorder
Zebra fish model	?	✓	?	?	?	?
Mouse model	X	X	✓	✓	✓	X
Rat model	X	X	✓	✓	✓	X
Rabbit model	?	✓	?	✓	✓	?
Ferret model	✓	✓	✓	✓	✓	✓
Pig model	✓	✓	✓	✓	✓	✓
Sheep model	?	✓	?	✓	✓	?

Alternatives to animal models include organoid cultures and differentiated tissue models **[129]**. One such example is the airway transmigration model **(Figure 1.5)** which was developed by our laboratory **[101, 106]** and offers the most physiologically experimental setup to our knowledge for conditioning recruited blood leukocytes into airway leukocytes. Much of the work showcased in the subsequent

chapters deals with the cellular outputs provided by this *in vitro* model. The ability to create GRIM neutrophils or other reprogrammed cell types on demand, which mimic the phenotypes seen *in vivo,* allows our group to investigate specific characteristics of our cells of interest. As a result of the work done utilizing this model, we can mass-produce CF airway-like monocytes in this *in vitro* platform to analyze changes in phenotype and function as well as conduct drug screening experiments.

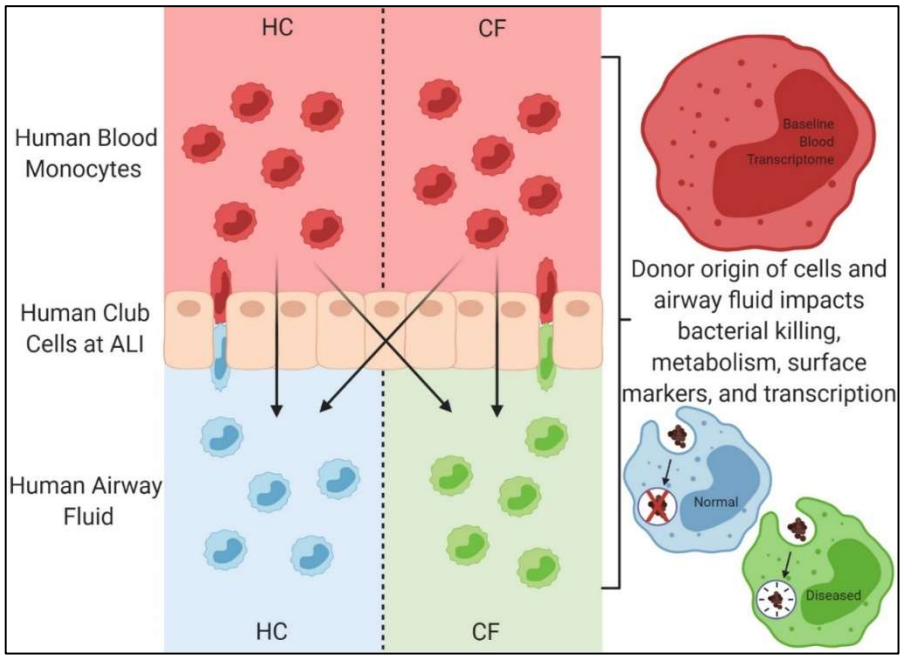

Figure 1.5. In vitro transmigration model mimicking CF airway inflammation. Layout of our *in vitro* model, which has initially been utilized **[101, 106]** to phenocopy the GRIM fate that we discovered in airway secretions of CF patients **[101-107]**. Herein, we use this *in vitro* model to condition naïve blood monocytes from healthy control (HC) and CF donors via transmigration into HC and CF airway fluid and study the impact of this transmigration on the functional and transcriptional properties of monocytes.

Sections of this chapter have been published as an original research article

in the International Journal of Molecular Sciences

Chapter 2: Monocyte phenotype and function after recruitment to CF airways

Introduction

Cystic Fibrosis (CF) is an autosomal recessive genetic disease, impacting an estimated 80,000 individuals worldwide, and still without cure. This disease disproportionately affects people of Caucasian descent [130], with over 1,800 confirmed mutations divided into 6 classes impacting the structure and localization of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) [87]. The dysfunction of CFTR affects multiple organs, with lung disease, featuring a pathological triad of obstruction, infection and inflammation, the primary cause of morbidity and death in CF patients [131].

Regarding inflammation, it is generally accepted that neutrophils are the first leukocytes to be recruited from blood into CF airways [132]. The role of blood monocytes in the process of CF airway inflammation is less clear. Monocytes express functional CFTR [133], as CFTR agonists induce depolarization of monocytes form non-CF donors, with lower activity in heterozygotes and no activity in patients homozygote for the delta508 mutation [120]. In addition, lack of CFTR function may hamper the ability to dock onto the endothelium and diapedese into tissues [134]. Chronic bacterial infection in CF patients also has been associated with an LPS-tolerant state which negatively affects the immune poise of monocytes in the periphery and downregulates their immune response to bacteria [135]. This may in turn negatively impact T cells due to insufficient recruitment or activation by monocytes, or even inhibition via PD-1 ligation by PD-L1 expressing monocytes [136].

In this study, we set out to characterize cell surface expression of select scavenger receptors and activation markers on blood monocytes prior to and after transmigration into *in vitro* airway model of airway inflammation. We then explored whether these transmigrated monocytes exhibited differential bacteria killing ability based on blood donor (healthy control versus CF patient) and transmigration condition (healthy control airway supernatant -HCASN- or CF airway supernatant -CFASN-), respectively.

Methods and Materials

Blood Collection and Processing

Blood was collected by venipuncture in EDTA tubes, and the cells and plasma isolated as previously described.

Monocyte Isolation

Monocytes were isolated from whole blood by negative selection, using the Stem Cell Technologies RosetteSep isolation kit. Red blood cell (RBC) lysis of cell suspension was conducted via hypotonic shock, comprised of a sequential 30-second incubation in ice cold, filtered water, followed by an equal volume of 1.8% NaCl. Cells were centrifugated at 400g for 10 minutes, supernatant was removed, and the process was repeated until no visible redness was present in the cell pellet. Cell viability was assessed by microscopy following staining with ethidium bromide and acridine orange live/dead staining added to cell suspension.

BAL Collection and Processing

Bronchoscopies with BALs were performed in the right middle lobe where three 1 mL/kg volumes of sterile saline were instilled and recovered. The first fraction was used for microbial culture analysis, and the second and third fractions were combined and used for leukocyte counts by Cytospin. The remainder of the combined 2 and 3 fractions was further processed with the addition of phosphate buffered saline with 2.5 mM ethylenediamine tetracetic acid (PBS-EDTA). The BAL sample is stored at 4ºC until processing, within one hour of collection. For processing, cells are dissociated by passing through an 18-gauge needle, followed by sequential centrifugation at 800 g and 3,000 g for 10 min at 4ºC. Resulting BAL fluid (BALF) was stored at -80°C.

Metabolic Analysis

Cells were freshly isolated from blood or harvested from transmigrated conditions and resuspended in a pH-controlled Seahorse medium supplemented with glutamine. Next, cells were plated on Seahorse

XFp plates, at 2x10|^5 cells per well and loaded onto Seahorse XFp analyzer. Plated cells were exposed to sequential injections of metabolic drugs, based on the provided mito cell stress test kit (Agilent), which includes oligomycin, FCCP, rotenone/antimycin, and an additional injection of 2-DG.

Transepithelial migration (TM) model

PMN transepithelial migration and subsequent inflammation occur first in the small airways of CF patients, i.e., the bronchiolar region, which is lined with a microvilli-covered epithelial monolayer dominated by Club cells (3-5). Therefore, to mimic PMN transmigration into the small airway lumen, we selected the H441 human Club cell line (20) to grow epithelial monolayers at air-liquid interface (ALI). To enable PMN loading in the lamina propria and transepithelial migration **(Fig. 2.1A)**, we used Alvetex (Reinnervate) 200 μm-thick inert 3D scaffolds with >90% porosity (pore sizes of 36-40 μm, with interconnects of 12-14 μm). In brief, inserts were activated with 70% ethanol, coated overnight at 37ºC with rat-tail collagen I (3 mg/mL, Sigma) and seeded with H441 cells at 2.5x10^5 cells per 12-well insert. Cells were first grown in submerged cultures with DMEM/F12 supplemented with 10% heat-inactivated serum, penicillin, and streptomycin. After 2 days, cells were supplemented basally with serum-free DMEM/F12 with 10% Ultroser G (Pall Life Sciences) to establish ALI. Cultures were grown for 2 weeks at ALI and supplemented basally with fresh medium every 48 hours. For TM experiments, the ALI cultures were placed with the apical compartment exposed to RPMI, leukotriene B4 (LTB4, 100 nM), CXCL8 (100 ng/mL), formyl-methionine-leucine-phenylalanine (fMLF,100 ng/mL), lipopolysaccharide (LPS, 500 ng/mL), or airway supernatant (ASN) from CF, HC, COPD, and LD subjects. TM experiments with 0.5-1 ×10^6 PMNs loaded onto the 200 μm-thick basal compartment of the Alvetex scaffold (situated upside) and allowed to migrate at 37°C at 5% CO2 through the collagen and epithelial layers into the apical compartment (situated downside, and bathed with either control medium with chemoattractant, or ASN). In some experiments, drugs were added to apical ASN and/or basal PMN suspensions. In other experiments, LPS-RS (competitive inhibitor of LPS binding to TLR4) was added to apical LPS or CF ASN. LPS and LPS-RS were purchased as ultrapure reagents from InvivoGen.

Flow Cytometry

Blood and airway cell samples were supplemented with 5 µl of flow cytometry antibody / 50 µl sample, of which stained for the following respective markers: CD36 (Biolegend, cat# 336204), CD91 (Invitrogen, cat# 46091942), CD47 (Biolegend, cat# 323114), CD172a (Biolegend, cat# 372106), CD63 (Biolegend, cat# 353026), PD-1 (Biolegend, cat# 329952), Calcein Violet Live/dead (ThermoFisher Scientific, cat# C34858), PD-L1 (Biolegend, cat# 329738), CD66b (Biolegend, cat# 392904), HLA-DR (Biolegend, cat# 307626), CD33 (Biolegend, cat# 366614). Samples were then incubated with antibody stains in the dark, at 4ºC|, for 30 minutes, before subsequent washing of sample (400g for 10 minutes). Following wash step, supernatant was removed, and samples were resuspended in 300 µl PBS plus 10 µl counting beads for enumeration purposes, prior to immediate acquisition. Otherwise, next day acquisition required samples to be fixed in lyse fix buffer (1 ml for cells, 2 ml for whole blood) after washing and stored at 4ºC overnight, with resuspension the next day.

Results

2.1 In vivo monocyte and in vitro transmigrated monocyte surface phenotype.

Whole blood was drawn from donors via venipuncture and subsequently processed by RosetteSep to isolate monocytes by negative selection **(Figure 1A)**. These cells were then profiled by flow cytometry to provide a baseline expression profile. An aliquot of blood monocytes was transmigrated through our *in vitro* transmigration model toward a CF airway-like microenvironment **(Figure 1B)** with profiling conducted to compare pre- and post-transmigrated cells. Airway cells from CF patients **(Figure 1C)** were collected by sputum induction and separated from the supernatant through established laboratory procedures to phenotype them like the blood cells. This shows that this population is relevant physiologically in the CF airway, and phenotypically, our target population of monocytes can be recapitulated *in vivo*.

