bjh review

Modern management of chronic granulomatous disease

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Summary

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disorder of phagocytic cells resulting in failure to kill a characteristic spectrum of bacteria and fungi and in defective degradation of inflammatory mediators with concomitant granuloma formation. Current prophylaxis with trimethoprim-sulfamethoxazole, itraconazole and in selected cases additional interferon gamma is efficient, but imperfect. A significant recent progress towards new antibiotic (e.g. linezolid) and antifungal (e.g. voriconazole and posaconazole) therapy will allow survival of most patients into adulthood. Adolescent and adult CGD is increasingly characterized by inflammatory complications, such as granulomatous lung and inflammatory bowel disease, requiring immunosupressive therapy. Allogeneic haematopoietic stem cell transplantation from a human leucocyte antigen identical donor is currently the only proven curative treatment for CGD and can be offered to the selected patients. Gene-replacement therapy for patients lacking a suitable stem cell donor is still experimental and faces major obstacles and risks. However, it may offer some transitory benefits and has helped in a few cases to overcome life-threatening infections.

Keywords: antifungal agents, interferon gamma, corticosteroids, stem cell transplantation, gene therapy.

Aetiology and pathogenesis of the disease

Chronic granulomatous disease (CGD) is an inherited immunodeficiency disorder which results from the *absence or malfunction of NADPH oxidase subunits* in phagocytic cells, e.g. in neutrophils, monocytes, macrophages and eosinophils. This oxidase is directly responsible for production of superoxide (the so-called respiratory burst), converted into microbicidal reactive oxygen species (such as hydrogen peroxide, hydroxylanion and hypochlorous acid), and indirectly for liberation and activation of complementary microbicidal azurophil granule proteases (cathepsin G and elastase) (Fig 1) (Reeves *et al*, 2002; Rada *et al*, 2004) as well as microbicidal neutrophil extracellular traps (Fuchs *et al*, 2007). NADPH oxidase deficiency renders the patient susceptible to *recurrent life-threatening infections by a spectrum of bacteria and fungi* (see *Infections*). Microorganisms are phagocytosed normally, but persist within cells, which form a barrier to antibodies and extracellularly acting antibiotics. The resulting infectious foci stimulate *granuloma formation*, partly through release and persistence of chemoattractants, which require oxygen metabolites for their degradation (Clark & Klebanoff, 1979; Hamasaki *et al*, 1989). Chronic granulomatous inflammation may compromise vital organs and account for additional morbidity. CGD affects between 1/200 000 and 1/250 000 live-births (Winkelstein *et al*, 2000), although the real incidence might be higher as a result of the underdiagnosis of milder phenotypes.

The NADPH oxidase is a multicomponent system (Roos *et al*, 2003), including a *membrane-bound flavocytochrome b558* comprised of a large subunit, gp91phox, and a small subunit, p22phox (phox, phagocyte oxidase). Phagocytosis of microorganisms leads to translocation of *four cytosolic factors* (p47, p67, p40phox and Rac 2) to the cell membrane to form the activated NADPH oxidase complex, which then binds NADPH and generates the respiratory burst (Fig 1). Defects in the genes that encode any of the NADPH oxidase components may abolish the electron transport from cytoplasmic NADPH to FAD, haem and on to intraphagosomal molecular oxygen. CGD is therefore a genetically heterogeneous disease. About 60% of CGD cases are because of mutations in the gene encoding gp91phox residing at Xp21.1 (*CYBB*). About 30% of patients have autosomal recessive (a/r) CGD because of lack of the cytosolic p47phox protein. Defects in another cytosolic factor, p67phox, and in the membrane-associated p22phox account for the remaining a/r CGD patients described to date (Roos *et al*, 2003). Mouse-knockout models with a phenotype resembling human CGD have been created for gp91phox and p47phox (Jackson *et al*, 1995; Pollock *et al*, 1995).

The functional diagnosis of CGD is based on demonstration of a defective respiratory burst. The quantitative *dihydrorhodamine 123 flow cytometry assay* is today's most accurate diagnostic test for CGD (Vowells *et al*, 1996), although the qualitative (microscopical) and less discriminant

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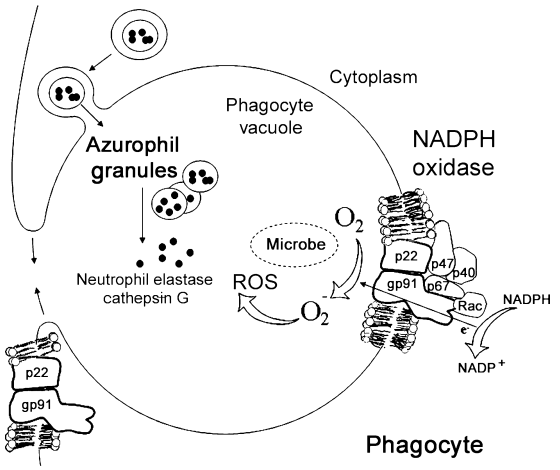

Fig 1. Phagosome formation and oxidative killing of microbes by phagocytic cells.

nitroblue tetrazolium dye test is still in clinical use. *Genotype testing* of patients by immunoblotting or direct gene sequencing is possible in research laboratories. Genotyping is not necessary for routine medical management, except for genetic counselling and prenatal diagnosis or gene therapy studies.

