Chronic Granulomatous Disease (CGD: An Update on Pathogenesis and Management for Internists

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Abstract

Inborn errors of immunity are emerging in the medical practice of adult physicians. The median life expectancy of once fatal pediatric primary immunodeficiencies (PIDs) was extended into adulthood by increasing awareness , shortening diagnostic delays and developing modern treatment regimens, incl. hemopoietic stem cell transplantation (HSCT). A prime example of the impressive progress is chronic granulomatous disease (CGD), an inherited deficiency of the innate immune system, with a median patient survival of 40 years and more.

The last few years have witnessed four major advances in our knowledge of CGD:

1. Realization of an increased burden of inflammatory complications in adult life requiring specialist medical care.
2. Better understanding of the physiologic anti-inflammatory function of the defective NADPH system opening new avenues for targeted drug interventions
3. Advances in reduced intensity conditioning (RIC) for allogeneic HSCT making it a safe procedure even for patients with severe infection or hyperinflammation.
4. Encouraging early data of an ongoing trial of gene-replacement therapy using a self-inactivated lentiviral vector.

Combining targeted antiinfectious/antiinflammatory measures and considering extended indications for curative HSCT are key to improving patient outcome further. Long-term assessment of gene therapy is not yet possible.

Abbreviations

AR – autosomal recessive; CGD – chronic granulomatous disease;
GT – gene therapy; GvHD – Graft-versus-host disease;
HSCT – hematopoietic stem cell transplantation; IFNg – interferon gamma;
IL1 – interleukin 1 beta; MA – myeloablation: NET – neutrophil extracellular trap; O2- superoxide anion; oxPS – oxidized phosphatidylserine;
PHOX – phagocyte NADPH oxidase; PIO – Pioglitazone; PT/CY – posttransplant cyclophosphamide; RIC – reduced intensity conditioning; ROS – reactive oxygen species; TDM – targeted drug monitoring; TMP-SMX - trimethoprim-sulfamethoxazole; TNFa– tumor necrosis factor alpha; XR – X-linked recessive

1. Introduction to Chronic Granulomatous Disease (CGD)

CGD is a rare inherited deficiency of the innate immune system affecting 4 to 5 per million live births in the US. The disease is caused by loss-of-function (LOF) mutations in one of the five genes encoding the subunits of the phagocyte NADPH oxidase (phox) complex. The phox deficiencies result in defective production of reactive oxygen species (ROS) leading to impaired microbial killing and excessive inflammation. CGD is thus characterized both by severe recurrent bacterial and fungal infections and by hyperinflammatory complications (most evident in the pulmonary, gastro-intestinal and urinary tracts). Diagnostic biologic tests for CGD rely on measurement of defective superoxide (O2-) generation. Conventional management is based on the combination of antimicrobial prophylaxis, prolonged treatment of bacterial and fungal infections after appropriate imaging/pathogen identification and cautious use of corticosteroids for inflammatory manifestations. Allogeneic hemopoietic stem cell transplantation (allo-HSCT) with an HLA-compatible donor is the only known cure of CGD and indicated early in patients with no residual O2- production, predicting a severe clinical phenotype.

2.Etiology and Pathophysiology of CGD

New knowledge of the multiple physiologic NADPH oxidase functions can now define CGDs both as genetic defects of pathogen clearance by neutrophils and as defects in the clearance of dying host cells by macrophages.
This insight is important for understanding the seemingly paradoxical association between primary immunodeficiency and autoinflammation and is opening new opportunities for targeted drug treatments.

2.1.Genetic Defects and Impaired Microbial Killing

CGD is an inherited disorder of assembly/activation of the NADPH oxidase complex. After stimulation of neutrophils, monocytes and macrophages the activated oxidase transfers electrons from NADPH to molecular oxygen generating superoxide (O2-), the so-called oxidative burst, which is converted into microbicidal oxygen species (ROS, e.g. hydrogen peroxide) (fig.1).