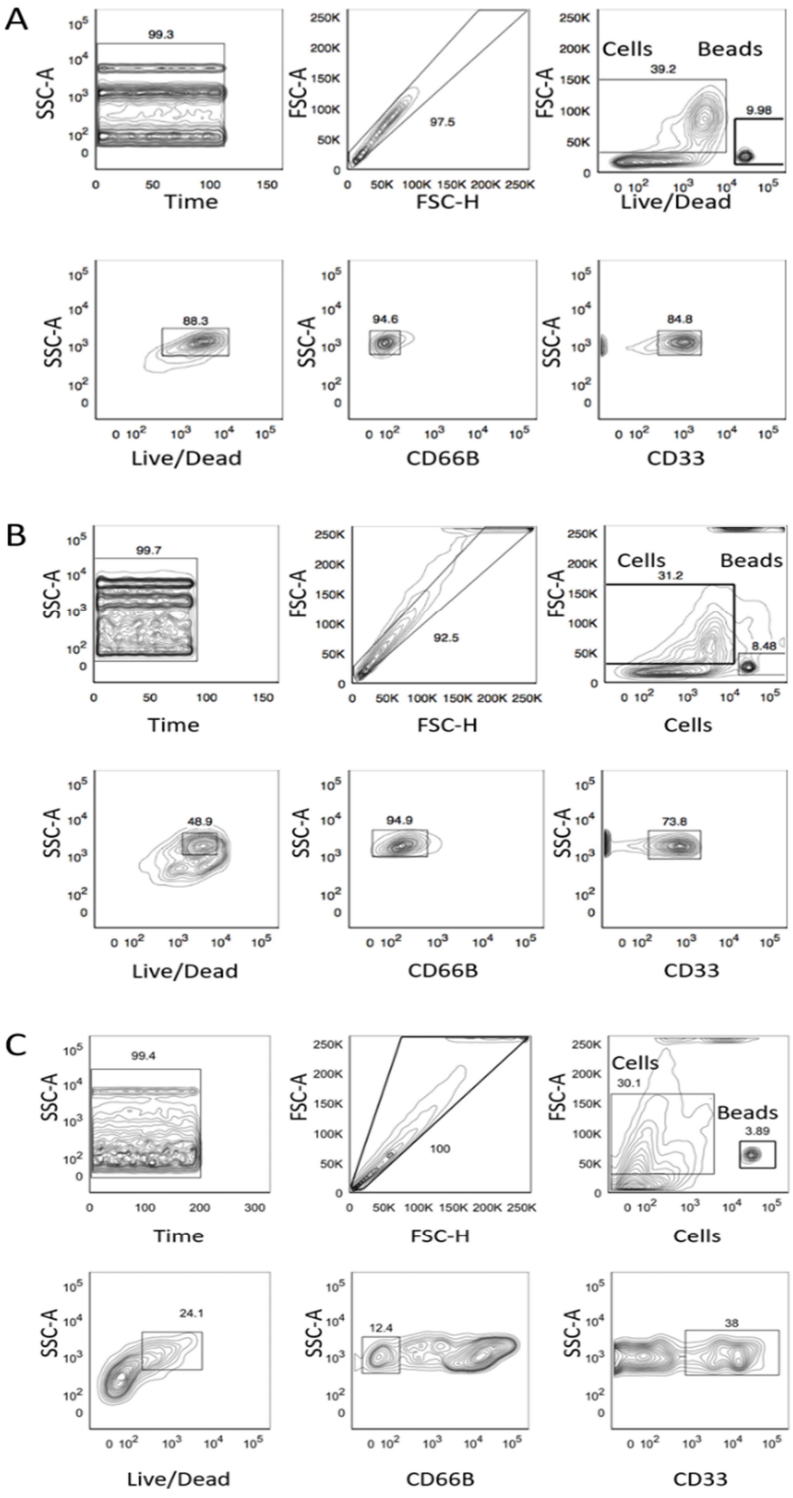

Figure 2.1. ***In vivo*** **monocytes present in adult sputum are similar to those produced upon transmigration of blood monocytes** ***in vitro.*** **(A)** Monocytes isolated from adult blood, as identified by flow cytometry. **(B)** After transmigration of isolated blood monocytes to CFASN, *in vitro*, this population shows a similar phenotype to that seen *in vivo.* **(C)** Monocytes identified in adult CF sputum *in vivo*.

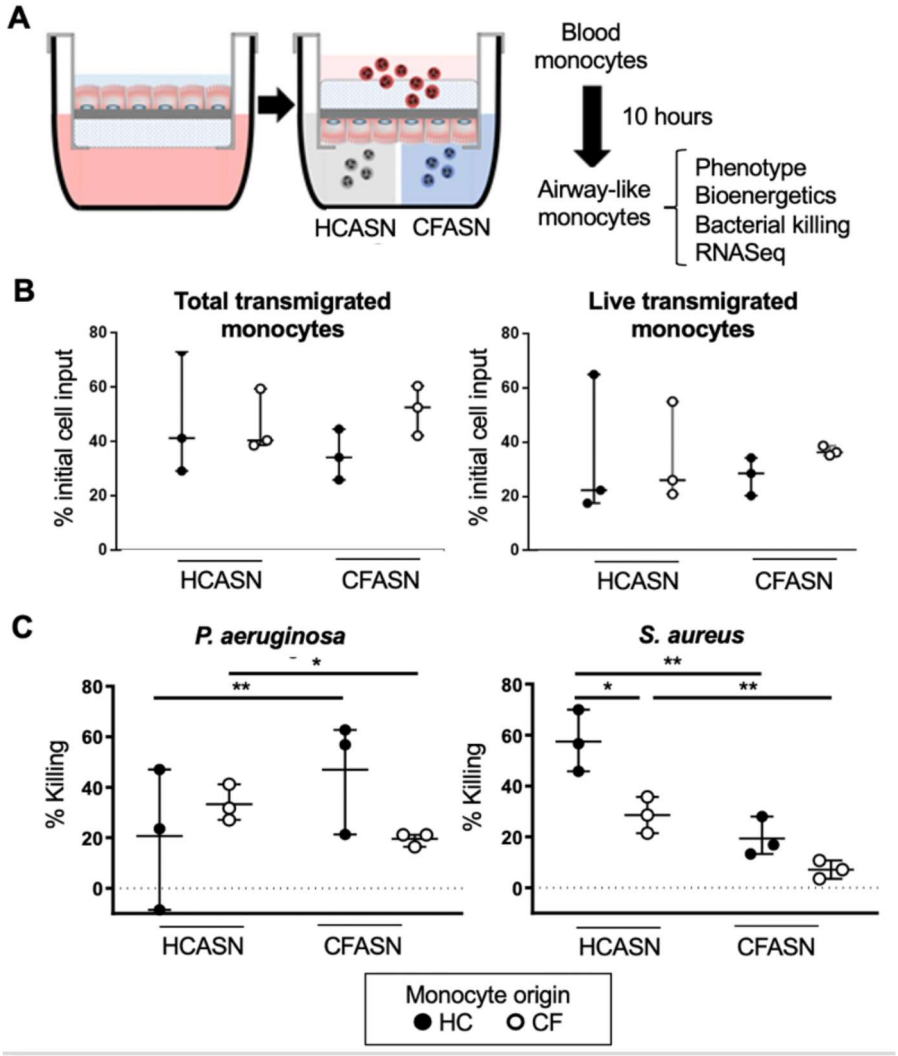

Figure 2.2. Yield and bactericidal activity of transmigrated monocytes in our model. (A) Our model enables transmigration of blood monocytes from HC or CF donors into HCASN or CFASN (mimicking recruitment into HC or CF airways, respectively), after which airway-like monocytes are analyzed functionally and transcriptionally. **(B)** Yields of total transmigrated monocytes (left) and live transmigrated monocytes (right) are shown as % of initial blood monocyte input. **(C)** Bactericidal activity of transmigrated monocytes against *P. aeruginosa* (left) and *S. aureus* (right) are shown as % of initial bacterial input. Statistics: Mann-Whitney test for different monocytes (HC vs. CF) recruited to the same ASN (HC or CF) and Wilcoxon signed rank test for the same monocytes (HC or CF) recruited to different ASN (HC vs. CF). * and ** mark $p < 0.05$ and $p < 0.01$, respectively. Non-significant differences are not indicated.

2.2 Blood monocyte transmigration into CFASN vs. HCASN results in similar metabolic activity.

Next, we collected CF and HC blood monocytes transmigrated to CFASN or HCASN and subjected them to analysis of mitochondrial respiration using a Seahorse XFp system. We derived from the oxygen consumption rate (OCR) measure **(Figure 2A)** five distinct metrics: basal respiration **(Figure 2B)**, maximal respiratory capacity **(Figure 2C)**, ATP-linked respiration **(Fig 2D)**, reserve capacity **(Figure 2E)**, and non-mitochondrial respiration **(Figure 2F)**. We did not observe any difference in the five metrics based on the origin of blood monocytes or ASN used.

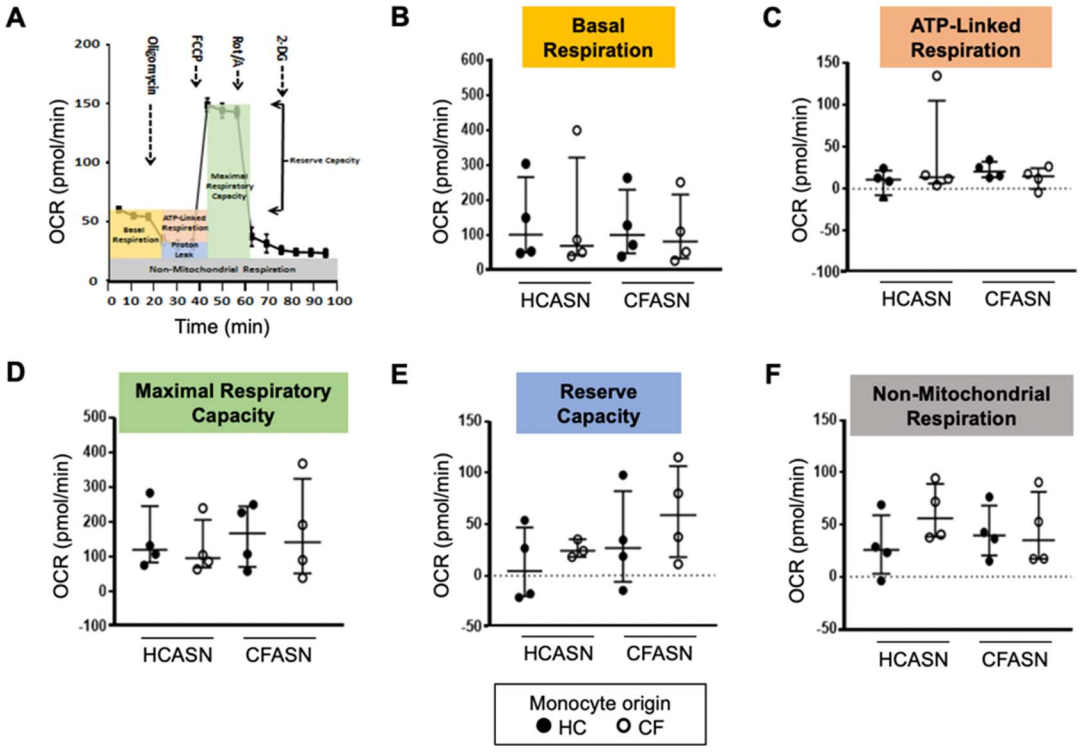

Figure 2.3. Metabolic activity of transmigrated monocytes. (A) Oxygen consumption rate (OCR) tracing upon sequential addition of oligomycin; fluoro-carbonyl cyanide phenylhydrazone (FCCP); rotenone and antimycin A (Rot/A); and 2-deoxyglucose (2-DG). OCR is used to compare **(B)** basal respiration, **(C)** ATP-linked respiration, **(D)** maximal respiratory capacity, **(E)** reserve capacity, and **(F)** non-mitochondrial respiration across types of transmigrated monocytes. Statistics: Mann-Whitney test for different monocytes (HC vs. CF) recruited to the same ASN; Wilcoxon signed rank test for the same monocytes recruited to different ASN (HC vs. CF). Non-significant differences not shown.

2.3 Blood monocyte transmigration into CFASN vs. HCASN results in minimal differences in expression of scavenger receptors and activation markers.

We next turned our attention to surface phenotyping. After transmigration, scavenger receptors CD36, CD91, CD47 and its cognate receptor CD172a did not show a unifying pattern in their change of expression based on origin of the monocytes and ASN (**Figure 3**).

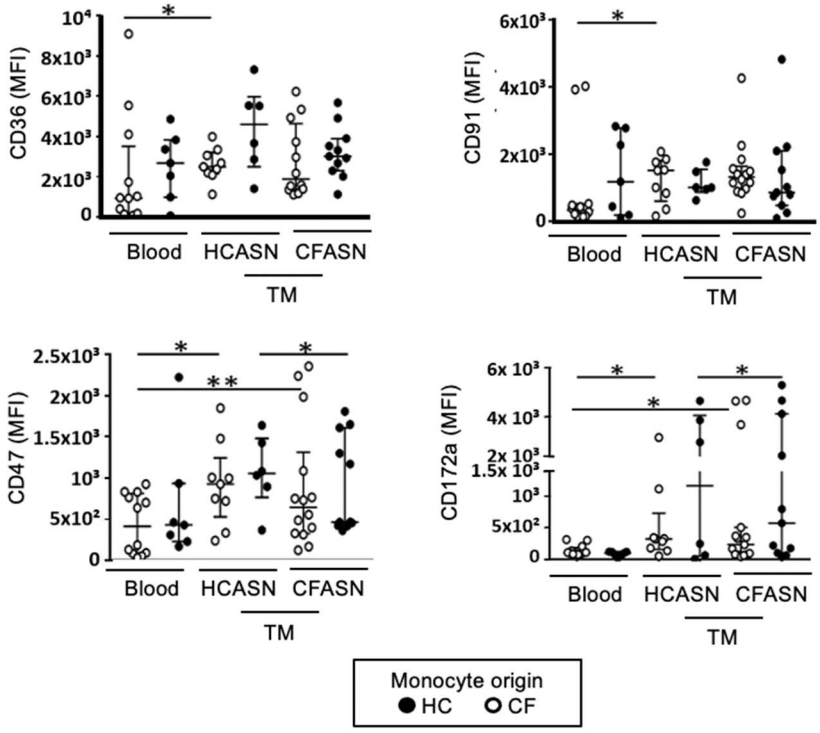

Figure 2.4. Expression of select scavenger receptors on transmigrated monocytes. Blood monocytes from HC or CF donors transmigrated into HCASN or CFASN were subjected to flow cytometric analysis to compare expression of scavenger receptors across subsets of transmigrated monocytes. Statistics: Mann-Whitney test for different monocytes (HC vs. CF) recruited to the same ASN (HC or CF) and Wilcoxon signed rank test for the same monocytes (HC or CF) recruited to different ASN (HC vs. CF). * and ** mark $p < 0.05$ and $p < 0.01$, respectively. Non-significant differences not shown.

