Arch Immunol Ther Exp, 2004, 52, 113–120
PL ISSN 0004-069X

WWW.AITE–ONLINE.ORG
Review

Received: 2003.11.10
Accepted: 2003.12.11
Published: 2004.04.15

Genetic and biochemical background of chronic granulomatous disease

Monika Jurkowska[1, 2], Ewa Bernatowska[3] and Jerzy Bal[1]

[1] National Research Institute of Mother and Child, Warsaw, Poland
[2] Postgraduate School of Molecular Medicine, Medical University of Warsaw, Poland
[3] Children's Memorial Health Institute, Warsaw, Poland

Source of support: self financing

Summary

Chronic granulomatous disease (CGD) is a rare inherited immunodeficency syndrome caused by a profound defect in the oxygen metabolic burst machinery. Activity of NADPH oxidase is absent or profoundly diminished, as at least one of its components ($gp91^{phox}$, $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$) is lacking or non-functional. This review explains the molecular basis of NADPH oxidase dysfunction by the effects of mutations in genes coding for particular oxidase components. Among the four types of CGD, the most common is X--linked CGD (approximately 65%), with defects in the *CYBB* gene encoding $gp91^{phox}$. A wide spectrum of mutations has been described in the *CYBB* gene with no predominant genotype. The second most common subtype of CGD caused by *NCF1* mutation accounts for 30% of CGD patients and is inherited in an autosomal recessive manner, with predominance of a homozygotous ΔGT deletion in the genotype. The other two CGD subtypes having an autosomal recessive pattern together account for no more than 10% of CGD cases. A strategy for the molecular diagnostics in CGD patients is proposed and principles of genetic counseling are discussed here.

Key words: **CGD • NADPH oxidase • genes • molecular diagnostics**

Full-text PDF: http://www.aite-online/pdf/vol_52/no_2/5240.pdf

Author's address: Monika Jurkowska, Department of Medical Genetics, Institute of Mother and Child, Kasprzaka 17a, 01-211 Warsaw, Poland, tel./fax: +48 22 32 77 200, e-mail: mjurkowska@imid.med.pl or mjurkowska@gazeta.pl

CLINICAL FEATURES OF CHRONIC GRANULOMATOUS DISEASE

Chronic granulomatous disease (CGD; OMIM (http://www.ncbi.nlm.nih.gov/Omim/) 306400, 233690, 233700, 233710) is a rare inherited immunodeficiency syndrome seen in approximately 1 in 250 000 individuals without major regard to ethnic background. The disease is caused by a profound defect in the oxygen metabolic burst that normally accompanies phagocytosis in all myeloid cells: neutrophils, eosinophils, monocytes and macrophages.

Biochemically, CGD is characterized by the inability of phagocytic leukocytes to generate the reactive oxygen compounds which are needed for the intracellular killing of phagocytized microorganisms[9]. The oxygen derivatives created by NADPH oxidase during the burst (superoxide, hydrogen peroxide, hypohalous acids, and hydroxyl radical) play a critical role in the killing of pathogenic bacteria and fungi. Therefore, when the system fails the most common pathogens encountered in CGD patients are catalase-positive organisms, because catalase prevents the CGD phagocytes from using microbial-generated hydrogen peroxide to kill these pathogens.

Typical infections are pneumonia, lymphadenitis, cutaneous, hepatic and perirectal abscesses, osteomyelitis, sepsis caused by *Staphylococcus aureus, Aspergillus spp., Candida albicans, Escherichia coli, Salmonella spp., Brukholderia cepacia (Pseudomonas cepacia)*, and other Gram-negative bacteria. In addition, CGD patients suffer from diffuse granulomas of the esophagus, stomach, biliary system, brain, ureters, or urinary bladder, presumably caused by microbes. Granulomas are an important cause of chronic complications in CGD. Most CGD patients develop first symptoms during early childhood; for some, residual respiratory burst activity CGD can present fully in adolescence[18].

MOLECULAR BACKGROUND OF THE CGD PHENOTYPE

The heterogeneous group of CGD patients lacks the activity of NADPH oxidase, defined by immunohistochemistry as the absence one or more of its components, i.e. gp91^{phox}, p22^{phox}, p47^{phox} and p67^{phox} (Table 1). In rare cases, the functional activity of one of these components is significantly decreased, also resulting in the CGD phenotype. Generally, the phenotype of NADPN oxidase deficiency is quite easy to detect, although sometimes other host defense system defects or a mild course of the disease may be confusing.

Biochemical differential diagnosis of CGD is based on respiratory burst measurement manifested in oxygen consumption, superoxide (O_2^-) generation in the nitroblue tetrazolium test (NBT), or hydrogen peroxide production. Chemiluminescence and flow cytometry can also follow these parameters.

