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The Cystic Fibrosis 'Gender Gap': Past Observations Present Understanding and Future Directions

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1. Introduction

Cystic Fibrosis (CF) is a systemic disease impacting upon several organ systems. These include gastrointestinal, reproductive, endocrine and pulmonary manifestations of which the latter contributes the heaviest burden of disease morbidity, mortality and impact on quality of life. The defective protein in the disease state is the Cystic Fibrosis Transmembrane Regulator (CFTR) that normally functions as an ion channel permitting intracellular chloride escape. Regulated by cyclic adenosine monophosphate (cAMP) and localized to the apical membrane of epithelial cells, CFTR's function is diminished or absent in CF precipitating a cycle of events including sodium hyper-absorption, mucus hypersecretion, impaired mucociliary clearance and pathogenic colonization with microorganisms. This in turn leads to the clinical picture of recurrent infections, bronchiectasis and airway destruction culminating in respiratory failure and premature death (Davis et al., 1996).

Several hundred mutations of the CFTR gene have been described with a sub-group causing disease. Differing mutations impact upon CFTR channel expression, localization and activity and in some cases a combination of these important functions. Dependent on these factors, CFTR mutations have been stratified into six distinct classes for example the class II ΔF508 mutation encodes a CFTR protein that is both defectively folded and processed resulting in disease (Rowe et al., 2005). Whilst the Republic of Ireland has both the highest incidence and carrier rates of CF worldwide, ΔF508 accounts for >95% alleles identified (Farrell, 2008).

An important long-standing observation in CF remains the fact that a gender dichotomy has been described. Females have poorer survival, worse lung function and earlier colonization with *Pseudomonas aeruginosa* when compared to males without adequate explanation. The following chapter will initially outline past epidemiological observations that have been made with regard to the CF 'gender gap' followed by our present understanding of potential explanations for these gender differences. A special focus on the major female sex hormone estrogen will be emphasized particularly its impact on the inflammatory and immune state within the female CF airway. Finally, directions for future basic science and clinical research in the area will be outlined.

2. Past observations

The female gender disadvantage has been observed in CF morbidity and mortality and is reported throughout the early literature. Differences encompass survival, lung function, frequency of infections and microbiological variation however despite investigation explanations have remained elusive. With the lack of adequate explanation persisting over the last decade, controversy has emerged as to whether such disparities in fact ever existed and if such variation could be explained by therapeutic factors or treatment differences alone that exist between countries and care centers worldwide.

The gender dichotomy outlined within early work in CF states that CF females have poorer survival, worse lung function and earlier colonization with *P. aeruginosa*. Early work by Corey *et al* (Corey & Farewell, 1996) assessing almost four thousand individuals with the disease from the Canadian registry illustrated that although regional differences in survival were identified that females had diminished survival of greater than five years when compared to males utilizing cohorts from both the 70's and 80's. The poorer survival in females was associated with poorer weight however the close inter-relation between declining pulmonary function, weight maintainence, gender and mortality was recognized and put forward for further investigation (Corey & Farewell, 1996). Similar results to these were uncovered from an Italian registry and then further confirmed in an independent United Kingdom assessment of mortality and survival in CF extending from 1947-2003 (Bossi et al., 1999; Dodge et al., 2007). In the early 90's, an important demographic shift in CF survival was noted. CF patients who previously were not surviving past childhood were now progressing to young adults. The proportion of adults with CF increased fourfold between the 70's and 90's together with a doubling of median survival from 14 to 28 years of age. Such changes in the age distribution of CF survival at this time provided clinicians further insight into the natural history of the disease that was previously unknown. Despite these improvements to CF survival, female patients continued to show a lower median survival age compared to males (25 versus 30 years of age in 1990) (FitzSimmons, 1993). Subsequent study involving >20,000 patients with CF and utilizing Cox proportional hazards regression analysis to compare age-specific mortality rates between genders again confirmed gender differences in median CF survival (25.3 versus 28.4 years for females versus males respectively) (Rosenfeld et al., 1997). Additionally, this work aimed to identify particular risk factors that may serve as potential explanatory variables for the observed gender related survival differences (Rosenfeld et al., 1997). Despite analysis using a variety of risk factors for death in CF including poor nutritional status, lung function, airway microbiology, pancreatic insufficiency, age at diagnosis, mode of presentation and race, none could account for the gender disparity identified further confirming the existence of a genuine 'gender gap' (Rosenfeld et al., 1997). Consequential to this and other works, it was unsurprising to note that gender was included as a major parameter in a predictive 5-year CF survivorship model developed to help both researchers and clinicians evaluate therapies, improve prospective study design, monitor practice patterns, counsel individual patients and aid determination of suitable candidates for lung transplantation (Liou et al., 2001).