CD36 and CD91 were only significantly increased compared to baseline in CF monocytes transmigrated to HCASN. CD47 and CD172a were significantly increased compared to baseline in CF

monocytes transmigrated to CFASN and HCASN, with significant differences between HC monocytes transmigrated to CFASN vs. HCASN for both (**Figure 4**). After transmigration, activation markers CD63, HLA-DR (class II major histocompatibility complex), programmed death-1 (PD-1) and its cognate receptor programmed death-ligand 1 (PD-L1) did not show a unifying pattern in their change of expression based on origin of monocytes and ASN (**Figure 5**).

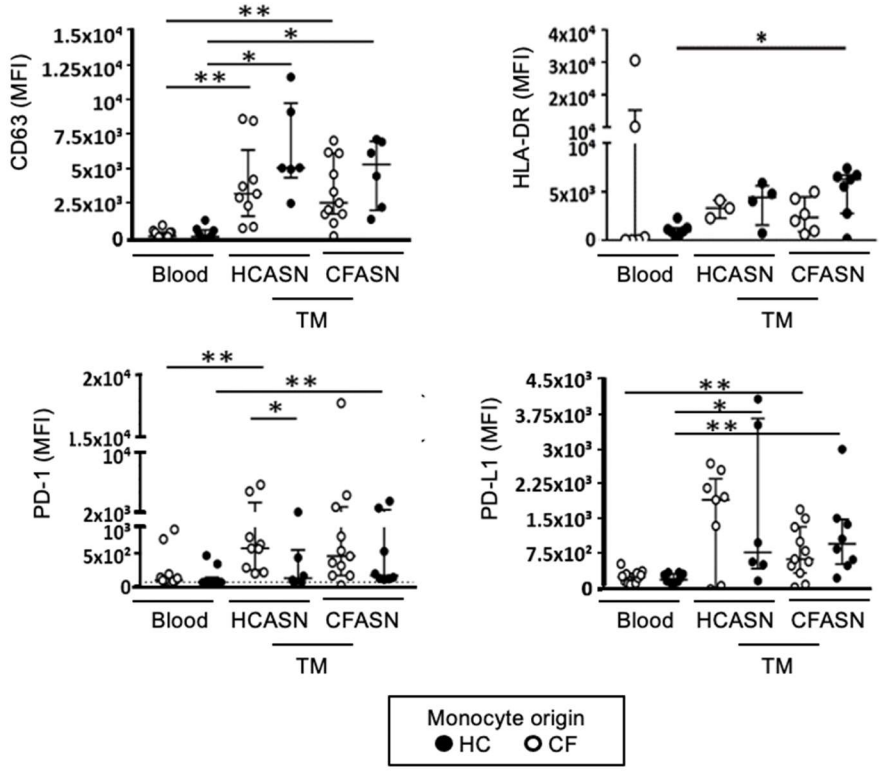

Figure 2.5. Expression of select activation markers on transmigrated monocytes. Blood monocytes from HC or CF donors transmigrated into HCASN or CFASN were subjected to flow cytometric analysis to compare expression of activation markers across subsets of transmigrated monocytes. Statistics: Mann-Whitney test for different monocytes (HC vs. CF) recruited to the same ASN (HC or CF) and Wilcoxon signed rank test for the same monocytes (HC or CF) recruited to different ASN (HC vs. CF). * and ** mark $p < 0.05$ and $p < 0.01$, respectively. Non-significant differences not shown.

CD63 was increased compared to baseline for both CF and HC monocytes transmigrated to CFASN and HCASN, but there was no difference between CF and HC monocytes within each ASN condition, or between ASN conditions within each type of monocyte; thus, the process of transmigration was responsible for significantly increasing CD63 expression. HLA-DR was only significantly increased compared to baseline to HC monocytes transmigrated to CFASN. PD-1 was increased compared to baseline in CF monocytes transmigrated to HCASN and HC monocytes transmigrated to CFASN, with a significant difference between CF and HC monocytes transmigrated to HCASN. Finally, PD-L1 was increased compared to baseline in CF monocytes transmigrated to CFASN and HC monocytes transmigrated to both CFASN or HCASN, and HC monocytes transmigrated to HCASN.

Discussion

In this study, we mimicked the recruitment of primary human blood monocytes to CF versus HC airways using a recently developed model which uses a well-differentiated small airway epithelium and human airway supernatant as conditioning medium for transmigrated leukocytes. Our findings suggest that monocytes transmigrated into CFASN do not demonstrate broadly different changes in their surface phenotype and metabolism. However, they are less efficient at killing bacteria. While previous studies have explored the role of monocytes in CF through analysis of those cells in blood, understanding their role in airway inflammation has been challenging, due to their low yield in airway samples and practical difficulties of replicating that microenvironment in a physiologically relevant manner. To our knowledge, our transmigration model is unique in its ability to enable migration and conditioning of naive primary human blood monocytes in primary airway fluid supernatant from patients with CF and control subjects. We initially focused on the analysis of the surface phenotype, metabolism and bacterial killing of transmigrated monocytes, since all these functional correlates were altered in the context of CFASN for transmigrated neutrophils.

For surface phenotyping, we focused our attention on select activation markers important in extrusion of monocyte granules (CD63), antigen presentation (HLA-DR), and immune poise (PD-1 and PD-L1) and select scavenger receptors involved in uptake of apoptotic cells, small vesicles, and debris such as CD36, CD91, CD47 and its binding partner CD172a. While transmigration to either CFASN or HCASN altered expression of several of these markers compared to baseline (blood monocytes pre-transmigration), only CD47 and CD172 were different between the two transmigration conditions, and only when HC monocytes were used in the transmigration. A broad lack of difference in metabolic activity based on real-time analysis of mitochondrial respiration was also observed between the two conditions. Therefore, monocytes do not undergo the same functional activation observed previously for neutrophils recruited in the CF airway environment *in vitro*, and *in vivo*. This is consistent with the seemingly limited role of these cells in CF airway pathogenesis.

When comparing the rate of transmigration conducted by monocytes that passed through our *in vitro* model, there was no appreciable difference between HC ASN and CF ASN conditions, nor between donor types. Additionally, the viability of these monocytes after 10 hours of transmigration was similar between conditions, donor types, based on cell counts, percentage of live monocytes from the original seeded number of cells, supporting the premise that monocytes are able to survive and function regardless of disease state. Expression of CD47 is also very similar between transmigration conditions, and this is maintained between donor types. Ultimately, CFTR mutation state does not appear to impact the ability to transmigrate to the airway.

We observed differential bacterial killing in transmigrated monocytes recruited to CFASN. It would be worthwhile to assess if mere exposure to the fluid influences bacterial killing or if the act of transmigration is required to induce this CF phenotype, as we have shown for neutrophils. More work is needed to explore this question. Nevertheless, our data are consistent with the notion that not just neutrophils, but also monocytes may underlie the paradoxical coexistence of bacteria and inflammatory cells in the CF airway microenvironment.

Sections of this chapter have been published as an original research article

in the International Journal of Molecular Sciences

Chapter 3: Monocyte transcriptional adaptation after recruitment to cystic fibrosis airways

Introduction

Neutrophils are avid phagocytes with strong antibacterial capacity. In cystic fibrosis (CF), neutrophils are recruited from blood into the lung and fail to control bacterial infection which affect CF aptients chronically. In a recent study, our group established that this paradox is explained by the active downregulation of bacterial-killing activity of neutrophils due to active transcriptional inhibition [106]. Whether similar downregulation exists in monocytes recruited from blood into CF airways is unknown [137].

Comparing sputum between healthy controls and CF patients with mild to severe disease, flow cytometry showed that monocytes made up approximately 20% of sputum cells in CF patients, while they only made up about 7% of sputum cells in healthy controls, while neutrophils amounted to 64% and 2%, respectively [138]. Transcripts for cytokines were increased in monocytes from CF versus healthy controls for *IL1B*, *CXCL2*, *CCL3*, *CCL4*, *CCL20*, *VEGFA*, *EREG*, and calprotectin. This also included increased expression in NLRP3 and anti-apoptotic proteins *MCL1* and *BCL2L1.* Conversely, there was notable downregulation in the MARCO scavenger receptors. Most striking was the vast phenotypic heterogeneity expressed in these activated monocyte populations in sputum.

However, CF monocyte studies thus far fall short due to the lack of pairing of surface, metabolic, and transcriptional phenotype with the functional phenotype. In addition, discriminating the relative effects of CFTR expression in monocytes (high in healthy controls and low to none in CF patients) and/or of the extracellular milieu (healthy vs. CF) is difficult unless one is able to separate these components experimentally.

In this study, we set out to investigate which transcription factors may be leading to the deficiency in bacterial killing in monocytes recruited to CF airways, and determine how blood monocytes transmigrating through our *in vitro* model differ in their transcriptional programming, based on the airway microenvironment they travel to.

Methods and Materials

Blood Collection and Processing

Blood was collected by venipuncture in EDTA tubes, and the cells and plasma isolated as previously described.

Monocyte Isolation

Monocytes were isolated from whole blood by negative selection, using the Stem Cell Technologies RosetteSep isolation kit. Red blood cell (RBC) lysis of cell suspension was conducted via hypotonic shock, comprised of a sequential 30-second incubation in ice cold, filtered water, followed by an equal volume of 1.8% NaCl. Cells were centrifugated at 400g for 10 minutes, supernatant was removed, and the process was repeated until no visible redness was present in the cell pellet. Cell viability was assessed by microscopy following staining with ethidium bromide and acridine orange live/dead staining added to cell suspension.

Transepithelial migration (TM) model

PMN transepithelial migration and subsequent inflammation occur first in the small airways of CF patients, i.e., the bronchiolar region, which is lined with a microvilli-covered epithelial monolayer dominated by Club cells. Therefore, to mimic PMN transmigration into the small airway lumen, we selected the H441 human Club cell line (20) to grow epithelial monolayers at air-liquid interface (ALI). To enable PMN loading in the lamina propria and transepithelial migration **(Fig. 2.1A)**, we used Alvetex (Reinnervate) 200 μm-thick inert 3D scaffolds with >90% porosity (pore sizes of 36-40 μm, with interconnects of 12-14 μm). In brief, inserts were activated with 70% ethanol, coated overnight at 37ºC with rat-tail collagen I (3 mg/mL, Sigma) and seeded with H441 cells at 2.5x105 cells per 12-well insert. Cells were first grown in submerged cultures with DMEM/F12 supplemented with 10% heat-inactivated serum, penicillin, and streptomycin. After 2 days, cells were supplemented basally with serum-free DMEM/F12 with 10% Ultroser G (Pall Life Sciences) to establish ALI. Cultures were grown for 2 weeks at ALI and supplemented basally with fresh medium every 48 hours. For TM experiments, the ALI

cultures were placed with the apical compartment exposed to RPMI, leukotriene B4 (LTB4, 100 nM), CXCL8 (100 ng/mL), formyl-methionine-leucine-phenylalanine (fMLF,100 ng/mL), lipopolysaccharide (LPS, 500 ng/mL), or airway supernatant (ASN) from CF, HC, COPD, and LD subjects. TM experiments with 0.5-1 ×106 PMNs loaded onto the 200 μm-thick basal compartment of the Alvetex scaffold (situated upside), and allowed to migrate at 37°C at 5% CO2 through the collagen and epithelial layers into the apical 57 compartment (situated downside, and bathed with either control medium with chemoattractant, or ASN). In some experiments, drugs were added to apical ASN and/or basal PMN suspensions. In other experiments, LPS-RS (competitive inhibitor of LPS binding to TLR4) was added to apical LPS or CF ASN. LPS and LPS-RS were purchased as ultrapure reagents from InvivoGen.

RNA extraction and transcriptomic analysis

RNA was isolated utilizing the NucleoSpin RNA isolation kit (Takara Biosciences) and stored at -80°C until use. RNA quality was assessed using the Bioanalyzer (Agilent technologies), while RNA libraries were prepared following the Illumina True seq manufacturer's protocol. Samples were subsequently run on Nextseq 550 sequencing system at 25 million single-ended reads per sample. Produced Fastq files from single end reads were aligned to the human reference genome (GRCh38.p13-Ensembl) using the alignment tool HISAT2 (version 2.1.0), using the default settings. Then, BAM files were sorted using SAMtools. Finally, to generate read counts expressed per gene, the tool FeatureCounts (1.5.2) was used. All processed counts were analyzed using DEseq2 to obtain differentially expressed genes (DEGs) between pre and post-transmigration. DEGs were defined as genes with fold changes > 2 folds, false discovery rate < 0.1, and p-value < 0.05. To understand the functions of the DEGs the Metacore server was used against pathway maps and pathway networks. To identify unique dysregulations of gene expression, all DEGs were intersected between conditions using the UGent bioinformatic webtool or Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/). Subsequent enrichment of distinctive gene terms was conducted in the MSigDB v7.2 database (https://www.gsea-msigdb.org/gsea/index.jsp) for molecular signature pathway outputs.

Results

3.1 Blood monocyte transmigration into CFASN vs. HCASN results in significant differences in transcriptional profile

There were significant transcriptional differences between CFASN and HCASN transmigration conditions. By principal component analysis (**Figure 1**), there was complete discrimination in transcriptional profiles of HC monocytes comparing baseline (pre-transmigration), HCASN, and CFASN transmigration conditions. As visualized in volcano plots and pathway analyses for differentially expressed genes (DEGs) in monocytes transmigrated to HCASN (**Figure 2A**) and CFASN (**Figure 2B**), respectively (both in comparison to baseline), multiple critical pathways were significantly different between the two conditions. These included pathways related to cell signaling, contractility, cytoskeleton, immune response, mucin expression and stem cell regulation.