The complex structure of the bust machinery enables numerous possible defect sites. In fact, it is not reflected in the uniform phenotype represented by CGD patients. No matter which element fails, complete lack of or (in rare cases) a profound decrease in oxidase burst is observed with all clinical consequences.

NADPH oxidase assembly

In resting, non-phagocytizing leukocytes, NADPH oxidase lacks activity and the enzyme components are localized in different parts of the cell. Phagocyte activation, e.g. by the binding of opsonized microorganisms to cell-surface receptors, leads to assembly of the active enzyme complex bound together with Src homology 3 (SH3) domains that interact with proline-rich regions (Fig. 1). Oxidase activation needs at least five different protein factors. In fact, during the course of neutrophil stimulation, the cytosolic factors p47^{phox}, p67^{phox}/p40^{phox}, and GTPase Rac1/2 translocate to the plasma membrane and associate with cytochrome b_{558}[11]. Cytochrome b_{558} is a flavohemoprotein consisting of p22^{phox} and heavily glycosylated gp91^{phox} subunits: this is the redox oxidase center that catalyzes the electron transfer from NADPH to oxygen ($NADPH + 2O_2 \rightarrow NADP^+ + 2O_2^- + H^+$)[35]. It binds two non-identical hemes with two pairs of histidines[8] and at least one flavin-adenine-dinucleotide

Table 1. Characteristic of NADPH oxidase components

Gene	Protein	Cellular location	Assembling domains	Aberrant in CGD (%)
CYBB	gp91^{phox}	membrane	–	~ 65
NCF1	p47^{phox}	cytosol	two proline-rich, two SH3 motifs	~ 25
NCF2	p67^{phox}	cytosol	one proline-rich, two SH3 motifs	~ 5
CYBA	p22^{phox}	membrane	one proline-rich motif	~ 5
NCF4	p40^{phox}	cytosol	one SH3 motif	–

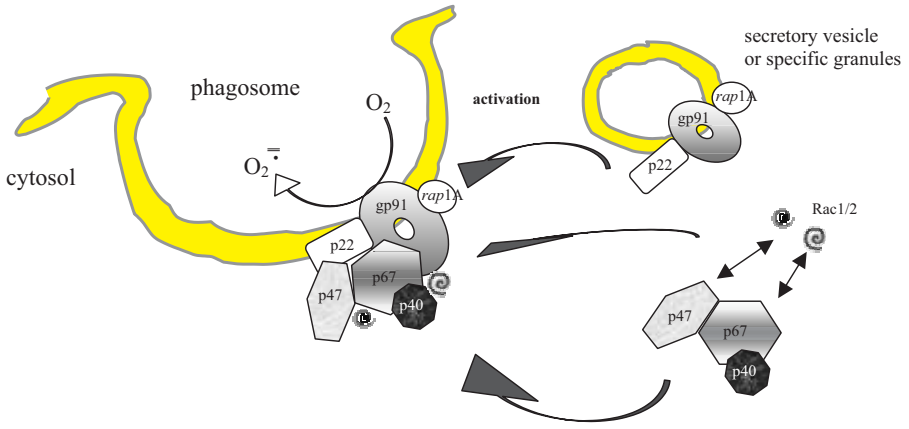

Figure 1. Hypothetical assembly of the NADPH oxidase in phagocytic cells[32].

(FAD). The identity of the cytosolic component(s) responsible for causing the conformational change in gp91^{phox} is a still an unsettled issue, as is the question of whether this is the consequence of direct interaction of the cytosolic component(s) with gp91^{phox} or an event secondary to its binding to the p22^{phox} subunit.

In resting neutrophils, this flavocytochrome is localized together with *rap*1A protein in the membranes of specific granules or a secretory vesicle. During cell activation, fusion of these organelles with the plasma membrane leads to re-allocation of the oxidase. At the same time, the p47-p67-p40 complex translocates to the plasma membrane and forms a complex with the cytochrome b_{558}. p47^{phox} and p67^{phox} are phosphorylated upon activation of the enzyme complex[17]. In addition, one or more cytosolic GTP-binding proteins appear to be required for oxidase activity[16]. For example, Rac-GTP not only binds to the regulatory p67^{phox}, but also interacts directly with the oxidase flavocytochrome and can initiate a signaling pathway leading to the translocation of cytosolic subunits and assemblage of the enzyme complex[15]. Binding of p67^{phox} to the cytochrome *b* permits electron flow from NADPH to FAD, whereas p47^{phox}-binding is necessary for electron flow from FAD via the hemes groups to the oxygen[5].

The NADPH oxidase enzyme system forms a small transmembrane electron-transport system that results in the oxidation of NADPH on the cytoplasmic surface and the generation of superoxide on the outer surface of the membrane, which in turn becomes the inner surface of the phagosome when invagination occurs during phagocytosis.