A lack of explanation for such gender differences in CF disease together with a narrowing of this gap recently has ignited controversy with regard to whether the CF 'gender gap' ever existed. Some arguments have been put forward against the phenomena of the female disadvantage in CF which attempt to explain the gender differences based on therapeutic

advances occurring over the last decade in the management of the disease. For instance, retrospective cross sectional analysis of annual assessment data from a single center has shown that during childhood and adolescence, the lung function and nutrition of CF patients should be equal between the genders and that individual clinic practice should be reviewed if a gender gap persists. The authors argue that prior studies that have shown poorer prognosis in CF females have generally combined data from several centers and that their aim was to determine whether with modern aggressive treatment of CF this gender difference remains when care is standardized within a single center (Verma et al., 2005). Whilst some of the arguments put forward by this work may be plausible, an important point to consider remains that the gender differences observed in CF were most clearly observed post-puberty and during adulthood and not during the adolescent years, the group assessed by this work. To lend further argument to this, recent data from an Italian registry confirmed the absence of a gender gap in CF survival however this assessment only included patients up to 16 years of age, too early to identify the previously described gender dichotomy (Viviani et al., 2011). As a result, one of the conclusions drawn from this dataset was that the emergence of mortality differences between the genders could not be excluded if this cohort was to be followed into adulthood, the timeframe of interest in older studies (Viviani et al., 2011). Additionally, although Olsen *et al* did acknowledge that females with CF are at higher risks of *P. aeruginosa* and *Burkholderia* colonization, require more intensified treatments with antibiotics and have greater rates of hospitalizations compared to males, they found no gender effect upon survival. It was again reiterated in the conclusions to this work that gender survival differences may in fact follow adolescence, the age group studied (Olesen et al., 2010).

To overcome some of the weaknesses in comparing CF survival data between institutions, a case mix adjustment method may be employed (O'Connor et al., 2002). Such a method accounts for baseline differences in both patient and disease characteristics and although no consensus with regards its use in CF currently exists, the characteristics utilized for such an adjustment should include those that differ across institutions and are associated with patient survival. By accounting for characteristics of disease severity that may be a consequence of treatment effectiveness, this analysis can abolish the argument put forward that improvements to treatment account for gender discrepancies observed in CF disease. Using such case mix adjustment methodologies, O'Connor *et al* have shown that female gender in CF remains associated with an increased risk of death (O'Connor et al., 2002). This conclusion is reached using a model encompassing patient and disease characteristics that are present at the time of diagnosis and not influenced by subsequent treatment strengthening the argument for the existence of a true 'gender gap'.

An alternative argument put forward to refute a true 'gender gap' is greater compliance to therapy among female patients with CF. Masterson *et al* studied adherence to infection control guidelines and medical therapy in a CF cohort and found that although age-related differences exist, gender was importantly not a significant factor for treatment adherence (Masterson et al., 2010).

In view of disagreement about the existence for gender variation in CF, a re-assessment for these differences was performed in early 2000, a time where survival rates were noted to have exponentially improved since the mid 80's. Although survival of both genders was discovered to have benefitted from this trend, females had consistently poorer survival rates

compared to male patients re-iterating the existence of genuine gender differences (Kulich et al., 2003). This has been more recently confirmed by data published from our own Irish registry (Jackson et al., 2011). As a consequence, it is clear that female gender is a negative prognostic factor in CF – a finding illustrated across several countries, registries and CF care centers.

