Detection of gp91-*phox* precursor protein in B-cell lines from patients with X-linked chronic granulomatous disease as an indicator for mutations impairing cytochrome *b*₅₅₈ biosynthesis

Colin D. PORTER*†‡¶, Futoshi KURIBAYASHI†§, Mohamed H. PARKAR*||, Dirk ROOS† and Christine KINNON*

*Division of Cell and Molecular Biology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, U.K. and †Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam, The Netherlands

NADPH oxidase cytochrome *b*₅₅₈ consists of two subunits, gp91-*phox* and p22-*phox*, defects of which result in chronic granulomatous disease (CGD). The nature of the interaction between these subunits has yet to be determined. Absence of p22-*phox* in autosomal CGD patient-derived B-cell lines results in detectable levels of an incompletely glycosylated gp91-*phox* precursor. We have detected this same precursor species in four cell lines from

patients with the X-linked form of the disease due to mutations in gp91-*phox*. Such mutations should delineate regions of gp91-*phox* important for its biosynthesis, including stable association with p22-*phox*. One mutation mapped to the putative FAD-binding domain, one mapped to a potential haem-binding domain, and two involved the region encoded by exon 3.

INTRODUCTION

Four specific components comprise the phagocyte NADPH oxidase, the function of which is to generate superoxide (O₂⁻) anions at the cell surface and within the phagocytic vacuoles of macrophages and neutrophils. Two of the components, p47-*phox* and p67-*phox*, are cytosolic and are thought to have a regulatory role, while p22-*phox* and gp91-*phox* are respectively the small and large subunits of the membrane cytochrome *b*₅₅₈ (also known as *b*₋₂₄₅). Electron transfer, resulting in the reduction of molecular oxygen, takes place via cytochrome *b*₅₅₈, which contains binding sites for the cofactors FAD and haem in addition to that for NADPH [1,2]. The C-terminal non-transmembrane domain of gp91-*phox* contains putative binding sites for FAD and NADPH and has been structurally modelled, based on homologies to the ferredoxin-NADP reductase family [3]. However, the binding sites for haem within the cytochrome and the nature of the interaction between p22-*phox* and gp91-*phox* have yet to be determined. Defects in any of the four oxidase components cause chronic granulomatous disease (CGD), a primary immunodeficiency resulting from inability to cope with bacterial and fungal infections due to the failure of phagocytes to kill ingested micro-organisms [4,5]. The most common form of the disease is X-linked (X-CGD) and is due to mutations in gp91-*phox*.

Although phagocytosis is not a feature of B cells, such cells express functional NADPH oxidase, albeit at low levels relative to neutrophils. Epstein-Barr virus (EBV)-transformed B-cell lines from unaffected individuals express NADPH oxidase while those derived from CGD patients display the molecular phenotype of their disease and fail to make O₂⁻ [6,7]. Such cell lines have

been valuable for the study of CGD and NADPH oxidase. Thus, the B-cell system is known to consist of the same components [7], and patient cell lines have been used as targets for functional reconstitution following gene transfer. We have previously used retroviral vectors for the expression of p22-*phox* and gp91-*phox* to restore cytochrome *b*₅₅₈ and NADPH oxidase function in appropriate patient-derived B-cell lines [8,9]. The reconstituted oxidase in these transduced cells is correctly regulated in response to protein kinase C agonists.

In the course of our studies to correct B-cell lines from CGD patients with a rare autosomal form (A-CGD) due to p22-*phox* deficiency, we identified a 65 kDa biosynthetic precursor of gp91-*phox* [8]. This was present in two such lines lacking p22-*phox*, at an abundance of approximately 5% relative to a control cell line. Additionally, the precursor was detectable, at less than 1% relative abundance, in a neutrophil extract from one of these patients. By means of glycosidase digestion analysis the precursor was determined to be a high-mannose modification of the core protein. Reconstitution of p22-*phox* expression in these cell lines resulted in the generation of mature gp91-*phox* with the complex modification, implying that p22-*phox* association with the gp91-*phox* precursor is a prerequisite for maturation of the latter through the Golgi apparatus. We postulated that a subset of X-CGD point mutations generating amino-acid substitutions in gp91-*phox* might similarly prevent its maturation.

