

Activation of H⁺ Conductance in Neutrophils Requires Assembly of Components of the Respiratory Burst Oxidase but Not Its Redox Function

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Abstract

In phagocytes, superoxide generation by the NADPH oxidase is accompanied by metabolic acid production. Cytoplasmic acidification during this metabolic burst is prevented by a combination of H⁺ extrusion mechanisms, including a unique H⁺ conductance. NADPH oxidase is deficient in chronic granulomatous disease (CGD) patients. The burst of acid production is absent in CGD patients lacking the 47-kD (p47-phox) or the 91-kD (gp91-phox) subunits of the oxidase. Activation of the H⁺ conductance is also defective in these patients suggesting that (a) the oxidase itself undertakes H⁺ translocation or (b) oxidase assembly is required to stimulate a separate H⁺ conducting entity. To discern between these possibilities, three rare forms of CGD were studied. In neutrophils expressing nonfunctional cytochrome b, the conductance was activated to near-normal levels, implying that functional oxidase is not required to activate H⁺ extrusion. CGD cells expressing diminished amounts of cytochrome displayed H⁺ conductance approaching normal levels, suggesting that the oxidase itself does not translocate H⁺. Finally, the conductance was only partially inhibited in patients lacking the 67-kD subunit, indicating that this component is not essential for stimulation of H⁺ transport. We propose that normal assembly of the oxidase subunits is required for optimal activation of a closely associated but distinct H⁺ conducting entity. (*J. Clin. Invest.* 1994. 93:1770–1775.) Key words: pH regulation • leukocytes • NADPH oxidase • channel • chronic granulomatous disease

Introduction

Polymorphonuclear leukocytes (neutrophils) migrate to inflammatory sites, where they synthesize a variety of microbicidal oxygen metabolites by a process termed the respiratory burst. The primary metabolite produced during the burst, superoxide anion (O₂⁻), is generated by the one-electron reduction of molecular oxygen in a reaction catalyzed by the NADPH oxidase, an enzymatic complex composed of at least five polypeptides (1–6). In dormant (unstimulated) neutrophils, three of these polypeptides, of molecular masses 47 kD (p47-phox), 67 kD (p67-phox), and 22 kD (rac-1, rac-2, or Krev-1), are recovered from the cytosolic fraction. A cy-

tochrome b, consisting of two polypeptides of 91 kD (gp91-phox) and 22 kD (p22-phox), is constitutively present in the plasma membrane. Upon neutrophil stimulation, the cytosolic components apparently migrate to the plasma membrane, where they associate with the cytochrome, thereby rendering the complex enzymatically active (1, 2). The importance of this enzyme in host defence is best exemplified by chronic granulomatous disease (CGD)¹ patients, in which one component of the oxidase is defective or missing (1, 2). The failure of these phagocytes to assemble a functional oxidase compromises their ability to mount a respiratory burst, and CGD patients consequently suffer severe and recurrent bacterial infections (1).

NADPH is the source of electrons for the reduction of oxygen during the respiratory burst. The oxidized products, NADP⁺ and H⁺, are released into the cytosol during superoxide synthesis. NADPH is then rapidly regenerated by the hexose monophosphate shunt, a pathway that also produces vast quantities of H⁺ (7). Collectively, the acid equivalents liberated by these reactions challenge the intracellular pH homeostasis of the activated neutrophil. To cope with this burst of acid production, neutrophils extrude H⁺ equivalents by at least three independent mechanisms: an electroneutral Na⁺/H⁺ antiport, an ATP-driven vacuolar type H⁺ pump, and a passive H⁺ conductance (8–11). Because activation of these H⁺ transport pathways occurs concurrently with activation of the NADPH oxidase, an association between these events seemed possible. However, using CGD neutrophils, we recently showed that activation of the Na⁺/H⁺ antiport and the V type H⁺ pump occur independently of the presence of a functional NADPH oxidase (12). In contrast, the stimulation of the H⁺ conductance was markedly reduced (<15% of control) in neutrophils lacking either the p47-phox or gp91-phox subunits (12). These findings suggested that assembly of a functional oxidase was required for activation of the conductive pathway.