In addition to survival variances, differences in the acquisition and conversion of the major pathogen and colonizer *P. aeruginosa* have been described in CF. Females with CF acquire *P. aeruginosa* in advance of males and furthermore convert to their mucoid phenotype earlier conferring worse clinical outcomes (Demko et al., 1995). Longitudinal assessment of this organism in CF has shown a relatively short transition from no *P. aeruginosa* to non-mucoid *P. aeruginosa* however a much more prolonged period, sometimes over a decade preceding mucoid conversion. Transition to mucoid correlated with a deterioration in cough and chest radiography scores along with pulmonary function (Li et al., 2005).

Interestingly, an assessment specifically designed to evaluate whether earlier acquisition of *P. aeruginosa* by females in CF, the greater impact of the organism on lung function or a combination of both factors contributed to the poorer survival in females found that although acquisition of mucoid organism was associated with an increased rate of decline of pulmonary function in both genders males had consistently better FEV_1 (% predicted) and survival rates compared to females. This suggests that alternative factors and not *P. aeruginosa* alone contribute to the gender differences observed in female CF patients (Demko et al., 1995). Separate and independent analysis of the risk factors for initial acquisition of *P. aeruginosa* in children with CF identified by newborn screening found that female gender amongst others represents an important risk for early detection (Maselli et al., 2003). Further study has additionally shown that whilst chronic mucoid *P. aeruginosa* can have prognostic implications in disease outcomes, that female gender again remains an important risk factor for its early detection (Levy et al., 2008).

Whilst these past observations illustrate that gender differences are an important consideration in CF disease, they importantly do not explain why they exist. One important difference between genders and a potential avenue for exploration is the circulating female hormone estrogen. Estrogens are the primary female sex hormone and have fundamental roles during the menstrual cycle. They circulate in three forms: estrone (E_1), estradiol (E_2) and estriol (E_3), with E_2 being the predominant and most active form in the non-pregnant state. Both E_2 and progesterone fluctuations throughout the menstrual cycle have been implicated to impact upon pulmonary function however explanations to address such differences in CF remain lacking (Tam et al., 2011). Our group and others have conducted studies examining E_2 and its effects on infectious, inflammatory and immune consequences in the CF airway. Although such work remains ongoing, its results may provide insight for the first time into reasons for these fundamental gender differences that so far have not been adequately explained.

2.1 Pulmonary innate immunity

The lung constitutes a large surface of the body in contact with the outside environment. It is continuously exposed to a large number of airborne microbes or microbial molecules, and can also be confronted with pathogens approaching via the blood stream. A number of

factors including lung structure and physiology and components of the pulmonary innate immune system interact to contribute to effective pulmonary defenses. Individual key factors of pulmonary innate immunity will be outlined following which the effects that E_2 may have on each component will be discussed. These include, but are not limited to the following:

2.1.1 Airway surface liquid (ASL)

The ASL is a protective layer of fluid that covers the airway epithelium. It contains electrolytes, soluble proteins and importantly provides an interface within which cilia can beat and move mucus up through the respiratory tract. The mucociliary escalator together with pulmonary surfactant provides a barrier material at the air-liquid interface of the lungs. Surfactant contains the surfactant proteins A (SP-A), SP-B, SP-C and SP-D which help to lower the surface tension and participate in innate immune defence.

2.1.2 Pattern recognition receptors (PRRs)

Toll-like receptors (TLRs) comprise a major family of PRRs that fulfil key roles in recognising, discriminating and responding to microbial infection (Greene & McElvaney, 2005). They are associated principally with macrophages and dendritic cells, however their expression is widespread and includes, but is not limited to cells of myeloid and lymphoid origin. TLRs are also expressed by pulmonary epithelial cells (Greene et al., 2005). Activation of TLRs by their cognate ligands can lead to induction of proinflammatory cytokine and antimicrobial peptide expression or up regulation of type 1 interferons. TLRs can also communicate with the adaptive immune response via modulation of cell adhesion and co-stimulatory molecules to induce longer term immunity and a range of non-microbial endogenous factors can also activate certain TLRs.