In the present study we screened such X-CGD-patient-derived B-cell lines for the presence of the 65 kDa gp91-*phox* precursor. Of a total of eight cell lines in which the gene defect was either known or presumed to be a point mutation, four had little or no gp91-*phox* while three expressed this precursor and one had both precursor and mature forms. The precursor detected in these X-

Abbreviations used: CGD, chronic granulomatous disease; EBV, Epstein-Barr virus; MoAb, monoclonal antibody; X-CGD, X-linked CGD; endoH, endoglycosidase H; PNGaseF, peptide:N-glycosidase F; A-CGD, autosomal CGD.

‡ Present address: Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, U.K.

§ Present address: Department of Biochemistry, Kyushu University School of Medicine, Fukuoka 812, Japan.

|| Present address: Eastman Dental Institute, 256 Grays Inn Road, London WC1X 8LD, U.K.

¶ To whom correspondence should be addressed.

CGD cell lines was indistinguishable from that detected in A-CGD cell lines due to p22-*phox* deficiency [8]. The gp91-*phox* mutations were determined. The region encoded by exon 3 (amino acids 48–84), and positions 244 and 338 were found to be important determinants of gp91-*phox* biosynthesis.

MATERIALS AND METHODS

CGD-patient-derived B-cell lines

The EBV-transformed B-cell lines used in this study were derived from eight unrelated X-CGD patients. The clinical features and nature of the mutations in these patients are described below. A cell line derived from a patient with A-CGD due to p22-*phox* deficiency (patient R.H. [8]) was used for comparison: the deficiency results from a homozygous splice donor site mutation leading to skipping of exon 5 [10].

A luminol-enhanced chemiluminescence assay was used to assess NADPH oxidase activity in these B-cell lines. Cells (2.5×10^6) were resuspended in 1 ml of Hanks' balanced salt solution containing 13 μ M luminol (5-amino-2,3-dihydro-1,4-phthalazonedione), 10 units/ml horseradish peroxidase and assayed at 37 °C, as described [7]. The cells were stimulated with 200 ng/ml phorbol myristate acetate.

Western-blot analysis of p22-*phox* and gp91-*phox* expression

Cellular membrane fractions were prepared following hypotonic lysis at 4 °C in 10 mM Tris/HCl, pH 7.4, 2 mM MgCl₂, 1 mM CaCl₂ and 2 mM PMSF. Nuclei were pelleted at 500 *g*, and membranes in the supernatant were pelleted by ultracentrifugation at 100 000 *g* for 30 min at 4 °C [9]. Membrane proteins (50 μ g) were fractionated by SDS/PAGE (6% acrylamide gels for the analysis of gp91-*phox*, or 12% for the analysis of p22-*phox*). Western-blot analysis, following transfer to Hybond-C_{extra} (Amersham International, U.K.) by semi-dry blotting, was as described [9], using either monoclonal antibody (MoAb) 48 (for gp91-*phox*) or 449 (for p22-*phox*) [11] and enhanced chemiluminescence detection.

To examine the glycosylation status of proteins detected with MoAb 48, membrane preparations were denatured and treated with peptide:N-glycosidase F (PNGaseF) or endoglycosidase H (endoH) (both obtained from New England Biolabs) before SDS/PAGE and Western-blot analysis [9]. A sample (45 μ g) of protein was digested with 2000 units for 2 h at 37 °C.