Two mechanisms can be envisaged to account for the relationship between the NADPH oxidase and the H⁺ conductance. It is conceivable that the oxidase itself undertakes H⁺ translocation, as has been suggested for the mitochondrial cytochrome oxidase (13). Alternatively, assembly of the oxidase may be an essential step in a sequence of events leading to the stimulation of a separate H⁺ conducting entity. In the latter case, electron flow through a functional oxidase may not be required for activation of H⁺ transport. In this report, three rare forms of CGD were studied in an attempt to discern between these alternative mechanisms. We analyzed cells from five patients deficient in p67-phox and two others with point

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Received for publication 10 June 1993 and in revised form 5 January 1994.

J. Clin. Invest.

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0021-9738/94/04/1770/06 \$2.00

Volume 93, April 1994, 1770–1775

1. Abbreviations used in this paper: BCECF, 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; CGD, chronic granulomatous disease; DPI, diphenylene iodonium; TPA, 4β-phorbol 12-tetradecanoate 13-acetate.

mutations in the gene encoding gp91-*phox*. In the latter patients, the cytochrome is expressed normally but is nonfunctional. Neutrophils from these two types of patients differ from those studied earlier in that association of the cytosolic and membrane-bound components of the NADPH oxidase is at least partially preserved. This experimental paradigm enabled us to test whether assembly, rather than function, of the oxidase is required for activation of the H⁺ conductance. In addition, patients with greatly diminished yet still detectable levels of cytochrome b were also analyzed. Cells from these patients were used to establish the quantitative correlation between the oxidase and the H⁺ transporters.

Methods

Bafilomycin A₁ was the kind gift of K. Altendorf (University of Osnabrück, Osnabrück, Germany). Diphenylene iodonium (DPI) was synthesized in our laboratory as described (14). All other reagents were commercially available. K⁺ medium contained 140 mM KCl, 1 mM MgCl₂, 10 mM glucose, and 20 mM Hepes titrated to pH 7.5 with concentrated potassium hydroxide. The osmolarity was adjusted to 295±5 mosM with KCl.

Neutrophils were isolated from freshly drawn blood of normal and CGD donors as described (15). The genetic subtype of each of the nine CGD patients studied was determined as previously described (1, 2, 16), including immunoblot analysis to determine p67-*phox* levels (17). The biochemical defects in five of these patients have also been reported earlier (Table I and references 15, 16, 18, 19). All experiments were performed within 8 h of neutrophil isolation. Translocation of p47-*phox* to the cytoskeleton was determined by an adaptation of the method of Nauseef et al. (20), as described under Table I. Cytoplasmic pH (pH_i) was measured fluorimetrically using the pH-sensitive fluorophore 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) as described (12). Activity of the H⁺ conductance was estimated as described in detail (11 and 12). Briefly, BCECF-loaded neutrophils were suspended in K⁺ medium to preclude H⁺ extrusion by the Na⁺/H⁺ antiporter. The activity of the NADPH oxidase was inhibited by treating the cells with 3 μM DPI, to prevent the generation of metabolic acid that would otherwise obscure detection of the H⁺ transport pathway. Finally, bafilomycin A₁ (100 nM) was added to inhibit H⁺ extrusion by the V type H⁺ pump (10) and valinomycin (1 μM) was included to provide a mobile counter-ion, ensuring that H⁺ fluxes would not be limited by the endogenous counterion permeability of the cells. Upon stabilization of pH_i, the neutrophil suspension was stimulated with the protein kinase C agonist 4β-phorbol 12-tetradecanoate 13-acetate (TPA 100 nM) to stimulate the conductive pathway. Because the extracellular medium is more alkaline than the cytosol and the transmembrane potential is near zero, activation of the conductance under these conditions facilitates net H⁺ efflux (see reference 11 for details). The activity of the H⁺ conductance was, therefore, estimated as the TPA-induced net rate of intracellular alkalinization. The contribution of pathways other than the conductance to the TPA response was assessed by repeating the experiments in the presence of 50 μM Zn²⁺. This dose of the cation was shown earlier to block the conductive pathway selectively (12). All estimates of the H⁺ conductance given below refer to the Zn²⁺-sensitive component of the pH change: the rate of change of pH_i in the presence of Zn²⁺ was subtracted from that in its absence. Buffering power was estimated by pulsing with weak electrolytes, as detailed in (21). In the pH_i interval studied (~6.8–7.4), the buffering power ranged from 25–28 mM/pH. Moreover, the buffering capacity of cells from normal and CGD donors was similar, thus validating the comparison of the rates of ΔpH and precluding the need to convert the data to H⁺ equivalent fluxes. Unless indicated otherwise (e.g., Table I), data are presented as means±SE of the number of experiments indicated in parentheses.

