Springer Semin Immunopathol (1998) 19:417–434

Springer Seminars
in Immunopathology

The molecular basis of chronic granulomatous disease

Christof Meischl, Dirk Roos

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service
and Laboratory of Experimental and Clinical Immunology, Academic Medical Center,
University of Amsterdam, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands

Introduction

Chronic granulomatous disease (CGD) is a rare inherited immunodeficiency syndrome, caused by the inability of the patients' phagocytic leukocytes to produce (sufficient) superoxide [14]. Phagocytic cells (neutrophils, eosinophils, monocytes and macrophages) constitute a central part of the innate (nonspecific) immune system, forming as such a first line of defense against various invading microorganisms, principally bacteria and fungi. These leukocytes phagocytose the pathogens and then kill them intracellularly by means of reactive oxygen species and a number of oxygen-independent microbicidal agents. The highly reactive oxygen species (such as superoxide, hydrogen peroxide, hypochloric acid and others) not only attack the ingested microorganisms directly, they also optimize the intraphagosomal milieu for the cationic microbicidal peptides and proteins there present.

The enzyme responsible for the generation of those oxygen metabolites is the phagocyte-specific NADPH oxidase [13], and it are the defects of this multi-component redox-center that lead clinically to the severe immunodeficiency syndrome of CGD.

Clinical presentation

CGD is a rare disease, with an estimated incidence of 1 : 250,000, all ethnic groups being equally affected. While the most common form of the syndrome is X-linked, different modes of autosomal inheritance are also possible. The overwhelming majority of patients with X-linked disease is, obviously, male; in the autosomal forms no sex preference is discernible.

As an inherited and severe immune defect, CGD presents itself early in life in the form of acute or chronic infections, two-thirds of the patients showing the first symptoms already in their first year of life [39]. Nevertheless, one of the surprising features of the disease is its highly variable severity; thus, in some patients the diagnosis is established only in their adult years. As a general rule, the organs that represent the in-

Correspondence to: D. Roos

dividual's border against the outside world, or the lymph stations downstream of those organs, are most frequently affected: lungs, skin and gastrointestinal tract. From here hematogenous spread to almost any other organ or organ system may take place, as demonstrated by studies in over 500 patients.

The isolated pathogens are usually catalase positive and/or relatively resistant to the other, non-oxidative killing mechanisms of the phagocytes. Catalase degrades hydrogen peroxide, which is also produced in small amounts by the microbes themselves, and thus deprives the phagocytes of the possibility to use this microbe-generated oxygen metabolite for killing. The microorganisms most frequently found in the above-mentioned studies are *Staphylococcus aureus*, various *Aspergillus* species, enteric gram-negative bacteria (including *Serratia marcescens* and various *Salmonella* species) and *Burkholderia cepacia*. When analyzing the culture and/or serological results, it should be kept in mind that pathogens that are harmless to a normal host may well be responsible for infections in a CGD patient.

Pneumonia is the most frequent serious infection in all age groups and is most commonly caused by *S. aureus*, *Aspergillus* species, *B. cepacia* and enteric gram-negative bacteria. Because sputum cultures are rarely informative, empiric treatment should be initiated the moment the clinical diagnosis of pneumonia has been established. In the case of severe pneumonia or of progression of disease under treatment, more aggressive diagnostic steps, such as bronchoscopy, needle biopsy or even open lung biopsy, may be warranted to determine rarer pathogens.

Burkholderia species (mainly *B. cepacia*, but also *B. gladioli*, *B. mallei*, *B. pseudomallei* and *B. picketii*) are unusually virulent in the CGD patient and one of the main causes of fatal pneumonias [83]. These microorganisms, which are ubiquitously present in the environment and need free oxygen radicals to be effectively killed, grow slowly in cultures and may, therefore, be diagnosed (too) late.

The next most frequent sorts of infection are cutaneous abscesses and suppurative lymphadenitis, the later affecting often the cervical nodes. The most commonly found pathogens are *S. aureus* and – in the case of lymphadenitis – various gram-negative bacteria (including *B. cepacia* and *Serratia marcescens*). Perirectal abscesses occur frequently and may persist for years in spite of adequate antibiotic treatment and meticulous care.

Hepatic and perihepatic abscesses are unexpectedly common and typically caused by *S. aureus*. They are frequently painless, even on palpation, and present with such unspecific symptoms as fever, malaise, anorexia and weight loss. The laboratory findings are often normal. Osteomyelitis may be due to hematogenous spread of the pathogens (*S. aureus*, *Salmonella* species, *Serratia marcescens*) or to contiguous invasion of bone, as can be seen in Aspergillus pneumonia with consecutive destruction of the adjacent ribs or vertebral bodies.

While the above affections normally represent acute disease states, CGD – as the name implies – is typically characterized by a chronic struggle of the immune system with the pathogens. Thus the granulomas, which can be found in a large variety of organs and to which CGD owes its name, are the result of chronic inflammatory cell reactions, involving mainly lymphocytes and histiocytes. The cytoplasm of the histiocytes is typically foamy and brown. These characteristic granulomas, while not pathognomic, should at least make CGD part of the differential diagnosis [44].

Clinically, the granulomas can become symptomatic by pain or signs of obstruction. When narrowing the upper gastrointestinal tract, they may present as dysphagia, stomach pain or recurrent vomiting, which, in the first months of life, can be misdiag-

nosed as pyloric stenosis. When affecting the urinary tract, the granulomas may cause dysuria, penile pain, a decreased urine volume or hydronephrosis [2].

Another chronic affection is the inflammatory bowel disease of CGD, which closely resembles Crohn's disease. Found in ten percent of the CGD patients, it typically involves the colon, but involvement of other parts of the gastrointestinal tract has also been described. The severity of this complication can vary widely, with symptoms ranging from mild diarrhea to a debilitating state of bloody diarrhea and malabsorption, necessitating colectomy [3].

Other manifestations of chronic inflammation are an eczematoid dermatitis, which may be present already at birth and mainly affects infants and children, gingivitis, chorioretinitis, glomerulonephritis and destructive white matter lesions of the brain. Very rarely, discoid or, even more rarely, systemic lupus erythematosus (SLE) is observed.

The children are often underweight and of short stature. Especially the X-linked patients tend to grow beneath the 5th percentile, which may be partially corrected in adolescence, when some catch-up growth can occur. The end of the first decade of life represents anyhow a milestone, since thereafter the infections are often less severe and occur less frequently, and the anemia typically found in those patients tends to resolve spontaneously. This anemia, with Hb values of 8–10 g/100 ml and with microcytosis, is an expression of the chronic disease state and does not respond therefore to therapies of iron deficiency, a common misdiagnosis.

Lymphadenopathy, hepatosplenomegaly and hypergammaglobulinemia can all be found in CGD independently of any acute disease process. Very rarely, other clinical syndromes, such as Duchenne muscular dystrophy, retinitis pigmentosa or McLeod's syndrome, may be associated with the X-linked form of CGD. As a rule, carriers of CGD are symptomfree. The proverbial exception to this rule are the carriers of X-linked CGD, who may present with a few syndrome-related conditions. Roughly half of them are troubled by recurrent stomatitis and/or moderately severe gingivitis and about one-fourth of these women will develop discoid lupus erythematosus. The latter condition manifests itself typically in the second decade of life, with (sun)light-sensitive, discoid lesions on face, arms and upper torso, is usually mild, rarely severe, and does not progress to SLE [7, 80].

Finally, extremely lyonized carriers of X-CGD can have a – usually mildly – increased risk of infection [56]. This is, however, exceptionally rare since as little as ten percent, or sometimes even less, of the phagocytes expressing a normally functional NADPH oxidase are sufficient to keep those individuals asymptomatic.