Mutation analysis of gp91-*phox* gene

Mutations for four of the X-CGD patients were previously identified, either by reverse transcription and PCR (RT-PCR) from peripheral blood RNA (patients F.B. and J.L.) [12], or by PCR amplification of individual exons from peripheral blood DNA (patients H.R. and R.L.; M. de Boer and D. Roos, unpublished work), followed by sequence analysis. The mutations for the other four patients were not previously characterized beyond the exclusion of detectable gene deletion by Southern-blot analysis (C. Porter, unpublished work). They were identified as part of this study, using DNA obtained from the B-cell lines, by amplification and sequence analysis of individual exons [13]. Every mutation was checked for the possibility of being a polymorphism by analysis of 100 normal alleles. RT-PCR products were sequenced to confirm the skipping of whole exons for patients P.C. and N.G.

RESULTS

X-CGD-patient-derived B-cell lines

We selected for study B-cell lines that were available either from patients with known single amino acid substitutions (patients J.L., H.R., F.B. and R.L.), or for whom the lesion was expected to be a point mutation on the basis of exclusion of detectable deletion (patients D.D., P.C., N.G. and G.S.). The latter group and patient R.L. had no neutrophil NADPH oxidase activity and were considered to have the 'classical' X-CGD phenotype (X91⁰). The remainder were 'variant' X91⁻ X-CGD patients on the basis of residual neutrophil haem spectrum and NADPH oxidase activity. Studies on neutrophils from patients J.L. and F.B. have been reported previously [12,14].

Superoxide production by each of the patient-derived EBV-transformed B-cell lines used in this study was assessed by luminol-enhanced chemiluminescence. All lines failed to respond above background in this assay, regardless of any residual activity shown by neutrophils from the same patient.

Western-blot analysis of p22-*phox* and gp91-*phox* expression in X-CGD patient B-cell lines

Membrane preparations of EBV-transformed B-cell lines from one unaffected individual and from seven X-CGD patients were analysed by Western blotting with the MoAbs 48 and 449, for detection of gp91-*phox* and p22-*phox* respectively. The signal of heterogeneous molecular mass with MoAb 48 in the control sample reflects variable glycosylation of mature gp91-*phox*. Of the seven X-CGD cell lines, three had very low (F.B.) or no

Figure 1 Western-blot analysis of gp91-*phox* and p22-*phox* expression

Upper panel: Western-blot analysis of gp91-*phox* expression in membrane preparations from EBV-transformed B-cell lines derived from seven patients with X-CGD and one unaffected individual (N). The positions of protein size markers in kDa are indicated. Lower panel: Western-blot analysis of p22-*phox* expression, as in upper panel.

Figure 2 Glycosidase analysis of gp91-phox expression in cell lines from X-CGD patients R.L., P.C., J.L. and H.R., from p22-phox-deficient A-CGD patient R.H., and from the unaffected control (N)

Cellular membrane preparations were digested with PNGaseF (F) or endoH (H) prior to Western-blot analysis as in the upper panel of Figure 1. Controls (C) were not digested.

(N.G., G.S.) detectable material, three had a readily detectable species of approximately 65 kDa (P.C., J.L., H.R.), and the last had both mature and 65 kDa species (R.L.) (Figure 1, upper panel). One further X-CGD cell line (D.D.) was previously studied and shown to be negative for gp91-phox expression [8]. Patient F.B. was of interest because an earlier study suggested the presence of a species of approximately 65 kDa in a neutrophil extract [12]. However, re-examination of this material showed that the neutrophils expressed protein of normal molecular mass but at very low abundance. This was shown to be true also for the B-cell line in that, following deglycosylation with PNGaseF, but not endoH, the core protein was faintly detectable, consistent with the low abundance of fully glycosylated gp91-phox (results not shown).

All cell lines expressed significant levels of p22-phox, regardless of gp91-phox status (Figure 1, lower panel). The levels of p22-phox were equivalent for all lines lacking mature gp91-phox: the level was approximately double for R.L., which expresses mature protein, equivalent to that of the control cell line. Thus, in this panel of B-cell lines, as we have previously reported for D.D. [8], the level of p22-phox does not reflect that of gp91-phox, as is the case in neutrophil extracts.

