Understanding chronic granulomatous disease biology essay

Science, Biology

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Abstract

Chronic Granulomatous disease is a case of primary immunodeficiency with x-linked or autosomal recessive inheritance pattern. It is characterized by inability of phagocytes to kill the ingested narrow spectrum catalase positive bacteria and fungi. The failure in lysis of phagocytosed particle occurs due to synthesis of a faulty copy of NADPH oxidase in host. The outcome of CGD is recurrent and deadly fungal and bacterial infections, other inflammatory diseases as well as granulomatous conditions in affected individuals. With the high mortality and morbidity rates for CGD, advances in the field of science and technology have worked towards altering the clinical scenario and life expectancy of CGD patients.

Introduction

Chronic granulomatous disease is a myeloid primary immunodeficiency. A myeloid primary immunodeficiency impairs function of innate immunity by loss of phagocytic activty of immune cells. CGD occurs at an average frequency of 1/250000 live births with life expectancy of the patient upto 25-30 years of age. [1] Chronic granulomatous disease occurs due to a defect in oxidative metabolic pathway in phagocytes by which they fail to produce microbicidal oxygen metabolites (superoxide). Thus an affected phagocytic cell ingests a foreign particle but fails to degrade it. CGD patients show high suscepbility to bacterial and fungal infections, loss of ability of mononuclear cells to function as phagocytes, skin absessions, formation of granulomas etc. The main cause of CGD is a point mutation event in genes that code for

NADPH oxidase. The conventional treatment for CGD is the use of certain antibiotics to curb infection, IFN-gamma treatment etc. Gene therapy, Haematopoietic stem cell transplants (HSCT) are novel therapies that are being developed to reduce the occurrence and adverse conditions commonly observed in CGD.[1][2][3][4]

Discussion

Link between CGD and NADPH Oxidase

The inability of phagocytes to produce oxygen metabolites in CGD lies in a defect of 1 of the 6 subunits commonly involved in formation of an active NADPH oxidase. Four proteins together form a single enzyme complex that brings about transfer of an electron from cytoplasmic NADPH to molecular oxygen. This enzyme complex is called as NAPDH oxidase, which is phagocyte specific. It is made up of a membrane bound cytochrome b and cytoplasmic components. Cytochrome b consists of a membrane bound x linked gp91Phox coded by CYBB gene, p22Phox coded by CYBA gene and two cytosolic components namely, p47Phox coded by NCF1 gene and P67Phox coded by NCF2 gene. Deficiency in gp91Phox results in X linked CGD (X-CGD) while malfunction of other components leads to autosomal recessive CGD. Both gp91 Phox and p22Phox depend upon each other for expression in a phagocyte. In addition to these components certain regulatory components e. g. p40Phox and rac are also involved in the enzyme complex formation. On stimulation, the cytosolic components get phosphorylated and bind tighly to each other. In presence of the regulatory components the cytosolic components attach to membrane bound

components to form an active NADPH oxidase. An electron is taken by NADPH and transferred to molecular oxygen to form superoxide. Superoxide dismutase converts superoxide to hydrogen peroxide which is further converted to bleach (Hypoclorous acid) in presence of Myeloperoxidase and Chlorine. Reactive oxygen species (superoxide) indirectly kill ingested particle by activation of non oxidative pathway that involves a primary granule protein (azurophile) and enzyme Elastase inside phagocytic vacuole. [2]Neutrophils also release these granule proteins and chromatin in ECM which associate with each other to form Neutrophil extracellular traps (NETs). These NETs trap extracellular fungi/ bacteria, degrade their virulence factor etc. In CGD patient, neutrophils are deficient in production of NETs. [1]Other roles of NADPH oxidase outside a netrophil include signaling for NFkB activation, increasing pulmonary permealibilty, improving intelligence quotient etc. Thus in CGD patient down regulation of these traits is seen. [2]

Biological Effect of CGD and its treatment

Neutrophil Apoptosis and Efferocytosis

Apoptotic cells have phosphatidyl serine (PS) on their external surface which is recognized by PS receptors. Due to this interaction, a phagocytic cell easily takes up an apoptotic cell; this process is called as Efferocytosis. The process leads to secretion of TGF-β by macrophages thus causing resolution of acute inflammation. Macrophages of CGD patient lack this functions leading to nechrosis and release of oxidants/proteases causing lupus disease in CGD patient. This condition can be reversed by IFN-gamma treatment. IFN-γ stimulates NO production, endogenous TNF-alpha production and Rac

activation. Further studies indicated that generation of 12/15 lipoxygenase and activation of a transcription factor PRAR■ was reduced due to impaired PS-PSR dependent IL-4 production. This hampers internal programming within a macrophage causing reduction in their efferocytosis activity. In vivo mice model proved that this impact could be reversed by injecting PS in CGD mice.