2.1.3 Antimicrobial proteins

A selection of antimicrobial peptides including the human beta-defensins (HBDs), cathelicidin/hCAP-18/LL-37, lactoferrin and lysozyme can be found in the respiratory tract. In addition to their direct bacterial killing activity some of these proteins also have anti-biofilm, anti-inflammatory and anti-viral properties (Rogan et al., 2006).

2.1.4 Proteases

In the healthy lung proteases fulfil basic homeostatic roles and regulate processes such as regeneration and repair. The principal classes of protease present in the lung are the serine, cysteinyl, aspartyl and metalloproteases. These can function to regulate processes as diverse as tissue remodelling, mucin expression, neutrophil chemotaxis and bacterial killing. Members of these protease classes orchestrate a diverse range of changes with respect to infection and inflammation in the lung (Greene & McElvaney, 2009).

2.1.5 Antiprotease protection in the lung

The activity of pulmonary proteases is regulated by antiproteases. There are three major serine antiproteases in the lung – alpha-1 antitrypsin, secretory leucoprotease inhibitor

(SLPI) and elafin. In addition to their anti-protease activities they also possess other intrinsic immunomodulatory, anti-inflammatory and antimicrobial properties (Greene & McElvaney, 2009).

2.2 Dysfunctional pulmonary innate immunity in cystic fibrosis

Pulmonary infection and inflammation in the CF lung are multifaceted processes. Defective chloride ion conductance due to mutant CFTR causes a decrease in the height of the ASL and an increase in both the volume and viscosity of mucus. The most important airway mucins are the secreted mucins, Muc5AC and Muc5B which are produced by goblet cells of the superficial airway epithelium. Their expression is increased in the CF lung and the overall composition of CF mucus is altered due to an increased content of macromolecules such as DNA, filamentous actin, lipids, and proteoglycans. Together these contribute to mucus plugging within the CF lung (Rose & Voynow, 2006; Voynow & Rubin, 2009). This also facilitates microbial colonisation with *P. aeruginosa, Staphylococcus aureus, Burkholderia cepacia*, *Prevotella* spp, *Candida* spp, and *Aspergillus* spp. One remarkable consequence of these events is an exaggerated influx of activated neutrophils into the lung. Due to the large numbers of infiltrating neutrophils higher than normal levels of neutrophil-derived reactive oxidant species and proteases are reached. The consequences of these events include (i) an imbalance in the lung's redox balance which can be further exaggerated due to reduced glutathione levels (Roum et al., 1993) that together lead to oxidative damage, and (ii) an imbalance in the normal protease-antiprotease balance due to the combined effects of oxidative inactivation of the normal anti-protease defences and the presence not only of excess neutrophil-derived but also bacterial-derived proteases (Greene & McElvaney, 2009).

Neutrophil elastase (NE) is the major protease released by neutrophils in the CF lung. It has significant effects (Kelly et al., 2008). Not only does it up regulate the expression of other proteases including metalloproteases and cysteinyl cathepsins (Geraghty et al., 2007), it can also inactivate certain serine antiproteases (elafin and SLPI) and abrogate their anti-inflammatory and immunomodulatory properties (Kelly et al., 2008; Weldon et al., 2009). In concert with proteinase-3, macrophage-derived metalloelastases and elastolytic proteases expressed by *P. aeruginosa*, NE can promote secretion of mucus and degrade surfactant proteins and antimicrobials. NE can also directly injure epithelial cells and reduce ciliary beat frequency, cleave haemoglobin, complement components and immunoglobulins and interfere with effective neutrophil killing of microbes (Kelly et al., 2008). NE also contributes not only to the intracellular killing of gram-negative bacteria by neutrophils but also, once released extracellularly, can play a role in bacterial killing by comprising a key component of neutrophil extracellular traps (NETs). NETs are involved in host defense (Brinkmann et al., 2004). They bind gram-positive and gram-negative bacteria and allow neutrophils to directly deliver high concentrations of serine proteases that degrade virulence factors and kill bacteria.

The high protease milieu of the CF lung can also impact deleteriously on antimicrobial protein activity. Defensins, lactoferrin, LL-37 and SLPI are all susceptible to proteolytic degradation particularly by cysteinyl cathepsins (Bergsson et al., 2009).