Analysis of the defects responsible for CGD has helped to define many of the biochemical and molecular features of this complex system. Individual protein constituents and their genes have been identified and cloned.

The *CYBB* gene (GenBank see http://www.ncbi.nlm.nih.gov/Entrez/) accession number X04011), which encodes gp91^{phox}, was one of the first to be identified by positional cloning[34] following chromosomal localization to Xp21.1[1]. Other human genes encoding the major components of NADPH oxidase are mapped each to a different chromosome: *NCF1* (p47^{phox}) to 7q11.23, *NCF2* (p67^{phox}) to 1q25, *CYBA* (p22^{phox}) to 16q24, and *NCF4* encoding p40^{phox} to 22q13.1. Up to now, mutations in four genes of the NADPH oxidase complex (except in *NCF4*) have been associated with CGD. A single missense mutation in Rac2 has recently been reported to cause a CGD-like condition in one individual. The patient was heterozygous for the mutation but severely affected, suggesting that the amino acid substitution acts in a dominant, negative fashion[37].

gp91^{phox}

Several studies have probed indirectly the structure of the gp91^{phox} glycoprotein molecule and its molecular interactions, but the locations of its functional domains have been inferred mostly by sequence homology rather than by direct demonstration.

gp91^{phox} functions as a flavodehydrogenase, and from sequence comparison between the C-terminal half of gp91^{phox} and the ferredoxin-NADP1 reductase flavoenzyme family the putative location of the FAD--binding and NADPH-binding domains within gp91^{phox} have been deduced[33]. A recent report, showing that inward H^+ currents are absent in cells from a gp91^{phox}-lacking patient, suggested that gp91^{phox} behaves as an unusual H^+ channel that allows H^+ influx and cytosolic acidification[2]. Expression of full-

Table 2. Properties of genes involved in CGD

Gene	Location	Size (kb)	Exons	mRNA size (kb)	Amino acids	Modifications
CYBB	Xp21.1	30	13	4.7	570	glycosylated
NCF1	7q11.23	18	9	1.4	390	phosphorylated during activation
NCF2	1q25	40	16	2.4	526	phosphorylated during activation
CYBA	16q24	8.5	6	0.8	195	–

-length or N-truncated gp91^{phox} and mutagenesis experiments were consistent with gp91^{phox} being the H^+ channel itself[13].

Among the four types of CGD, the most common is X-linked CGD (approximately 65%), with defects in the *CYBB* gene encoding gp91^{phox}. The *CYBB* gene encompasses 13 exons spanning 30 kb of genomic DNA (Table 2). A wide spectrum of mutations (substitutions, deletions, insertions, and splice-site)[32] has been described in the *CYBB* gene, representing more than 200 different changes (http://www.uta.fi/imt/bioinfo/CYBBbase/).

The heterogeneity of mutations and the lack of any predominant genotype indicate that the worldwide incidence of the disease represents many different mutational events, with no evidence for a founder effect. Such a pattern is expected for a disorder with the phenotype of a severe immune-system defect and recurrent infections. Mutations lead to complete lack of cytochrome (gp91^0) or partial loss of *CYBB* expression (gp91$^-$). In a very few rare cases, missense mutations resulting in normal but nonfunctional levels of cytochrome b_{558} have been identified (X91$^+$). Some of these have provided interesting information about the NADPH oxidase structure and activation mechanisms. Rare cases describing X91$^+$ CGD patients have either mutations leading to substitutions in the N-terminal part of gp91^{phox}, which affect heme binding and stable interaction with p22^{phox} [6], or bear mutations in the cytosolic C-terminal part of gp91^{phox}. This region of gp91^{phox} is important for FAD- and NADPH-binding and is also involved in the recruitment of cytosolic phox proteins[27] (Fig. 2). One case was reported where cytochrome b_{558} displayed abnormal distribution within the leukocytes: it was present in the lysate but not on the surface of the cells. The mutation responsible for that phenotype was located within a probable FAD-binding sequence (His338Tyr)[26].

Several mutations were identified in the promoter region of the *CYBB* gene, one leading to the interesting phenomenon of oxidase activity restricted to eosinophils with a mild course of CGD[36]. This indicates the different mechanism of gp91^{phox} expression regulation in eosinophils.