This review addresses recent progress in supportive and curative treatments for CGD, delineates several areas of controversy and points to future therapeutical developments. The last comprehensive reviews on clinical care date from 2000 (Segal *et al*, 2000) and 2002 respectively (Goldblatt, 2002). Present treatment modalities are summarized in Table I.

Clinical manifestations

Infections

Chronic granulomatous disease patients suffer from severe recurrent bacterial and fungal infections of body surfaces, e.g. the skin, the airways and the gut, as well as in the draining lymph nodes. Following contiguous and haematogenous spread, a wide range of internal organs can be affected, e.g. the liver and the bones. The major clinical manifestations of CGD are therefore pyoderma, pneumonia, inflammation of the gastrointestinal tract, lymphadenitis, liver abscess and osteomyelitis (Winkelstein *et al*, 2000). A high level of vigilance is necessary in searching for these infections. Infections are indolent and both suppurative and granulomatous. Their clinical severity can be underestimated by the non-specialist.

Infections at the portals of entry Lungs: The five main groups of organisms responsible for the pneumonias of CGD comprise *Aspergillus* spp., including the particularly virulent *A. nidulans* (Segal *et al*, 1998), other fungi, *Burkholderia* spp. (Speert *et al*, 1994), other Gram-negative bacteria, *S. aureus* and *Nocardia* spp. (Dorman *et al*, 2002) and, prevalent in developing countries, *Mycobacteria* spp., tuberculous or non-tuberculous (Movahedi *et al*, 2004; Bustamante *et al*, 2007). *Focal invasive fungal pneumonias* are insidious in onset (with malaise and chronic cough), but have the highest mortality: local extension from the lungs to the pleura and the bones of the chest wall occurs in one-third of the patients (Cohen *et al*, 1981). Fever and/or neutrophilia is more common in *inhalation-related acute miliary fungal pneumonias* (Siddiqui *et al*, 2007) as well as in *Burkholderia* and *Nocardia infections*. C-reactive protein

Table I. CGD: main treatment modalities.

Modality	Indication	Duration	Drug	Paediatric dosage
Antibiotic prophylaxis	Bacterial infections	Lifelong	Trimethoprim-sulfamethoxazole	6 + 30 mg/kg/d
	Fungal infections	Lifelong	Itraconazole	5 mg/kg/d*
Empiric antibiotic treatment	Gram$^+$ infections	Until pathogen ident.	Teicoplanin	10 mg/kg/d
	Gram$^-$ infections	Until pathogen ident.	Ciprofloxacin	15 mg/kg/d
	Fungal infections	Until pathogen ident.	Voriconazole	14 mg/kg/d
Interferon γ prophylaxis	Recurrent infections	Lifelong	γ-Interferon	3×50 μg/m^2 /week (s.c.)
White cell transfusions	Severe refractory infections	Until recovery or antibody formation	G-CSF stimulated leucocytes	10 μg/kg (s.c.) 12 h before leukapheresis
Antiinflammatory treatment	Obstructing granuloma	7–10 d → taper	Prednisolone	0·5–1 mg/kg/d
Stem cell transplantation	Recurrent serious manifestations (see Table III)	LAF-isolation *c.*2 months, isolation at home *c.*6–9 months	HLA identical marrow transplant	$>2 \times 10^6$/kg CD34$^+$ cells

*Oral solution.

ident., identification; LAF, laminar air flow; G-CSF, granulocyte colony-stimulating factor; HLA, human leucocyte antigen; CGD, chronic granulomatous disease; s.c., subcutaneously.

and erythrocyte sedimentation rate are useful parameters to assess infection and treatment responses. As clinical and radiological [X-ray, computed tomography (CT) and positron emission tomography (PET)-scan] findings are often unspecific, and rare organisms and mixed infections a possibility, *a microbiological diagnosis* should be vigorously pursued. This requires bronchoalveolar lavage or, with higher diagnostic yield, transthoracic needle aspiration under CT guidance. Diagnosis of pneumonia caused by fungi or *Nocardia* spp. necessitates also exclusion of their dissemination, e.g. of bone metastasis and silent brain abscess, by bone and central nervous system (CNS)-CT scans.

Infections at internal sites Lymphnodes: Cervical lymphnodes are frequently infected. Spontaneous rupture or drainage of the abscesses may lead to fistula formation. Together with granulomas on histology this can result in the erroneous diagnosis of tuberculosis. Lymphadenitis is mostly caused by *S. aureus* and by *Gram-negative bacteria*, including a newly identified, Ceftriaxone sensitive pathogen, *Granulobacter bethesdensis* (Greenberg *et al*, 2006), causing a 'culture negative' necrotizing lymphadenitis.

Liver: Liver abscesses are again difficult to diagnose clinically, as a violent inflammatory reaction is absent. If suspected (e.g. by unexplained fever, malaise and weight loss) the diagnosis is best made by CT scans (Garcia-Eulate *et al*, 2006). Needle biopsy may be used for microorganism isolation (mostly *S. aureus*) and susceptibility testing.

Bone: Osteomyelitis may involve the small bones of the hands and feet and affect multiple sites. Aspiration of pus is mandatory for microorganism isolation (frequently encountered organisms are *Serratia marcescens, Aspergillus* spp. and *S. aureus*).