Molecular basis of the disease

The NADPH oxidase is a multi-component enzyme with a redox center that transfers electrons from intracellular NADPH onto extracellular (or intraphagosomal) molecular oxygen, thereby generating superoxide (Fig. 1):

$$NADPH + 2O_2 \rightarrow NADP^+ + 2O_2^- \cdot + H^+$$

The weakly microbicidal superoxide is then – spontaneously or enzymatically catalyzed – converted into hydrogen peroxide and other, more potent metabolites.

The electron transfer itself is a multistep process, during which the electrons are transported sequentially along several moieties of the oxidase:

$$NADPH \rightarrow FAD \rightarrow 2\ Heme \rightarrow 2O_2$$

Although FAD and the two heme groups are part of the redox center of the enzyme, cytochrome b_{558}, NADPH cannot bind to it unless the complete enzyme has been assembled during activation, and only then can electron transfer actually take place.

Cytochrome b_{558}, a flavo-hemoprotein, is composed of two of the enzyme's subunits, gp91^{phox} and p22^{phox}, in a 1 : 1 stoichiometry [36, 63, 77, 88]. Incorporated in the membranes of specific granules and secretory vesicles in resting cells, cytochrome b_{558} becomes expressed on the phagolysosome and on the cell surface when the granules/vesicles fuse with those larger membrane systems during cell activation (Fig. 1). The stimulus for this activation is the binding of opsonized microorganisms or high concentrations of chemoattractants to phagocyte surface receptors.

As part of this activation, the enzyme's three cytosolic components, p47^{phox}, p67^{phox} and p40^{phox}, as well as several low-molecular weight GTP-binding proteins, translocate to the cytochrome b_{558} in the membrane to form there the complete and active form of the NADPH oxidase (Fig. 1).

A defect in any one of the four components gp91^{phox}, p22^{phox}, p47^{phox} or p67^{phox} abolishes (or reduces) the activity of the oxidase and leads thus to CGD. Defects in the other enzyme components are not known. While p40^{phox} might not be essential for the function of the enzyme (and mutations, therefore, would not become symptomatic), a defect in one of the GTP-binding proteins (rac1/2, rap1A), which are in-

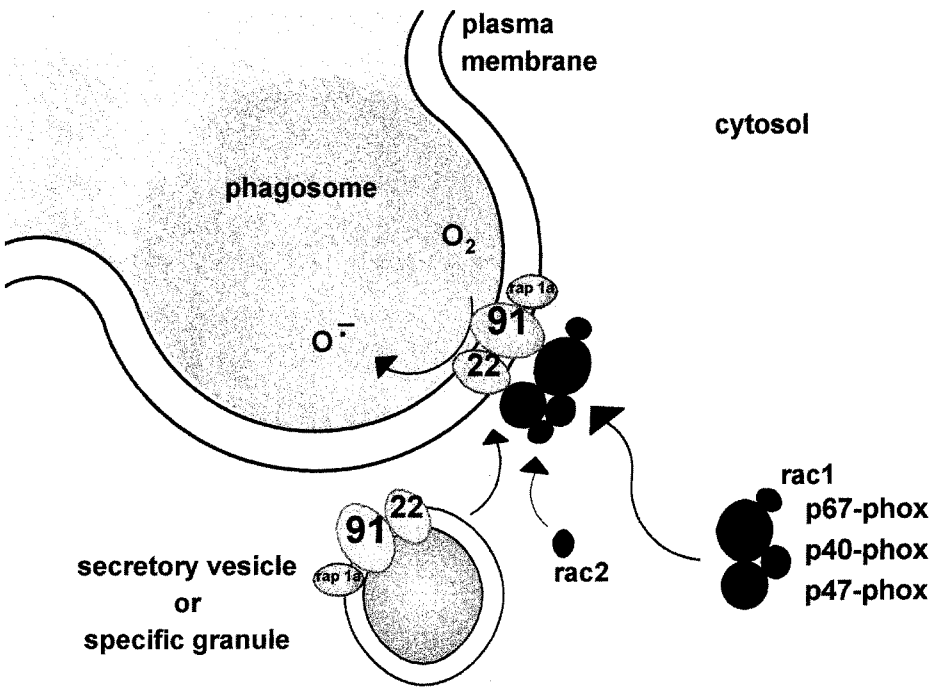

Fig. 1. Activation of the NADPH oxidase. Assembly of the activated enzyme and phagosome formation are concomitant processes. Translocation of cytosolic proteins is initiated by serine phosphorylation in p47^{phox} and controlled by small GTP-binding proteins (rac1, rac2, rap1A). This translocation leads to a conformational change in gp91^{phox} that permits NADPH binding, thus activating the NADPH oxidase enzyme

volved in the regulation of a great number of cellular functions, might be imcompatible with life.

gp91^{phox}

The CYBB gene, which codes for gp91^{phox}, is located on the X-chromosome (Xp21.1), has a length of 30 kb and comprises 13 exons [20, 75, 86]. The translation product, a protein of 570 amino acids, needs for its further maturation and stabilization the presence of p22^{phox} – in such a way, that abolished expression of one protein automatically leads to simultaneous absence of the other [67]. Post-translational modifications are the glycosylation of three of its five potential N-linked glycosylation sites [89]. The N-terminal half of the mature protein contains four or five hydrophobic, probably membrane-spanning domains, while the hydrophilic C-terminal part is thought to represent the cytosolic side of the protein. The hydrophobic part contains the heme moieties (one shared with p22^{phox}) [68] and is probably also involved in the interaction with p22^{phox}. For the C-terminal part a three-dimensional model has been deduced from sequence homology with the ferridoxin $NADP^+$ reductase flavoenzyme family [79]. Putatively, therefore, this part of the protein contains one binding site for NADPH and the FAD-binding site. In the inactive state of the enzyme, the NADPH-binding site is probably covered by a loop of 20 amino acids [85].

p22^{phox}

p22^{phox} is encoded by the gene CYBA, which is located on chromosome 16q24 and spans 8.5 kb and six exons [21, 64]. The resulting protein of 195 amino acids is thought to share one heme moiety with gp91^{phox}, and has one proline-rich region involved in interaction with SH3 (src homology region 3) domains.

p47^{phox}

NCF1, the gene on chromosome 7q11.23 coding for p47^{phox}, has a length of 15 kb, encompassing 11 exons [29]. Its product, a protein of 390 amino acids, contains nine serine phosphorylation sites, two SH3 domains and one proline-rich region.

p67^{phox}

NCF2 on chromosome 1q25 codes for p67^{phox}, is 40 kb long and comprises 16 exons [46]. p67^{phox} itself, with its 526 amino acids possesses a (higher affinity) binding-site for NADPH [81], two SH3 domains and one proline-rich region.

p40^{phox}

Very little is known yet about p40^{phox}, a protein of 339 amino acids. Its gene, NCF4, spans 18 kb and 10 exons and is located on chromosome 22q13.1 [96]. The protein contains one SH3 domain [94].

Activation of the enzyme

Responding to as yet only partially unravelled upstream events that transmit the signals originating from the cell surface receptors, the activation of the NADPH oxidase itself seems to be initiated by a change in the phosphorylation of $p47^{phox}$ [78]. The different protein kinases implicated in this process apparently phosphorylate different groups of serines [25], with each different state of phosphorylation corresponding to a different three-dimensional conformation of the protein. This process would disrupt the cytosolic complex of $p47^{phox}/p67^{phox}/p40^{phox}$ in the resting cell, exposing until then inaccessible SH3 and/or proline-rich domains [32, 45]. This change results in translocation of these three proteins to the membrane, where they associate themselves with cytochrome b_{558} (Fig. 1). The interactions of the cytosolic components with each other and with the cytochrome are mediated by SH3 domains binding specifically to certain proline-rich regions [18, 26, 31, 45, 47, 84], but other types of protein-protein interaction seem to play a role as well [17, 66].