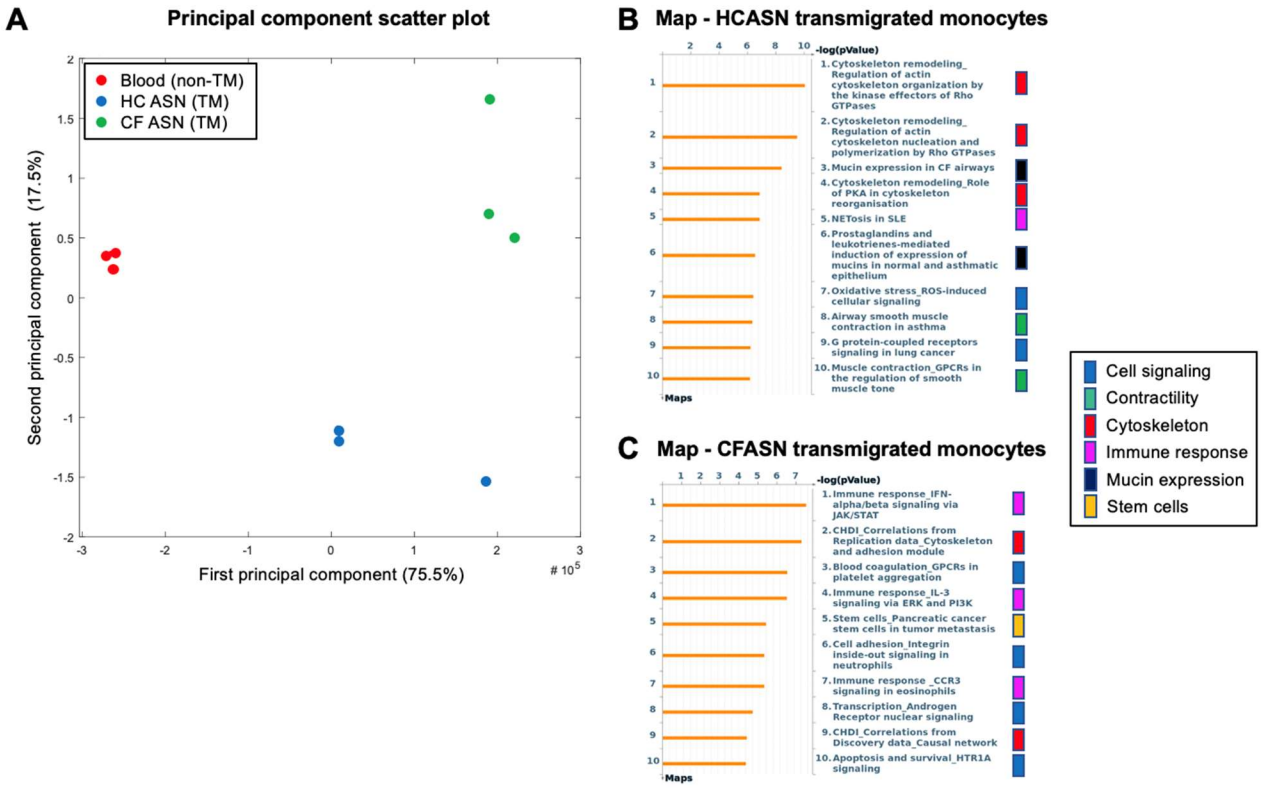

Figure 3.1. Transcriptomic analysis of transmigrated monocytes. (**A**) Principal component analysis of RNASeq data from healthy control blood monocytes prior to (non-transmigrated, red)

and after transmigration (TM) to HC (blue) or CFASN (green) shows clear subsetting by condition (n = 3 independent biological replicates each). Pathway analysis by Metacore (right) illustrating differentially expressed genes and pathways in monocytes transmigrated to (**B**) HCASN and (**C**) CFASN, respectively, compared to blood monocytes.

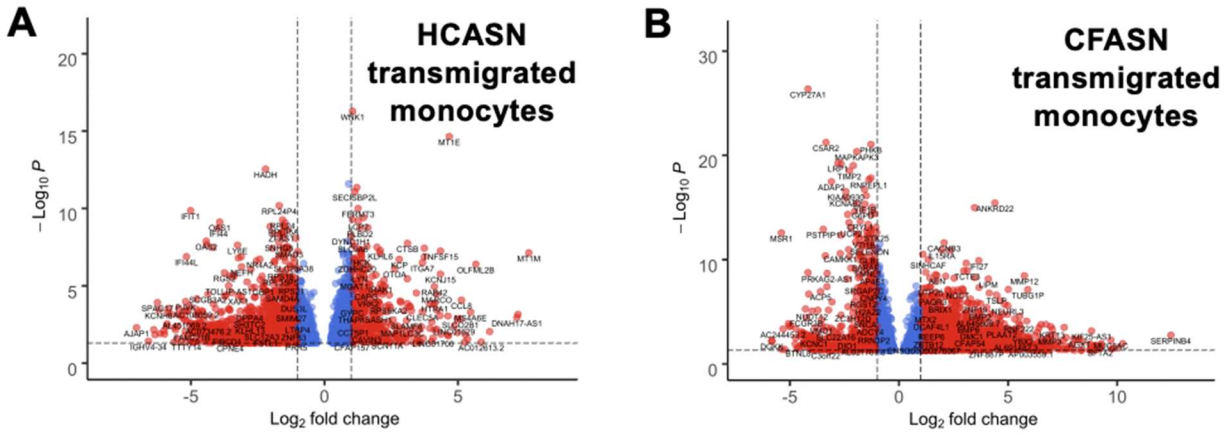

Figure 3.2. Transcriptomic analysis of transmigrated monocytes. Volcano plots (log2 fold change greater or lower than 2 or -2, and a p-value less than 0.05) illustrating differentially expressed genes and pathways in monocytes transmigrated to **(A)** HCASN and **(B)** CFASN, respectively, compared to blood monocytes.

To better visualize which pathways were commonly or uniquely altered in CFASN and HCASN transmigration conditions, Venn diagrams were computed based on significantly dysregulated genes. The resulting gene signatures were overlapped to KEGG Biocarta, Reactome, and Hallmark gene sets. As a result, we identified 3,578 shared upregulated genes (**Figure 3A**) associated with small molecular transport, post-translational modification, and lipid metabolism, whose upregulation was caused by the process of transmigration *per se*. Monocytes transmigrated to HCASN uniquely upregulated 1,863 genes related transcription and translation (**Figure 3B**), while those transmigrated to CFASN uniquely upregulated 740 genes related to intracellular trafficking and transport and cytokine signaling (**Figure**

3C). We also identified 3,254 shared downregulated genes (**Figure 4A**) related to translation and lipid metabolism. This matches the scale of change and pathways seen for shared *upregulated* genes, indicative of bidirectional regulation of these functions upon transmigration. Monocytes transmigrated to HCASN uniquely downregulated 1,244 genes related to oxidative metabolism and innate immunity (**Figure 4B**), while those transmigrated to CFASN uniquely downregulated 1,616 genes related to translation (**Figure 4C**).

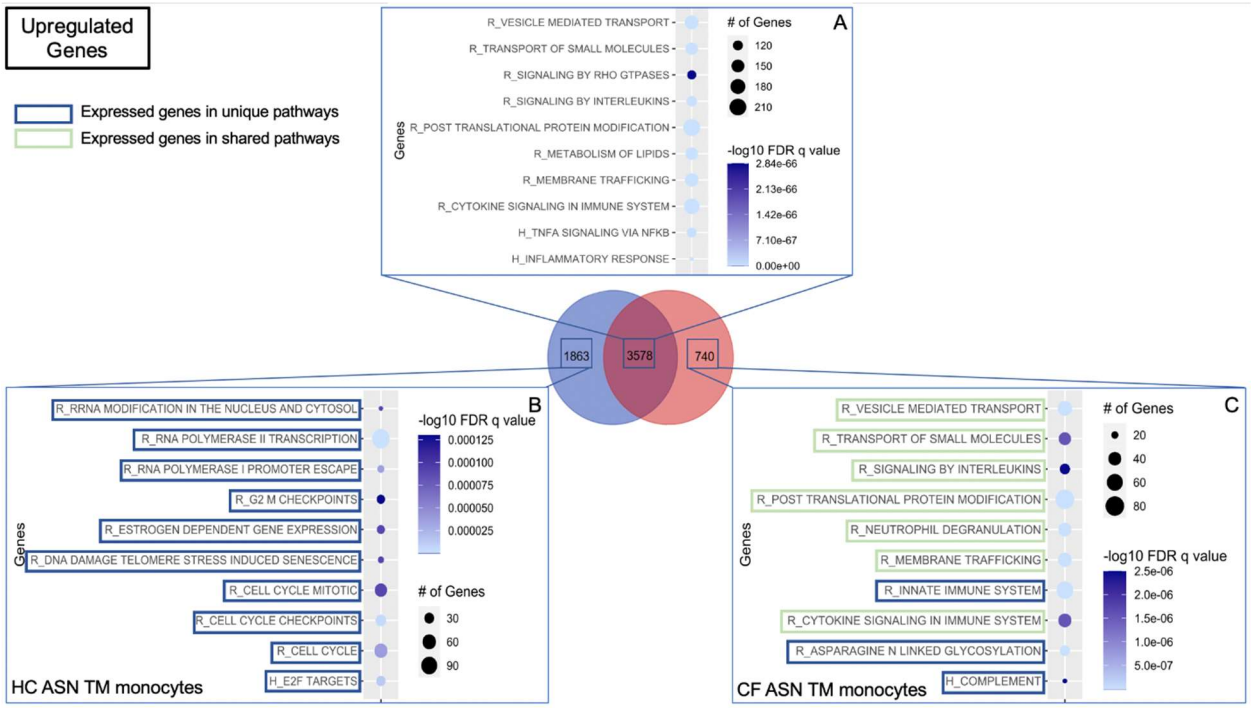

Figure 3.3. Pathway upregulation in HCASN-migrated and CFASN-migrated monocytes. The transcriptome of monocytes was analyzed at 10 hours post-transmigration towards HCASN or CFASN. Pathway enrichment analysis was conducted with MSigDB (Molecular Signatures Database v7. 2) using hallmarks, biocarta, reactome, and KEGG for the overlaps with our data sets, and FDR <0.05 on upregulated genes compared to blood monocytes. Shown are **(A)** shared upregulated pathways and those upregulated more significantly in **(B)** HCASN-migrated monocytes and **(C)** CFASN-migrated monocytes. Pathways are marked as H for hallmarks; K for Kegg; and R for reactome.

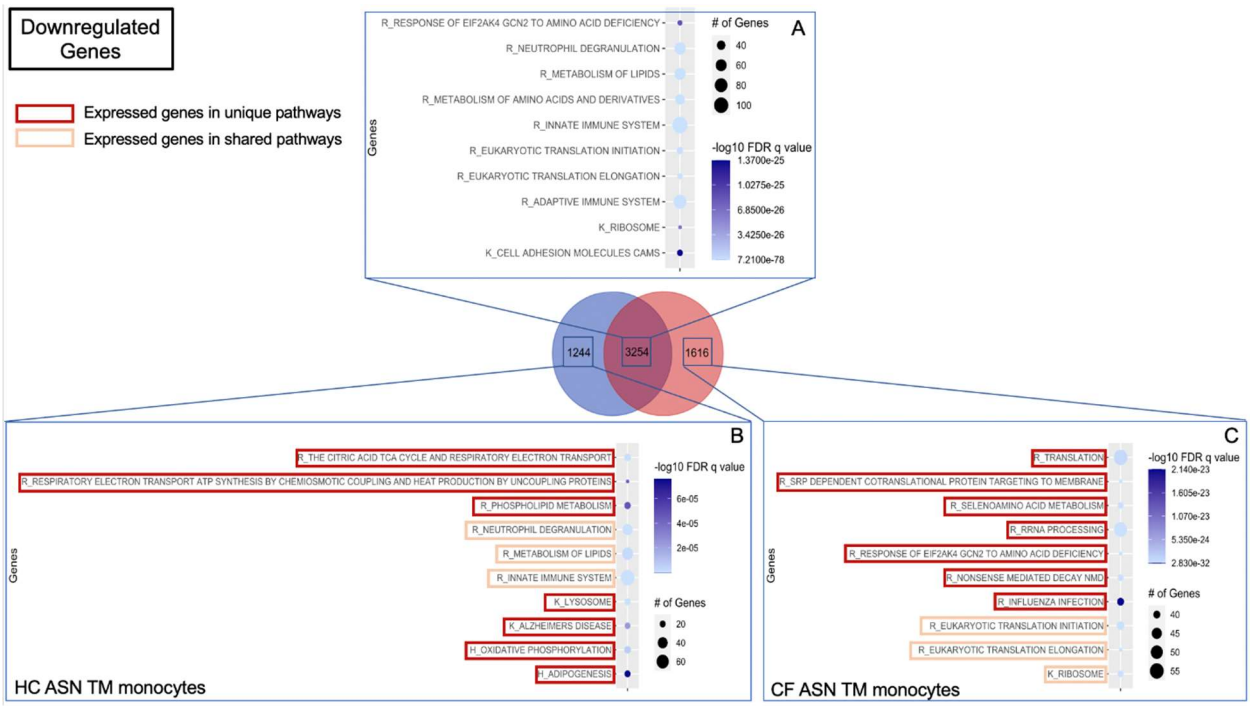

Figure 3.4. Pathway downregulation in HCASN-migrated and CFASN-migrated monocytes. The transcriptome of monocytes was analyzed at 10 hours post-transmigration towards HCASN or CFASN. Pathway enrichment analysis was conducted with MSigDB (Molecular Signatures Database v7. 2) using hallmarks, biocarta, reactome, and KEGG for the overlaps with our data sets, and FDR <0.05 on downregulated genes compared to blood monocytes. Shown are **(A)** shared downregulated pathways and those downregulated more significantly in **(B)** HCASN-migrated monocytes and **(C)** CFASN-migrated monocytes. Pathways are marked as H for hallmarks; K for Kegg; and R for reactome.