Septicaemia: Although localized infections are the rule in CGD, patients may also develop septicaemia, the most common causes being *Salmonella* spp. and other Gram-negative bacteria (e.g. *Burkholderia cepacia* and *Serratia marcescens)* and *S. aureus. B. cepacia* can typically manifest as necrotizing pneumonia with septicaemia resulting in rapid decline of the clinical status and, occasionally, death (Speert *et al*, 1994).

Inflammation

Another important manifestation of CGD is an enhanced and persistent inflammatory response, reflected by *hypergammaglobulinaemia* and *anaemia* (in the 8–10 g/l Hb range). Persistent inflammation at drainage sites and surgical wounds may lead to dehiscence. Granuloma formation can also result in occlusion of hollow viscera, e.g. the upper gastrointestinal or the urinary tract. In the stomach granulomas can cause *gastric outlet obstruction* with persistent vomiting (Danziger *et al*, 1993). In the urinary tract the commonest manifestation is *inflammatory cystitis* (Collman & Dickerman, 1990). Granulomas in the bladder wall can lead to obstruction of the urethral and ureteric orifices and subsequently cause *hydronephrosis* (Korman *et al*, 1990). About 20% of patients are affected by *granulomatous colitis* mimicking Crohn's disease (Marciano *et al*, 2004). Persistent inflammation in both CGD patients and mouse models of CGD can occur independently of infection (Morgenstern *et al*, 1997), so that inflammatory sites are frequently sterile. One possible explanation for the apparent failure to resolve inflammation, is the inability of CGD phagocytes to degrade chemotactic factors (Clark & Klebanoff, 1979; Hamasaki *et al*, 1989).

Prevention of infection

General health care

Common sense measures in reducing exposure to potentially infectious agents are sometimes neglected and have to be instructed to patients and parents. Very useful information and fact sheets can be downloaded from http://www.cgd.org.uk. CGD patients should receive all routine immunizations (including measles and varicella live vaccines as well as yearly influenza vaccine to prevent potentially lethal bacterial superinfections). Avoidance of Bacille Calmette-Guérin (BCG) vaccination is advocated because of risk of local BCGitis or rarely disseminated BCG-osis (Bustamante *et al*, 2007). Wounds should be washed well and rinsed with antiseptic solutions (e.g. 2% H_2O_2 or Betadine). Professional dental cleaning, flossing and antibacterial mouth washes can help prevent gingivitis. Extensive dental work and surgery, associated with bacteraemia, should be covered with additional antibiotics e.g. amoxicillin/clavulanic acid. Pulmonary infections can be prevented by refraining from smoking, not using bedside humidifiers and avoiding sources of *Aspergillus* spores (e.g. animal stables, hay, mulch, rotten plants, compost piles, wood chips and construction sites). The risk of perirectal abscesses can be diminished by avoiding constipation and rectal manipulations, e.g. suppositories or taking rectal temperature.

Outpatient visits are used to emphasize the importance of continuous exposure and antimicrobial prophylaxis as well as the early recognition of potentially serious infections despite a paucity of symptoms (Roesler *et al*, 2005). In the latter case an elevated C-reactive protein is often present. In the case of fever or persistent cough, a liver abscess, *Salmonella* and *Burkholderia* septicemia as well as the two types of *Aspergillus* pneumonia (inhalational miliary and focal invasive) have to be excluded first. If no infectious focus is found, a combined PET/CT scan can be very helpful to localize occult infections (Gungor *et al*, 2001).

Antibiotic prophylaxis

The cornerstone of clinical care is lifelong antibiotic and antifungal prophylaxis with intracellularly active microbicidal agents. The most commonly used antibiotic is the lipophilic *trimethoprim/sulfamethoxazole (co-trimoxazole)*, which has

a broad activity against Gram-negative bacteria (including *Serratia marcescens* and *Burkholderia* spp.) and *Staphylococci* and is concentrated inside host cells (Gmunder & Seger, 1981). It is well tolerated and very rarely leads to overgrowth of resistant pathogens, probably because it leaves the non-pathogenic anaerobic gut flora intact, which prevents colonization by resistant strains (van der Waaij *et al*, 1972). No randomized, controlled trial has been performed, but several retrospective studies justify long-term Co-trimoxazole prophylaxis of all CGD patients (Weening *et al*, 1983; Mouy *et al*, 1989; Margolis *et al*, 1990). A marked reduction of serious bacterial infections and surgical interventions (namely abscess drainages) and consequently a large reduction in the number of hospitalization days were observed. Benefits were seen both in X-linked and in a/r CGD. The recommended dosage for bacterial prophylaxis is 6 mg/kg/d of trimethoprim and 30 mg/kg/d of sulfamethoxazole in two divided doses. In case of sulphonamide allergy *ciprofloxacine* or an extended-spectrum oral cephalosporin are suitable alternatives.