Innate Immune Receptors

Neutrophils are the key phagocytes whose functionality is controlled by innate immune receptors e. g. toll like receptor and complement receptor. Neutrophils from CGD patients show low levels of TLR-5, TLR-9, CD11b, CD-18, CD-35 etc. Reduced TLR-5 results in improper neutrophil activation against bacterial flagella, while reduced levels of CD11b/CD18 impair phagocytosis of s. aureus. Reduction in expression of these receptors correlates to severity of CGD in patients.

Oxidation of T-cell membrane protein

This process is reactive oxygen species (ROS) dependent. It controls activation and proliferation of T cells. Low ability of ROS production in immune cells is related to increase in thiol groups on T cell membrane. In recent studies it has now been proved that ROS deficient macrophages initiate autoimmunity like conditions.

Th17 cells

These highly proinflammatory cells are involved in inflammatory process of autoimmunity. These IL17 producing cells are counterbalanced by T

regulatory cells and are also involved in dealing with pathogens. In CGD this balance in not met and several complications thus get amplified.

Indolamine-2, 3-Dioxygenase (IDO)

IDO controls immune responses and is involved in synthesis of intermediates with immunosuppressive properties e. g. L-kynurenine. These intermediates produced by IDO have also shown to activate Th-17 proinflammatory cells. IDO's function depends on concentration of superoxide produced by NADPH oxidase. In CGD since the enzyme is faulty, excessive inflammatory responses are induced. [5]

Accelerated calcium influx

Change in membrane potential followed by activation of neutrophil is absent in CGD. Membrane depolarization activates several other immune cells and at the same time stops calcium influx, thus preventing hyperactivation. Improper regulation of calcium balance within a cell may leads to additional abnormality in CGD. This phenomenon of deporalization is controlled by NADPH oxidase. Since the enzyme is non functional in CGD patient, the pool of calcium ion in cell is uncontrolled leading to hyper inflammatory response and thus granuloma formation. [6]

Granuloma formation

Granulomas are characteristic feature of CGD. They are commonly seen on spleen, GI tract, urinary tract, skin, lymph node etc. and cause obstruction of these tracts. However the causative agent for granuloma formation is not

completely known and it has been observed that appropriate therapeutic doses of corticosteroid works towards controlling this effect.

Aspergillosis

Acute pneumonitis condition due to inhalation of mulch or decayed organic matter containing fungal spores/load is a characteristic feature of CGD. The causative agent i. e. Aspergillus in most of the cases can be detected using Bronchoscopy and lung biopsies. Treatment with antifungal agents e. g. Prednisone etc heals the problem.

Inflammatory lesions

These pulmonary inflammations normally occur in lungs of CGD patients which on progression cause hypoxia and other functional abnormilties. Treatment with Methotrexate is used to heal this condition. [2][8]

Manifestations in CGD

Pleuropulmonay manifestations

These conditions should be carefully treated as it shows risk of spreading into chest wall and could also cause osteomyelitis. Manifested infection is treated with proper clinical management or by surgical removal of affected area. The condition can be diagnosed using CT scan or magnetic resonance (MR) imaging.