The nature of the 65 kDa species was studied by glycosidase enzyme digestion, using PNGaseF (which removes N-linked sugar moieties regardless of modification type) and endoH (which

is specific for high-mannose modifications). A membrane preparation from cell line R.H., previously shown to lack p22-phox and express a 65 kDa high-mannose precursor of gp91-phox [8], was included in this analysis to determine whether the species in the X-CGD and A-CGD cell lines were equivalent (Figure 2). The three X-CGD cell lines (P.C., J.L. and H.R.) and the A-CGD cell line (R.H.) all showed species of indistinguishable molecular mass and digestion sensitivities. Thus, this gp91-phox precursor can result either from mutations in gp91-phox itself or from absence of p22-phox. Additionally, the precursor abundance in all these cell lines was equivalent. This was previously determined for cell line from R.H. to be approximately 5% of the unaffected control [8], based on quantification of deglycosylated core protein, and probably reflects the reduced stability of the precursor in the absence of p22-phox. Glycosidase analysis also demonstrated the presence of the precursor for patient R.L. in addition to mature fully glycosylated gp91-phox (Figure 2): the precursor abundance is similar to that in the other CGD cell lines.

Nature of gp91-phox mutations

The mutations in patients F.B. and J.L. were previously determined to result in a Gly³⁸⁹ → Ala or Cys²⁴⁴ → Ser substitution respectively [12]. Those for R.L. and H.R. were identified as His³³⁸ → Tyr and Pro⁵⁶ → Leu substitutions respectively (M. de Boer and D. Roos, unpublished work). The mutations for the remaining four patients were determined as part of this study, subsequent to characterization of their molecular phenotypes. Those for D.D. and G.S. were nonsense mutations resulting in truncations of the protein at amino acids 130 and 290, respectively, consistent with the lack of detectable gp91-phox protein in B-cell lines derived from these patients. Those for N.G. and P.C. both involve splicing defects, with skipping of exon 5 or exon 3 respectively. The exon 5 skip results in a frameshift after codon 112, leading to a stop at codon 133. Deletion of exon 3 (amino acids 48–84), however, does not result in frameshift. Moreover, the analysis above indicates that the resultant deleted protein is synthesized, although its maturation is impaired. This deletion of 37 amino acids would reduce the core protein molecular mass by 4 kDa. No such change was apparent from Western-blot analysis but this is not inconsistent with the anomalous electrophoretic mobility of the core protein (55 kDa, compared with a calculated molecular mass of 65 kDa).

Table 1 Summary of mutation and gp91-phox status for X-CGD B-cell lines

Abbreviation: aa, amino acids.

Cell line	Phenotype	Nucleotide change*	Amino acid change	gp91-phox status
D.D.	X91 ⁰	C-400 → T	Arg ¹³⁰ → STOP	Absent
F.B.	X91 [−]	G-1178 → C	Gly ³⁸⁹ → Ala	Low abundance 91 kDa
G.S.	X91 ⁰	C-880 → T	Arg ²⁹⁰ → STOP	Absent
H.R.	X91 [−]	C-179 → T	Pro ⁵⁶ → Leu	65 kDa precursor
J.L.	X91 [−]	G-744 → C	Cys ²⁴⁴ → Ser	65 kDa precursor
N.G.	X91 ⁰	Skip exon 5†	Frameshift, STOP	Absent
P.C.	X91 ⁰	Skip exon 3‡	Loss of aa 48–84	65 kDa precursor
R.L.	X91 ⁰	C-1024 → T	His ³³⁸ → Tyr	91 kDa and 65 kDa precursor

* Mutation analysis for F.B. and J.L. [12]; H.R. and R.L. (M. de Boer and D. Roos, unpublished work); D.D., G.S., N.G. and P.C. (this study).

† Mutation of intron 5 splice donor site (gt → tt), leading to exon 5 skip.