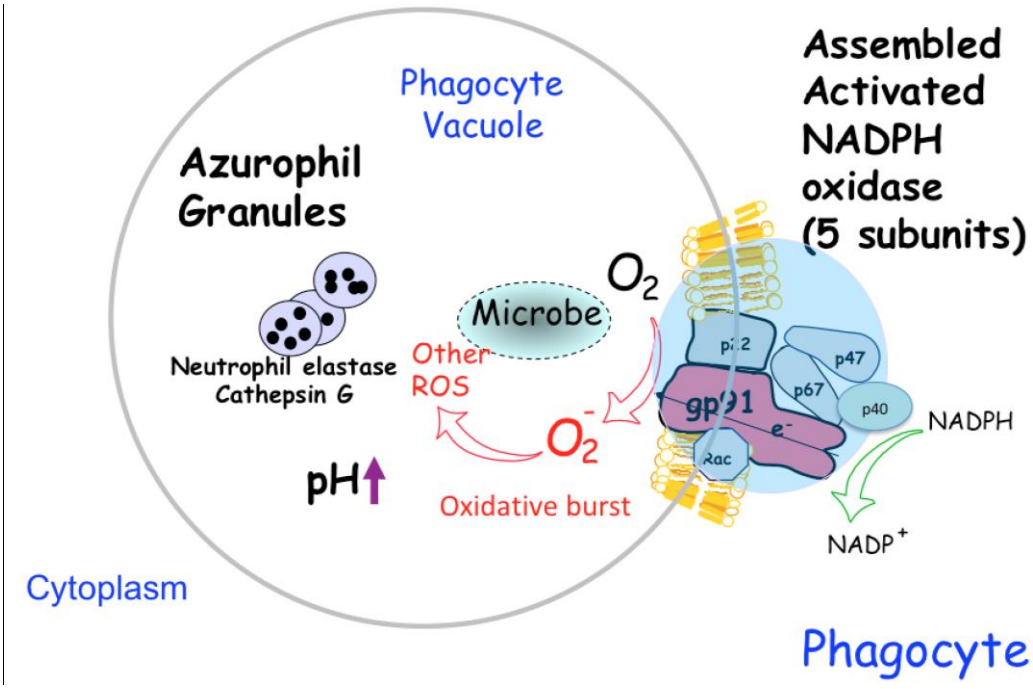


Fig.1 Phagocyte NADPH oxidase dependent intracellular microbial killing by ROS and proteases.

In the US 2/3 of CGD cases are caused by mutations in the X-linked gene encoding gp91phox, the electron transferase subunit of the NADPH complex. 1/3 of cases are caused by biallelic mutations in either of three A/R genes encoding cytosolic proteins (p47, p67, p40 phox) regulating gp91 phox after their translocation to the plasma membrane or in the gene coding for the membrane-bound p22phox protein stabilizing gp91 phox. A/R mutations in the gene encoding p47phox are the second most common cause of CGD observed in 25% of cases in the US.

The disease-causing mutation should be determined in every CGD patient. This is essential for genetic counseling and important for prognostication in CGD (1): Since residual O2- generation (as in most p47 phox deficiencies) presents a lower risk for infections and mortality compared to absent O2- production (as in most cases of gp91phox deficiency).

Apart from being directly responsible for production of microbicidal ROS, the NADPH oxidase in neutrophils is indirectly responsible for liberation and activation of complementary microbicidal proteases (e.g. elastase and cathepsin G) from primary (azurophilic) granules (fig.1). Both mechanisms collaborate in killing/ digestion of pathogens entrapped by neutrophils in phagocytic vacuoles, a process deficient in CGD patients (2) and reversed following gene therapy.

Neutrophils producing O2- finally disintegrate their own intracellular membranes and release decondensed

chromatin (DNA/histones) together with >20 microbicidal granule proteins into the extracellular space, forming weblike Neutrophil Extracellular Traps (NETs) (3). Released NETs continue the killing process for hours and can also trap and kill microbes that are too big to be phagocytosed (e.g. bacterial aggregates and fungal hyphae) (4), a process again deficient in CGD patients (5), that can be reversed by gene therapy (4).