In the membrane-associated complex, $p47^{phox}$ appears to stabilize the interaction of $p67^{phox}$ – and possibly rac1 – with the cytochrome [10, 11]. $P67^{phox}$ with its high-affinity binding site for NADPH, on the other hand, could bind to $gp91^{phox}$, which contains a lower-affinity NADPH-binding site, to form the catalytically efficient binding site of the active enzyme [81]. The loop of 20 amino acids that covers the NADPH-binding site in $gp91^{phox}$ in the resting state, is thought to move out of the way, as a result of either the complex formation or of an independent control mechanism. NADPH then

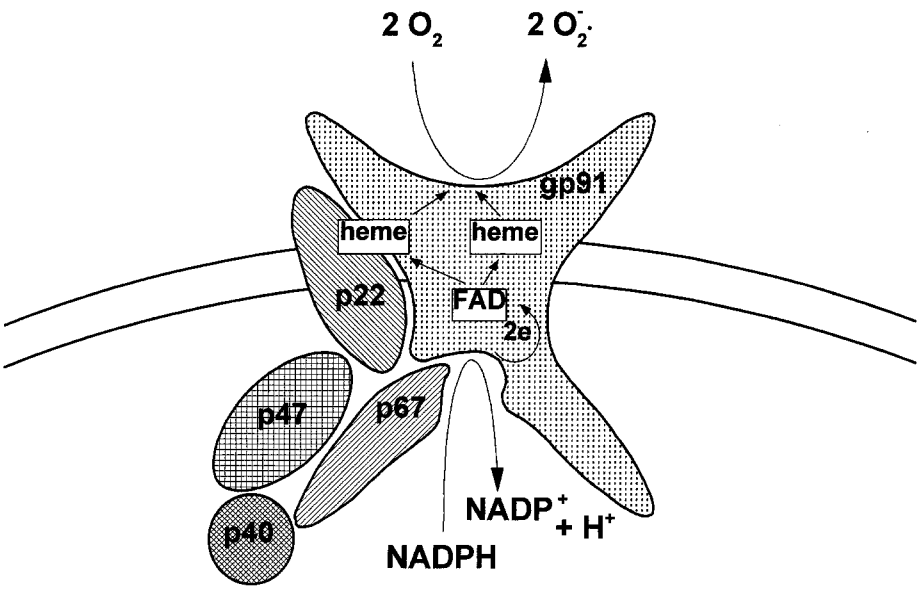

Fig. 2. Schematic illustration of the electron transfer mechanism of the NADPH oxidase (see text). After assembly of the NADPH oxidase complex, NADPH from the cytosol can bind to the enzyme and donate its electrons. These electrons are then transmitted via FAD and heme groups to molecular oxygen on the other side of the plasma membrane, thus generating superoxide in either the phagosome or in the extracellular environment

binds to the completely assembled oxidase, electron transfer will start and superoxide generation starts (Fig. 2).

Both the assembly of the enzyme and the electron flow itself are subject to a complex network of regulating and modifying influences. $P40^{phox}$, for instance, besides stabilizing the cytosolic complex in resting cells, seems to down-regulate NADPH oxidase activity through competition of its SH3 domain with that of other, essential oxidase components [76].

Furthermore, three low-molecular weight GTP-binding proteins, rac1, rac2 and rap1A, are known to play an important role in the activation and function of the enzyme [1, 41]. Functioning as molecular switches in signalling cascades, with an inactive GDP-bound and an active GTP-bound state, they themselves are again under the control of GDP-dissociation inhibitor (GDI) and GDP-dissociation stimulator (GDS) proteins [1, 57]. The activated rac1 appears to translocate together with $p47^{phox}/p67^{phox}$ and to influence the electron flow through the active oxidase [19, 24, 49]; rac2 is thought to translocate independently of the other cytosolic proteins [34]. Rap1A, on the other hand, is associated with the membrane-bound cytochrome b_{558} [69] and may indirectly link the activity of the oxidase to intracellular cAMP levels [6].

Results obtained with a cell-free system indicate, finally, that the presence and polymerization of actin enhance the activation of the NADPH oxidase [58]. The impressive complexity of the activation of the enzyme and its intricate control mechanisms point to the importance of a tightly controlled, place- and time-restricted release of free oxygen radicals, since an uncontrolled release of these products would have devastating effects for the affected individual. This complexity, on the other hand, is the explanation for the fact that defects in different genes can lead to the same cellular dysfunction and disease.

Molecular defects

Defects in $gp91^{phox}$

Defects in $gp91^{phox}$ comprise about two-thirds of the cases, and are as such the most frequent cause of CGD. All possible types of mutation, except gene conversions, have been found in CYBB, with single nucleotide substitutions accounting for 65% of the defects, and deletions and insertions for the remaining 35% [74]. Very large deletions, extending over other coding genes localized on the X-chromosome, can result in various clinical entities, such as Duchenne muscular dystrophy, retinitis pigmentosa or McLeod's syndrome, being associated with CGD.

In a recent multicenter review of the mutations found in 261 X-linked CGD kindreds, 65% of these mutations were found to be family specific, with the other 35% being clustered around a few hotspots, mainly around CpG sequences [72]. The large majority of X-linked mutations in CGD leads to a complete lack of $gp91^{phox}$, due to instability of the mRNA or of the translated protein. These are called $X91^0$ variants, to differentiate them from the (few) cases with reduced or normal protein expression, called $X91^-$ and $X91^+$, respectively. In the cases of $X91^-$, the reduced protein expression is accompanied by a roughly proportional decrease of superoxide production, whereas the $X91^+$ variants express normal amounts of a non-functional protein.

While being clinically indistinguishable from the $X91^0$ variants, the cases of $X91^+$ CGD are of great interest for the understanding of the working mechanism of the oxi-

dase, because they allow analysis of how different defects block various steps of the activation process or in the electron transport. The ten X91$^+$ mutations known so far have contributed in this way to our knowledge of gp91 phox, by identifying regions important for the binding of one of the heme groups, NADPH or the cytosolic oxidase components [48, 74, 79].

Two patients have been found with single nucleotide substitutions in the 5′promotor region of CYBB. Interestingly, both patients possessed small subsets (5%) of neutrophils with normal NADPH oxidase activity. The mutations abolished the binding of an undefined protein to the DNA of the promotor sequence, but enhanced the association of another, larger one. The investigators theorized that this might be an indication for different subsets of neutrophils controlling the gene for gp91^{phox} by means of different DNA-binding proteins [62, 95]. Only one (rare) polymorphism has been found in CYBB to date, further illustration of the extreme sensitivity of this protein for mutations.

Recently a regularly updated database (X-CGDbase) has been established, which is freely available for anonymous file transfer protocol (ftp) at ftp.csb.ki.se and ftp.helsinki.fi(in the directory pub/x-cgdbase). The WWW site is at http://www.helsinki.fi/science/signal/databases/x-cgdbase [72].

Defects in p22^{phox}

About 5% of the cases of CGD are caused by defects of p22^{phox}. In the nine families with A22-CGD investigated so far, ten different mutations were found in the 18 alleles involved [74]. The only A22$^+$ mutation known, a substitution of glutamic acid for one of the prolines in its proline-rich region, apparently destroys the interaction with the SH3 domain of p47^{phox}, thereby interrupting the activation of the enzyme [22, 45, 47, 84]. In CYBA four polymorphisms are known.

Defects in p47^{phox}

Mutations in NCF1 account for about 30% of the cases of CGD. In strong contrast with the variation in the mutations found in the other subtypes of CGD, only four different mutations have been reported in A47 CGD to date. In 15 unrelated patients described, 11 were homozygotes and 4 compound heterozygotes for a dinucleotide deletion in the first four nucleotides of exon 2 (GTGT → GT) [74].

This situation has long remained unexplained, but the finding of the deletion-containing gene sequence (in addition to the wild-type sequence) also in healthy individuals has hinted at the existence of one or more pseudogenes in the human genome. It is now believed that recombination events between NCF1 and a pseudogene, which is a highly homologous but non-functional gene copy, cause the extremely high uniformity of mutations found in A47 CGD [33, 71].