Lymph nodes

This infection involves formation of suppurative (pus) abscesses or non suppurative granulomas due to chronic inflammation. The causative agent is mostly S. aureus. [7]

Skeletal manifestations

Osteomyelitis, bone infection occurs in 1/3rd of CGD individuals. The causative agent is Aspergillus and the site of infection is mostly ribs and vertebrae. Pain, tenderness, swellings etc are signs of infection. [1][2]

Gastrointestinal manifestations

The condition is commonly found in X-CGD and results in obstruction of GI tract. Inflammation of internal antral wall and accumulation of lipid laden histocytes are commonly observed in this condition. Clinical management of this condition involves use of antibiotics, steroids etc. Surgical therapy e. g. colostomy with ileostomy is another alteration in treatment of GI infections. However use of Cyclosporine is an alternative treatment to colostomy in CGD patients wherein other medical therapies fail to mount a response. Other sites of manifestations involve urinary tract, hepatic and spleenic areas, central nervous system etc. [7]

Treatments involved in CGD

Prophylactic approach

Treatment for inflammatory and autoimmune complication face a problem as the approaches used induce immunosuppressions in an already immunocompromised CGD patient. However certain therapies used to curb down the effects and occurrence of CGD are given below, TNF ✔ inhibitors e. g. Infliximad are good anti-inflammatories but have many side effects. [1] [8]Infections in CGD are commonly caused due to organism like, S. aureus, Serratia marcescens, Nocardia, Aspergillus etc. Thus prophylactic treatment against these infections includes certain antibiotics specific to the causative

agents. Trimethoprim/sulfamethoxazole prophylaxis is used against S. aureus infections. Treatments with Intaconazole, Voriconazole etc are used to heal Aspergillus infections. In case of local infection, local treatment such as steroid Enemas can be used, but use of steroids have many risk factors involved e. g. osteoporosis, growth retardation etc. For acute infections with Nocardia, Linezolid is highly effective. Efficacy of antibiotic used depends on the therapeutic index, time of exposure, selection of the antibiotic, host condition etc. Certain antibiotics need to be taken indefinitely to prevent relapse of infection. [1][2]Surgery plays an important role in management of CGD. In fungal infection of chest wall and vertebrae, excision of fungi infected site by surgery is recommended when use of antibiotic fails to show an impact. The surgical sutures should be left untouched for prolonged period of time as the treated areas are weak and take time to heal. Certain lung infection that cannot be eradicated by other prophylactic cure can be treated using surgery. [7]Ganulocyte transfusion is other alternative in severe or refractory infections in CGD patients. Hydrogen peroxide synthesized by tranfused cells can diffuse into affected cells and get converted to bleach thus maintaining normal activity of phagocytes in CGD patients. Adverse effect of granulocyte transfusion includes induction of alloimmunization for next transplant, pulmonary leukocytosis, and fever etc. Treatment with cord blood has been gaining importance as a therapy in increasing life expectancy of CGD patients. For White cell transfusion, leukapheresis (movement of neutrophils), neutrophil function, their survival time is improved by administration of G-CSF in healthy donor before transfusion. This treatment is usually accepted and shows positive results

however certain side effects include, leucocystasis, leucoagglutination, risk of alloimmunization towards next allogenic stem cell transplants etc. [9]IFN-■ treatment is a good alternative to otherwise harsh effects of other prophylactic measures. A combination of IFN-■ with prophylactic antibiotics is a recommended treatment for CGD patients. IFN-gamma is a main macrophage activating factor. It enhances synthesis of ROS and killing of foreign particle. IFN-■ works in a patient regardless of his age, past use/nonuse of prophylactic antibiotic, inheritance of disease etc. IFN gamma shows hardly any toxicity in host and is well accepted by the host body. IFN-■ was used to successfully treat CGD children below the age of 10 years. [10][11]

Therapeutic approach

Antimicrobial prophylaxis involve treatments that could give rise to resistance to the curative agent involved and it also involves need for frequent hospitalization, To overcome these drawbacks certain new method as CGD therapies are devised. These approaches are implemented in patients who are chronically ill and in whom other therapies show no effect. In gene therapy one of the approaches is to transform patient’s cell with use of autologous hematopoietic CD 34+ cells conditioned with Fludarabine, Busulfan etc and engineered with a retroviral vector expressing normal gp91Phox gene. However the possible outcome is a low population of transduced cells. This could be an outcome of low humoral immunity, less hematopoietic stem cell (HSC) carrying the viral vector etc. To overcome this, certain cellular markers are used to identify tranduced stem cell