‡ Mutation of last nucleotide of exon 3 (G-264 → A), disrupting intron 3 splice donor site and leading to exon 3 skip.

Table 1 summarizes the mutation and gp91-*phox* status for each of the B-cell lines.

DISCUSSION

We previously hypothesized that a subset of gp91-*phox* amino acid substitutions leading to X-CGD might disrupt its biosynthesis and prevent completion of the modification of the 65 kDa high-mannose precursor, analogous to the situation in p22-*phox*-deficient A-CGD [8]. The gp91-*phox*/p22-*phox* subunit interaction is apparently necessary for transport through the Golgi complex, since maturation of gp91-*phox* glycosylation is dependent upon the presence of p22-*phox* [8]. In order to test this hypothesis we analysed the cytochrome subunits in eight X-CGD B-cell lines, since the precursor is relatively stable in such cells. For five cell lines the mutation in gp91-*phox* did not cause premature termination of protein synthesis. Of these, four were identified for which there was partial (one example) or total disruption of maturation of a gp91-*phox* precursor indistinguishable from that detected for a p22-*phox*-deficient A-CGD B-cell line.

Our results indicate that the regions of gp91-*phox* delineated by mutations at Pro⁵⁶, Cys²⁴⁴ and loss of amino acids 48–84 (encoded by exon 3) are critical for its biosynthesis. The consequence of the mutation at His³³⁸ is less severe. If it is assumed that interactions that are important for biosynthesis are maintained in the mature protein, then it is possible to relate these mutations to the structure of cytochrome *b*₅₅₈. One possible explanation is that these mutations affect the gp91-*phox*/p22-*phox* subunit interaction, which is a prerequisite for maturation of the gp91-*phox* precursor [8]. Affecting some other aspect of biosynthesis, such as cofactor binding, could also be responsible. However, that the precursor abundance is in each case similar to that due to expression of wild-type gp91-*phox* in the absence of p22-*phox* (as for p22-*phox*-deficient A-CGD) argues against gross structural changes, such as altered membrane insertion, for which reduced stability would be expected. Mutations affecting the subunit interaction could, in principle, involve either gp91-*phox* molecular surface features that make contact with p22-*phox*, or positions at other sites of the molecule necessary for maintenance of the structural integrity of the cytochrome. The latter might be expected to include the binding domains for FAD and haem.

The His³³⁸ → Tyr mutation occurs within the putative FAD-binding domain, as determined from molecular modelling based on ferredoxin-NADP reductase [3]. The motif His³³⁸-Pro-Phe-Thr interacts with the flavin, with His³³⁸ making extensive contacts with both sugar and phosphate moieties. Based on the structural model this mutation is predicted to exclude FAD. The partial disruption of protein maturation is reminiscent of the situation in 3T3 fibroblasts transduced for expression of gp91-*phox* (C. Porter, unpublished work), in which the mature fully glycosylated form would be dependent on interaction with endogenous murine p22-*phox*. These observations could be interpreted in each case as being due to a weakened subunit interaction.

The other positions implicated in gp91-*phox* maturation are not included in the gp91-*phox* structural model, which covers only the cytoplasmic C-terminal domain from position 279 [3]. The N-terminal domain of the molecule contains several potential membrane-spanning regions and potential N-glycosylation sites. Analysis with an anti-peptide antibody has shown that an epitope contained within residues 150–172 is extracellular [15], but the topology of this domain is otherwise unknown. While the positions of haem attachment within cytochrome *b*₅₅₈ are un-

gp91- <i>phox</i>	232-	T A E S L A V H N I T V C E
		: :
Cyt b ₅₅₉	15-	T V R W L A V H T L A V P S

Figure 3 Amino-acid-sequence alignments of a potential haem-binding domain in gp91-*phox* with the known haem-binding domain of the β -subunit of *Synechocystis* 6803 photosystem II cytochrome *b*₅₅₉ [21]