Defects in p67^{phox}

With around 5% of the described cases, A67 CGD also represents a rare subtype of the disease. In the 11 A67 CGD patients characterized so far, 12 different mutations were

found among the 22 alleles [74]. While the level of mRNA is usually normal, no protein expression has been found in A67 CGD, with the exception of one $A67^+$ patient, whose $p67^{phox}$ protein is apparently non-functional due to the deletion of one amino acid. This deletion causes a strongly diminished binding of rac1, and thereby a disturbance in NADPH oxidase activation [49].

Defects in $p40^{phox}$

There are no known defects in this protein.

Correlations between genotype and phenotype

In general, the $X91^0$, $X91^+$, A22 and A67 subforms of CGD present with similar clinical severity. It might be expected, on the other hand, that the severity of the clinical symptoms in $X91^-$ CGD correlates with the amount of residual superoxide production (3–30%) found in these patients' phagocytes. This is, while often true, not a dependable rule [73]. Possibly, variabilities in other host defense systems play an important role here.

A47 CGD, as borne out by a few clinical studies, seems to follow in general a more benign clinical course [53, 91]. Given the observations that $p47^{phox}$ under certain in vitro conditions seems not to be essential for oxidase activity [30, 42, 43], it might be speculated that some residual superoxide generation is also possible in the in vivo situation [11].

Molecular diagnosis

CGD – once clinically suspected – can be diagnosed in the laboratory by the phagocytes' failure to produce reactive oxygen species. A number of methods are available for the assessment of superoxide production. The classical, and still widely used, test for this purpose is the so-called NBT slide test, where a yellow dye in solution (nitroblue tetrazolium) is reduced by superoxide to insoluble blue formazan. The fraction of stained and unstained cells and even the staining intensity in each cell are then evaluated under a microscope [55].

The activity of the NADPH oxidase can, furthermore, be measured by oxygen consumption (oxygen electrode), superoxide generation (reduction of ferricytochrome c, chemiluminescence) or hydrogen peroxide production (oxidation of homovanillic acid).

Today, flow cytometry is frequently used to assess the neutrophils' NADPH oxidase activity [70]. This method, which uses fluorescent dyes for the detection of hydrogen peroxide, is sensitive, measures at the single cell level (and can thus distinguish active and non-active cell fractions) and has the additional advantage that non-purified leukocyte suspensions can be used. Once the diagnosis of CGD is established, the missing subunit of the enzyme, and thereby the subgroup of the disease, can be determined by immunoblot analysis of neutrophil fractions. This simple-sounding principle is marred by the fact that the two components of cytochrome b_{558} need each other for stable expression, so that the lack of one protein automatically

leads to absence of the other. In that case, the family history and a carrier pattern in the mother's neutrophils (see below) may indicate an X-linked type of disease. Still, since one-third of the X-linked defects are new mutations in the parental germline cells, X-linked CGD cannot be excluded in this way. If all four subunits of the NADPH oxidase are present on Western blot, an X^+ or A^+ variant, with a dysfunctional protein, is probable. In that situation, the cell-free assay, an in vitro system that reconstitutes the NADPH oxidase from its individual components, has to be employed, to localize the defective subunit in either the membrane ($gp91^{phox}$, $p22^{phox}$) or the cytoplasm ($p47^{phox}$, $p67^{phox}$) [12, 54]. The causative mutation is then determined by sequencing of polymerase chain reaction (PCR)-amplified cDNA or genomic DNA.

Carrier detection

Carriers of X-linked CGD have two subpopulations of neutrophils, one with the "healthy" X-chromosome in an active form and therefore rendering the cells capable of expressing a functional NADPH oxidase, and another one with the mutated X-chromosome active and therefore incapable of inducing superoxide generation. The ratio between these two populations is determined by the process of lyonization, the random inactivation of one of the two X-chromosomes in all female cells.

The resultant mosaic pattern for superoxide production can best be analyzed by the NBT slide test or by flow cytometry. Because extreme lyonization can result in seemingly unaffected carriers, carrier analysis is nowadays most often performed by sequencing. In some circumstances, when the family-specific mutation is known, other techniques, such as allele-specific restriction enzyme analysis, single-strand conformational polymorphism (SSCP) or restriction fragment length polymorphism (RFLP), may also be employed. Autosomal carrier detection, which is best done by sequencing, is still difficult for A47 CGD.

Prenatal diagnosis

Prenatal diagnosis is best performed by sequencing of DNA from amniotic fluid cells or chorionic villi obtained during the 10th week of gestation. It should be performed when the known family-specific mutation is known. In principle, all subtypes of CGD can be diagnosed prenatally, but often the combined heterozygous mutations of autosomal diseases render such a diagnosis more difficult.

After verification of the fetal origin of the cells and determination of the fetus' sex, the sequencing is carried out on PCR-amplified fragments of the relevant genomic region. The results are compared to the simultaneously obtained sequences of the indicator patient in the family, the pregnant woman and a healthy control [16].

If the family-specific mutation is not known, allele-specific markers can be used, for which several have been described. If these markers are informative in the affected family, they offer a more than 50% reliable method for prenatal diagnosis.

Treatment

The prognosis of the syndrome originally called "fatal granulomatous disease of childhood" in its first description in 1957 [5, 44] has since dramatically improved, due, in part, to the emergence of several specialized centers that assembled larger groups of patients to improve the knowledge and treatment of this disease (e.g., the National Institutes of Health, Scripps Clinic/Stanford University, the Paediatric Clinics of the University of Amsterdam and of the University of Zürich).

While one retrospective study in 1989 found a survival rate of 50% at 10 years of age (with an improved prognosis thereafter) [59], nowadays most patients survive well into adulthood, especially those with A47 CGD.

Modern treatment of CGD rests on five pillars:

1. Prevention of infections through immunization of the patients and avoidance of probable sources of pathogens
2. Prophylaxis with trimethoprim-sulfamethoxazole or dicloxacillin
3. Prophylactic administration of recombinant human interferon-gamma (rIFN-γ)
4. Most important: early and aggressive use of parenteral antibiotics
5. Surgical drainage and/or resection of infectious foci

CGD patients should receive all routine immunizations (including the live-virus vaccines) on schedule, as well as a yearly influenza vaccination. Any skin damage should be promptly washed with soap and water and rinsed with antiseptic agents (e.g., 2% hydrogen peroxide, betadine solution). Since perirectal abscesses represent a common and often stubborn problem in CGD, careful rectal hygiene, including frequent soaking in warm soapy baths, and avoidance of constipation are recommended. The risk of pulmonary infections can be reduced by not smoking, not using bedside humidifiers and avoiding possible sources of Aspergillus (e.g. decaying plants, rotting wood, sawdust, hay or straw). Finally, attention should be paid to optimal dental cleaning, including flossing and antibacterial mouthwashes.

Prophylaxis with trimethoprim-sulfamethoxazole (5 mg trimethoprim/kg per day, given orally in one or two doses) can reduce the number of bacterial infections by more than half, as was borne out by three studies representing a total of 95 patients [53, 59, 90]. In case of allergy against sulfamethoxazole, dicloxacillin (25–50 mg/kg per day) may be given, but the prophylactic efficacy of this drug has been less well documented.

The merits of an antifungal therapy are not yet well established. While ketoconazole was shown in one study to convey no protection against Aspergillus [59], in another study itraconazole proved to be effective prophylactically [60]. However, the long-term safety of this latter drug in CGD-related prophylaxis remains to be investigated.

In 1991, a phase III multi-center, double-blind, randomized placebo-controlled study, encompassing 128 patients, demonstrated a by 70% reduced risk to develop a serious infection under prophylaxis with rIFN-γ, as compared with placebo [37]. This benefit is maintained over longer periods of time, as has since been shown in two phase IV studies [4, 92]. The side effects of this treatment – mild headaches, low-grade fevers a few hours after administration – are generally negligible. Recommended are 0.05 mg/m^2, given subcutaneously three times a week. (For infants with < 0.5 m^2 the recommended dose is 0.0015 mg/kg, given subcutaneously three times a week.)