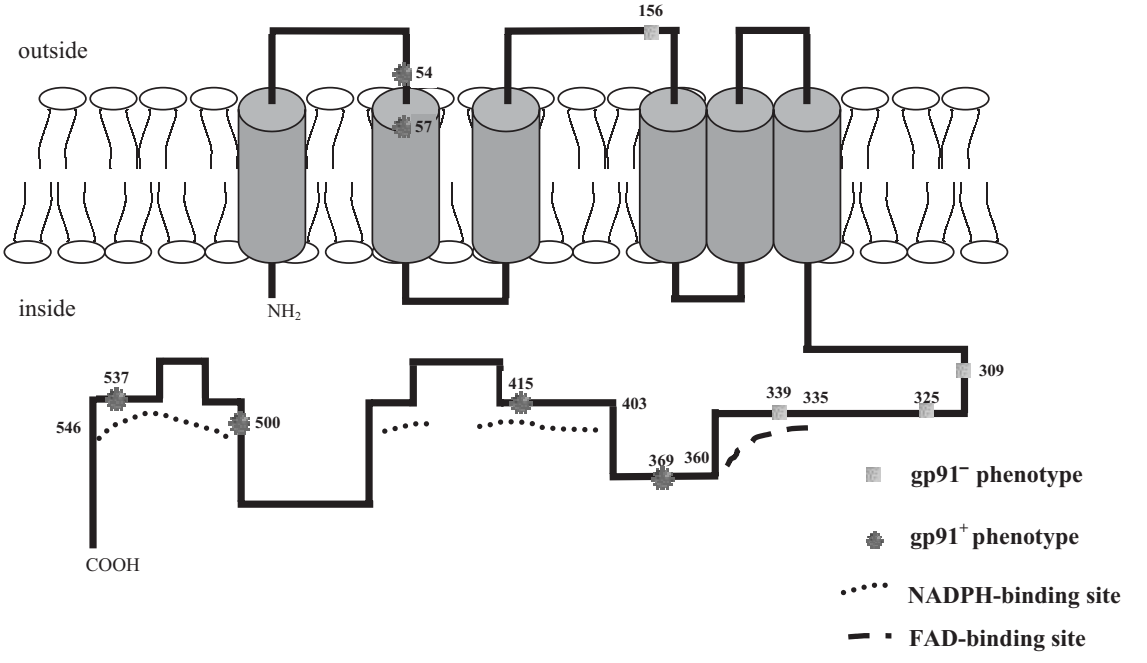

Figure 2. Schematic representation of gp91^{phox} unit of cytochrome b_{558} [29] (modified).

p47^{phox}

The second most common subtype of CGD is caused by p45^{phox} deficiency and is inherited in an autosomal recessive manner. Interestingly, it accounts for 30% of CGD patients, whereas the other two CGD subtypes that have an autosomal recessive pattern together account for no more than 10% of CGD cases.

p47^{phox} protein contains two SH3 motifs and at least one proline-rich region, both involved in oxidase assembly. In resting cells it forms a cytosolic complex with p40^{phox} and p67^{phox}, also proved to be essential for Rac2 translocation during oxidase activation.

The *NCF1* gene is around 15 kb long and consists of 11 exons varying from 55 to 165 bp in length. The product of translation is 390 amino acids. The *NCF1* promoter lacks TATA and CCAAT boxes, but several possible binding-sites for PU.1, SP-1, AP-1 and Oct1 transcription factors have been identified in the promoting region[4].

The genomic region of 7q11.23, which contains both the gene and several pseudogenes, is notable for several closely spaced, duplicated segments of DNA[12]. In fact, 50.37% of the p47 gene is a repetitive sequence. Pseudogenes of high homology (>98%) differ in only a few sites[20], including the absence of the GT sequence (ΔGT) at the beginning of exon 2, the commonest mutation in p47^{phox}-deficient patients. This mutation predicts a frameshift and premature stop codon at amino acid 51 [3]. Few additional pathogenic substitutions were identified in A47 CGD patients, and the large number of polymorphic sites suggests little dependence of p47^{phox} on a strict conformation.

Because of the redundancy in large blocs of the sequence, a physical map of the locus has been difficult to construct. Currently, published mapping studies suggest that the wild-type gene is telomeric, and two or more pseudogenes are at a maximum of 1.5–2.0 cM in the direction of the centromere, which is close by genomic standards[22]. Within the 15 kb gene there are 21 Alu sequences and nine Chi-like sequences. Interestingly, nine Alu sequences are contained within the flanking introns 1 and 2 and all are orientated in the 3' to 5' direction. The high density of Alu sequences, particularly orientated in one direction, has been associated with recombination mutational events in several disorders. Unlike the *NCF1* gene, conversions there were detected in a minority of patients, whereas they are almost exclusively detected in p47^{phox}-deficient CGD[34]. Only approximately 10% of *NCF1* mutations are of *de novo* origin.

p22^{phox}

Despite the fact that the p22^{phox} subunit does not contain redox centers, its presence is essential for NADPH oxidase assembly. In myeloid cells, the absence of p22^{phox} protein due to genetic defects also results in the loss of gp91^{phox} expression and vice versa, indicating that each of these proteins requires the other for mutual stability. p22^{phox} might serve as a linker protein between cytosolic components and gp91^{phox}. The primary structure of p22^{phox} suggests it contains 4 membrane-spanning domains in the N-terminal two-thirds of the molecule and a proline-rich domain in the C-terminal cytoplasmic tail (Fig. 3). In fact, the proline-rich domain of p22^{phox} binds the N--terminal SH3 domain of p47^{phox} [23], and this interaction is believed to play a dominant role in promoting the association of the cytosolic complex.