Pulmonary inflammation in CF is also mediated by proinflammatory molecules such as C5a, LTB4, ceramide, and the chemotactic tripeptide Proline-Glycine-Proline (Greene, 2010),

which together contribute to the highly proinflammatory milieu in the CF lung. Furthermore the CF lung is a TLR agonist-rich milieu, represented by microbial-derived factors (bacterial, viral and fungal) and neutrophil elastase (NE), respectively (Greene et al., 2008). The chronic inflammatory phenotype evident in CF airway epithelial cells is likely due in large part to activation of TLRs (Greene et al., 2008). In the CF lung NE–induced activation of TLR signalling is mediated via EGFR ligand generation and EGFR activation (Bergin et al., 2008).

Overall the CF lung is highly prone to exaggerated inflammation. Although it is a neutrophil-rich environment, neutrophil degranulation and killing activity are dysfunctional. With inadequate anti-inflammatory mechanisms due to oxidation and proteolytic inactivation, incomplete resolution of infection occurs, bacterial biofilms remain established and infective exacerbations induce more severe symptoms. In females with CF these events may be further complicated due to gender-specific effects mediated in part by the female sex hormone estrogen which will now be discussed.

3. Present understanding

Estrogen, the predominant female hormone is released from the ovaries and subsequently circulates bound to sex hormone binding globulin (SHBG). Free E_2 interacts with its specific estrogen receptors (ERs) to affect human physiological responses. ERs are predominantly based in the cell cytoplasm however more recently, an association with the cell plasma membrane has been described (Levin, 2009). The major ERs are ERα and ERβ, which share structural similarities however can effect opposing responses based on tissue type and location (Weihua et al., 2003).

Traditional effector mechanisms associated with ERs are genomic where the hormone following binding to its receptor within the cell cytoplasm shuttles as a complex into the cell nucleus to induce gene transcription of estrogen-responsive genes (Metivier et al., 2006). Membrane associated ERs however act through non-genomic pathways that are more rapid affecting protein kinases and mobilizing intracellular calcium stores (Morley et al., 1992; Pedram et al., 2006; Pietras & Szego, 1975; Pietras et al., 2005; Razandi et al., 2000, 2004). Genomic and non-genomic pathways may interact with one another resulting in modification of gene transcription as the end-event.

ERs are distributed throughout the human body however proportions vary by organ system. In some settings, both receptors are expressed whereas in others one subtype predominates. For example, ERα is related to reproductive tissues, bone, liver and the kidney whilst ERβ is more abundant in the colon, bladder and lung. Its role in the lung, particularly one that is chronically inflammed is a subject of continuing research in the context of CF (Chotirmall et al., 2010). Emerging inflammatory, immune and microbiological data suggests a potential role for E_2 in the cause and course of chronic inflammatory lung diseases such as CF.

Over a monthly menstrual cycle, *in vivo* E_2 concentrations fluctuate with highest levels preceding ovulation with the lowest around menstruation. In view of its physiological role, ability to fluctuate in concentration, coupled to its capability to modulate cellular functions, responses and gene expression in those containing estrogen response elements (EREs), E_2

represents an attractive avenue for investigation in terms of the gender differences observed in CF disease.

Our current understanding of the role that E_2 plays within the female CF lung is driven by investigations focused on its effects upon the dysfunctional pulmonary innate immune system and specifically some of its key components as described above. One such component is the ASL which is already known to be compromised in the setting of CFTR dysfunction. Coakley *et al* (Coakley et al., 2008) have recently shown that in the setting of E_2 exposure ASL is further compromised by dehydration and an increased risk of infection and subsequent exacerbation during high circulating E_2 states. Therefore the two-week period of a single menstrual cycle where E_2 concentrations are highest represents a high risk time-frame of acquiring infection and promoting exacerbation (Coakley et al., 2008). To date however, this proposed relationship between E_2 concentration, menstrual cycle phase and infective exacerbations is yet to be illustrated by *in vivo* study and represents a future direction for clinical research.