The position of the sequence within these proteins is indicated at the left. Identical and homologous amino acids are indicated by '|' and ':' respectively. The histidine residue known or proposed to co-ordinate haem is in bold. Cys²⁴⁴ of gp91-*phox*, mutation of which results in CGD, is underlined.

known it has been shown that haem is associated with both subunits. It is likely that there are two non-identical [16] bishistidiny haems, one within gp91-*phox* and a second shared between the subunits, and that the haem-binding domains are within the membrane [17]. The only non-polymorphic His residue in p22-*phox* is at position 94, within a region showing homology to the haem-binding site of mitochondrial cytochrome *c* oxidase polypeptide I [18] and a region of the chromaffin granule cytochrome *b*₅₆₁ that may be involved in co-ordinating haem [19]. Mutation of this His residue results in p22-*phox*-deficient A-CGD [20]. A motif surrounding gp91-*phox* His²³⁹ shows homology to the haem-binding domain of the β -subunit of *Synechocystis* 6803 photosystem II cytochrome *b*₅₅₉ (Figure 3). For this molecule the haem is axially co-ordinated by two His residues, one in each subunit, and is responsible for holding the two subunits together within the membrane [21]. If His²³⁹ does bind haem then the nearby Cys²⁴⁴ → Ser substitution may prevent maturation of the gp91-*phox* precursor by disrupting this association. One possibility is that a haem molecule is co-ordinated between p22-*phox* His⁹⁴ and gp91-*phox* His²³⁹, and that the mutation at gp91-*phox* Cys²⁴⁴ disrupts the interaction of the two subunits. As discussed above, this would be analogous to the expression of wild-type gp91-*phox* in the absence of p22-*phox*.

Deletion of residues 48–84 (by skipping of exon 3 due to a splice site mutation), or mutation of Pro⁵⁶ → Leu both abolish gp91-*phox* maturation. No data are available concerning the structure of the N-terminus of gp91-*phox*. However, on the basis of hydrophobicity plots, amino acids 48–78 have previously been considered to be membrane-spanning. If this were so then deletion of this region would be likely to have gross structural consequences. That such a mutant protein can form the gp91-*phox* precursor argues against such an involvement. In contrast to the consequence of the Pro⁵⁶ → Leu mutation, recent studies of neutrophils from an X-CGD patient with an Arg⁵⁴ → Ser mutation have shown that this substitution does not affect processing of the cytochrome subunits but results in a non-functional haem centre with reduced mid-point potential [16,22]. The implication of this region in haem-binding suggests that one possibility for disruption of gp91-*phox* maturation by both Pro⁵⁶ → Leu and the deletion of residues 48–84 is via an effect on haem incorporation. A further possibility is that Pro⁵⁶ is within a region interfacing with p22-*phox* and stabilizing the subunit interaction. Both Ala⁵³ → Asp and Ala⁵⁷ → Glu also result in X-CGD [5,23], but no data concerning the subunits are available for these mutations.

We have validated our hypothesis that observation of the gp91-*phox* precursor in B-cell lines from X-CGD patients can map their mutations to sites of known or predicted functional importance. The success of this approach, based on naturally occurring mutations, implies that mutagenesis of p22-*phox* or gp91-*phox* and expression in B-cell lines null for the respective

subunit is likely to be a fruitful line of research to delineate further functional domains of cytochrome b_{558} .

This work was supported by grants from Action Research, The Welton Trust, the Netherlands Organization for Scientific Research and the Foundation for Preventive Medicine. We are grateful to Professor A. Segal and Dr. N. Keep for helpful discussions concerning computer modelling of gp91-*phox*.