This improved host defense, which is found independently of the subtype of the disease, is not parallelled by an improvement in superoxide production by purified

neutrophils or monocytes [37, 61]. Apparently rIFN-γ boosts other, oxygen-independent defense mechanisms.

One of the central guidelines in treating CGD is to initiate anti-infectious treatment promptly and to continue it until certain eradication of the pathogens. Later, in CGD, may be too late. Not every low-grade fever or minor infection needs maximal therapy, but patients should be observed closely, and action taken at the first signs of deterioration.

Before initiating treatment, reasonable (and rapid) efforts should be made to localize the infection and isolate the causative agent. However, since treatment in those situations should begin without delay, it often has to be an empirical one, to be modified later if shown to be necessary by the culture results.

Empirical treatment should provide strong coverage for *S. aureus* and gram-negative bacteria, including *B. cepacia* (e.g., a combination of nafcillin and ceftazidime). If there is no improvement within 24–48 h, more aggressive attempts at defining the responsible pathogen should be made. Additionally, empirical modifications of the treatment might be warranted, such as adding high-dose intravenous trimethoprim-sulfamethoxazole to cover ceftazidime-resistant *B. cepacia*.

While the basic strategy remains the same when a fungal infection is suspected, the necessity to expose the patient to a long and intensive treatment with amphotericin B should intensify the efforts to obtain a definite diagnosis. Fungal infections most often affect the lungs and/or bone, and are treated with amphotericin B for 5–6 months, even longer if warranted by the clinical data. For the first 2–3 months the drug is given on a daily basis, thereafter on an alternate-day schedule. After discontinuation of amphotericin B, oral itraconazole should be given over several years, to prevent a recurrence or reactivation of the infection.

Local foci of infection, such as abscesses, empyemas, necrotic infected tissue and bone infected by fungi, are often very resistant against anti-infection therapy, especially in an immunecompromised host, and warrant surgical resection or drainage. This is certainly true for pulmonary fungal infections, where cavity or necrotic lesions of the lung, continuous spread to the ribs or vertebral bodies or brain metastases all need the attention of the surgeon. But also cutaneous or hepatic/perihepatic abscesses require surgical or needle drainage, followed, especially in the latter case, by several months of parenteral antibiotics.

If all the medical and surgical measures outlined above fail, an attempt with granulocyte transfusions may be made. Additionally, the therapy with rIFN-γ can be intensified to a daily form of administration.

If the McLeod's syndrome is associated with the X-linked form of CGD (or has not been excluded by DNA analysis) special care has to be taken with the transfusion of erythrocytes, thrombocytes or granulocytes. Since certain Kell antigens are only weakly expressed in this mild form of hemolytic anemia, the patients are very rapidly sensitized against those antigens, which are ubiquitously present in the general population. As long as McLeod's syndrome has not been excluded, therefore, erythrocyte antigen phenotyping should be done prior to transfusing the patient for the first time, to administer, if necessary, only Kell-negative blood products.

Although the immunosuppressive corticosteroids should be avoided in CGD patients, they may sometimes be warranted by symptomatic granuloma formation or severe forms of CGD inflammatory bowel disease. Both complications respond well to these agents (e.g., 0.5–1 mg prednisone/kg per day) [8, 15]. After a few weeks treatment, the dose of corticosteroids should be tapered to prevent a rapid relapse.

CGD, as a defect of the hematopoietic stem cells, is, in principle, amenable to bone marrow transplantation. While this is a curative approach and has been successful in several cases [27, 35, 40], the overall results have been problematic, due to serious problems with failure of engraftment. Newer regimens, however, which make use of busulfan to achieve adequate myeloid suppression, may help to solve this problem, so that the place of bone marrow transplantation among the treatment options for CGD has to be reconsidered ([35] and Seger, personal communication).

Animal models

While there is no known natural animal model for CGD, recently two murine models have been presented. The group of M. Dinauer succeeded in constructing a model for X-CGD through gene targeting of murine embryonic stem cells [65], and the group of S. M. Holland created a $p47^{phox}$ knockout mouse, using comparable techniques [38].

Those models will allow to study possible clinical differences between the genetic subgroups in detail, as well as to test the efficacy and safety of new therapeutic techniques, including gene therapy [23, 52].

Prospectives of new therapeutic advances

Treatment of CGD, for all the great progress seen since the first description of the disease, remains to this day largely symptomatic. With the advent of the new genetic technologies, however, a cure for (some) inherited diseases seems possible.

Gene therapy seeks to introduce a functional copy of the defective gene into the genome of the affected cells. For CGD, partial correction of the defect in vitro has been described, by treating CGD peripheral blood progenitor cells, B lymphocytes, monocytes or genetically modified myeloid cell lines with retroviral or adenoviral vectors that contain cDNA of one of the four genes involved in the pathogenesis of CGD [50, 82, 87, 93]. Only one attempt at clinical gene therapy has been published so far, involving five patients with the $A47^0$ subtype of CGD. Modifying the patients' $CD34^+$ hematopoietic progenitors, mobilized to the peripheral blood by granulocyte colony-stimulating factor, maximal levels of one corrected neutrophil in 1500 neutrophils analyzed were obtained after retransfusion of the transduced cells. These levels then declined below the detection limit during the following 3–6 months [51].

The optimism with regard to these trials is based on the observations that in some $X91^-$ patients 3–5% of oxidase activity can result in a mild clinical phenotype, and that in some extremely lyonized X-CGD carriers as few as 5% fully functioning neutrophils are sufficient to confer substantial anti-infectious protection. A complete (100%) reconstitution of the oxidase activity seems, therefore, not necessary.

The major limitation of all gene therapy trials so far, CGD or otherwise, is the duration of expression of the transduced gene product. Nevertheless, in the case of CGD, the existing vector constructs might already be used to treat infections resistant to other forms of treatment. Another nascent therapeutic possibility is the in utero transplantation of hematopoietic stem cells, which has already been used in various circumstances and with mixed results [9], most successfully in a case of X-linked SCID [28]. This technique remains as yet limited to those instances where the donor cells have a

natural growth advantage over their fetal counterparts – which would not be the case in CGD – but future developments are likely to relativize this precondition.

Both methods are, of course, only at the beginning of their development, and much work remains to be done, but CGD, as a very well-characterized inherited affection of the hematopoietic stem cells, is likely to be among the first syndromes to profit from those advances.

Summary and conclusions

CGD is a rare inherited immunodeficiency syndrome, caused by the phagocytes' inability to produce (sufficient) reactive oxygen metabolites. This dysfunction is due to a defect in the NADPH oxidase, the enzyme responsible for the production of superoxide. It is composed of several subunits, two of which, gp91^{phox} and p22^{phox}, form the membrane-bound cytochrome b_{558}, while its three cytosolic components, p47^{phox}, p67^{phox} and p40^{phox}, have to translocate to the membrane upon activation. This is a tightly and intricately controlled process that involves, among others, several low-molecular weight GTP-binding proteins. Gp91^{phox} is encoded on the X-chromosome and p22^{phox}, p47^{phox} and p67^{phox} on different autosomal chromosomes, and a defect in one of these components leads to CGD. This explains the variable mode of inheritance seen in this syndrome.

Clinically CGD manifests itself typically already at a very young age with recurrent and serious infections, most often caused by catalase-positive pathogens.

Modern treatment options, including prophylaxis with trimethoprim-sulfamethoxazole and rIFN-γ as well as early and aggressive anti-infection therapy, have improved the prognosis of this disease dramatically.

CGD, as a very well-characterized inherited affection of the hematopoietic stem cells, is predestined to be among the first diseases to profit from the advances in cutting-edge therapeutics, such as gene therapy and in utero stem cell transplantation.