2.2. Excessive Inflammation

Dying (apoptotic) neutrophils are rapidly cleared by macrophages in an „immunologically silent“ way preventing hyperinflammation by the debris. Neutrophils externalize oxidized phosphatidyl serine (oxPS) on their surface membrane which is then recognized through oxPS receptors on macrophages triggering neutrophil uptake into phagocytic vacuoles (fig 2) (6).

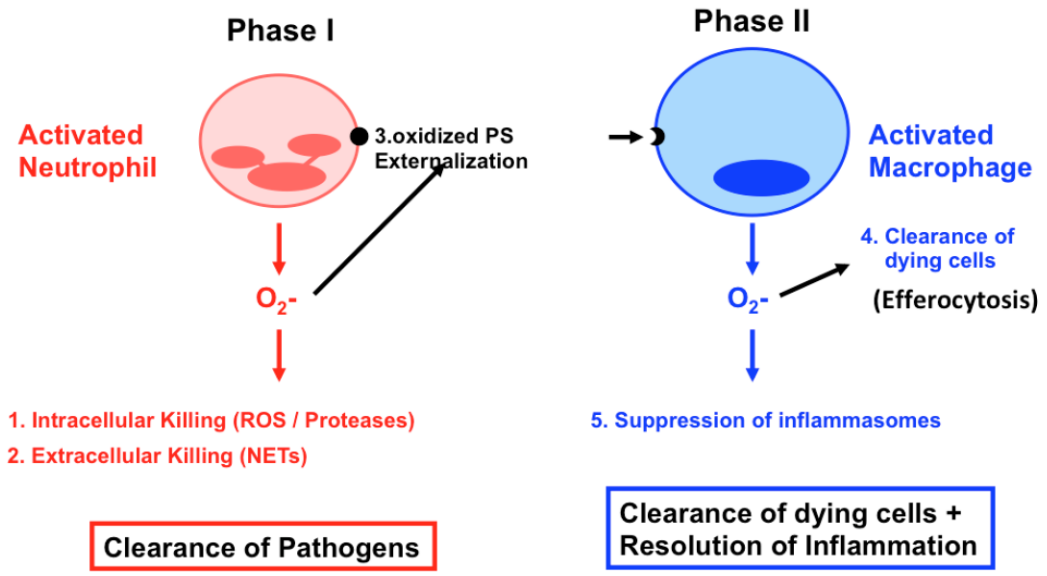


Fig.2 Summary of physiologic NADPH oxidase functions:
Clearance of pathogens by activated neutrophils +
clearance of dying neutrophils by activated macrophages.

The subsequent clearance process of apoptotic neutrophils within macrophages, also called efferocytosis (Greek „to carry to the grave“), ultimately resolves inflammation. Uptake is followed by NADPH oxidase mediated recruitment of LC3-proteins of the autophagy system to the phagosomes enhancing their fusion with lysosomes and facilitating controlled degradation of their cargo (fig.3) (7). LC3-associated phagocytosis is accompanied by release of antiinflammatory cytokines (e.g. IL10).