Antimycotic prophylaxis

After introduction of antibacterial prophylaxis, fungal infections persisted with an incidence of 0·15 episodes per patient year (Winkelstein *et al*, 2000). For antifungal prophylaxis the lipophilic *itraconazole* is the drug of choice, displaying high activity against *Aspergillus* spp. The molecule is taken-up by neutrophils and exerts intracellular activity (Perfect *et al*, 1993). In an open-label study on itraconazole prophylaxis in 30 CGD-patients the rate of *Aspergillus* infections could be reduced to one-third in comparison with historical controls (Mouy *et al*, 1994). A randomized, double-blind, placebo-controlled crossover study has recently confirmed these observations (Gallin *et al*, 2003). In 39 enrolled CGD patients one serious fungal infection occurred in the itraconazole group compared with seven cases in the placebo recipients. Both studies support routine itraconazole prophylaxis of all CGD-patients.

The erratic absorption of the capsule form has been overcome with the introduction of a liquid formulation in cyclodextrin, which does not require the concomitant intake of food, and is not affected by reduced gastric acidity. A steady-state plasma level is reached after 2 weeks of itraconazole oral solution at a single daily dose of 5 mg/kg (de Repentigny *et al*, 1998). The oral solution is generally well tolerated and safe. Future developments in antifungal prophylaxis include a powder formulation of amphotericin B delivered via an inhaler directly to the lungs once weekly.

Interferon-gamma prophylaxis

Interferon-gamma (IFNγ) is a macrophage-activating cytokine produced by T cells and natural killer cells. A subgroup of *variant* X-CGD patients, who have splice site mutations, have been shown to be responsive to IFNγ (Condino-Neto & Newburger, 2000; Ishibashi *et al*, 2001). Treatment for 2 d with 100 μg/m^2 IFNγ s.c. improved splicing efficiency, so that a small amount of normal gp91phox transcript was generated and exported from the nucleus. This resulted in an increase in cytochrome b expression, allowing near normal levels of O_2^- production and bactericidal activity of neutrophils and monocytes (Ezekowitz *et al*, 1988). The improvement in phagocyte function peaked at 2 weeks and was sustained for 4–6 weeks (Ezekowitz *et al*, 1990), indicating that IFNγ acted at the level of myeloid progenitor cells.

Based on these important findings a multicenter, transatlantic, randomized, double-blind, placebo-controlled phase III study was conducted to evaluate efficacy and potential toxicity of IFNγ in infection prophylaxis in 128 patients with *classical CGD* (The International Chronic Granulomatous Disease Cooperative Study Group, 1991). While the study demonstrated significant efficacy in the IFNγ arm with a reduction in the frequency of severe infections of >70%, regardless of age and inheritance of CGD, several confounding issues arose. The clinical improvements, stably maintained in patients treated for longer time-periods in two phase IV studies (Bemiller *et al*, 1995; Weening *et al*, 1995), were not accompanied by improvements in NADPH oxidase function (Muhlebach *et al*, 1992; Woodman *et al*, 1992). A significant efficacy in preventing *Aspergillus* infections could not be demonstrated during the study period. Finally the benefit of IFNγ for relatively 'healthy' *versus* 'chronically ill' CGD patients had not been addressed separately. In addition, the drug is expensive, requires repeated injections (3 × 50 μg/m^2/week s.c.) and has some side effects (mainly headaches and fever within a few hours after administration). Thus controversy remains about its routine administration in CGD. IFNγ prophylaxis is offered only in selected CGD cases by most European physicians, while it is rather universally prescribed in the USA.

The therapeutic use of IFNγ after the onset of infection, when natural IFNγ levels are already elevated, has not been investigated by controlled studies and remains controversial. Some experts suggest that ongoing IFNγ prophylaxis should be interrupted in periods of high fever, or in the perioperative period of major surgery to avoid side effects. As IFNγ upregulates human leucocyte antigen (HLA)-expression, IFNγ prophylaxis has to be stopped at least 4 weeks before haemopoietic stem cell transplantation.

Treatment of acute infections

Antibiotic therapy

The cornerstone of the treatment of acute infections in CGD patients is prompt and prolonged therapy, with the appropriate parenteral antimicrobials aiming at eradication of the causative organism(s). Before culture results are available, initial antibiotic therapy has to be based on the most likely infectious agents expected. Antibiotics chosen should cover a broad spectrum of Gram-negative bacteria including

Burkholderia spp., *S. aureus* and *Nocardia* spp. *Ciprofloxacin* is one of the useful first-line agents with an appropriate antimicrobial spectrum. A course of oral ciprofloxacin may also be taken along as reserve on journeys or holidays. Being lipophilic, it is concentrated within neutrophils and reduces *in vitro* the survival of *Serratia marcescens* (Canton *et al*, 1999) and of intracellular *S. aureus* (Peman *et al*, 1994). Additional antistaphylococcal cover is provided by combining *Ciprofloxacin* with *Teicoplanin*. Teicoplanin is avidly concentrated into neutrophils and has good intracellular activity against *S. aureus* (Carlone *et al*, 1989). In case of failure to respond within 24–48 h empirical changes in antibiotic coverage may be needed before definitive pathogen identification, including the administration of an antimycotic drug, e.g. Voriconazole, if not administered from the very beginning.