References

1. Abo A, Pick E, Hall A, Totty N, Teahan CG, Segal AW (1991) Activation of the NADPH oxidase involves the small GTP-binding protein p21^{rac1}. Nature 353 : 668
2. Aliabadi H, Gonzalez R, Quie PG (1989) Urinary tract disorders in patients with chronic granulomatous disease. N Engl J Med 321 : 706
3. Ament ME, Ochs HD (1973) Gastrointestinal manifestations of chronic granulomatous disease. N Engl J Med 288 : 382
4. Bemiller LS, Roberts DH, Starko KM, Curnutte JT (1995) Safety and effectiveness of long-term interferon gamma therapy in patients with chronic granulomatous disease. Blood Cells Mol Dis 21 : 239
5. Berendes H, Bridges RA, Good RA (1957) Fatal granulomatous disease of childhood: clinical study of new syndrome. Minn Med 40 : 309
6. Bokoch GM, Quilliam LA, Bohl BP, Jesaitis AJ, Quinn MT (1991) Inhibition of Rap1A binding to cytochrome b558 of NADPH oxidase by phosphorylation of Rap1A. Science 254 : 1794
7. Brandrup F, Koch C, Petri M, Schiodt M, Johansen KS (1981) Discoid lupus erythematosus-like lesions and stomatitis in female carriers of X-linked chronic granulomatous disease. Br J Dermatol 104 : 495
8. Chin TW, Stiehm ER, Falloon J, Gallin JI (1987) Corticosteroids in treatment of obstructive lesions of chronic granulomatous disease. J Pediatr 111 : 349
9. Cowan MJ, Golbus M (1994) In utero hematopoietic stem cell transplants for inherited diseases. Am J Pediatr Hematol Oncol 16 : 35
10. Cross AR, Curnutte JT (1995) The cytosolic activating factors p47^{phox} and p67^{phox} have distinct roles in the regulation of electron flow in NADPH oxidase. J Biol Chem 270 : 6543

11. Cross AR, Yarchover JL, Curnutte JT (1994) The superoxide-generating system of human neutrophils possesses a novel diaphorase activity. Evidence for distinct regulation of electron flow within NADPH oxidase by p67-phox and p47-phox. J Biol Chem 269 : 21 448
12. Curnutte JT (1985) Activation of human neutrophil nicotinamide adenine dinucleotide phosphate, reduced (trisphosphopyridine nucleotide, reduced) oxidase by arachidonic acid in a cell-free system. J Clin Invest 75 : 1740
13. Curnutte JT, Kipnes, RS, Babior BM (1975) Defect in pyridine nucleotide dependent superoxide production by a particulate fraction from the granulocytes of patients with chronic granulomatous disease. N Engl J Med 293 : 628
14. Curnutte JT, Whitten DM, Babior BM (1974) Defective superoxide production by granulocytes from patients with chronic granulomatous disease. N Engl J Med 290 : 593
15. Danziger RN, Goren AT, Becker J, Greene JM, Douglas SD (1993) Outpatient management with oral corticosteroid therapy for obstructive conditions in chronic granulomatous disease. J Pediatr 122 : 303
16. De Boer M, Bolscher BGJM, Sijmons RH, Scheffer H, Weening RS, Roos D (1992) Prenatal diagnosis in a family with X-linked chronic granulomatous disease with the use of the polymerase chain reaction. Prenat Diagn 12 : 773
17. De Leo FR, Ulman KV, Davis AR, Jutila KL, Quinn MT (1996) Assembly of the human neutrophil NADPH oxidase involves binding of $p67^{phox}$ and flavocytochrome b to a common functional domain in $p47^{phox}$. J Biol Chem 271 : 17 013
18. De Mendez I, Garrett MC, Adams AG, Leto TL (1994) Role of p67-phox SH3 domains in assembly of the NADPH oxidase system. J Biol Chem 269 : 16 326
19. Diekmann D, Abo A, Johnston C, Segal AW, Hall A (1994) Interaction of Rac with p67phox and regulation of phagocytic NADPH oxidase activity. Science 265 : 531
20. Dinauer MC, Orkin SH, Brown R, Jesaitis AJ, Parkos CA (1987) The glycoprotein encoded by the X-linked chronic granulomatous disease locus is a component of the neutrophil cytochrome b complex. Nature 327 : 717
21. Dinauer MC, Pierce EA, Bruns GA, Curnutte JT, Orkin SH (1990) Human neutrophil cytochrome b light chain (p22-phox). Gene structure, chromosomal location, and mutations in cytochrome-negative autosomal recessive chronic granulomatous disease. J Clin Invest 86 : 1729
22. Dinauer MC, Pierce EA, Erickson RW, Mühlebach TJ, Messner H, Orkin SH, Seger RA, Curnutte JT (1991) Point mutation in the cytoplasmic domain of the neutrophil p22-phox cytochrome b subunit is associated with a nonfunctional NADPH oxidase and chronic granulomatous disease. Proc Natl Acad Sci USA 88 : 11 231
23. Ding CJ, Kume A, Björgvinsdóttir H, Hawley RG, Pech N, Dinauer MC (1996) High-level reconstitution of respiratory burst activity in a human X-linked chronic granulomatous disease (X-CGD) cell line and correction of murine X-CGD bone marrow cells by retroviral-mediated gene transfer of human $gp91^{phox}$. Blood 88 : 1834
24. Dusi S, Donini M, Rossi F (1996) Mechanisms of NADPH oxidase activation: translocation of p40-phox, Rac1 and Rac2 from the cytosol to the membranes in human neutrophils lacking p47-phox or p67-phox. Biochem J 314 : 409
25. El Benna J, Faust LP, Johnson JL, Babior BM (1996) Phosphorylation of the respiratory burst oxidase subunit p47phox as determined by two-dimensional phosphopeptide mapping. Phosphorylation by protein kinase C, protein kinase A, and a mitogen-activated protein kinase. J Biol Chem 271 : 6374
26. Finan P, Shimizu Y, Gout I, Hsuan J, Truong O, Butcher C, Bennett P, Waterfield MD, Kellie S (1994) An SH3 domain and proline-rich sequence mediate an interaction between two components of the phagocyte NADPH oxidase complex. J Biol Chem 269 : 13 752
27. Fischer A, Griscelli C, Friedrich W, Kubanek B, Levinsky R, Morgan G, Vossen J, Wagemaker G, Landais P (1986) Bone-marrow transplantation for immunodeficiencies and osteopetrosis: European survey, 1968–1985. Lancet II : 1080
28. Flake AW, Roncarolo MG, Puck JM, Almeida Porada G, Evans MI, Johnson MP, Abella EM, Harrison DD, Zanjani ED (1996) Treatment of X-linked severe combined immunodeficiency by in utero transplantation of paternal bone marrow. N Engl J Med 335 : 1806
29. Francke U, Hsieh CL, Foellmer BE, Lomax KJ, Malech HL, Leto TL (1990) Genes for two autosomal recessive forms of chronic granulomatous disease assigned to 1q25 (NCF2) and 7q11.23 (NCF1). Am J Hum Genet 47 : 483
30. Freeman JL, Lambeth JD (1996) NADPH oxidase activity is independent of $p47^{phox}$ in vitro. J Biol Chem 271 : 22 578