population, and also the invitro transduction protocol is shortened. Gene therapy tough a promising approach to eradicating CGD may fail because of low selective advantage in corrected cells, it is thus necessary to find a new safe vector with high transducing efficiency of HSC. [12][13]Since gene therapy is in its developmental stage, Allogenic hematopoetic stem cell transplant (HSCT) is gaining importance. The major drawback of this method is Graft vs host reaction (GVHD). [14]Factors to be considered in HSCT, Infectious area should be specifically identified using PET/CT scan and biopsy. Granulocytes used in transfusion should be G-CSF primed to increase their shell life and activity within the host system. To prevent GVHD, TNF-✔ antagonists and/or certain immunosuppresants should be given to the host. Patient being considered for graft shouldn’t be exposed to donor’s granulocyte prior/during transplantation, this is done to prevent alloimmunization occurrence. The phase of disease, clinical history of recipient, availability of donor should be considered before transplantationHCST should be performed in genoidentical individual and thus HLA typing becomes essential. Myeloablative HSCT in closely matched related or unrelated donor is a positive therapy for children with CGD if performed in early stages of the disease. Further graft combination with ATG/Busulfan/Treosulfan etc can be used to secure the myeloid graft which is to be transplanted. [4][8][15]

Common Signs and symptoms of CGD

OsteomyletisSkin problems e. g. impetigo, skin abscesses and furuncles, eczema etcPersistent diarrheaFrequent and refractory pneumoniaJoint problemLung problems etc. [1][2][3]

Diagnosis

Diagnostic methods should be rapid and practical with respect to their analytical performance with appropriate validation and evaluation capacities. Following are certain diagnostic methods implemented in diagnosis of CGD condition, Functional analysis of neutrophils of the newborn in CGD can be done by Flow cytometric detection by conversion of dihydrorhodamine-1, 2, 3 (DHR) to rhodamine-1, 2, 3. It is a rapid and sensitive method of diagnosis. In DHR the leukocytes are separated from, the sample and cultured separately. These cells are then loaded with a set volume of non fluorescent DHR and incubated at 37oc in presence of catalase. After this the cells are stimulated in Phorbol myristate acetate (PMA), incubated again at 37oc and quantified under flow cytometry. Stimulation index was determined as the ratio of fluorescence in stimulated cells to the fluorescence in non stimulated cells. DHR analysis can also be done at antenatal stage. DHR assay is rapid and highly sensitive for low sample of blood. It is a simple and also cheap laboratory test. Certain cytogenetic assays are also done for prenatal analysis in CGD. Chronionic villus sampling is a good method for early detection of CGD in fetal stage of growth. Prenatal analysis of DNA of suspected CGD prone fetus can also be done by direct sequencing which detects family mutation. This test recommended only if the suspect has a

family history of CGD. [16]Other effective measures in detecting mutation in CYBB gene are single strand conformation polymorphism analysis, denaturing high temperature liquid chromatography etc. Mutations can also be detected using RT-PCR techniques. A new technique proposed for analysis of CYBB gene is high resolution melting technology. By this method even carriers can be detected. HRM technology involves an automated DNA extractor and a high resolution instrument as the only cost factor. CYBB appears to be a good subject for genetic analysis as its polymorphisms are rare, mutations are small i. e. either missence or nonsense. Most of the time CGD shows recurrent GT deletion in patient's DNA, this is presented as

GT. It has been recently found out that this GT is due to homologous recombination between NCF1 gene and its pseudogene counterpart. Carriers with this defect can be tested by a new method called as gene scan technology. [17]Erythrocyte sedimentation rate is also a parameter tested in studying the diseased state. Immuno blot techniques make further genetic analysis of patient's DNA possible. [3]Superoxide production can be directly measured to study NADPH oxidase activity in phagocytes of patients. [2]In Nitroblue tetrazolium test (NBT), activated neutrophils are incubated with NBT dye, this causes accumulation of formazan, a blue pigment in cells. Failure in colour change indicates CGD condition. NBT test may give a false normal result in p47PHox deficient CGD or in certain form of X-CGD. [7] [16]Diagnosis using computerized tomography (CT) scan, biopsy, bone scan can be done to image site of infection. Ultrasonographic or CT can be used in performing percutaneous drainage of abscesses. [7]Complete blood count can also be performed to detect CGD. [1][2]