As infections often respond slowly, intravenous antibiotic treatment must be followed by prolonged oral treatment sometimes continued over months. Therapy must be extended further, if serum indicators of inflammation (e.g. C-reactive protein) suggest ongoing infection, or if special organisms are isolated (e.g. *Aspergillus* spp. and *Nocardia* spp.). A novel antibiotic, *Linezolid*, has proven effective as a second-line drug in Nocardiosis with excellent penetration of the cerebrospinal fluid after i.v. administration every 12 h and 100% oral bioavailability (Moylett *et al*, 2003).

Antifungal therapy

In the past, prior to the advent of the new azoles, prolonged and repeated treatments of fungal infections with the conventional nephrotoxic amphotericin B has led to progressive renal insufficiency in some CGD patients. *Renal transplantation* had to be performed in three patients, has had a successful long-term outcome and was combined with a haemopoietic stem cell transplant from the same or a different donor in two of them (Bolanowski *et al*, 2006).

In the last few years there have been important new developments in antifugal therapy with promise of improved cure rates for invasive infections. The second generation azole, *voriconazole*, was shown to be superior to conventional amphotericin B as initial treatment for invasive aspergillosis in an open, randomized study, with a rate of successful outcome of 53% vs. 32% (Herbrecht *et al*, 2002). In a compassionate use study voriconazole appeared to be safe and efficient in children with aspergillosis or scedosporidiosis: Of 13 CGD patients, eight (62%) had a successful outcome. Response rate in the difficult-to-treat CNS fungal infections was as high as 55% (Walsh *et al*, 2002).

Based on these data voriconazole is recommended as new standard of care for invasive aspergillosis (including amphotericin B resistant *Asp. terreus* infections) and for many *Scedosporidium* infections in CGD. In patients with renal failure the intravenous Voriconazole formulation should be used with caution, because of accumulation of the nephrotoxic cyclodextrin vehicle (Johnson & Kauffman, 2003). Oral formulation can be used instead, has excellent bioavailability and is cheaper than the intravenous one.

Posaconazole has proven efficacy as salvage therapy against a broad spectrum of invasive fungal infections. In a pilot study of eight CGD patients with fungal infections refractory to voriconazole, posaconazole was safe and efficient (Segal *et al*, 2005). *Echinocandins* (e.g. Caspofungin) have not yet been evaluated as initial therapy for invasive aspergillosis in clinical trials. Equally the benefit of combination antifungal therapy (e.g. of an echinocandin with an azole) has not been definitively assessed, so that recommendations for CGD patients cannot yet be made.

In addition to systemic antifungal treatment, *surgical debridement or excision of a dominant consolidated focal fungal infection* is advisable, especially when chest-wall structures and vertebrae are involved (Pogrebniak *et al*, 1993). Supportive therapy with white cell transfusions in case of therapy-refractory infections (e.g. caused by *Asp. nidulans*) is discussed below.

Fungal infections typically require prolonged treatment (e.g. for 4–6 months). Once the infection is in remission, patients should continue prophylaxis with oral itraconazole or voriconazole indefinitely to prevent recurrence or reactivation of infection.

Surgical interventions

Surgery still plays an important role in the management of CGD. Procedures in CGD comprise drainage of abscesses (e.g. in skin, lymph nodes and rectum wall), relief of obstruction (e.g. in hydronephrosis), and excision of consolidated suppurative and granulomatous lesions (e.g. in lung and liver). One has to remember that *operative sites* in CGD invariably become infected, heal very slowly and often form fistulas. Sutures therefore should not be removed early and drains be left in place for a prolonged period (Eckert *et al*, 1995).

Hydronephrosis secondary to ureteral granulomas may be successfully decompressed by percutaneous nephrostomy under ultrasound guidance until parenteral methylprednisolone therapy takes effect, obviating the need for more extensive surgery (Korman *et al*, 1990). Larger *liver abscesses* (e.g. >5 cm) require surgical excision and drainage in addition to a 1–2 months course of antibiotic therapy, as liver abscesses in CGD are not simply an encapsulated collection of pus, but rather a semisolid, multiloculated mass of microabscesses and granulomas (Garcia-Eulate *et al*, 2006), cure by percutaneous drainage alone is rare and the relapse rate is high (Lublin *et al*, 2002). In a few cases, when surgery was contraindicated, several experimental approaches have been tried successfully: Intralesional granulocyte instillation (Lekstrom-Himes *et al*, 1994), percutaneous transhepatic alcoholization (Alberti *et al*, 2002) and percutaneous radiofrequency thermal ablation as used for treatment of liver cancer (Haemmerich & Wood, 2006). Surgery may also be necessary for excision (e.g. by segmentectomy) of a *dominant consolidated focal lung infection* that cannot be eradicated by antimicrobial agents alone. Risks include bleeding,

bronchopleural fistula formation and pleural contamination with empyema. Postoperative management of these patients is demanding, requiring prolonged use of antibiotics and sometimes white cell transfusions (Pogrebniak *et al*, 1993).

White cell transfusions

White cell transfusions have been used in selected CGD patients for the treatment of life-threatening bacterial and fungal infections (von Planta *et al*, 1997). Their value, however, has not been evaluated in a prospective controlled trial, so that their clinical use remains somewhat controversial. Progress in the mobilization of neutrophils in healthy donors by administration of granulocyte colony-stimulating factor (G-CSF) has enhanced leukapheresis yields (Briones *et al*, 2003), neutrophil functions (Leavey *et al*, 2000) and survival time after transfusion (Ozsahin *et al*, 1998). *In vitro* a small proportion of normal neutrophils mixed with a large amount of CGD neutrophils synergizes in killing extracellular *Aspergillus* hyphae (Rex *et al*, 1990).