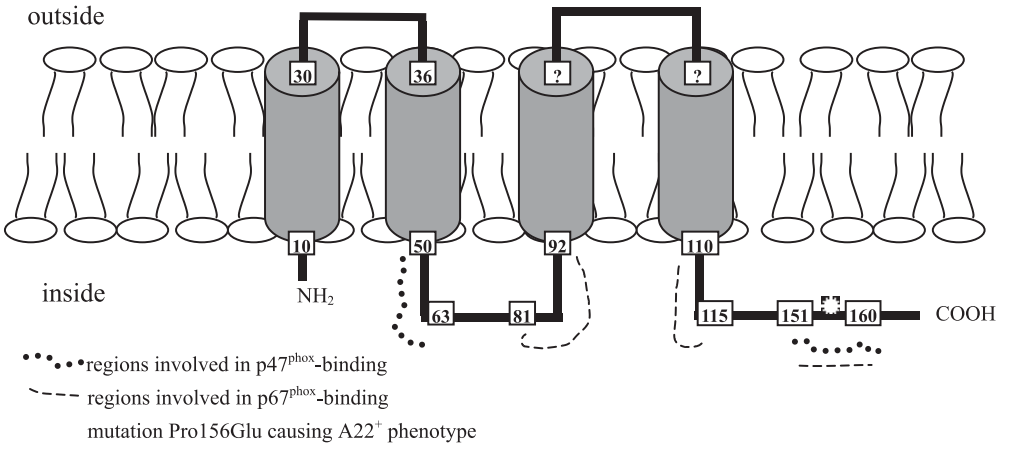

Figure 3. The membrane topology of p22^{phox} subunit, as based on "peptide walking" approach[10] (modified).

Missense mutations, resulting in complete lack of protein, cause amino acid substitutions within the transmembrane regions, whereas mutations that do not affect the synthesis of $p22^{phox}$, as well as polymorphisms, involve residues located outside the membrane[30]. Of the 10 different missense mutations known by the year 2000, only 1 results in the expression of stable protein (the $A22^{+}$ phenotype)[7]. In this case, the patient was homozygous for the substitution of proline 156 with glutamine[14]. This particular substitution was very informative functionally, as biochemical analysis showed that it resulted in the failure of $p47^{phox}$ to translocate to the membrane. Proline 156 is within a short proline-rich region in the cytoplasmic tail of $p22^{phox}$ (amino acids 151-160) and the profound effect of its alteration to glutamine highlights the importance of this region of the $p22^{phox}$ molecule in interactions with an SH3 domain in $p47^{phox}$.

$p67^{phox}$

$p67^{phox}$ deficiency is the rarest form of the disease, accounting for fewer than 6% of cases. The *NCF2* gene spans approximately 40 kb and contains 16 exons[21]. Translation results in a 526-amino-acid protein. $p67^{phox}$ contains two SH3 domains: one was found to interact with a proline-rich domain in the C terminus of $p47^{phox}$, and the other was possibly engaged in an intramolecular bond with a proline-rich domain at the center of the molecule.

As in $gp91^{phox}$ and $p22^{phox}$ deficiencies, $p67^{phox}$ CGD patients show a high degree of heterogeneity in the genetic defects that underlie their disease. The mutations in the $p67^{phox}$ gene identified in the patients reported lead to marked instability of the $p67^{phox}$ mRNA or protein (or both). That results in undetectable levels of $p67^{phox}$, a profound loss of respiratory burst activity, and a relatively severe clinical phenotype, despite the fact that the presence of other cytochrome *b* subunits remains intact[28].

These findings are in good agreement with the proposal that $p67^{phox}$ is the protein directly responsible for the induction of a conformational change in $gp91^{phox}$ and with the identification of an "activation domain" in $p67^{phox}$ responsible for interaction with cytochrome b_{558} [27]. It was implied that $p67^{phox}$ interacts directly with the $gp91^{phox}$ subunit and exclusively transports Rac1 to the complex.

$p40^{phox}$ resides in a complex with $p67^{phox}$ in the cytosol of resting neutrophils. Although it is not required for oxidase activity in a cell-free assay, it is thought to play a role in stabilizing $p67^{phox}$ as well as $p47^{phox}$ in intact cells. The level of $p40^{phox}$ is decreased in p67--deficient patients.