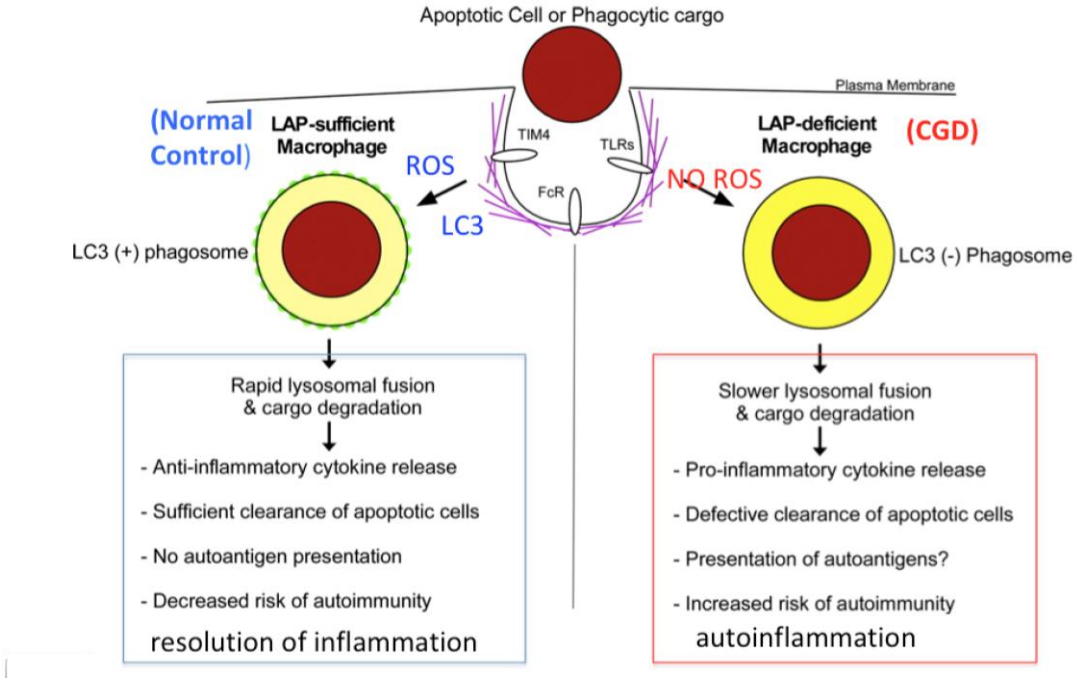


Fig.3 LC3-associated phagocytosis and inflammation:
Consequences of defective clearance of dying cells
in CGD (adopted from Heckmann, J Mol Biol 2017 (7)

In CGD patients apoptosis of neutrophils (8) and their clearance by macrophages are delayed (9). Defective clearance of dying cells leads to excess generation of pro-inflammatory cytokines (e.g. IL1 beta and TNF alpha) and autoinflammation (fig.3) (7). In CGD patients defective efferocytosis of human monocytes can be restored during experimental short term treatment with pioglitazone (PIO), a licensed antidiabetic drug mediating resolution of inflammation. This treatment is accompanied by enhanced phagocyte mitochondrial ROS production bypassing the NADPH oxidase defect (10). Since PIO also restored the bactericidal capacity in murine CGD (11) it was used in an infant with CGD to overcome refractory bacterial lung abscesses before succesful HSCT (12). Efficacy and safety of this „bridging effect“ require further exploration of PIO in therapy-refractory CGD patients with an indication for HSCT.

3. Clinical Presentation of CGD

CGD is characterized both by serious infections and hyperinflammatory manifestations. During the course of CGD the first infection occurs much earlier than the first inflammatory manifestation (at median ages of o.9 vs 11.3 years) (13). While infection remains a concern in adults as primary cause
of death, inflammatory events emerge and predominate during adolescence and adulthood.

3.1. Bacterial and Fungal Infections

In N. America (14) and Europe (15) infections in CGD arise from a limited number of five organisms: *Staphylococcus aureus* (lymphadenitis,

liver abscess osteomyelitis, pneumonia, sepsis), *Burkholderia cepacia* (necrotizing pneumonia, sepsis), *Serratia marcescens* (sepsis, osteomyelitis), *Nocardia* and *Aspergillus* spp (subacute pneumonia, dissemination to brain and bone).
Invasive fungal infections are acquired through inhalation of spores resulting in subacute pneumonia that can spread to ribs, spine and brain. *Asp.fumigatus* is the most frequent cause, while *Asp.nidulans* causes more inflammatory and refractory disease (20). Mortality is reduced by treating with azoles (voriconazole or posaconazole).