White cell transfusions are generally well tolerated, but adverse events include development of *leucoagglutinins* with rapid neutrophil consumption and, rarely, *pulmonary leucostasis* (Stroncek *et al*, 1996). The risk of *alloimmunization to HLA antigens* may complicate subsequent allogeneic stem cell transplantation. Erythrocyte antigen phenotyping should always be carried out before a CGD patient's first transfusion. A handful of X-CGD patients with a very rare deletion of the Xp21.1 region resulting in the absence of the K^x protein and other Kell antigens (*McLeod erythrocyte phenotype with acanthocytosis and haemolysis*) may become quickly sensitized to the Kell antigens of normal red cells (Brzica *et al*, 1977). With the advent of potent new antifungal drugs the use of white cell transfusions is likely to decrease in the near future.

Treatment of inflammatory complications

Chronic inflammatory bowel disease (e.g. colitis) and acute granulomatous exacerbations of the bowel (e.g. gastric outlet obstruction), the urinary tract (e.g. ureteral and urethral obstruction) and the lung (e.g. inhalative acute miliary pneumonia) require cautious use of immunosuppressive therapy. In a single case report, severe anaemia of chronic inflammation in a CGD-patient with the McLeod erythrocyte phenotype was reversed by high-dose recombinant human erythropoietin, combined with steroids (Aouba *et al*, 2007). Although corticosteroids should generally be avoided in CGD, *low-dose prednisolone* is the mainstay of therapy for the obstructive complications. *Granulomatous cystitis* quickly responds to corticosteroids (e.g. 0·5–1 mg/kg/d prednisolone for the first week, to be tapered over 6 weeks), but may relapse after steroid withdrawal, requiring long-term maintenance on very low-dose oral prednisolone (e.g. 0·1–0·2 mg/kg every other day) (Collman & Dickerman, 1990). *Inhalational acute miliary pneumonia*, often because of *Aspergillus* spp. inhaled from massive exposure to garden mulch up to 10 d before the first symptoms, is a life-threatening medical emergency with development of hypoxia because of rapidly increasing infiltrates. It requires immediate combined antimycotic and steroid medication (voriconazole plus 1 mg/kg methylprednisolone i.v. for a week, followed by gradual tapering) (Siddiqui *et al*, 2007). Determination of the optimal therapy of *granulomatous colitis* secondary to CGD remains an urgent need. CGD-associated colitis resembles Crohn disease, so that today's therapy follows treatment options for this disorder

Table II. Chronic granulomatous disease: drugs for treatment of granulomatous colitis.

	Mild to moderate	Severe to fulminant colitis	Proctitis	Fistula*
I. Topical treatments				
5-Aminosalicylate				
Oral (40–50 mg/kg/d)	+ (induction ± maintenance)	−	−	−
Rectal (1×/d)	−	−	+	−
Budesonide				
Oral (9 mg/d)	+ (induction)	−	−	−
Rectal (1×/d)	−	−	+	−
II. Systemic Treatments				
Prednisolone				
i.v. (1 mg/kg/d initially, then taper)	−	+ (induction ±	−	−
oral (1 mg/kg/d initially, then taper)	−	maintenance)	−	−
Infliximab (5 mg/kg at 0, 2, 6 weeks)	−	+ (induction if steroid refractory)	−	+ (induction)
Azathioprine (3 mg/kg/d)	−	+ (maintenance if steroid dependent or refractory)	−	+ (maintenance)

*Add metronidazole/ciprofloxacine.

(Table II). First-line therapy in severe cases is prednisone (e.g. 1 mg/kg/d), with gradual tapering over several months to alternate day treatment (e.g. 0·25 mg/kg every other day) (Marciano *et al*, 2004). In steroid-dependent patients long-term *azathioprine* has been used for its steroid-sparing effect (Zanditenas *et al*, 2004). In steroid-refractory patients *anti-tumour necrosis factor-α* (Infliximab) may be administered for remission induction (Sandborn, 2003). Steroid-dependent or -refractory colitis can be cured by *stem cell transplantation* with rapid induction of complete and stable remission (Seger *et al*, 2002). If an HLA identical stem cell donor is available, transplantation should be considered in such cases to avoid long-term conventional immunosuppression in an already immunodeficient patient.

Outcome of conventional management

Prospective survival data of the US CGD registry created in 1992 indicate that patients with X-CGD have a higher rate of infection and higher mortality (about 5%/year) than p47phox deficient a/r patients (about 2%/year) (Winkelstein *et al*, 2000). Infections caused by *Aspergillus* spp. accounted for over a third of the deaths. Exciting new developments in antifungal agents now provide hope for better survival in the near future.

Recent experience from centres specializing in the care of CGD patients suggests that the current mortality has fallen to under 3% and 1% respectively (H. L. Malech, personal communication). Better dissemination of expert management protocols and the routine involvement of CGD specialists in important therapeutic decisions should be strongly encouraged to improve also the outcome in treatment sites caring only occasionally for affected individuals. The problem of compliance with lifelong medication in adolescents however will remain.