REFERENCES

- 1 Segal, A. W., West, I., Wientjes, F., Nugent, J. H. A., Chavan, A. J., Haley, B., Garcia, R. C., Rosen, H. and Scrace, G. (1992) *Biochem. J.* **284**, 781–788
- 2 Rotrosen, S., Yeung, C. L., Leto, T. L., Malech, H. L. and Kwong, C. H. (1992) *Science* **256**, 1459–1462
- 3 Taylor, W. R., Jones, D. T. and Segal, A. W. (1993) *Protein Sci.* **2**, 1675–1685
- 4 Thrasher, A. J., Keep, N. H., Wientjes, F. and Segal, A. W. (1994) *Biochim. Biophys. Acta* **1227**, 1–24
- 5 Roos, D. (1994) *Immunodef. Rev.* **138**, 121–157
- 6 Volkman, D. J., Buescher, E. S., Gallin, J. I. and Fauci, A. S. (1984) *J. Immunol.* **133**, 3006–3009
- 7 Porter, C. D., Parkar, M. H., Collins, M. K. L., Levinsky, R. J. and Kinnon, C. (1992) *J. Immunol. Methods* **155**, 151–157
- 8 Porter, C. D., Parkar, M. H., Verhoeven, A. J., Levinsky, R. J., Collins, M. K. L. and Kinnon, C. (1994) *Blood* **84**, 2767–2775
- 9 Porter, C. D., Parkar, M. H., Levinsky, R. J., Collins, M. K. L. and Kinnon, C. (1993) *Blood* **82**, 2196–2202
- 10 Porter, C. D., Parkar, M. H. and Kinnon, C. (1996) *Human Mutation*, in the press
- 11 Verhoeven, A. J., Bolscher, B. G. J. M., Meerhof, L. J., van Zwieten, R., Keijer, J., Weening, R. S. and Roos, D. (1989) *Blood* **73**, 1686–1694
- 12 Bolscher, B. G. J. M., de Boer, M., de Klein, A., Weening, R. S. and Roos, D. (1991) *Blood* **77**, 2482–2487
- 13 de Boer, M., Bolscher, B. G. J. M., Dinauer, M. C., Orkin, S. H., Edvard Smith, C. I., Ahlin, A., Weening, R. S. and Roos, D. (1992) *Blood* **80**, 1553–1558
- 14 Roos, D., de Boer, M., Borregaard, N., Bjerrum, O. W., Valerius, N. H., Seger, R. A., Muhlebach, T., Belohradsky, B. H. and Weening, R. S. (1992) *J. Leuk. Biol.* **51**, 164–171
- 15 Imajoh-Ohmi, S., Tokita, K., Ochiai, H., Nakamura, M. and Kanegasaki, S. (1992) *J. Biol. Chem.* **267**, 180–184
- 16 Cross, A. R., Rae, J. and Curnutte, J. T. (1995) *J. Biol. Chem.* **270**, 17075–17077
- 17 Quinn, M. T., Mullen, M. L. and Jesaitis, A. J. (1992) *J. Biol. Chem.* **267**, 7303–7309
- 18 Parkos, C. A., Dinauer, M. C., Walker, L. E., Allen, R. A., Jesaitis, A. J. and Orkin, S. H. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 3319–3323
- 19 Perin, M. S., Fried, V. A., Slaughter, C. A. and Sudhof, T. C. (1988) *EMBO J.* **7**, 2697–2703
- 20 de Boer, M., de Klein, A., Hossle, J.-P., Seger, R., Corbeel, L., Weening, R. S. and Roos, D. (1992) *Am. J. Hum. Genet.* **51**, 1127–1135
- 21 Pakrasi, H. B., De Ciechi, P. and Whitmarsh, J. (1991) *EMBO J.* **10**, 1619–1627
- 22 Cross, A. R., Heyworth, P. G., Rae, J. and Curnutte, J. T. (1995) *J. Biol. Chem.* **270**, 8194–8200
- 23 Ariga, T., Sakiyama, Y., Tomizawa, K., Imajoh-Ohmi, S., Kanegasaki, S. and Matsumoto, S. (1993) *Eur. J. Pediatr.* **152**, 469–472