Modern approaches

Recent studies showed partial correction of affected phagocytes in patients with X-CGD as well as autosomal recessive CGD by subcutaneous administration of recombinatnt IFN-gamma (rIFN-gamma). Treatment showed 5-10 fold increase in superoxide production by neutrophil and increase even in their bactericidal activity. Thus rIFN-gamma with further use of technology can make a treatment better. [18]The normal retroviral agents used in gene therapy aren’t reliable in terms of their specificity as they also harbor risk of insertional mutagenesis. To overcome this, self inactivating (SIN)-LTR Retro viral (RV) vectors are used with internal promoter and deleted LTR enhancer activity. In order to achieve safety with the use of retroviral vector system insulators and suicide gene constructs are used. In spite of these modification in a vector it still poses threat by integration within 5’ regulatory region of coding areas on gene that are actively expressed in target cells e. g. protooncogenes.[13]Lentiviral vectors (LV) are safer as they don’t preferentially integrate within 5’ regulatory portion of the gene. Certain non integrating lentiviral systems have been made which don’t integrate into the host and are thus get diluted during cell division. However this system cannot be used in treatment of disorders that involve proliferating cells. Other novel methods to obtain locus specific targeting are gene correction and safe harbor. Gene correction is replacing a faulty gene with a DNA fragment containing wild type sequence while leaving the rest of the sequences in the host intact. This strategy uses endogenous promoter and regulatory elements thus maintaining normal expressions of the gene. Targeting safe harbor involves targeting of safe loci in the genome that lack

oncogenes and whose disruption does not affect normal functioning of the cell. Both of these methods involve introduction of exogenous DNA template into the host. Different approaches have been designed to obtain locus specific DNA break and specific integration of exogenous DNA in host. Homing endonucleases are sequence specific enzymes that cause double stranded break (DSB) at a specific site. Zinc finger nucleases are artificially made fusion proteins that are a combination product of zinc finger DNA binding domain specific to a site on DNA and nuclease domain of endonuclease Fok1. ZFN can be made specific to a mutated site on DNA and the affected gene could be rectified by homologous recombination or by non homologous end joining mechanism (NHEJ). Another novel gene delivery system similar to transpospons is made containing components that allow expression of transposons and vector containing gene of interest which needs to be introduced in host genome. Sleeping beauty transposons, piggyback transposons system etc are used for this purpose. However even with use of these system risk stills persists and can lead to chromosomal alteration, altered gene expression etc. [1][12][13]Certain antifungal prophylaxis now uses a powdered form of Amphotericin B introduced directly into the lungs on weekly basis via an inhaler. [19]A recently discovered mulch pneumonitis, a severe CGD infection uses both antifungal therapy and corticosteroids in treatment. Further studies of critical pathway and functions of NADPH oxidase can be carried out by considering incident of infections by new emerging uncommon pathogens in CGD e. g. G. bethesdensis, N. udagawae etc.[8][9][20]

Prevention and precautions

An individual who has a family history of CGD and is planning on starting a family should go for genetic counseling. Genetic screening and chorionic villus sampling for early detection of CGD is recommended. [3][16]For precaution sake certain general health care should be followed by CGD patients. Few measures are mentioned below, An affected individual should receive full immunizations. On infection the wounds should be washed well and treated with antiseptic. Patient should follow good oral hygience to prevent gingitivitis. Pulmonary infection should be avoided by refraining from smoking, staying away from sources of fungal spores e. g. mulch, decaying matter etc.[9]

Conclusion

Even if the overall survival rate in CGD has improved over last decade, the annual mortality is 2-5% and about 50% of patients live up to the age of 30 years. CGD is thus still a lethal disease especially at the adult age. The future goals are to develop better curative measures, from which HSCT and gene therapy have better scope for improvement. HSCT could be improved if the side effects of conditioning and pre existing infection in CGD patient at the time of transplant are reduced. However further knowledge about donor selection, time of transplantation, efficacy of transplant needs to be studied for better advancement of HSCT as a therapy. Similarly gene therapy can improved if efficient method for inducing growth advantage for corrected cell is achieved in vivo. Thus in spite of its rarity and age, GCD is developing in w. r. t. its outlook, management, prognosis etc. Thus Chronic Granulomatous

disease serves as a good platform for medicinal, gene therapy, genetics and transplantation studies.