Several emerging pathogens endemic in other regions of the world have to be added to this list and may infect also travelling CGD patients from Western countries:

- TB constitutes a high risk for children with CGD resulting in a complicated clinical course (16). Routine *BCG* vaccination in CGD causes severe disease, e.g. draining skin lesions at the vacination site, regional lymphadenitis („BCGitis“) and more rarely disseminated disease (BCG sepsis) (17).
- *Burkholderia pseudomallei* , a water/soil bacterium causing systemic melioidosis has been identified in CGD children of South East Asia. They acquire the infection during work in rice-fields via skin wounds, inhalation or drowing (18).
- *Leishmania infantum*, an intracellular protozoon endemic on the Mediterranian coast, has caused visceral leishmaniasis-associated hemophagocytic syndrome in Portuguese and Spanish CGD patients (19

3.2. Inflammatory Manifestations

The classic inflammatory complications of CGD are most prominent in the pulmonary, gastrointestinal and urinary tracts (e.g. as *interstitial lung disease*, granulomatous colitis and *granulomatous cystitis*).
Granulomatous colitis in CGD mimicks Crohn`s disease and affects up to one half of CGD patients (24). Colonoscopy permits diagnostic biopsies revealing epitheloid granulomas and pigment-laden macrophages. Empiric initial therapy is based on corticosteroids.

To this list two emerging complications have to be added:

- *Fulminant mulch pneumonitis*, an emergency caused by massive inhalation of aerosols from mulch, compost, dead leaves containing *Aspergillus* spores. The excessive inflammatory response to the spores results in miliary infiltrates and hypoxia requiring ventilation. An iv combination of voriconazole and methylprednisolone is life-saving (21).
- *Non-cirrhotic portal hypertension* following liver abscesses: Obliteration of central/portal veins and nodular regenerative hyperplasia can ultimately lead to portal hypertension (22). Liver abscesses, mostly by *Staph.aureus*, are now treated by antibiotics in combination with steroids avoiding surgical excision (23), hopefully reducing the portal hypertension rate

Surgical sites in CGD often become infected and heal very slowly *with fistulas*. Sutures should not be removed early and drains be left for a prolonged

period. Excessive wound granulation with dehiscence responds to steroids.

3.3. Clinical Presentation in Adults

In the first nation-wide retrospective study, focusing on the long-term outcome of the French pediatric CGD cohort of 80 patients, the grown-up CGD patients displayed similar rates and characteristics of severe infections and inflammatory episodes as in childhood (13). Main sequelae of pediatric CGD observed in adulthood were

1. *growth failure* as consequence of serious infections and inflammation and of repeated steroid treatments.
2. *chronic dyspnea* from restrictive respiratory failure after repeated lung infections.
3. c*hronic digestive complications* from episodes of inflammatory colitis, bowel stenosis and perianal fistulas with substantial impact on quality of life.

The social consequences of the above complications and repeated hospital admissions were serious with poor educational achievement in half of the patients (13).

Proper surveillance and follow up are required for adult CGD patients especially during the crucial transition from pediatric to adult medical departments.
Regular follow up should be performed in order to detect and properly manage occult infections, effectively treat inflammatory events and prevent long-term complications (25).

4.Conventional Treatment of CGD

Clinical management of CGD has been reviewed before(26,27).

4.1. Antimicrobial Prophylaxis

The cornerstone of clinical care is mainly based on retrospective studies and consists of lifelong antibacterial and antifungal prophylaxis with intracellularly active microbicidal agents. Lipophilic *co-trimoxazole (=TMP/SMX)* results in a marked reduction of serious bacterial infections and abscess drainages (28).
It is recommended at 5mg/kg/day TMP up to 160 mg daily.

For antifungal prophylaxis the lipophilic *itraconazole* is the drug of choice with high activity against *Aspergillus spp*. (29). Itraconazole is recommended up to a maximum of 200 mg once daily. For optimal bioavailability, itraconazole capsules should be taken at 5 mg/kg/day with food, while itraconazole solution should be taken at 2.5 mg/kg/day in fasting condition.