To investigate whether the altered antibacterial activity of monocytes transmigrated to CFASN may be associated with changes in subcategories of immune response genes, a directed analysis was conducted (**Figure 5**).

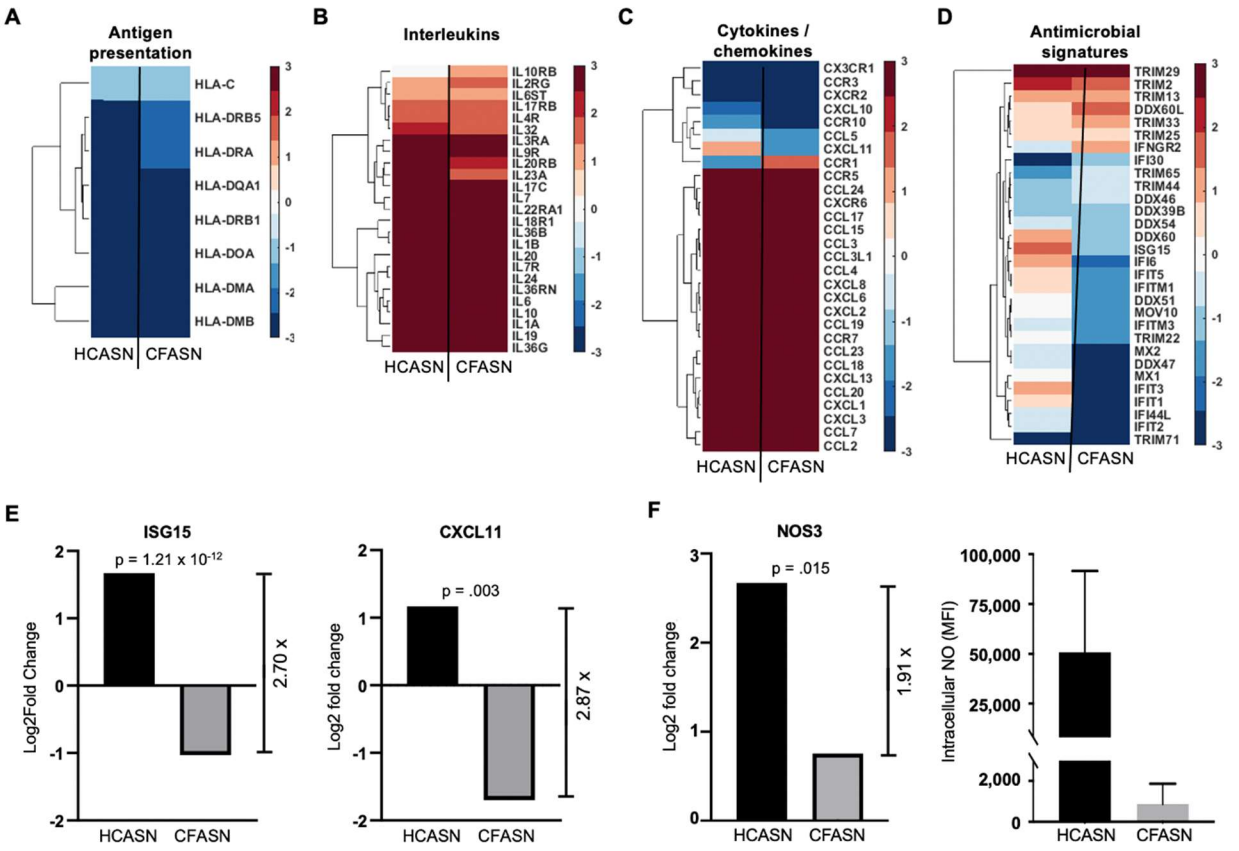

Figure 3.5. Analysis of differentially expressed genes (DEGs) related to immunity in monocytes transmigrated to HCASN vs. CFASN. Shown are chosen DEGs representative of (**A**) antigen presentation, (**B**) interleukins, (**C**) cytokines/chemokines, and (**D**) antimicrobial signatures. Shown in (**E**) are ISG15 and CXCL11, two genes with direct antibacterial functions, which are significantly downregulated in CFASN-recruited monocytes (Log2 scale for both), and in (**F**) the nitric oxide producing enzyme NOS3 (left panel, Log2 scale) and corresponding intracellular NO levels (right panel, shown as MFI).

Genes involved in antigen presentation (**Figure 5A**) and encoding interleukins (**Figure 5B**) and cytokines/chemokines (**Figure 5C**) showed very similar profiles in HCASN and CFASN transmigrated monocytes, with the exception of CXCL11 (increased in the HCASN condition and decreased in the CFASN condition) and CCR1 (decreased in the HCASN condition and increased in the CFASN condition).

There were more pronounced differences in the expression pattern of antimicrobial genes (**Figure 5D**), notably interferon-stimulated gene 15 (ISG15) and C-X-C motif chemokine 11 (CXCL11), two critical effectors of antibacterial immunity which were significantly downregulated in monocytes transmigrated to CFASN (**Figure 5E**). Comparing genes encoding nitric oxide (NO) synthase (NOS) enzymes, we found significantly higher induction of NOS3 in HCASN vs. CFASN transmigrated monocytes (**Figure 5F**). This increased expression of NOS3 coincided with the increased production of intracellular NO in the former (**Figure 5F**).

For completeness and to enable independent reanalysis of our RNASeq data, we posted transcriptomic comparisons between blood monocytes, HCASN-recruited monocytes, and CFASN-recruited monocytes in an online repository at: https://data.mendeley.com/datasets/gcp66ch34c/3

Table 3.1. Gene sets modulated in transmigrated monocytes. Shown are genes whose expression was significantly changed compared to blood monocytes.

Amino Acid Transporters			
SLC10A6	SLC26A2	SLC39A7	SLC7A11
SLC11A2	SLC26A9	SLC39A8	SLC7A2
SLC13A3	SLC27A2	SLC3A2	SLC7A5
SLC16A10	SLC2A1	SLC41A1	SLC8A2
SLC16A3	SLC2A14	SLC41A2	SLC9A5
SLC16A7	SLC2A3	SLC43A2	SLC9A8
SLC17A5	SLC2A6	SLC44A3	SLC9B1
SLC19A2	SLC30A5	SLC44A4	SLC9B2
SLC1A1	SLC30A6	SLC4A2	SLCO4A1
SLC1A2	SLC33A1	SLC5A3	SLC22A5
SLC1A3	SLC34A2	SLC6A13	SLC25A12
SLC22A1	SLC36A4	SLC6A14	SLC27A3
SLC24A3	SLC38A1	SLC6A6	SLC6A12
SLC25A18	SLC39A1	SLC6A7	SLC8A1
SLC25A4	SLC39A14	SLC6A9	SLC9A3R1
SLC26A11	SLC39A6	SLC7A1	
Antiviral Genes			
TRIM71	IFITM3	DDX47	IFNGR2
TRIM29	IFIT2	DDX39B	IFI30
TRIM2	IFIT3	DDX51	TRIM65
MOV10	IFI44L	DDX54	TRIM44
MX1	IFITM1	TRIM25	DDX46
MX2	IFIT1	TRIM13	DDX60

TRIM22 ISG15	IFI6 IFIT5	TRIM33 DDX60L	
Antigen Presentation			
HLA-C HLA-DMA	HLA-DMB HLA-DOA	HLA-DQA1 HLA-DRA	HLA-DRB1 HLA-DRB5
Apoptosis/Engulfment			
ELMO3	FAS	ELMO1	
Chemokine and Cytokines Receptors			
CCR3 CX3CR1 CXCL10 CXCR2 CCR1 CCR9 CXCL11	CCL5 CCR10 CCL2 CCL20 CCL4 CCL19 CCL3	CCL3L1 CCL17 CCL7 CCL24 CCL23 CCL15 CCL18	CCR5 CCR7 CXCR6 CXCL1 CXCL6 CXCL3 CXCL8 CXCL13
Circadian Rhythm			
CLOCK	FBXL18	FBXL2	PER2
Clusters of Differentiation			
CD2 CD200R1 CD300A CD300LB	CD36 CD4 CD86 CD80	CD81 CD69 CD44 CD68	CD300E CD300C
Cytosolic Transporters			
Rab3A Rab3D Rab44 Rab9B Rab40B	RabAC1 Rab6D Rab42 Rab20 Rab2B	Rab35 Rab31 Sec22A Sec22C Sec16A	Rab4A RabL6 Rab22A Rab12
Endogenous Retroviruses			
ERVE-1	HHLA2	HHLA3	
Interleukins			
IL-10RB IL-2RG IL-10 IL-17C IL-17RB IL-18R1 IL-19	IL-1A IL-1B IL-20 IL-20RB IL-22RA1 IL-23A	IL-24 IL-32 IL-36B IL-36G IL-36RN IL-3RA	IL-4R IL-6 IL-6ST IL-7 IL-7R IL-9R
Intracellular Signaling			
STAT6 STAT5B RIPK1	DICER1 STAT2	SOCS1 SOCS3	STAT3 STAT4
Lipid-Related Molecules			
LRP8 LRP10	VLDLR	OLR1	CD36
Proteases			

MMP25 ADAM1B	ADAM19 ADAMTS1	ADAM17	MMP9
Surface Receptors and Coreceptors			
TIMP1 VEGFB TREM1 SIGLEC9 SIGLEC12	SIGLEC1 SIGLEC6 NRP1 FOSL1	SEMA3A C-MYC VEGFA VEGFC	SIGLEC7 TREM2 TREML2 HIF-1A
Toll-Like Receptors			
TLR2	TLR5	TLR6	TLR7

Discussion

Much of the current literature has focused on the characteristics of monocytes from the peripheral blood before subsequently assessing the killing capacity, phenotype, and immune poise of these cells in their baseline state. Our work has advanced the characterization of monocytes in CF by not only assessing these outcomes, but also tying them to transcriptional programming. We compared the effects on transmigrated peripheral blood monocytes based on exposure to airway fluid derived from healthy controls and CF patients and detailed transcriptional pathways expressed in addition to the differentially expressed genes that comprise them.

By PCA analysis, we were able to visualize the transcriptional differences between monocytes freshly isolated from blood, those transmigrated to HCASN, and those transmigrated to CFASN, with clear segregation between these three conditions **(Figure 1A)**. This analysis also shows that the process of transmigration, whether to HCASN or CFASN, exerts a dominant effect difference on the expression of genes, as most of the genes that are expressed and modulated are shared between the two transmigration conditions. When looking at differentially expressed genes, there are still hundreds of genes that are uniquely expressed in each of the transmigration conditions. Some of these uniquely expressed pathways include cytoskeletal rearrangement in monocytes transmigrated to HCASN, vs. immune signaling and regulation in monocytes transmigrated to CFASN.

The most notable upregulated pathways composed of 3,578 expressed genes shared between the two transmigration conditions included those relevant to lipid metabolism, small molecule/vesicular transport, and cytokine signaling. This is consistent with the literature as transmigration of monocytes into inflammatory microenvironments generally poises them to become more activated themselves. Downregulated pathways composed of 3,254 expressed genes shared between the two conditions pertained to cell adhesion, signaling to the adaptive immune system, amino acid metabolism, and translation machinery and processes. We also noted a reduction in antimicrobial signatures in monocytes transmigrated to CFASN vs. HCASN, suggesting that the CF milieu actively inhibits bacterial killing by monocytes. Specifically, transcripts for ISG15, CXCL11 and NOS3 were downregulated. In conjunction with this finding, we saw at the functional level, that intracellular nitric oxide was decreased in monocytes transmigrated to CFASN. In addition, antiviral signatures are downregulated in monocytes transmigrated to CFASN versus HCASN. This finding coincides with the known depletion of IFN-γ in the CF airway microenvironment, considering this cytokine is an inducer of these antiviral signatures. In monocytes transmigrated to CF ASN, the transcripts differentially expressed downstream of IFN-γ signaling include a variety of IFIT and TRIM genes, which were predominately downregulated compared to blood monocytes and lower compared to monocytes transmigrating to HCASN.

Further experiments assessing the importance of iNOS signaling in bacterial killing for monocytes in CF and how the ability to activate the IFN-γ pathway may modulate the killing capacity of the cells. Collectively, these results confirm that the CF milieu downregulates antimicrobial activity in monocytes recruited from blood just as it does to neutrophils **[106]**, which may explain the propensity of CF patients to develop chronic infections.