Cure of the disease

Haemopoietic stem cell transplantation

Over recent years the results of haemopoietic stem cell transplantation (HSCT) have improved considerably. Conventional *myeloablative marrow conditioning* followed by transplantation of normal unmodified haematopoietic stem cells can cure CGD, which is a stem cell disease. A European collaborative study reported the outcome of 27 CGD patients who mostly received a busulfan-based regimen (busulfan at 16 mg/kg total dose), followed by a marrow graft from an HLA identical sibling donor (Seger *et al*, 2002). Severe side effects, graft-versus-host disease and inflammatory flare-up, were almost exclusively seen in the subgroup of nine patients with pre-existing, ongoing infection, mainly aspergillosis. Overall survival of the 27 patients was 85%, with 81% of patients cured of CGD. Survival in the patients without infection at transplantation was excellent. Most cured patients had >95% circulating donor myeloid cells. Pre-existing infections and chronic inflammatory lesions cleared in all engrafted survivors. Of special note, even children with severe lung restriction following chronic granulomatous lung disease profited, slowly normalizing decreased oxygen saturation, reversing clubbing of fingers and toes and manifesting a growth spurt.

The *decision for or against HSCT* should be made early in life, when HSCT is best supported and when there is still a paucity of CGD sequelae. As there exist no predictive laboratory parameters, this decision has to be based on the individual clinical course. Uncomplicated CGD is not considered an indication for HSCT. In contrast, HSCT may be most useful in CGD patients who either have recurrent serious infections despite correct antimicrobial (and in some IFNγ) prophylaxis or have severe steroid-dependent or steroid-resistant inflammatory complications plus a suitable stem cell donor (Table III). Recent introduction of *anti-CD52* (Campath 1H at 1 mg/kg) into the European CGD BMT protocol for *in vivo* T cell and monocyte depletion has enabled transplants from molecularly matched unrelated donors with a similarly good outcome as transplants from sibling donors (The European Group for Blood and Marrow Transplantation Working Party for Inborn Errors, unpublished observations).

Ideally, infections ought to be under control before starting conditioning for HSCT. In chronically infected patients HSCT still remains an option. Morbidity can be reduced by employing a less toxic conditioning regimen compared with conventional myeloablative conditioning. A first trial of *non-ablative mini-conditioning* [using cyclophosphamide 120 mg/kg, fludarabine 125 mg/m^2 and antithymocyte globulin (ATG) 40 mg/kg] followed by a T-cell depleted HLA-genoidentical stem cell allograft in 10 stable CGD-patients resulted in low engraftment and the need for donor lymphocyte infusions to improve donor

Table III. Chronic granulomatous disease: indications for stem cell transplantation.

Standard risk patient (absent infection/inflammation)	High risk patient (ongoing infection/inflammation)
≥1 life-threatening infection in the past	Intractable infection (e.g. aspergillosis)
Severe granulomatous disease with organ dysfunction (e.g. lung restriction)	Steroid-dependent or refractory granulomatous disease (e.g. colitis)
Non-availability of specialist care	
Non-compliance with antibiotic prophylaxis	

Plus human leucocyte antigen identical donor.

chimerism (Horwitz *et al*, 2001). A small trial of *subablative reduced-intensity conditioning* (RIC) (using busulfan 8 mg/kg, fludarabine 180 mg/m^2 and ATG 40 mg/kg) in three high-risk adult CGD patients, in contrast, led to full donor chimerism and cure in all cases (Gungor *et al*, 2005). Another type of subablative RIC (4 Gy of total body irradiation, cyclophosphamide 50 mg/kg and fludarabine 200 mg/m^2) followed by a two HLA-mismatched cord blood transplantation in a single adult McLeod phenotype CGD patient with invasive aspergillosis also resulted in full donor engraftment and cure (Suzuki *et al*, 2007). RIC with subsequent HSCT is thus a promising treatment modality for fragile CGD patients with intractable infection or inflammation. RIC should now be further tested in children with high-risk CGD.

In the absence of an HLA identical sibling or unrelated donor, haploidentical HSCT has been performed only twice (Kikuta *et al*, 2006; Miki *et al*, 2006), and is considered rather risky because of delayed immune reconstitution and graft failure. At least one family has therefore resorted to *in vitro* fertilization (IVF) and preimplantation HLA-testing (Van de Velde *et al*, 2004) to select an HLA genoidentical, disease-free sibling embryo as a '*saviour baby*' for successful stem cell transplantation of a brother suffering from severe X-CGD and lacking such a donor (Duke, 2006). This treatment option would require a severe clinical course of the disease in the index patient, absence of any HLA-identical donor, a young maternal age (<36 years), and – most importantly – the firm wish of the parents to have another healthy child. As the probability of a successful pregnancy in the most experienced IVF centres is around 10%, this demanding treatment modality, legal in some European countries and in the USA, has to be approached with sober judgement. It is likely to be superseded by successful gene therapy.