We have added to the understanding of E_2's role within the innate immune system in CF by demonstrating that high circulating E_2 states confer a TLR hyporesponsiveness to a range of bacterial agonists manifested by an inhibition of IL-8 release. We found that the mechanism by which this phenomenon occurs is through ERβ-mediated upregulation of SLPI, an important anti-protease/anti-inflammatory described above that is widely expressed within the respiratory tract (Chotirmall et al., 2010). SLPI, in separate work has been shown to competitively inhibit NF-κB p-65 subunit binding to DNA inhibiting the transcription of NF-κB regulated genes such as IL-8 (Taggart et al., 2005). It is also important to note that in the non-CF context NF-κB has long been described to affect E_2 signaling pathways, for example within circulating monocytes or tissue macrophages E_2 can block LPS-induced NF-κB activity (Ghisletti et al., 2005). As we have demonstrated E_2 to have an anti-inflammatory role in the context of the female CF lung, we hypothesized that although an environment of chronic uncontrolled inflammation may be damaging over the course of lifelong disease, acute surges in inflammation particularly in the setting of an acute infection may in fact provide protection and play a crucial role in facilitating bacterial clearance. Our published data would suggest however that in CF females this acute inflammatory response to an infective exacerbation is blunted by the presence of circulating E_2 and when taken together with the compromised ASL, high circulating E_2 states create an environment prone to both acquisition of infection and a subsequently compromised response to it (Chotirmall et al., 2010). It is becoming clearer with studies such as those described that E_2 plays an important role in some of the observed gender differences in CF disease probably in tandem with other factors influencing clinical outcomes. One important offshoot from our work illustrating the hyporesponsive state induced by E_2 exposure is in potentially elucidating why anti-inflammatory agents such as the leukotriene B4 antagonist have been unsuccessful in clinical trial, in fact causing premature trial termination (trial registration: NCT00060801) owing to increased infection within the treatment arm (Schmitt-Grohe & Zielen, 2005). Despite this, it remains important to highlight that clinical benefit has been shown following use of high-dose Ibuprofen however mechanisms to explain these outcomes are still sought (Konstan et al., 1995). Notably, we detected ERβ to be the predominant ER within the CF airway by use of bronchial brushings obtained via bronchoscopy (Chotirmall et al., 2010). An increased ERβ expression is associated with oxidative stress and hypoxic conditions explaining why it probably predominates in the CF context (Chotirmall et al., 2010; Schneider et al., 2000).