In addition to the above antimicrobials *interferon gamma (IFNg)* is part of the routine prophylaxis regimen in US centers, while most European experts use IFNg only in selected cases. The dose is 50 ug/m2 sc 3x/week. This prophylactic regimen is based on a prospective multicenter randomized placebo-controlled trial of IFNg in 128 classic CGD patients performed before the advent of antifungal prophylaxis with itraconazole. The trial resulted in reduction of the frequency of mainly severe bacterial infections >70% (30). There were no improvements in NADPH oxidase function nor a significant efficacy in preventing *Aspergillus* infections during the limited study period. A later prospective controlled non-randomized longterm (lasting 5 years) Italian multicenter study of 35 CGD patients , comparing treatment with TMP/SMX

and itraconazole alone versus addition of IFNg, showed no difference in the rates of severe infection (31). The exact mechanism of how IFNg exerts its effect in CGD is still unknown adding to the debate over its utility. Since IFNg upregulates HLA expression, IFNg prophylaxis has to be stopped at least 4 weeks before HSCT.

Immunization with live bacterial vaccines (BCG and live Salmonella typhi vaccines) is contraindicated in CGD. Other routine vaccines incl annual influenza vaccination can be given safely.

4.2. Treatment of Serious Infections

Significant rises in CRP should prompt evaluation for infection, incl. Appropriate imaging and pathogen identification. CT or MRI imaging should be followed till resolution of infections. A definitive microbiological diagnosis by tissue biopsies is essential for proper treatment. An appropriate sample may require fine needle aspiration or percutaneous drainage of liquid pus.

Recommended empiric initial therapy is based on limited clinical data because of the rarity of CGD. Consultation with an experienced ID specialist is strongly advised. Initial therapy is tailored once culture/ susceptibility and histopathology are known. Longer courses of antimicrobials are needed for adequate therapy.
Consider addition of steroids in case of fulminant Aspergillosis or severe Nocardia infection not responsive to appropriate antibiotic therapy.

4.2. Treatment of Inflammatory Complications

Antimicrobial prophylaxis decreases the risk of severe infections, but not the risk of inflammatory manifestations which can affect >50% of patients (especially those with XR-CGD). Management of inflammatory complications is challenging, as treatment with antiinflammatory/ immunosuppressive agents increase the risk of infection. Treatment of inflammatory manifestations in CGD has been reviewed before (32).

Short courses of *corticosteroids* followed by gradual tapering are required in acute granulomatous exacerbations of the bowel (e.g. gastric outlet obstruction), the urinary tract (e.g. ureteral obstruction) and the lung (inhalative acute miliary pneumonia). Long-term treatment of granulomatous colitis follows treatment options for Crohn`s disease under the cover of the routine antimicrobial prophylaxis in CGD. First-line therapy in severe CGD-colitis cases is prednisone (1mg/kg/day) for 1-2 weeks with slow tapering over 1-2 months to o.1-o.25 mg/kg/day (26). Steroids inhibit granuloma formation by suppressing proinflammatory cytokine production and TNFa-dependent fusion of macrophages into multinucleated giant cells (33). Steroid dosage during taper can be adjusted to the severity of colonic inflammation by following fecal levels of calprotectin released from apoptotic neutrophils.

Steroids are effective in severe CGD-colitis, but when taken off treatment relapse is high. Some patients become steroid-dependent requiring second line therapies (incl. immunosuppressants and biologic agents). Patients with refractory colitis

necessitate a multistep approach and may become eligible for allo-HSCT.

Azathioprine, an immunosuppressant, is used as second line therapy to maintain remission in steroid-dependent cases and to improve outcomes in patients with fistulating disease.

Thalidomide blocks nuclear localization of Nf-KB inhibiting the production of inflammatory cytokines. It was used by the Paris group for treatment of refractory colitis with complete clinical responses in4/6 patients after 6 months (34) and was not associated with increased risk of infections.

Infliximab, a monoclonal antibody to TNF alpha, has been used by the NIH group in five CGD patients with steroid-refractory colitis. It is effective, but resulted in severe intercurrent infections with CGD pathogens in all five and death in two, precluding long-term therapy in CGD (35).

Use of *Anakinra*, an IL1-receptor antagonist, has also been reported in CGD patients with refractory colitis. Its efficacy has been contested by the NIH group, when treatment of five CGD patients with severe colitis led only to marginal or no benefit (36).