Stem cell gene therapy

Experimental gene therapy trials for CGD are ongoing in Frankfurt, London, Zurich and in the USA. At first sight CGD seems a good candidate for such an approach, as the genes encoding the relevant subunits of the NADPH oxidase complex are metabolic genes not involved in cell proliferation. Furthermore functional correction of as few as 10% of neutrophils should be sufficient to alleviate the symptoms of the disease based on the experience in X-linked CGD carriers (Mills *et al*, 1980) and on gene therapy studies in murine CGD (Dinauer *et al*, 2001). In addition, the expression of small amounts of gp91phox can lead to considerable reconstitution of superoxide generation (Bjorgvinsdottir *et al*, 1997).A major obstacle however remains: the *lack of a selective growth advantage of gene transduced cells*, e.g. of the ability of corrected cells to survive and replace uncorrected cells *in vivo* (Stein *et al*, 2006). In CGD the first two phase I gene therapy trials performed without marrow conditioning achieved only very low percentages of functionally corrected cells *in vivo* (<1%) persisting for a few months after reinfusion (Malech *et al*, 1997, 2004). Similar results have been observed in another phase I trial with a different γ-retroviral vector (Barese *et al*, 2004), suggesting that the protocols had to be combined with either submyeloablative conditioning or a co-expressed drug-resistance gene allowing for *in vivo* expansion of the corrected cells. The report on successful gene marking of up to 10% of myeloid cells in the adenosine deaminase severe combined immunodeficiency gene therapy study (Aiuti *et al*, 2002) prompted the use of a similar conditioning regimen (busulfan i.v.) for the treatment of X-CGD patients. Two adults with X-CGD were recently treated according to a protocol administering a *submyeloablative dose of busulfan* (4 mg/kg/d × 2 i.v.) followed by reinfusion of CD34$^+$ cells. The latter had been isolated from the peripheral blood of the patients after G-CSF, and gene-transduced *in vitro* with a *spleen focus forming* γ-retrovirus vector. This treatment provided a substantial therapeutic benefit early after transplantation (within 50 d) to the two CGD patients, suffering from an otherwise incurable bacterial (*S. aureus* liver) or fungal (*Asp. fumigatus* lung cavity) infection. In both patients a limited expansion in the number of gene-corrected cells from initially 10–30 to 40–60% was observed starting 5 months after transplantation. The expansion resulted from *activating retroviral insertions into three proto-oncogenes*, namely *MDS1/EVI1, PRDM16 and SETBP1* (Ott *et al*, 2006). Thereafter *silencing of the gp91phox transgene expression* took place, leading to barely detectable O_2^- generation in the gene-corrected cells, and death of one patient from severe sepsis with multiple organ failure (European Society of Gene Therapy, 2006). The risk of insertional mutagenesis and transactivation of proto-oncogenes from retrovirus-mediated gene therapy with unknown long-term consequences (none? myelodysplastic syndrome? leukaemia?) revealed in this recent trial clearly points to the necessity of developing next generation vectors with improved safety. *Self-inactivating* (SIN) vectors lacking the potent retroviral enhancer elements within the long terminal repeats (LTR) show much less transactivation potential than conventional LTR-driven vectors (Modlich *et al*, 2006). Transgene expression in SIN vectors is driven by an *internal, tissue-(myelo) specific, cellular promoter*, further reducing the probability of oncogene activation at the stem cell level. After extensive preclinical testing, the first clinical trial using an improved SIN-vector for CGD is expected in about 1 years' time. *Lentiviral vectors* could offer additional safety by integrating into transcriptional units, as opposed to γ-retroviral vectors integrating in proximity to promoter regions. Moreover lentiviral vectors allow gene transfer into quiescent cells and do not require extensive preculture of CD34$^+$ cells with cytokines. Preclinical testing for lentiviral gene therapy trials in CGD is ongoing (Roesler *et al*, 2002), but will take additional time. In summary, a gene therapy approach to CGD may become feasible to overcome recalcitrant or life-threatening infections. Although hitherto of limited transitory effect, the use of this technology in careful experimental studies may serve as salvage therapy to prepare selected patients with very

poor performance status for later allogeneic bone marrow transplantation. Cure of CGD by gene therapy alone remains a more distant goal for the future.

Future directions

The prognosis for CGD patients has markedly improved over the past 10 years. Nevertheless the prophylactic and therapeutical approaches routinely employed are only supportive, still imperfect, and demand lifelong patient compliance. CGD thus remains a lethal disease, nowadays at an adult age. The ultimate goal is to develop curative approaches. Allogeneic stem cell transplantation and, possibly, gene-replacement therapy are such options. Two major obstacles immanent to CGD have been identified and might be overcome in a not too distant future. More patients would be treated using HSCT, if the inflammatory complications (graft-versus-host disease and inflammatory flare-up) triggered by heavy conditioning and pre-existing infection in a CGD recipient, could be prevented (Yang *et al*, 2002). RIC combined with moderate *in vivo* depletion of inflammatory cells is worth pursuing, taking care to preserve some donor T cells needed for engraftment of unrelated grafts. Gene therapy would advance, if the missing selective survival advantage of CGD-corrected cells could be substituted in a safe and efficient manner. Submyeloablative conditioning might pave the way to engraftment, perhaps combined with a system for on-demand *in vivo* selection of the gene-corrected cells using a non-mutagenic drug (Rappa *et al*, 2007). Although only the future will assure whether HSCT or gene therapy offers the best cure for CGD, one can be cautiously optimistic about important advances in the next 10 years.

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