The Genes and Germs of Cystic Fibrosis

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Cystic Fibrosis is the most common autosomal recessive, potentially lethal genetic disorder, associated with pulmonary and pancreatic insufficiency. The abnormality lies in a mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene.

The Disease

The CFTR gene is located on chromosome 7 and comprises of 27 exons that encode an integral transmembrane glycoprotein of 1482 amino acids. The normal CFTR protein functions in chloride transport in cells that line the lungs, the liver, pancreas, intestine, reproductive tract and skin. These organ systems are therefore involved in the manifestations of the disease. More than 1300 mutations in this gene have been identified worldwide to date. The most frequent mutation identified is designated as the Delta F508 mutation. This is a 3 base deletion at the 508th codon causing the loss of a phenylalanine residue. This mutation presents in 70-100 % of Caucasian CF patients was found in only 33% of our Pakistani patients. The CF mutations in the remaining 66 % of Pakistani patients have yet to be defined.

The basic defect in CF is a decrease in chloride conductance across apical membranes. The CFTR receptor is made up of various structures. It has intracellular loops that have been shown to be important for the processing of CFTR and correct delivery to the cell membrane. There are also twelve transmembrane domains (M1 - M12) which play a major role in the regulation of pore function. Two of these transmembrane domains are associated with mutations R334Q/W and R347C/H/L/P causing CF. These mutations identified in the first nuclear binding domains (NBF1), while very few occur in NBF2. The NBFs are thought to bind and hydrolyse ATP. Many mutations identified in CF occur in the first nuclear binding domain (NBF1), while very few occur in NBF2. The delta F508 mutation occurs in NBF1. The activity of CFTR as an ion channel depends upon phosphorylation of the R domain and binding of ATP to the nuclear binding domains.

In a normal individual, phosphorylation of the R domain leads to generation of energy in the form of ATP. The generated ATP binds with the nuclear binding domains resulting in the opening of the chloride channel.

In CF there is a failure of the outward chloride channel to respond to phosphorylation by protein kinase (PK)-A or protein kinase C resulting in epithelial dysfunction. Increased cellular chloride and sodium concentrations further lead to the production of thick salty mucus secretions from the cells. This viscous mucus reduces the functional capacity of the lungs and increases the frequency of infection.

Cystic fibrosis is associated with pulmonary and pancreatic insufficiency depending on the particular mutation. Mucus plugging in the airways predisposes the patient to atelectasis and a vicious cycle of inflammation and infection that ultimately leads to bronchiectasis and fibrosis in the lungs. Similarly pancreatic insufficiency leads to fat malabsorption, hypoalbuminemia and failure to thrive. This is results in immunocompromise section and further predisposes the patient to frequent infections.

The Pathogens

The gram negative bacteria Pseudomonas aeruginosa and Burkholderia cepacia are opportunistic human pathogens that are responsible for severe nosocomial infections in immunocompromised patients and are the major pathogens in CF. It is suspected that the two pathogens form mixed biofilms in the same patient and most likely are capable of interacting with each other. Recent evidence suggests that both organisms have a unique communication system using N acetyl-homoserine lactone signal molecules. The airways in CF also have an incompletely characterized innate defense system that facilitates a bacterial phenotype switch to a more intractable mucoid form. The CF lung airways have a unique communication system using N acetyl-homoserine lactone signal molecules.

Burkholderia cepacia flagella also contribute to infection and inflammation by activation of NF-kappa B dependent on expression of TLR5 (Toll like receptor 5). A large percentage (80-90%) of isolates from CF patients produce the exopolysaccharide cepacian which has been hypothesized to play a role in the persistence and colonization of the bacteria in the CF lung. Burkholderia isolates from CF patients across Europe were studied in this regard. Sporadic and cross-infected strains were identified by random amplification of polymorphic DNA. While B. cepacia was identified as the greatest cause of cross infections amongst patients, B multivorans and B stabilis, B.cepacia and B.viennensis were also identified. A similar study from Italy demonstrated that patient to patient spread of B cepacia was stronger with strains belonging to the recA lineage. The mortality of patients infected with B cepacia was higher than that of those infected with B multivorans.
Pseudomonas aeruginosa was the major bacterial opportunistic pathogen in CF. It is responsible for pulmonary infections in almost 80% of CF patients. Pseudomonas aeruginosa regulates flagellin expression in the airways of CF patients. This allows the organism to avoid detection by the host mechanism and phagocytosis in the chronic phase of CF lung infection. The heterogeneous environment of the lung of the CF patient may also give rise to P. aeruginosa small colony variants (SCVs) with increased antibiotic resistance, autoaggregative growth behavior and an enhanced capacity to form biofilms. Genotyping of strains is therefore important as some strains may be associated with increased morbidity and mortality.

Staphylococcus aureus is often the first bacterial pathogen to colonize the respiratory tract of pediatric patients with CF and has been thought to play a major role in early lung disease. Infection with the pathogen leads to aggressive immunologic and inflammatory responses that lead to progressive airway obstruction with thick viscid mucus and scarring. This sets the stage for infections with other bacterial pathogens such as Pseudomonas and Burkholderia.

Other pathogens that have been implicated in pulmonary disease in CF patients include Adenovirus, Hemophilus influenzae, Mycobacteria, xylosoxidans and Stenotrophomans maltophilia.

Treatment

Progressive lung damage follows recurrent respiratory tract infections and results in frequent hospitalization and impairment in the growth and nutritional status of the child with CF. While new treatment modalities have helped improve lung function, treatment is geared to the delay or prevention of initial lung injury. Previous studies of antimicrobial prophylaxis against Staphylococcus aureus have shown mixed results. However, more recent data did not demonstrate an improved clinical outcome with this prophylaxis, rather an increased emergence of colonization with P. aeruginosa.

Improved lung function and decreased pulmonary exacerbations have been seen with the use of long-term macrolide antibiotics for the management of patients colonized by P. aeruginosa. Nebulized aminoglycosides are helpful in preventing or reducing infections with this pathogen. Tobramycin is one of the aminoglycosides with the lowest systemic toxicity which enables aerosol delivery of doses high enough to overcome the antagonistic effects of sputum. However, continual administration may result in the development of resistance. This resistance is transient and returns after a period off the antibiotic.

Aminoglycoside antibiotics such as gentamicin can also suppress premature termination codons, permitting transcription to continue to the normal end of the transcript, resulting in the expression of full-length CFTR at the apical cell membrane.

A vaccine against P. aeruginosa based on its recombinant outer membrane has been developed. Regular vaccination of young CF patients for a period of 10 years has been shown to reduce the frequency of chronic infections with the pathogen.

Other modalities that have been used for the treatment of infections include intravenous immunoglobulins, Rifampicin, sodium fusidate and fosfomycin.

Summary

Cystic fibrosis is a complex disease with myriad problems. A thorough understanding of the early natural history of lung disease in young CF patients is crucial for the development of effective treatment strategies. Careful evaluation of the various pathogens and their management is very important to improve longevity and the quality of life in our patients. It is also imperative that efforts be made to avoid excessive unwarranted antibiotic use and prevent the emergence of resistant pathogens.

References

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