Chapter 4: Conclusions and perspectives

This body of work has provided evidence that the function and transcriptional profile of blood monocytes are significantly modified upon transmigration toward a CF-like airway microenvironment. Although the CF airway microenvironment caused recruited monocytes to lose part of their bacterial killing ability, we observed little impact pertaining to surface and metabolic phenotypes compared to monocytes recruited to the HC airway microenvironment. This is in stark contrast with the major functional changes that our group has previously shown to occur in neutrophils recruited to CF vs HC airways, which included not only decreased bacterial killing ability, but also increased metabolism and degranulation **[101-107]**. However, we found similar transcriptional adaptation in monocytes recruited to CF airway fluid (this study) and in neutrophils recruited to that microenvironment **[106]**, featuring a major downregulation of antimicrobial pathways notably.

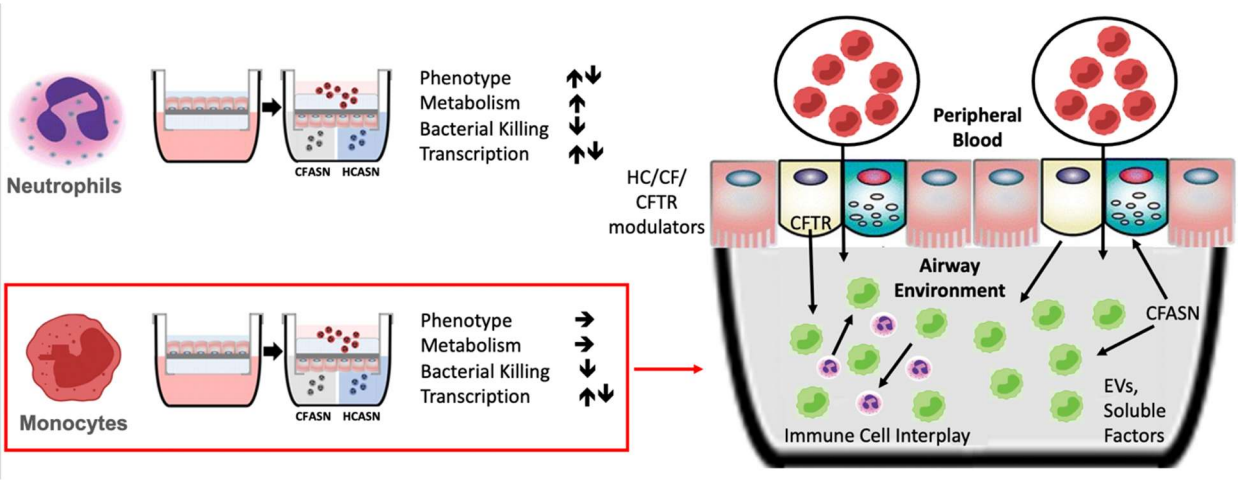

Figure 4.1. Graphical representation of findings presented in this manuscript. Compared to prior published data from our group on CF airway-recruited neutrophils (top left), CF-airway recruited monocytes showed differential impact of that pathological microenvironment (bottom left, red box). How this adaptation influences the cross-talk between recruited neutrophils and monocytes via direct interplay, interactions mediated by EVs and soluble factors will be addressed in future investigations (right).

We observed decreased killing of bacteria by CF monocytes, with a further reduction upon recruitment to the CF airway condition, particularly with the gram-positive bacterium *S. aureus*. Further experiments with other gram-positive bacteria are warranted to assess whether this effect is species-specific. Furthermore, we should investigate why healthy monocytes recruited to CF airways appear to have enhanced killing of the gram-negative bacterium *P. aeruginosa* compared to those recruited to healthy control airways. As these monocytes have transmigrated, one of two effects can be assumed: the factors responsible for the differential killing capacity relate either to the effect of the airway fluid on the epithelium and subsequently on the transmigrated monocytes, or to a direct effect of the fluid on the monocytes. Further studies are required to investigate the components in the CF airway fluid that are responsible for the relative loss of bacterial killing ability by recruited monocytes.

Current work in our lab is focusing on this question. Targeted analysis has identified a role for immunometabolic mediators such as resistin, which we found to correlate with structural lung damage in young children and loss of lung function in adults. Fluid fractionation based on molecular weight identified the 300 kDa retentate as necessary and sufficient to induce the reprogrammed phenotype of neutrophils. Subsequently, this fraction was shown to contain primarily extracellular vesicles or EVs. EVs are cell-derived 50 – 150 nm vesicles containing a wide array of information that can influence the reprogramming of cells in tissue environments. Main questions to be investigated include the contents and surface properties of EVs, the ability of recruited monocytes to take up EVs, and the potential effects of internalized EVs on monocytes.

Another perspective to consider is how monocytes are transcriptionally altered after CFTR modulator use, in particular, after transmigration into the airway microenvironment compared to healthy monocytes. This would allow us to not only see how these cohorts of cells compare after CFTR correction, but also what other molecular pathways are impacted by CFTR-independent mechanisms. This brings up again the impact of the airway fluid itself or the indirect influence of the airway epithelium during or after the process of transmigration. Focused investigations into how hypoxia may be influencing

the activity of these monocytes are also warranted. Previous work has shown that hypoxia has a pro-inflammatory impact on myeloid cells, and specifically macrophages, with HIF signaling enabling metabolic adjustments. Experimental conditions used for the work conducted in this thesis did not account for oxygen deprivation, which is a common occurrence in CF airways. In addition, pursuant to our finding that intracellular NO is differentially modulated in monocytes transmigrated to CFASN vs HCASN, exploring pathways that influence its levels and their possible impact on antimicrobial activity would be of interest. Prospective testing of supplementation with L-arginine in the milieu would be helpful to see if a surplus of substrate for conversion by iNOS into NO would aid in the rescue of antimicrobial activity in monocytes transmigrated into the CFASN.

As was mentioned before, numerous interferon-stimulated genes are downregulated in the CF airway microenvironment. Of these, a few stood out as potentially playing a significant role in the decreased bacterial killing displayed by transmigrated monocytes. For instance, we observed that ISG15, CXCL11, and NOS3 were downregulated in CF airway conditions for healthy monocytes. ISG15, in particular, is conventionally recognized as an antiviral effector, yet recent studies noted antibacterial roles this mediator, especially after it is released in its soluble form to act in paracrine fashion. CXCL11 similarly functions in a dual nature as either an antimicrobial or signaling factor. Conventionally, this marker is induced and activated by interferon gamma like the rest of the members in its interferon-inducible family (CXCL9 and CXCL10). The decreased expression in CF airways is consistent with the documented depletion of interferon gamma in the CF airway microenvironment. Prior studies have attempted to rescue macrophage function, for example, by supplementing *ex vivo* cultures with interferon gamma in the context of *B. cenocepacia* infection. These macrophages were able to reduce the bacterial burden by inducing autophagy and carrying out clearance of their internalized products. Similar positive effects of interferon gamma may be observed with recruited monocytes.

While the focus of this thesis work is in large part on CF, our findings relating to blood monocyte functional, metabolic, antibacterial, and transcriptional adaptations upon recruitment to airway fluid

extend to other airway diseases that share similar inflammatory profiles. Some of the relevant respiratory diseases include COPD, asthma, emphysema, and acute respiratory distress syndrome **[139-141]**. COPD, in particular, is one of the most closely related airway diseases to CF. Indeed, our group has previously noted a similar phenotype of neutrophils that transmigrated into COPD airway fluid to those transmigrated into the CF airway fluid **[106]**. Functionally, it appears that both monocytes and macrophages in COPD display a reduction in phagocytic / killing capacity required for clearing exogenous agents and endogenous apoptotic cells and debris. This results in tissue damage and persistent bacterial infections. Apart from the canonical monocyte subsets, within COPD and more specifically within acute exacerbation of chronic obstructive pulmonary disease (AECOPD), an atypical monocyte population of large monocytes (A Mo) arises in the peripheral blood that can serve as a prognostic marker **[142]**. This population is nearly negligible in healthy individuals, while 60% of monocytes in AECOPD patients display this expression profile. Most notably, when comparing A Mo from patients to typical monocytes from healthy controls, the latter subset had greater size and higher expression of CD16, CCR2, and ICAM. A Mo also express lower levels of the MHC-II presentation marker HLA-DR, suggesting potential modulation of immune function. A positive correlation was found between the % of A Mo in AECOPD patients and the length of hospital stay and disease duration. Further studies exploring fine monocyte subsets in patients with COPD, but also with CF, may shed further light onto pathogenesis.

Lastly, much of the work conducted in this thesis dealt with monocytes in isolation, without direct interactions from the network of cells they would be connecting with in these conditions. In particular, further investigation of how airway monocytes are influenced by neutrophils in the adult CF airway is warranted, as crosstalk / competition between these cells is well documented during transmigration and residency within the airways. Mimicking disease states by adjusting the proportion of transmigrating monocytes and neutrophils may provide a window into how the dynamics of myeloid cell recruitment from blood and subsequent activation profiles within the airway are modulated transcriptionally, phenotypically, and functionally.

References

1. Terry, R.L. and S.D. Miller, Molecular control of monocyte development. Cell Immunol, 2014. **291**(1-2): p. 16-21.
2. Dotta, L. and R. Badolato, Primary immunodeficiencies appearing as combined lymphopenia, neutropenia, and monocytopenia. Immunol Lett, 2014. **161**(2): p. 222-5.
3. Auffray, C., M.H. Sieweke, and F. Geissmann, Blood monocytes: development, heterogeneity, and relationship with dendritic cells. Annu Rev Immunol, 2009. **27**: p. 669-92.
4. Chong, S.Z., et al., CXCR4 identifies transitional bone marrow premonocytes that replenish the mature monocyte pool for peripheral responses. J Exp Med, 2016. **213**(11): p. 2293-2314.
5. Barve, R.A., et al., Transcriptional profiling and pathway analysis of CSF-1 and IL-34 effects on human monocyte differentiation. Cytokine, 2013. **63**(1): p. 10-17.
6. Chomarat, P., et al., IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. Nat Immunol, 2000. **1**(6): p. 510-4.
7. Boyette, L.B., et al., Phenotype, function, and differentiation potential of human monocyte subsets. PLoS One, 2017. **12**(4): p. e0176460.
8. Cappellari, R., et al., Shift of monocyte subsets along their continuum predicts cardiovascular outcomes. Atherosclerosis, 2017. **266**: p. 95-102.
9. Idzkowska, E., et al., The Role of Different Monocyte Subsets in the Pathogenesis of Atherosclerosis and Acute Coronary Syndromes. Scand J Immunol, 2015. **82**(3): p. 163-73.
10. Sampath, P., et al., Monocyte Subsets: Phenotypes and Function in Tuberculosis Infection. Front Immunol, 2018. **9**: p. 1726.
11. Wong, K.L., et al., The three human monocyte subsets: implications for health and disease. Immunol Res, 2012. **53**(1-3): p. 41-57.
12. Larson, S.R., et al., Ly6C(+) monocyte efferocytosis and cross-presentation of cell-associated antigens. Cell Death Differ, 2016. **23**(6): p. 997-1003.

13. Tsukamoto, M., et al., CD14(bright)CD16+ intermediate monocytes are induced by interleukin-10 and positively correlate with disease activity in rheumatoid arthritis. Arthritis Res Ther, 2017. **19**(1): p. 28.

14. Resende, D.P., et al., Non-classical circulating monocytes in severe obesity and obesity with uncontrolled diabetes: A comparison with tuberculosis and healthy individuals. Tuberculosis (Edinb), 2019. **114**: p. 30-41.

15. Lee, J., et al., The MHC class II antigen presentation pathway in human monocytes differs by subset and is regulated by cytokines. PLoS One, 2017. **12**(8): p. e0183594.

16. del Fresno, C., et al., Potent phagocytic activity with impaired antigen presentation identifying lipopolysaccharide-tolerant human monocytes: demonstration in isolated monocytes from cystic fibrosis patients. J Immunol, 2009. **182**(10): p. 6494-507.

17. Germic, N., et al., Regulation of the innate immune system by autophagy: monocytes, macrophages, dendritic cells and antigen presentation. Cell Death Differ, 2019. **26**(4): p. 715-727.

18. Auffray, C., et al., Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science, 2007. **317**(5838): p. 666-70.

19. Greiffo, F.R., et al., CX3CR1-fractalkine axis drives kinetic changes of monocytes in fibrotic interstitial lung diseases. Eur Respir J, 2020. **55**(2).

20. Rosseau, S., et al., Moraxella catarrhalis--infected alveolar epithelium induced monocyte [ppppp[[[precruitment and oxidative burst. Am J Respir Cell Mol Biol, 2005. **32**(2): p. 157-66.

21. Yang, J., et al., Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. Biomark Res, 2014. **2**(1): p. 1.

22. Menezes, S., et al., The Heterogeneity of Ly6C(hi) Monocytes Controls Their Differentiation into iNOS(+) Macrophages or Monocyte-Derived Dendritic Cells. Immunity, 2016. **45**(6): p. 1205-1218.

23. Polancec, D.S., et al., Azithromycin drives in vitro GM-CSF/IL-4-induced differentiation of human blood monocytes toward dendritic-like cells with regulatory properties. J Leukoc Biol, 2012. **91**(2): p. 229-43.

24. Gomez Perdiguero, E., et al., Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature, 2015. **518**(7540): p. 547-51.

25. van de Laar, L., et al., Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages. Immunity, 2016. **44**(4): p. 755-68.

26. Mass, E., Delineating the origins, developmental programs and homeostatic functions of tissue-resident macrophages. Int Immunol, 2018. **30**(11): p. 493-501.