Allo-HSCT is the only curative treatment for refractory colitis in CGD. HSCT can save patients from extensive colon surgery, risky because of poor wound healing, anastomosis complications and fistula formation. Inflammatory manifestations rapidly regress postHSCT permitting withdrawel of steroids with a subsequent growth spurt into predetermined percentile channels in children (37).

5.Curative Treatments

The above prophylactic and routine therapeutic approaches are mostly supportive and depend on lifelong patient compliance. CGD thus remains a lethal disease, nowadays at an adult age. The ultimate goal is to develop safe curative approaches, e.g. allo-HSCT regimens with reduced-intensity conditioning (RIC) and autologous stem cell gene-therapies (GT) for CGD patients without a suitable HLA-compatible donor.

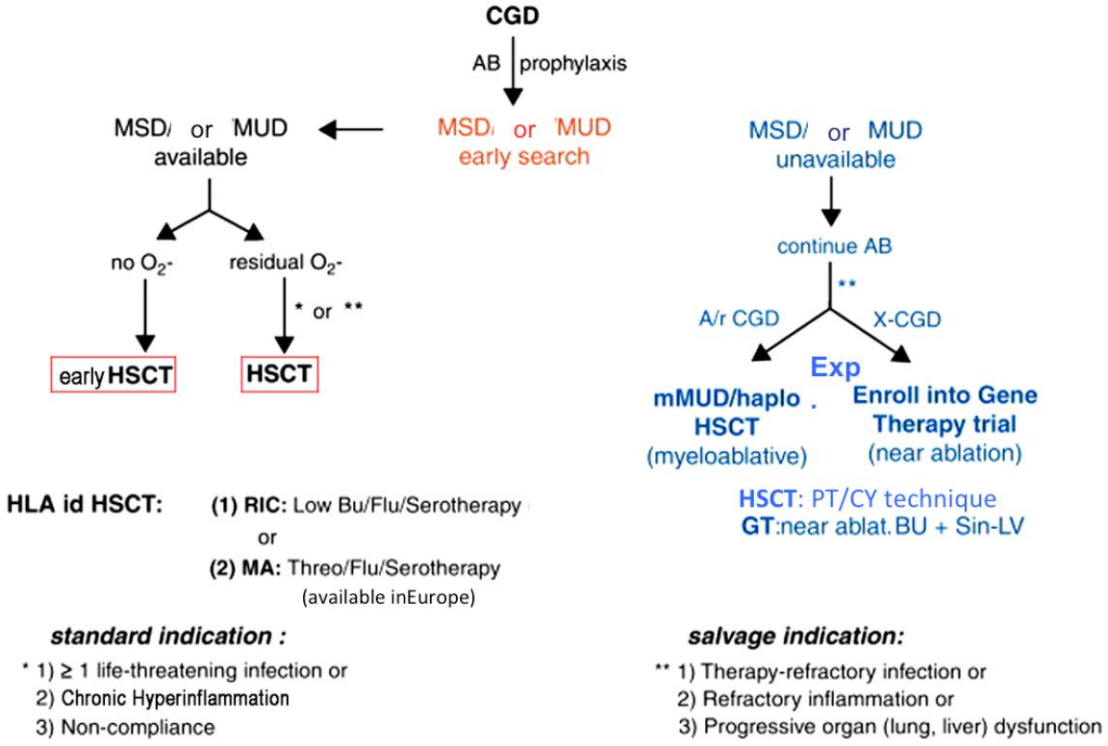
5.1. Allogeneic HSCT

CGD is a proinflammatory disease with a high risk of alloreactivity (rejection and GvHD) after myeloablative cytotoxic marrow conditioning.
Therefore antithymocyte globulin in HLA-matched sibling transplantation and alemtuzumab in matched unrelated (MUD) transplantation were introduced for in vivo depletion of alloreactive recipient and donor T-cells.
In addition the toxic myeloablative conditioning was replaced by a submyeloablative reduced intensity conditioning (RIC) regimen to decrease tissue damage and reduce release of proinflammatory cytokines. These two measures allowed safe transplantation even in high-risk CGD patients with ongoing infection/ inflammation and in younger adults. Preexisting infections and chronic inflammatory lesions cleared in all engrafted survivors. Even children with severe lung restriction improved their lung function slowly, normalizing decreased oxygen saturation, reversing clubbed fingers and toes and manifesting a growth spurt (37).