MOLECULAR DIAGNOSTICS OF CGD

CGD is an example of a genetic disorder where (except for X-CGD) only the discovery of a particular lesion guarantees precise molecular diagnosis and

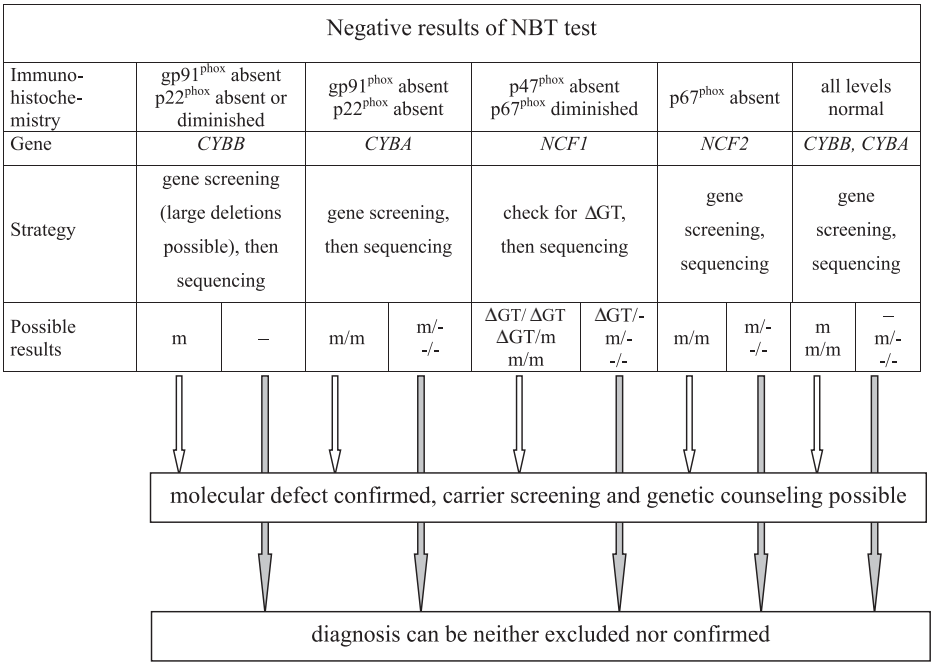

Figure 4. Proposed system of genetic testing in CGD.

enables establishment of the carrier status. This is performed by molecular biology techniques such as polymerase chain reaction, sequencing, or allele-specific restriction enzyme analysis. Also single-strand conformation polymorphism or simple restriction fragment length polymorphism can be informative in defined cases (Fig. 4).

Carrier detection is of great importance for genetic counseling and prenatal diagnosis of CGD. In the case of X-linked CGD, this is usually relatively easy, because female carriers (who are mostly healthy) generally exhibit two populations of cells (due to random X-chromosome inactivation): one positive for NADPH oxidase activity and the other negative. These distinct populations can readily be distinguished biochemically using the NBT slide test or flow cytometry.

In the case of the autosomal recessive CGD forms, the strategy is more complicated. Generally, carriers of autosomal recessive CGD have uniform populations of neutrophils that are capable of generating amounts of O_2^- within the normal range, and the individuals concerned have no obvious clinical manifestations. In the relatively few heterozygotes for p22^{phox} deficiency in whom cellular flavocytochrome b_{558} concentrations have been measured, these too appear within normal limits. Consequently, the only reliable way of testing for such carriers is by molecular genetic analysis.

The approach to carrier status establishment in prenatal diagnosis involves identifying the particular mutation in a given family and then analyzing fetal DNA directly for the mutant allele(s).

PERSPECTIVES

The mainstay of current CGD therapy is antibacterial and antifungal prophylaxis. Antibiotic dosages of trimethoprim sulfamethoxazole as the drug of choice prevent bacterial and intraconazole fungal infections[19]. In case of infection, therapy includes aggressive and prolonged application of antibiotics and prednisone and surgical drainage of abscesses or resection (when possible) of granulomas. Immunomodulatory agents, such as interferon γ, also play a role in the treatment and prevention of intractable infection. Bone marrow transplantation and gene therapy may offer the promise of complete cure in the future.

Some studies show that CGD in adults may be more common than previously assumed[25]. In view of the possibility of treatment, infection prophylaxis and genetic counseling for affected families, CGD should be excluded in any patient with unexplained infections or granulomas.