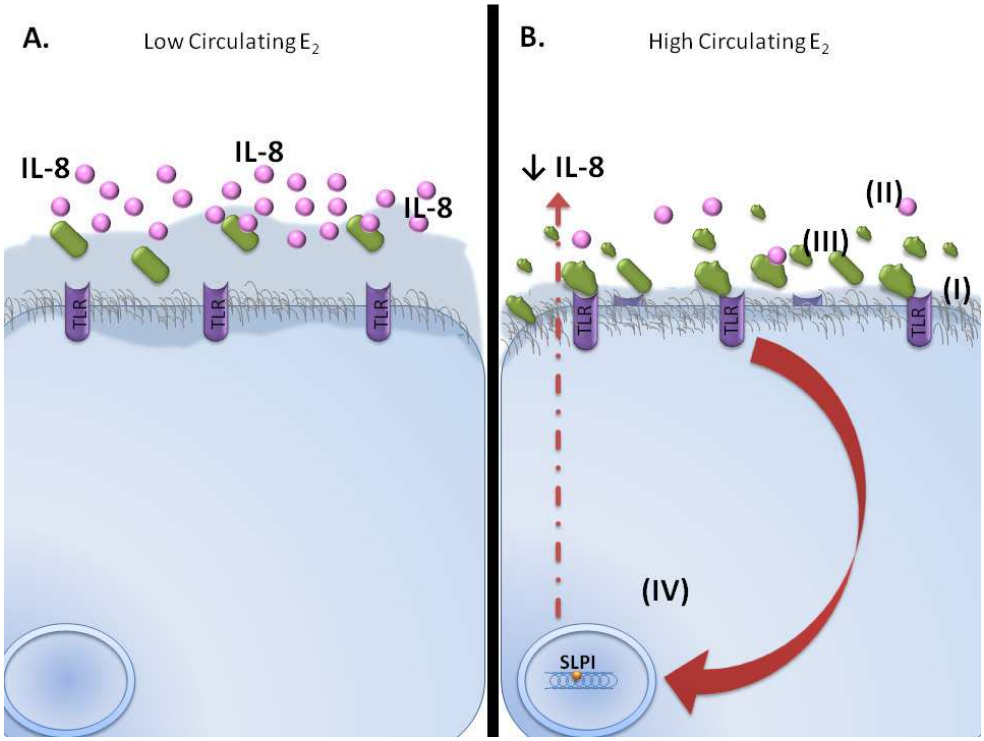

Fig. 1. The effect of 17β-estradiol (E_2) within the female CF airway. (A) In states of low circulating E_2 (luteal phase), a dehydrated airway surface liquid (ASL) overlies the CF female airway epithelium. Within the lumen, antimicrobials (pink) such as lactoferrin and neutrophil chemokines e.g. interleukin-8 (IL-8) are detected along-with potential *Pseudomonas aeruginosa* colonization. (B) During high circulating E_2 states (follicular phase), (I) a further disadvantaged and diminished ASL (Coakley et al; 2008) coupled with (II) an inhibitory effect and consequent dearth of anti-microbial peptides (Wang et al; 2010) combine to create an ideal environment primed for infection and exacerbation (III). (IV) Following infection, a blunted response to microbial agonists occur resulting in diminished luminal IL-8 and a hypo-responsive immune state (Chotirmall et al; 2010). These factors (I-IV) combine to confer an elevated risk of infection and subsequent exacerbation in the E_2 exposed CF airway.

More recent work additionally highlights a role for E_2 in *P. aeruginosa* infection. Proposed mechanisms include an enhanced Th17 regulated inflammatory response and suppression of innate antibacterial defences including the anti-microbial peptide lactoferrin (Wang et al., 2010). When assessed in unison, our work and others may provide an important mechanistic basis for some of the gender differences observed in CF disease. For instance, in CF, a dehydrated ASL overlies the female airway epithelium whilst within the lumen, antimicrobials and the neutrophil chemokines (e.g. IL-8, LTB4, C5a and Proline-Glycine-Proline) may be detected along with *P. aeruginosa* colonization. During high circulating E_2 states (follicular phase of the menstrual cycle), a further disadvantaged and diminished ASL (Coakley et al., 2008) coupled with impaired antimicrobial defences (Wang et al., 2010) combine to create an ideal environment primed for infection and exacerbation. Following infection, a blunted response to microbial agonists occurs resulting in diminished luminal IL-8 and a hypo-responsive immune state due to up-regulation of SLPI (Chotirmall et al., 2010). In tandem, these factors combine to confer an elevated risk of infection and subsequent exacerbation in the E_2 exposed CF airway (Figure 1). The effect of E_2 however on other antimicrobial peptides, proteases and anti-proteases within the pulmonary environment is yet to be fully established.

An alternate but critical avenue to further address gender differences in CF is by investigating the relationship between E_2 and microorganisms within the CF airway. The CF microbiome is complex and encompasses interplay between various bacteria, viruses and fungi that co-exist some to detrimental effect. Whether circulating hormones such as E_2 may influence this organism-rich milieu is an area of ongoing investigation. Microbial endocrinology, an emerging field has begun to address some of these important questions. This particular area of research focuses upon examining the effect of hormones on microorganisms such as *P. aeruginosa*. Thus far, published work in the field has described the effects of hormones such as noradrenaline and norepinephrine upon microorganisms that impact upon their adhesion proteins among other virulence factors (Freestone et al., 1999, 2008). Despite the crucial functions that E_2 carries out in its eukaryotic host, its impact upon prokaryotes are less clearly understood. Whilst E_2 mediates its effects in eukaryotes through its major receptors –α and –β, comparable structures have been sought within prokaryotes. Estrogen binding proteins have been identified in *P. aeruginosa* whilst *Escherichia coli* possesses enzymes with analogous homology to human ERs (Baker, 1989; Rowland et al., 1992; Sugarman et al., 1990). Additionally, it is known that *P. aeruginosa* actively breaks down E_2 to its major metabolite estriol (E_3) and whether such metabolites can impact upon microorganisms particularly in the CF context remains to be determined (Fishman et al., 1960). Importantly, certain fungi are capable of producing estrogenic-like substances termed myco-estrogens however, whether these have any relevance for pulmonary environment in CF remain undetermined.