27. Roszer, T., Understanding the Biology of Self-Renewing Macrophages. Cells, 2018. **7**(8).

28. Arfvidsson, J., et al., Monocyte subsets in myocardial infarction: A review. Int J Cardiol, 2017. **231**: p. 47-53.

29. Liao, X., et al., Distinct roles of resident and nonresident macrophages in nonischemic cardiomyopathy. Proc Natl Acad Sci U S A, 2018. **115**(20): p. E4661-e4669.

30. Min, Y., et al., Distinct Residential and Infiltrated Macrophage Populations and Their Phagocytic Function in Mild and Severe Neonatal Hypoxic-Ischemic Brain Damage. Front Cell Neurosci, 2020. **14**: p. 244.

31. Zigmond, E., et al., Infiltrating monocyte-derived macrophages and resident kupffer cells display different ontogeny and functions in acute liver injury. J Immunol, 2014. **193**(1): p. 344-53.

32. Tacke, F., et al., Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. J Clin Invest, 2007. **117**(1): p. 185-94.

33. Narasimhan, P.B., et al., Nonclassical Monocytes in Health and Disease. Annu Rev Immunol, 2019. **37**: p. 439-456.

34. Lacerte, P., et al., Overexpression of TLR2 and TLR9 on monocyte subsets of active rheumatoid arthritis patients contributes to enhance responsiveness to TLR agonists. Arthritis Res Ther, 2016. **18**: p. 10.

35. Rodriguez-Munoz, Y., et al., Peripheral blood monocyte subsets predict antiviral response in chronic hepatitis C. Aliment Pharmacol Ther, 2011. **34**(8): p. 960-71.

36. Selimoglu-Buet, D., et al., Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia. Blood, 2015. **125**(23): p. 3618-26.

37. Teer, E., et al., HIV and cardiovascular diseases risk: exploring the interplay between T-cell activation, coagulation, monocyte subsets, and lipid subclass alterations. Am J Physiol Heart Circ Physiol, 2019. **316**(5): p. H1146-h1157.

38. Zhou, J., et al., CD14(hi)CD16+ monocytes phagocytose antibody-opsonised Plasmodium falciparum infected erythrocytes more efficiently than other monocyte subsets, and require CD16 and complement to do so. BMC Med, 2015. **13**: p. 154.

39. Villani, A.C., et al., Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science, 2017. **356**(6335).

40. Tamura, T., Regulation of mononuclear phagocyte development by IRF8. Rinsho Ketsueki, 2017. **58**(7): p. 798-805.

41. Nowlin, B.T., et al., Monocyte subsets exhibit transcriptional plasticity and a shared response to interferon in SIV-infected rhesus macaques. J Leukoc Biol, 2018. **103**(1): p. 141-155.

42. Metcalf, T.U., et al., Human Monocyte Subsets Are Transcriptionally and Functionally Altered in Aging in Response to Pattern Recognition Receptor Agonists. J Immunol, 2017. **199**(4): p. 1405-1417.

43. O'Neill, L.A., R.J. Kishton, and J. Rathmell, A guide to immunometabolism for immunologists. Nat Rev Immunol, 2016. **16**(9): p. 553-65.

44. Palmer, C.S., et al., Glucose Metabolism in T Cells and Monocytes: New Perspectives in HIV Pathogenesis. EBioMedicine, 2016. **6**: p. 31-41.

45. Hall, C.J., et al., Mitochondrial metabolism, reactive oxygen species, and macrophage function-fishing for insights. J Mol Med (Berl), 2014. **92**(11): p. 1119-28.

46. Okura, Y., et al., Monocyte/macrophage-specific NADPH oxidase contributes to antimicrobial host defense in X-CGD. J Clin Immunol, 2015. **35**(2): p. 158-67.

47. Perez, D., et al., Eimeria ninakohlyakimovae induces NADPH oxidase-dependent monocyte extracellular trap formation and upregulates IL-12 and TNF-alpha, IL-6 and CCL2 gene transcription. Vet Parasitol, 2016. **227**: p. 143-50.

48. Aratani, Y., Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys, 2018. **640**: p. 47-52.

49. Lincoln, J.A., et al., Exogenous myeloperoxidase enhances bacterial phagocytosis and intracellular killing by macrophages. Infect Immun, 1995. **63**(8): p. 3042-7.

50. Oyenuga, A.O., et al., Association of monocyte myeloperoxidase with incident cardiovascular disease: The Atherosclerosis Risk in Communities Study. PLoS One, 2018. **13**(10): p. e0205310.

51. Ruhnau, J., et al., Stroke alters respiratory burst in neutrophils and monocytes. Stroke, 2014. **45**(3): p. 794-800.

52. Fernandez-Ruiz, I., et al., Differential lipid metabolism in monocytes and macrophages: influence of cholesterol loading. J Lipid Res, 2016. **57**(4): p. 574-86.

53. Yoon, B.R., et al., Role of SLC7A5 in Metabolic Reprogramming of Human Monocyte/Macrophage Immune Responses. Front Immunol, 2018. **9**: p. 53.

54. Gao, X. and H. Han, Jolkinolide B inhibits glycolysis by downregulating hexokinase 2 expression through inactivating the Akt/mTOR pathway in non-small cell lung cancer cells. J Cell Biochem, 2018. **119**(6): p. 4967-4974.

55. Palmer, C.S., et al., Regulators of Glucose Metabolism in CD4(+) and CD8(+) T Cells. Int Rev Immunol, 2016. **35**(6): p. 477-488.

56. Paschoal, V.A., et al., mTORC1 inhibition with rapamycin exacerbates adipose tissue inflammation in obese mice and dissociates macrophage phenotype from function. Immunobiology, 2017. **222**(2): p. 261-271.

57. Bosco, M.C., et al., Monocytes and dendritic cells in a hypoxic environment: Spotlights on chemotaxis and migration. Immunobiology, 2008. **213**(9-10): p. 733-49.

58. Kawai, T. and S. Akira, The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol, 2010. **11**(5): p. 373-84.

59. Nagashima, H. and Y. Yamaoka, Importance of Toll-like Receptors in Pro-inflammatory and Anti-inflammatory Responses by Helicobacter pylori Infection. Curr Top Microbiol Immunol, 2019. **421**: p. 139-158.

60. Yokoyama, K., et al., Rab27a negatively regulates phagocytosis by prolongation of the actin-coating stage around phagosomes. J Biol Chem, 2011. **286**(7): p. 5375-82.

61. Radtke, A.L., et al., Listeria monocytogenes exploits cystic fibrosis transmembrane conductance regulator (CFTR) to escape the phagosome. Proc Natl Acad Sci U S A, 2011. **108**(4): p. 1633-8.

62. Wu, Q., et al., Iron oxide nanoparticles and induced autophagy in human monocytes. Int J Nanomedicine, 2017. **12**: p. 3993-4005.

63. Castro-Dopico, T. and M.R. Clatworthy, IgG and Fcγ Receptors in Intestinal Immunity and Inflammation. Front Immunol, 2019. **10**: p. 805.

64. PrabhuDas, M.R., et al., A Consensus Definitive Classification of Scavenger Receptors and Their Roles in Health and Disease. J Immunol, 2017. **198**(10): p. 3775-3789.

65. Marcovecchio, P.M., et al., Scavenger Receptor CD36 Directs Nonclassical Monocyte Patrolling Along the Endothelium During Early Atherogenesis. Arterioscler Thromb Vasc Biol, 2017. **37**(11): p. 2043-2052.

66. Basu, S., et al., CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. Immunity, 2001. **14**(3): p. 303-13.

67. Zong, G., et al., SR-A1 suppresses colon inflammation and tumorigenesis through negative regulation of NF-kappaB signaling. Biochem Pharmacol, 2018. **154**: p. 335-343.

68. Jackson, W.D., T.W. Weinrich, and K.J. Woollard, Very-low and low-density lipoproteins induce neutral lipid accumulation and impair migration in monocyte subsets. Sci Rep, 2016. **6**: p. 20038.

69. Gardai, S.J., et al., By binding SIRPalpha or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. Cell, 2003. **115**(1): p. 13-23.

70. Vandivier, R.W., et al., Role of surfactant proteins A, D, and C1q in the clearance of apoptotic cells in vivo and in vitro: calreticulin and CD91 as a common collectin receptor complex. J Immunol, 2002. **169**(7): p. 3978-86.

71. Ferrer, D.G., et al., Standardized flow cytometry assay for identification of human monocytic heterogeneity and LRP1 expression in monocyte subpopulations: decreased expression of this receptor in nonclassical monocytes. Cytometry A, 2014. **85**(7): p. 601-10.

72. Lv, Z., et al., Loss of Cell Surface CD47 Clustering Formation and Binding Avidity to SIRPalpha Facilitate Apoptotic Cell Clearance by Macrophages. J Immunol, 2015. **195**(2): p. 661-71.

73. Yu, G.T., et al., PD-1 blockade attenuates immunosuppressive myeloid cells due to inhibition of CD47/SIRPalpha axis in HPV negative head and neck squamous cell carcinoma. Oncotarget, 2015. **6**(39): p. 42067-80.

74. Londino, J.D., et al., Cleavage of Signal Regulatory Protein α (SIRPα) Enhances Inflammatory Signaling. J Biol Chem, 2015. **290**(52): p. 31113-25.

75. de Vries, H.E., et al., Signal-regulatory protein alpha-CD47 interactions are required for the transmigration of monocytes across cerebral endothelium. J Immunol, 2002. **168**(11): p. 5832-9.

76. Duus, K., et al., Direct interaction between CD91 and C1q. Febs j, 2010. **277**(17): p. 3526-37.

77. Buechler, C., K. Eisinger, and S. Krautbauer, Diagnostic and prognostic potential of the macrophage specific receptor CD163 in inflammatory diseases. Inflamm Allergy Drug Targets, 2013. **12**(6): p. 391-402.

78. Wang, J., et al., Expression of serum sCD163 in patients with liver diseases and inflammatory disorders. Int J Clin Exp Pathol, 2015. **8**(7): p. 8419-25.

79. Lee, C.H. and T. Chun, Anti-Inflammatory Role of TAM Family of Receptor Tyrosine Kinases Via Modulating Macrophage Function. Mol Cells, 2019. **42**(1): p. 1-7.

80. Rothlin, C.V., et al., TAM receptors are pleiotropic inhibitors of the innate immune response. Cell, 2007. **131**(6): p. 1124-36.

81. Wagner, C.J., C. Schultz, and M.A. Mall, Neutrophil elastase and matrix metalloproteinase 12 in cystic fibrosis lung disease. Mol Cell Pediatr, 2016. **3**(1): p. 25.

82. DeLeon-Pennell, K.Y., et al., CD36 Is a Matrix Metalloproteinase-9 Substrate That Stimulates Neutrophil Apoptosis and Removal During Cardiac Remodeling. Circ Cardiovasc Genet, 2016. **9**(1): p. 14-25.

83. Jovic, S., et al., Expression of MIG/CXCL9 in cystic fibrosis and modulation of its activities by elastase of Pseudomonas aeruginosa. J Innate Immun, 2014. **6**(6): p. 846-59.

84. Suter, S., The role of bacterial proteases in the pathogenesis of cystic fibrosis. Am J Respir Crit Care Med, 1994. **150**(6 Pt 2): p. S118-22.

85. Riordan, J.R., et al., Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science, 1989. **245**(4922): p. 1066-73.

86. Bonadia, L.C., et al., CFTR genotype and clinical outcomes of adult patients carried as cystic fibrosis disease. Gene, 2014. **540**(2): p. 183-90.

87. Fanen, P., A. Wohlhuter-Haddad, and A. Hinzpeter, Genetics of cystic fibrosis: CFTR mutation classifications toward genotype-based CF therapies. Int J Biochem Cell Biol, 2014. **52**: p. 94-102.

88. McIlwaine, M., B. Button, and K. Dwan, Positive expiratory pressure physiotherapy for airway clearance in people with cystic fibrosis. Cochrane Database Syst Rev, 2015(6): p. Cd003147.

89. Kurbatova, P., et al., Model of mucociliary clearance in cystic fibrosis lungs. J Theor Biol, 2015. **372**: p. 81-8.

90. Wark, P. and V.M. McDonald, Nebulised hypertonic saline for cystic fibrosis. Cochrane Database Syst Rev, 2018. **9**: p. Cd001506.

91. Moheet, A. and A. Moran, CF-related diabetes: Containing the metabolic miscreant of cystic fibrosis. Pediatr Pulmonol, 2017. **52**(S48): p. S37-s43.

92. Regard, L., et al., [Ageing with cystic fibrosis: Classical and emerging comorbidities in adults with cystic fibrosis]. Rev Pneumol Clin, 2018. **74**(5): p. 279-291.

93. Cabrini, G., Innovative Therapies for Cystic Fibrosis: The Road from Treatment to Cure. Mol Diagn Ther, 2019. **23**(2): p. 263-279.

94. Flume, P.A., et al., Recovery of lung function following a pulmonary exacerbation in patients with cystic fibrosis and the G551D-CFTR mutation treated with ivacaftor. J Cyst Fibros, 2018. **17**(1): p. 83-88.

95. Robinson, K.A., O.A. Odelola, and I.J. Saldanha, Palivizumab for prophylaxis against respiratory syncytial virus infection in children with cystic fibrosis. Cochrane Database Syst Rev, 2016. **7**: p. Cd007743.