REFERENCES

1. Baehner R. L., Kunkel L. M., Monaco A. P., Haines J. L., Conneally P. M., Palmer C., Heerema N. and Orkin S. H. (1986): DNA linkage analysis of X chromosome-linked chronic granulomatous disease. Proc. Natl. Acad. Sci. USA, 83, 3398–3401.
2. Banfi B., Schrenzel J., Nusse O., Lew D. P., Ligeti E., Krause K. H. and Demaurex N. (1999): A novel H^+ conductance in eosinophils: unique characteristics and absence in chronic granulomatous disease. J. Exp. Med., 190, 183–194.
3. Casimir C. M., Bu-Ghanim H. N., Rodaway A. R. F., Bentley D. L., Rowe P. and Segal A. W. (1991): Autosomal recessive chronic granulomatous disease caused by deletion at a dinucleotide repeat. Proc. Natl. Acad. Sci. USA, 88, 2753–2757.
4. Chanock S. J., Roesler J., Zhan S., Hopkins P., Lee P., Barrett D. T., Christensen B. L., Curnutte J. T. and Gorlach A. (2000): Genomic structure of the human p47^{phox} (*NCF1*) gene. Blood Cells Mol. Dis., 26, 37–46.
5. Cross A. R. and Curnutte J. T. (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, 6543–6548.
6. Cross A. R., Heyworth P. G., Rae J. and Curnutte J. T. (1995): A variant X-linked chronic granulomatous disease patient (X91$^+$) with partially functional cytochrome b. J. Biol. Chem., 270, 8194–8200.
7. Cross A. R., Noac D., Rae J. and Heyworth P. G. (2000): Hematologically important mutations: the autosomal recessive forms of chronic granulomatous disease (first update). Blood Cells Mol. Dis., 26, 561–565.
8. Cross A. R., Rae J. and Curnutte J. T. (1995): Cytochrome b_{245} of neutrophils superoxide-generating system contains two non-identical hemes. Potentiometric studies of a mutant form of gp91^{phox}. J. Biol. Chem., 270, 17075–17077.
9. Curnutte J. T., Orkin S. H. and Dinauer M. C. (1994): Genetic disorders of phagocyte function. In Stamatoyannopoulos G., Neinhuis A. W., Majerus P. W. and Varmus H.: The molecular basis of blood diseases. WB Saunders, Philadelphia, 493–540.
10. Dahan I., Issaeva I., Gorzalczany Y., Sigal N., Hirshberg M. and Pick E. (2002): Mapping of functional domains in the of flavocytochrome b_{559} participating in the assembly of the NADPH oxidase complex by peptide walking. J. Biol. Chem., 277, 8421–8432.
11. De Leo F. R. and Quinn M. T. (1996): Assembly of the phagocyte NADPH oxidase: Molecular interaction of oxidase proteins. J. Leukocyte Biol., 60, 677–691.
12. De Silva U., Massa H., Trask B. and Green E. D. (1999): Comparative mapping of the region of human chromosome 7 deleted in Williams syndrome. Genome Res., 9, 428–436.
13. DeCoursey T. E., Cherny V. V., Zhou W. and Thomas L. L. (2000): Simultaneous activation of NADPH oxidase-related proton and electron currents in human neutrophils. Proc. Natl. Acad. Sci. USA, 97, 6885–6889.
14. Dinauer M. C., Pierce E. A., Erickson R. W., Muhlebach T. J., Messner H., Orkin S. H., Seger R. A. and Curnutte J. T. (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, 11231–11235.
15. Dinauer M. C. (2003): Regulation of neutrophil function by Rac GTPases. Curr. Opin. Hematol., 10, 8–15.

16. El Benna J. E., Ruedi J. and Babior B. (1994): Cytosolic guanine nucleotide-binding protein rac2 operates *in vivo* as a component of th eneutrophil respiratory burst oxidase. J. Biol. Chem., 269, 6729–6734.

17. Faust L., El Benna J., Babior B. and Chanock S. J. (1995): The phosphorylation targets of $p47^{phox}$, a subunit of the respiratory burst oxidase: Functions of the individual serines as evaluated by site-direct mutagenesis. J. Clin. Invest., 96, 1499–1505.

18. Finn A., Hadzik N., Morgan G., Strobel S. and Levinsky R. J. (1990): Prognosis of chronic granulomatous disease. Arch. Dis. Child, 65, 942–945.

19. Goldblatt D. (2002): Current treatment options for chronic granulomatous disease. Expert Opin. Pharmacother., 3, 857–863.

20. Görlach A., Lee P. L., Roesler J., Hopkins P. J., Christensen B., Green E. D., Chanock S. J. and Curnutte J. T. (1997): A $p47^{phox}$ pseudogene carries the most common mutation causing $p47^{phox}$--deficient chronic granulomatous disease. J. Clin. Invest., 100, 1907–1918.

21. Kenney R. T., Malech H. L., Epstein N. D., Roberts R. L. and Leto T. L. (1993): Characterization of the $p67^{phox}$ gene: genomic organization and restriction fragment length polymorphism analysis for prenatal diagnosis in chronic granulomatous disease. Blood, 82, 3739–3744.