4. Future directions

Our understanding of the role that E_2 may potentially play within the female CF airway has exponentially grown. Studies performed to date have implicated the hormone as a potential explanation for the long-observed gender differences in CF disease. With such advances to our understanding come further avenues for future exploration.

One such avenue is the role of modifying the endogenous concentration of E_2 to alleviate its detrimental effects. Use of the anti-estrogen agent Tamoxifen or the oral contraceptive pill (OCP) can achieve this albeit by differing mechanisms. Tamoxifen *in vitro* is able to re-instate the ASL to its pre-E_2 state confirming the importance of E_2 in mediating its negative effect (Coakley et al., 2008). Use of the OCP to modulate endogenous E_2 concentrations would be a preferred and safer option although no studies to date have examined use of the OCP in CF in terms of effects on ASL or infection frequency. Such studies will undoubtedly emerge in due course and may provide a valuable potential future therapeutic option for CF females.

Although E_2 in its most active form is the chosen compound for most studies to date, it must be considered that through its natural metabolic process that E_2 is broken down into metabolites such as E_3. Whether such metabolites have effects within the CF airway on immune, inflammatory or infectious consequences is another area for future focus. In terms of innate immunity, prior publications in the non-respiratory setting have shown that E_2 has a major influence on antimicrobial peptides such as lactoferrin, elafin and SLPI (Fahey et al., 2008).

Whilst we have shown in our work that E_2 up-regulates SLPI in CF bronchial epithelium, the effects of E_2 on other anti-microbials have yet to be fully established (Chotirmall et al., 2010). Furthermore, the functional consequence of E_2 exposure on the various anti-microbial peptides has not been addressed.

The complex effects of E_2 on inflammation continue to be deciphered and consequently ERβ agonists have emerged as anti-inflammatory candidates. The development of such a compound however remains a pharmacological challenge that the forthcoming decade should address. Other important estrogen-based chemical compounds include the estrogen dendrimer conjugate (EDC) that binds ERs but excludes them from accessing the cell nucleus and exerting genomic effects (Harrington et al., 2006). Future research utilizing compounds such as the EDC will evaluate the precise contributions of genomic versus non-genomic mechanisms in a variety of *in vitro* and *vivo* settings that will provide further insight into the mechanistic explanations for the gender disparities acknowledged.

Finally, an exciting new development in CF therapeutics is targeting of the basic genetic defect by the use of channel potentiators such as VX-770 (Accurso et al., 2010). Whether E_2 or gender appears to impact upon these emerging agents and their effects represents another exciting direction for both CF basic science and clinical research leading us into the next decade of CF care.

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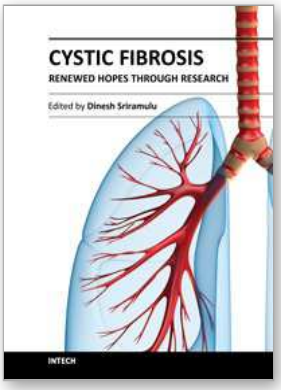

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Living healthy is all one wants, but the genetics behind creation of every human is different. As a curse or human agony, some are born with congenital defects in their menu of the genome. Just one has to live with that! The complexity of cystic fibrosis condition, which is rather a slow-killer, affects various organ systems of the human body complicating further with secondary infections. That's what makes the disease so puzzling for which scientists around the world are trying to understand better and to find a cure. Though they narrowed down to a single target gene, the tentacles of the disease reach many unknown corners of the human body. Decades of scientific research in the field of chronic illnesses like this one surely increased the level of life expectancy. This book is the compilation of interesting chapters contributed by eminent interdisciplinary scientists around the world trying to make the life of cystic fibrosis patients better.

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