96. Krivchenia, K., et al., Timing of Spirometry May Impact Hospital Length of Stay for Cystic Fibrosis Pulmonary Exacerbation. Lung, 2018. **196**(2): p. 207-211.

97. Cousin, M., et al., Rhinovirus-associated pulmonary exacerbations show a lack of FEV1 improvement in children with cystic fibrosis. Influenza Other Respir Viruses, 2016. **10**(2): p. 109-12.

98. Bellinghausen, C., et al., Viral-bacterial interactions in the respiratory tract. J Gen Virol, 2016. **97**(12): p. 3089-3102.

99. Conese, M., et al., Neutrophil recruitment and airway epithelial cell involvement in chronic cystic fibrosis lung disease. J Cyst Fibros, 2003. **2**(3): p. 129-35.

100. Giacalone, V.D., et al., Neutrophil Adaptations upon Recruitment to the Lung: New Concepts and Implications for Homeostasis and Disease. Int J Mol Sci, 2020. **21**(3).

101. Forrest, O.A., et al., Frontline Science: Pathological conditioning of human neutrophils recruited to the airway milieu in cystic fibrosis. J Leukoc Biol, 2018. **104**(4): p. 665-675.

102. Ingersoll, S.A., et al., Mature cystic fibrosis airway neutrophils suppress T cell function: evidence for a role of arginase 1 but not programmed death-ligand 1. J Immunol, 2015. **194**(11): p. 5520-8.

103. Laval, J., et al., Metabolic adaptation of neutrophils in cystic fibrosis airways involves distinct shifts in nutrient transporter expression. J Immunol, 2013. **190**(12): p. 6043-50.

104. Makam, M., et al., Activation of critical, host-induced, metabolic and stress pathways marks neutrophil entry into cystic fibrosis lungs. Proc Natl Acad Sci U S A, 2009. **106**(14): p. 5779-83.

105. Margaroli, C., et al. Transcriptional adaptation in neutrophils recruited to cystic fibrosis airways underlies changes in canonical immune functions. in 32nd Annual North American Cystic Fibrosis Conference. 2018. Denver, CO, USA: Pediatr Pulmonol.

106. Margaroli, C., et al., Transcriptional firing represses bactericidal activity in cystic fibrosis airway neutrophils. Cell Rep Med, 2021. **in press**.

107. Tirouvanziam, R., et al., Profound functional and signaling changes in viable inflammatory neutrophils homing to cystic fibrosis airways. Proc Natl Acad Sci U S A, 2008. **105**(11): p. 4335-9.

108. Chandler, J.D., et al., Myeloperoxidase oxidation of methionine associates with early cystic fibrosis lung disease. Eur Respir J, 2018. **52**(4).

109. Margaroli, C., et al., Elastase Exocytosis by Airway Neutrophils Is Associated with Early Lung Damage in Children with Cystic Fibrosis. Am J Respir Crit Care Med, 2019. **199**(7): p. 873-881.

110. Margaroli, C. and R. Tirouvanziam, Neutrophil plasticity enables the development of pathological microenvironments: implications for cystic fibrosis airway disease. Mol Cell Pediatr, 2016. **3**(1): p. 38.

111. Grunwell, J.R., et al., Neutrophil Dysfunction in the Airways of Children with Acute Respiratory Failure Due to Lower Respiratory Tract Viral and Bacterial Coinfections. Sci Rep, 2019. **9**(1): p. 2874.

112. Giacalone, V.D., et al., Immunomodulation in Cystic Fibrosis: Why and How? Int J Mol Sci, 2020. **21**(9).

113. Fernandez Fernandez, E., et al., CFTR dysfunction in cystic fibrosis and chronic obstructive pulmonary disease. Expert Rev Respir Med, 2018. **12**(6): p. 483-492.

114. Belchamber, K.B.R. and L.E. Donnelly, Macrophage Dysfunction in Respiratory Disease. Results Probl Cell Differ, 2017. **62**: p. 299-313.

115. Simonin-Le Jeune, K., et al., Impaired functions of macrophage from cystic fibrosis patients: CD11b, TLR-5 decrease and sCD14, inflammatory cytokines increase. PLoS One, 2013. **8**(9): p. e75667.

116. Tarique, A.A., et al., CFTR-dependent defect in alternatively-activated macrophages in cystic fibrosis. J Cyst Fibros, 2017. **16**(4): p. 475-482.

117. Wilson, G.B. and H.H. Fudenberg, Does a primary host defense abnormality involving monocytes-macrophages underlie the pathogenesis of lung disease in cystic fibrosis? Med Hypotheses, 1982. **8**(5): p. 527-42.

118. Bonfield, T.L., et al., Absence of the cystic fibrosis transmembrane regulator (Cftr) from myeloid-derived cells slows resolution of inflammation and infection. J Leukoc Biol, 2012. **92**(5): p. 1111-22.

119. Gao, Z. and X. Su, CFTR regulates acute inflammatory responses in macrophages. Qjm, 2015. **108**(12): p. 951-8.

120. Sorio, C., et al., Defective CFTR expression and function are detectable in blood monocytes: development of a new blood test for cystic fibrosis. PLoS One, 2011. **6**(7): p. e22212.

121. Guerra, L., et al., CFTR-dependent chloride efflux in cystic fibrosis mononuclear cells is increased by ivacaftor therapy. Pediatr Pulmonol, 2017. **52**(7): p. 900-908.

122. Hisert, K.B., et al., Ivacaftor-Induced Proteomic Changes Suggest Monocyte Defects May Contribute to the Pathogenesis of Cystic Fibrosis. Am J Respir Cell Mol Biol, 2016. **54**(4): p. 594-7.

123. Assani, K., et al., Correction: IFN-gamma Stimulates Autophagy-Mediated Clearance of Burkholderia cenocepacia in Human Cystic Fibrosis Macrophages. PLoS One, 2019. **14**(2): p. e0213092.

124. Bear, C.E., A Therapy for Most with Cystic Fibrosis. Cell, 2020. **180**(2): p. 211.

125. Griese, M., et al., Safety and Efficacy of Elexacaftor/Tezacaftor/Ivacaftor for 24 Weeks or Longer in People with Cystic Fibrosis and One or More F508del Alleles: Interim Results of an Open-Label Phase 3 Clinical Trial. Am J Respir Crit Care Med, 2021. **203**(3): p. 381-385.

126. McCarron, A., M. Donnelley, and D. Parsons, Airway disease phenotypes in animal models of cystic fibrosis. Respir Res, 2018. **19**(1): p. 54.

127. Rosen, B.H., et al., Animal and model systems for studying cystic fibrosis. J Cyst Fibros, 2018. **17**(2S): p. S28-S34.

128. Zhou, Z., et al., The ENaC-overexpressing mouse as a model of cystic fibrosis lung disease. J Cyst Fibros, 2011. **10 Suppl 2**: p. S172-82.

129. Crawford, K.J. and D.G. Downey, Theratyping in cystic fibrosis. Curr Opin Pulm Med, 2018. **24**(6): p. 612-617.

130. Cutting, G.R., Cystic fibrosis genetics: from molecular understanding to clinical application. Nat Rev Genet, 2015. **16**(1): p. 45-56.

131. Hartl, D., et al., Innate immunity in cystic fibrosis lung disease. J Cyst Fibros, 2012. **11**(5): p. 363-82.

132. Cabrini, G., et al., Role of Cystic Fibrosis Bronchial Epithelium in Neutrophil Chemotaxis. Front Immunol, 2020. **11**: p. 1438.

133. Ettorre, M., et al., Electrophysiological evaluation of cystic fibrosis conductance transmembrane regulator (CFTR) expression in human monocytes. Biochim Biophys Acta, 2014. **1840**(10): p. 3088-95.

134. Sorio, C., et al., Mutations of Cystic Fibrosis Transmembrane Conductance Regulator Gene Cause a Monocyte-Selective Adhesion Deficiency. Am J Respir Crit Care Med, 2016. **193**(10): p. 1123-33.

135. del Fresno, C., et al., Monocytes from cystic fibrosis patients are locked in an LPS tolerance state: down-regulation of TREM-1 as putative underlying mechanism. PLoS One, 2008. **3**(7): p. e2667.

136. Avendaño-Ortiz, J., et al., Pseudomonas aeruginosa colonization causes PD-L1 overexpression on monocytes, impairing the adaptive immune response in patients with cystic fibrosis. J Cyst Fibros, 2019. **18**(5): p. 630-635.

137. Garratt, L.W., et al., Small macrophages are present in early childhood respiratory disease. J Cyst Fibros, 2012. **11**(3): p. 201-8.

138. Schupp, J.C., et al., Single-Cell Transcriptional Archetypes of Airway Inflammation in Cystic Fibrosis. Am J Respir Crit Care Med, 2020. **202**(10): p. 1419-1429.

139. Ravi, A.K., et al., COPD monocytes demonstrate impaired migratory ability. Respir Res, 2017. **18**(1): p. 90.

140. Wang, Y., et al., Lung fluid biomarkers for acute respiratory distress syndrome: a systematic review and meta-analysis. Crit Care, 2019. **23**(1): p. 43.

141. Murakami, J., et al., Pulmonary emphysema and tumor microenvironment in primary lung cancer. J Surg Res, 2016. **200**(2): p. 690-7.

142. Yang, J., et al., Expansion of a Population of Large Monocytes (Atypical Monocytes) in Peripheral Blood of Patients with Acute Exacerbations of Chronic Obstructive Pulmonary Diseases. Mediators Inflamm, 2018. **2018**: p. 9031452.

Abbreviations

2-DG – 2-deoxy-D-glucose

A Mo – Airway monocytes

AECOPD – Acute exacerbation of chronic obstructive pulmonary disorder

APC – Antigen presentation cell

APE – Acute pulmonary exacerbation

ASN – Airway supernatant

Beta-ENaC – Beta subunit of the epithelial sodium channel

BrdU – Bromodeoxyuridine

CD – Cluster of differentiation

CF – Cystic fibrosis

CFRD – CF-related diabetes

CFTR – Cystic fibrosis transmembrane regulator

CLIP – Class II – associated invariant chain peptide

COPD – Chronic obstructive pulmonary disorder

CXCR4 – Chemokine receptor type 4

CX3CL1 – Fractalkine

DC – Dendritic cell

DEG – Differentially expressed genes

DMEM – Dulbecco's modified eagle medium

DNA – Deoxyribonucleic acid

ENAC – Epithelial sodium channel

EV – Extracellular vesicles

FCCP – Carbonyl cyanide p-trifluoro methoxyphenylhydrazone

FDR – False discovery rate

FEV – Forced expiratory volume

GLUT1 – Glucose transporter 1

GM-CSF – Granulocyte-macrophage colony-stimulating factor

GM-CSFR – Granulocyte-macrophage colony-stimulating factor receptor

HC – Healthy control

HCASN – Healthy control ASN

HIF – Heat inducible factor

HLA – Human leukocyte Antigen

ICAM – Intercellular adhesion molecule

IFN – Interferon

IL – Interleukin

iNOS – Inducible nitric oxide

ISG15 – Interferon stimulated gene 15

LDLR – Low-density lipid receptor

LPS – Lipopolysaccharide

LTB_4 - Leukotriene B4

M-MDSC – Myeloid derived suppressor cell

MCP-1 – Mouse chemoattractant protein - 1

M-CSF – Macrophage-colony stimulating factor

M-CSFR – Macrophage-colony stimulating factor receptor

MDM – Monocyte-derived macrophage

MHC II – Major histocompatibility complex II

MIF – Macrophage migration inhibitory factor

MMP – Matrix metalloprotease

MPO – Myeloperoxidase

mTOR – Mammalian target of rapamycin

NADPH – Nicotinamide adenine dinucleotide phosphate

NE – Neutrophil elastase

NET – Neutrophil extracellular traps

NK – Natural killer cells

NO – Nitric oxide

NOS – Nitric oxide synthase

NOX – NAPDH oxidase

Ox-LDL – Oxidized LDL

OxPhos – Oxidative phosphorylation

PARP1 – Poly-ADP-ribose polymerase 1

PBMC – Peripheral blood mononuclear cells

PD-1 – Programmed cell death 1

PD-L1 – Programmed cell death ligand 1

PCA – Principal component analysis

PMN – Polymorphonuclear cells

PPP – Pentose phosphate pathway

PRR – Pathogen recognition receptor

RA – Rheumatoid arthritis

ROS – Reactive oxidative species

Rot/A – Rotenone/Antimycin A

RUNX1 – Runt-related transcription factor 1

SDF-1 – Stromal cell-derived factor-1

SIRPA – Signal regulatory protein alpha

SLC7A5 – Solute carrier family 7 member 5

SR-A1 – Scavenger receptor A1

STAT – Signal transducer and activator of transcription

TAM – Tyro3, Axl, Mer

TCA – Tricarboxylic acid cycle

TLR – Toll-like receptor

TM – Transmigrated

TNF – Tumor necrosis factor

TRAIL – TNF-related apoptosis-inducing ligand

TRIM – Tripartite motif

VLDL – Very low-density lipid

WHIM – Warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis syndrome

Figures Index

Chapter 1: Introduction to monocyte development, phenotype, function, and relationship to cystic fibrosis airway disease

Chapter 2: Monocyte phenotype and function after recruitment to CF airways

Chapter 3: Monocyte transcriptional adaptation after recruitment to cystic fibrosis airways

Chapter 4: Conclusions and perspectives