22. Kourilsky P. (1986): Molecular mechanism for gene conversion in higher cells. Trends Genet., 2, 60.

23. Leto T. L., Adams A. G. and De Mendez I. (1994): Assembly of the phagocyte NADPH oxidase: binding of Src homology 3 domains to proline-rich targets. Proc. Natl. Acad. Sci. USA, 91, 10650–10654.

24. Leusen J. H., Verhoeven A. J. and Roos D. (1996): Interactions between the components of the human NADPH oxidase: intrigues in the phox family. J. Lab. Clin. Med., 128, 461–476.

25. Liese J. G., Jendrossek V., Jansson A., Petropoulou T., Kloos S., Gahr M. and Belohradsky B. H. (1996): Chronic granulomatous disease in adults. Lancet, 347, 220–223.

26. Lin S. J., Huang Y. F., Chen J. Y., Noack D., Wang J. Y., Lin C. Y., Chiang B. L., Yang C. M., Liu C. C. and Shieh C. C. (2002): Molecular quality control machinery contributes to the leukocyte NADPH oxidase deficiency in chronic granulomatous disease. Biochim. Biophys. Acta, 1586, 275–286.

27. Nisimoto Y., Motalebi S., Han C. H. and Lambeth J. D. (1999): The p67(phox) activation domain regulates electron flow from NADPH to flavin in flavocytochrome b(558). J. Biol. Chem., 274, 22999–23005.

28. Patino P. J., Rae J., Noack D., Erickson R., Ding J., de Olarte D. G. and Curnutte J. T. (1999): Molecular characterization of autosomal recessive chronic granulomatous disease caused by a defect of the nicotinamide adenine dinucleotide phosphate (reduced form) oxidase component $p67^{phox}$. Blood, 94, 2505–2514.

29. Rae J., Newburger P. E., Dinauer M. C., Noack D., Hopkins P. J., Kuruto R. and Curnutte J. T. (1998): X-linked chronic granulomatous disease: mutations in the *CYBB* gene encoding the $gp91^{phox}$ component of respiratory-burst oxidase. Am. J. Hum. Genet., 62, 1320–1331.

30. Rae J., Noack D., Heyworth P. G., Ellis B. A., Curnutte J. T. and Cross A. R. (2000): Molecular analysis of 9 new families with chronic granulomatous disease caused by mutations in *CYBA*, the gene encoding $p22^{phox}$. Blood, 96, 1106–1112.

31. Roesler J., Curnutte J., Rae J., Barrett D., Patino P., Chanock S. J. and Goerlach A. (2000): Recombination events between the $p47^{phox}$ gene and its highly homologous pseudogenes are the main cause of autosomal recessive chronic granulomatous disease (CGD). Blood, 95, 2150–2156.

32. Ross D., de Boer M., Kuribayashi F., Meischl C., Weening R. S., Segal A. W., Ahlin A., Nemet K., Hossle J. P., Bernatowska--Matuszkiewicz E. and Middleton-Price H. (1996): Mutations in the X-linked and autosomal recessive forms of chronic granulomatous disease. Blood, 87, 1663–1681.

33. Rotrosen D., Yeung C. L., Leto T. L., Malech H. L. and Kwong C. H. (1992): Cytochrome b558: the flavin-binding component of the phagocyte NADPH oxidase. Science, 256, 1459–1462.

34. Royer-Pokora B., Kunkel L. M., Monaco A. P., Goff S. C., Newburger P. E., Baehner R. L., Cole F. S., Curnutte J. T. and Orkin S. H. (1986): Cloning the gene for an inherited human disorder – chronic granulomatous disease – on the basis of its chromosomal location. Nature, 322, 32–38.

35. Stasia M. J., Lardy B., Maturana A., Rousseau P., Martel C., Bordigoni P., Demaurex N. and Morel F. (2002): Molecular and functional characterization of a new X-linked chronic granulomatous disease variant ($X91^{+}$) case with a double missense mutation in the cytosolic $gp91^{phox}$ C-terminal tail. Biochim. Biophys. Acta, 1586, 316–330.

36. Weening R. S., de Boer M., Kuijpers T. W., Neefjes V. M., Hack W. W. and Roos D. (2000): Point mutations in the promoter region of the CYBB gene leading to mild chronic granulomatous disease. Clin. Exp. Immunol., **122**, 410–417.

37. Williams D. A., Tao W., Yang F., Kim C., Gu Y., Mansfield P., Levine J. E., Petryniak B., Derrow C. W., Harris C., Jia B., Zheng Y., Ambruso D. R., Lowe J. B., Atkinson S. J., Dinauer M. C. and Boxer L. (2000): Dominant negative mutation of the hematopoietic-specific Rho GTPase, Rac2, is associated with a human phagocyte immunodeficiency. Blood, 96, 1646–1654.