To further optimize the RIC protocol targeted drug monitoring (TDM) was introduced. Low-dose busulfan was administered, serum busulfan levels were measured, and the cumulative area-under-the-concentration curve was individually adjusted real-time to a submyeloablative target range of 45-65 mg/lxh. An international prospective CGD/HSCT trial using this Zürich RIC protocol was then performed at 16 centers in 10 countries (38). 56 CGD patients aged 0-40 years were enrolled of whom 42 patients had high-risk features and 25 were adolescents or young adults. 2-year probability of overall survival (OS) was 96% and of event-free survival (EFS) 91%. Equivalent outcomes between matched siblings and MUDs were observed. Incidence of GvHD and of graft failure was low. Excellent myeloid donor chimerism (>90%) was documented in 93% of surviving patients.

Because of its efficacy and favorable toxicity the low busulfan RIC regimen is quite promising, but requires good lab facilities for real-time TDM. Alternatively a reduced toxicity conditioning (RTC) regimen based on myeloablative treosulfan (available in Europe) was used by the Newcastle group and has achieved good survival rates in a retrospective CGD study (39). Secondary graft failure in 12% of the 70 patients requires further investigation on durability of myeloid chimerism.

The decision for or against HSCT should be made early in life. Patients with gp91phox mutations and absent O2- production have a survival of only 50% beyond 40 years of age compared to patients with p47phox mutations and residual o2- generation (over 80% beyond 40 years) (1). Our algorithm based on O2- production and the individual clinical course reflects the present consensus for patient selection and HSCT timing in CGD (fig. 4)



Two recent reports from Sweden (40) and UK (41) comparing HSCT versus conventional treatment support HSCT as being the preferable treatment for severe CGD. Children not undergoing transplantation have more serious infections, surgical interventions, hospital admissions and less height for age compared with postHSCT children. Very long-term postHSCT survival and quality of life data in adult CGD are not yet available.

5.2. Therapies under Investigation

5.2.1. HLA-haploidentical HSCT

Transplants with haploidentical donors present greater risk of graft rejection and GvHD. The present technique of global donor T-cell depletion from the graft to avoid severe GvHD results in 6-9 months of profound T-cell deficiency. A new protocol of selective deletion of alloreactive T-cells in vivo on days +3/+4 postHSCT by high-dose cyclophosphamide (at 50 mg/kg/day) presents a promising alternative (42). Cyclophosphamide (Cy) is non-toxic to stem cells and kills rapidly deviding alloreactive T-cells post transplantation (PT), while sparing resting T-cells with specificity for infectious agents.
PT/Cy controls GvHD without affecting engraftment and allows more rapid reconstitution of donor immunity to opportunistic infections. This promising new technique has been successfully applied in a few CGD patients (43,44) and should now be investigated in a prospective multicenter study. The easy logistics of PT/Cy are an asset in countries with no access to an unrelated donor registry and lacking a sophisticated laboratory for graft manipulations required for conventional T-cell depletion of haploidentical grafts.

5.2.2. Stem Cell Gene Therapy

CGD remains an attractive though difficult target for autologous stem cell gene therapy (GT). The major obstacle is the lack of selective growth advantage of gene-transduced stem cells. Previous protocols have either resulted in graft failure or development of a myelodysplastic syndrome due to transactivation of the MDS1/EVI1 protooncogene by gammaretroviral insertions (45). Early data of an ongoing prospective clinical trial in XR-XGD (based on a new self-inactivating lentiviral vector (46)) indicate a safer integration profile with no evidence of mutagenesis and stable activity >20% in the first treated CGD patients (47). Taken the advances in GT and HSCT together, one can be cautiously optimistic about important advances in the next 5-10 years on the long road to optimal management for CGD.

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