31. Fuchs A, Dagher M-C, Vignais PV (1995) Mapping the domains of interaction of $p40^{phox}$ with both $p47^{phox}$ and $p67^{phox}$ of the neutrophil oxidase complex using the two-hybrid system. J Biol Chem 270 : 5695
32. Fuchs A, Dagher MC, Fauré J, Vignais PV (1996) Topological organization of the cytosolic activating complex of the superoxide-generating NADPH-oxidase. Pinpointing the sites of interaction between p47phox, p67phox and p40phox using the two-hybrid system. Biochim Biophys Acta 1312 : 39
33. Görlach A, Roesler J, Hopkins PJ, Christensen BL, Lee P, Green ED, Chanock SJ, Curnutte JT (1995) The p47-phox gene has a pseudogene carrying the most common mutation for p47-phox deficient chronic granulomatous disease. Blood 86 : 206 a
34. Heyworth PG, Bohl BP, Bokoch GM, Curnutte JT (1994) Rac translocates independently of the neutrophil NADPH oxidase components $p47^{phox}$ and $p67^{phox}$. Evidence for its interaction with flavocytochrome b_{558}. J Biol Chem 269 : 30 749
35. Ho CML, Vowels MR, Lockwood L, Ziegler JB (1996) Successful bone marrow transplantation in a child with X-linked chronic granulomatous disease. Bone Marrow Transplant 18 : 213
36. Huang J, Hitt ND, Kleinberg ME (1995) Stoichiometry of p22-phox and gp91-phox in phagocyte cytochrome b_{558}. Biochemistry 34 : 16 753
37. International Chronic Granulomatous Disease Cooperative Study Group (1991) A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease. N Engl J Med 324 : 509
38. Jackson SH, Gallin JI, Holland SM (1995) The p47phox mouse knock-out model of chronic granulomatous disease. J Exp Med 182 : 751
39. Johnston RB, Newman SL (1977) Chronic granulomatous disease. Pediatr Clin North Am 24 : 365
40. Kamani N, August CS, Campbell DE, Hassan NF, Douglas SD (1988) Marrow transplantation in chronic granulomatous disease: an update, with 6-year follow-up. J Pediatr 113 : 697
41. Knaus UG, Heyworth PG, Evans T, Curnutte JT, Bokoch GM (1991) Regulation of phagocyte oxygen radical production by the GTP-binding protein Rac 2. Science 254 : 1512
42. Koshkin V, Lotan O, Pick E (1996) The cytosolic component $p47^{phox}$ is not a sine qua non participant in the activation of NADPH oxidase but is required for optimal superoxide production. J Biol Chem 271 : 30 326
43. Koshkin V, Lotan O, Pick E (1997) Electron transfer in the superoxide-generating NADPH oxidase complex reconstituted in vitro. Biochim Biophys Acta 1319 : 139
44. Landing BH, Shirkey HS (1957) A syndrome of recurrent infection and infiltration of viscera by pigmented lipid histiocytes. Pediatrics 20 : 431
45. Leto TL, Adams AG, Mendez I de (1994) Assembly of the phagocyte NADPH oxidase: binding of Src homology 3 domains to proline-rich targets. Proc Natl Acad Sci USA 91 : 10 650
46. Leto TL, Lomax KJ, Volpp BD, Nunoi H, Sechler JM, Nauseef WM, Clark RA, Gallin JI, Malech HL (1990) Cloning of a 67-kD neutrophil oxidase factor with similarity to a noncatalytic region of p60c-src. Science 248 : 727
47. Leusen JHW, Bolscher BGJM, Hilarius PM, Weening RS, Kaulfersch W, Seger RA, Roos D, Verhoeven AJ (1994) ^{156}Pro → Gln substitution in the light chain of cytochrome b_{558} of the human NADPH oxidase (p22-phox) leads to defective translocation of the cytosolic proteins p47-phox and p67-phox. J Exp Med 180 : 2329
48. Leusen JHW, Boer M de, Bolscher BGJM, Hilarius PM, Weening RS, Ochs HD, Roos D, Verhoeven AJ (1994) A point mutation in gp91-phox of cytochrome b_{558} of the human NADPH oxidase leading to defective translocation of the cytosolic proteins p47-phox and p67-phox. J Clin Invest 93 : 2120
49. Leusen JHW, Klein A de, Hilarius PM, Åhlin A, Palmblad J, Smith CIE, Diekmann D, Hall A, Verhoeven AJ, Roos D (1996) Disturbed interaction of p21-rac with mutated p67-phox causes chronic granulomatous disease. J Exp Med 184 : 1243
50. Li F, Linton GF, Sekhsaria S, Whiting Theobald N, Katkin JP, Gallin JI, Malech HL (1994) CD 34^+ peripheral blood progenitors as a target for genetic correction of the two flavocytochrome *b*558 defective forms of chronic granulomatous disease. Blood 84 : 53
51. Malech HL, Sekhsaria S, Whiting-Theobald N, Linton GF, Vowells SJ, Li F, Miller JA, Holland SM, Leitman SF, Carter CS, Read EJ, Butz R, Wannebo C, Fleisher TA, Deans RJ, Spratt SK, Maack CA, Rokovich JA, Cohen LK, Maples PB, Gallin JI (1996) Prolonged detection of oxidase-positive neutrophils in the peripheral blood of five patients following a single cycle of gene therapy for chronic granulomatous disease. Blood 10 : 486 a
52. Mardiney M III, Jackson SH, Spratt SK, Li F, Holland SM, Malech HL (1997) Enhanced host defense after gene transfer in the murine $p47^{phox}$-deficient model of chronic granulomatous disease. Blood 89 : 2268

53. Margolis DM, Melnick DA, Alling DW, Gallin JI (1990) Trimethoprim-sulfamethoxazole prophylaxis in the management of chronic granulomatous disease. J Infect Dis 162 : 723
54. McPhail LC, Shirley PS, Clayton CC, Snyderman R (1985) Activation of the respiratory burst enzyme from human neutrophils in a cell-free system. J Clin Invest 75 : 1735
55. Meerhof LJ, Roos D (1986) Heterogeneity in chronic granulomatous disease detected with an improved nitroblue tetrazolium slide test. J Leukoc Biol 39 : 699
56. Mills EL, Rholl KS, Quie PG (1980) X-linked inheritance in females with chronic granulomatous disease. J Clin Invest 66 : 332
57. Mizuno T, Kaibuchi K, Ando S, Musha T, Hiraoka K, Takaishi K, Asada M, Nunoi H, Matsuda I, Takai Y (1992) Regulation of the superoxide-generating NADPH oxidase by a small GTP-binding protein and its stimulatory and inhibitory GDP/GTP exchange proteins. J Biol Chem 267 : 10 215
58. Morimatsu T, Kawagoshi A, Yoshida K, Tamura M (1997) Actin enhances the activation of human neutrophil NADPH oxidase in a cell-free system. Biochem Biophys Res Commun 230 : 206
59. Mouy R, Fischer A, Vilmer E, Seger R, Griscelli C (1989) Incidence, severity, and prevention of infections in chronic granulomatous disease. J Pediatr 114 : 555
60. Mouy R, Veber F, Blanche S, Donadieu J, Brauner R, Levron J-C, Griscelli C, Fischer A (1994) Long-term itraconazole prophylaxis against Aspergillus infections in thirty-two patients with chronic granulomatous disease. J Pediatr 125 : 998
61. Mühlebach TJ, Gabay J, Nathan CF, Erny C, Dopfer G, Schroten H, Wahn V, Seger RA (1992) Treatment of patients with chronic granulomatous disease with recombinant human interferon-gamma does not improve neutrophil oxidative metabolism, cytochrome *b*558 content or levels of four anti-microbial proteins. Clin Exp Immunol 88 : 203
62. Newburger PE, Skalnik DG, Hopkins PJ, Eklund EA, Curnutte JT (1994) Mutations in the promoter region of the gene for gp91-phox in X-linked chronic granulomatous disease with decreased expression of cytochrome *b*558. J Clin Invest 94 : 1205
63. Parkos CA, Allen RA, Cochrane CG, Jesaitis AJ (1987) Purified cytochrome *b* from human plasma membrane is comprised of two polypeptides with relative molecular weights of 91,000 and 22,000. J Clin Invest 80 : 732
64. Parkos CA, Dinauer MC, Walker LE, Allen RA, Jesaitis AJ, Orkin SH (1988) Primary structure and unique expression of the 22-kilodalton light chain of human neutrophil cytochrome *b*. Proc Natl Acad Sci USA 85 : 3319
65. Pollock JD, Williams DA, Gifford MA, Li LL, Du X, Fisherman J, Orkin SH, Doerschuk CM, Dinauer MC (1995) Mouse model of X-linked chronic granulomatous disease, an inherited defect in phagocyte superoxide production. Nat Genet 9 : 202
66. Ponting CP (1996) Novel domains in NADPH oxidase subunits, sorting nexins, and PtdIns 3-kinases: binding partners of SH3 domains. Protein Sci 5 : 2353
67. Porter CD, Parkar MH, Verhoeven AJ, Levinsky RJ, Collins MK, Kinnon C (1994) p22-phox-deficient chronic granulomatous disease: reconstitution by retrovirus-mediated expression and identification of a biosynthetic intermediate of gp91-phox. Blood 84 : 2767
68. Quinn MT, Mullen ML, Jesaitis AJ (1992) Human neutrophil cytochrome *b* contains multiple hemes. Evidence for heme associated with both subunits. J Biol Chem 267 : 7303
69. Quinn MT, Mullen ML, Jesaitis AJ, Linner JG (1992) Subcellular distribution of the Rap1A protein in human neutrophils: colocalization and cotranslocation with cytochrome *b*559. Blood 79 : 1563
70. Roesler J, Hecht M, Freihorst J, Lohmann Matthes ML, Emmendorffer A (1991) Diagnosis of chronic granulomatous disease and of its mode of inheritance by dihydrorhodamine 123 and flow microcytofluorometry. Eur J Pediatr 150 : 161
71. Roesler J, Görlach A, Rae J, Hopkins PJ, Lee P, Curnutte JT, Chanock SJ, (1995) Recombination events between the normal p47-phox gene and a highly homologous pseudogene are the main cause of autosomal recessive chronic granulomatous disease (CGD). Blood 86 : 260 a
72. Roos D (1996) X-CGDbase: a database of X-CGD-causing mutations. Immunol Today 17 : 517
73. Roos D, Boer M de, Borregaard N, Bjerrum OW, Valerius NH, Seger RA, Mühlebach T, Belohradsky BH, Weening RS (1992) Chronic granulomatous disease with partial deficiency of cytochrome *b*558 and incomplete respiratory burst: variants of the X-linked, cytochrome *b*558-negative form of the disease. J Leukoc Biol 51 : 164
74. Roos D, Boer M de, Kuribayashi F, Meischl C, Weening RS, Segal AW, Åhlin A, Nemet K, Hossle JP, Bernatowska-Matuszkiewicz E, Middleton-Price H (1996) Mutations in the X-linked and autosomal recessive forms of chronic granulomatous disease. Blood 87 : 1663

75. Royer-Pokora B, Kunkel LM, Monaco AP, Goff SC, Newburger PE, Baehner RL, Cole FS, Curnutte JT, Orkin SH (1986) Cloning the gene for an inherited human disorder-chronic granulomatous disease-on the basis of its chromosomal location. Nature 322 : 32
76. Sathyamoorthy M, Mendez I de, Adams AG, Leto TL (1997) $p40^{phox}$ down-regulates NADPH oxidase activity through interactions with its SH3 domain. J Biol Chem 272 : 9141
77. Segal AW (1987) Absence of both cytochrome b_{-245} subunits from neutrophils in X-linked chronic granulomatous disease. Nature 326 : 88
78. Segal AW, Heyworth PG, Cockcroft S, Barrowman MM (1985) Stimulated neutrophils from patients with autosomal recessive chronic granulomatous disease fail to phosphorylate a Mr 44,000 protein. Nature 316 : 547
79. Segal AW, West I, Wientjes F, Nugent JH, Chavan AJ, Haley B, Garcia RC, Rosen H, Scrace G (1992) Cytochrome b_{-245} is a flavocytochrome containing FAD and the NADPH-binding site of the microbicidal oxidase of phagocytes. Biochem J 284 : 781
80. Sillevis Smitt JH, Weening RS, Krieg SR, Bos JD (1990) Discoid lupus erythematosus-like lesions in carriers of X-linked chronic granulomatous disease. Br J Dermatol 122 : 643
81. Smith RM, Connor JA, Chen LM, Babior BM (1996) The cytosolic subunit $p67^{phox}$ contains an NADPH-binding site that participates in catalysis by the leukocyte NADPH oxidase. J Clin Invest 98 : 977
82. Sokolic RA, Sekhsaria S, Sugimoto Y, Whiting Theobald N, Linton GF, Li F, Gottesman MM, Malech HL (1996) A bicistronic retrovirus vector containing a picornavirus internal ribosome entry site allows for correction of X-linked CGD by selection for MDR1 expression. Blood 87 : 42
83. Speert DP, Bond M, Woodman RC, Curnutte JT (1994) Infection with *Pseudomonas cepacia* in chronic granulomatous disease: role of nonoxidative killing by neutrophils in host defense. J Infect Dis 170 : 1524
84. Sumimoto H, Kage Y, Nunoi H, Sasaki H, Nose T, Fukumaki Y, Ohno M, Minakami S, Takeshige K (1994) Role of Src homology 3 domains in assembly and activation of the phagocyte NADPH oxidase. Proc Natl Acad Sci USA 91 : 5345
85. Taylor WR, Jones DT, Segal AW (1993) A structural model for the nucleotide binding domains of the flavocytochrome b_{-245} beta-chain. Protein Sci 2 : 1675
86. Teahan CG, Rowe P, Parker P, Totty N, Segal AW (1987) The X-linked chronic granulomatous disease gene codes for the beta chain of cytochrome b_{-245}. Nature 327 : 720
87. Thrasher AJ, De Alwis M, Casimir CM, Kinnon C, Page K, Lebkowski J, Segal AW, Levinsky RJ (1995) Functional reconstitution of the NADPH-oxidase by adeno-associated virus gene transfer. Blood 86 : 761
88. Wallach TM, Segal AW (1996) Stoichiometry of the subunits of flavocytochrome b_{558} of the NADPH oxidase of phagocytes. Biochem J 320 : 33
89. Wallach TM, Segal AW (1997) Analysis of glycosylation sites on gp91phox, the flavocytochrome of the NADPH oxidase, by site-directed mutagenesis and translation in vitro. Biochem J 321 : 583
90. Weening RS, Kabel P, Pijman P, Roos D (1983) Continous therapy with sulfamethoxazole-trimethoprim in patients with chronic granulomatous disease. J Pediatr 103 : 127
91. Weening RS, Adriaansz LH, Weemaes CM, Lutter R, Roos D (1985) Clinical differences in chronic granulomatous disease in patients with cytochrome *b*-negative or cytochrome *b*-positive neutrophils. J Pediatr 107 : 102
92. Weening RS, Leitz GJ, Seger RA (1995) Recombinant human interferon-gamma in patients with chronic granulomatous disease – European follow-up study. Eur J Pediatr 154 : 295
93. Weil WM, Linton GF, Whiting-Theobald N, Vowells SJ, Rafferty SP, Li F, Malech HL (1997) Genetic correction of $p67^{phox}$ deficient chronic granulomatous disease using peripheral blood progenitor cells as a target for retrovirus mediated gene transfer. Blood 89 : 1754
94. Wientjes FB, Hsuan JJ, Totty NF, Segal AW (1993) p40phox, a third cytosolic component of the activation complex of the NADPH oxidase to contain src homology 3 domains. Biochem J 296 : 557
95. Woodman RC, Newburger PE, Anklesaria P, Erickson RW, Rae J, Cohen MS, Curnutte JT (1995) A new X-linked variant of chronic granulomatous disease characterized by the existence of a normal clone of respiratory burst-competent phagocytic cells. Blood 85 : 231
96. Zhan S, Vazquez N, Wientjes FB, Budarf ML, Schrock E, Ried T, Green ED, Chanock SJ (1996) Genomic structure, chromosomal localization, start of transcription, and tissue expression of the human p40-phox, a new component of the nicotinamide adenine dinucleotide phosphate-oxidase complex. Blood 88 : 2714