Results

Neutrophils from normal and CGD donors were compared to assess the roles of the NADPH oxidase in activation of the H⁺ conductance. A summary of the functional and biochemical properties of the cells isolated from the patients used in this study is presented in Table I. Patients with point mutations in the gene encoding gp91-*phox* were studied first. In patient 1, gp91-*phox* contained a Pro-415→His substitution (18), while patient 2 had an Arg-54→Ser substitution (unpublished observation). Although neutrophils from these patients were unable to synthesize superoxide, they contained normal levels of cytochrome b, as determined spectroscopically (Table I). These findings imply that while not affecting the expression of the glycoprotein or its association with p22-*phox*, the point mutations render the complex enzymatically inactive. This type of CGD is, therefore, designated as X91⁺, to distinguish it from the classic X91⁰ form, in which a stable gp91-*phox* protein is not synthesized, and cytochrome b is, therefore, not detectable by spectroscopic or immunoblotting techniques (1).

The metabolic acid production that accompanies activation of the oxidase in normal individuals was reported to be absent in CGD patients unable to mount a respiratory burst (12, 22). Consistent with the earlier findings, the pH_i drop induced by stimulation with TPA was largely absent in patient 2 (Table II). Metabolic acidification was not tested in patient 1. Despite the absence of functional oxidase in patients 1 and 2, the Zn²⁺-inhibitable H⁺ conductance, measured as described under Materials and Methods, showed a significant activation (Fig. 1 B and Table II). Patients 1 and 2 showed an average rate of alkalinization of 0.117 pH/min, corresponding to ~80% of the control response (0.144±0.021 pH/min, *n* = 7; see Table II). This behavior differs markedly from that of cytochrome-deficient (i.e., X91⁰) neutrophils, which display a greatly diminished conductance (<15% of control; Fig. 1 B and reference 12). Together, these data indicate that a functional cytochrome b is not required to activate the H⁺ conductance. Since superoxide generation is not a prerequisite for stimulation of the conductance, reactive oxygen species do not appear to be involved in activation of this pathway, as has been suggested for some ion channels (23). Instead, the physical presence of the cytochrome b protein suffices to support the TPA-induced activation of the H⁺ conductance.

Patients exhibiting a second type of point mutation in gp91-*phox*, a nonconservative Glu-309→Lys substitution, were studied next. This mutation, analyzed in a pair of maternal first cousins, gives rise to an unstable gp91-*phox*, leading to expression of only 16–18% of normal cytochrome b (16). Since oxidase activity is limited by the quantity of functional cytochrome, the rate of superoxide generation by these cells is greatly diminished compared to control levels (patients 3 and 4 in Table I). As a result of the reduced oxidase activity, the rate of metabolic acid production by these cells was marginal. Patient 4 showed a TPA-induced metabolic acidification of 0.010 pH/min (~19% of control), while the acidification in patient 3 was not detectable (Table II). Nevertheless, both patients displayed a substantial stimulated H⁺ conductance. Patient 3 showed a net rate of alkalinization, corresponding to the H⁺ conductance, of 0.141 pH/min while the alkalinization in patient 4 was 0.058 pH/min (Fig. 1 C and Table II). These rates correspond to an average of 70% of the control H⁺ conductance, substantially larger than those measured in X91⁰ CGD

Table I. Summary of Biochemical and Genetic Basis of the Respiratory Burst Defect in Nine CGD Patients Analyzed in this Study

Patient	Sex	O ₂ production*	Cytochrome b†	p67-phox‡	CGD type	Mutant oxidase gene	p47-phox translocation to cytoskeleton (% control)¶	Ref.
		nmol/min/10 ⁷ cells	pmol/10 ⁷ cells					
1. R.C.**	M	0.2	56.2	N	X91 ⁺	gp91-phox	83 (1)	18, 19
2. T.J.**	M	0	51.8	N	X91 ⁺	gp91-phox	70 (1)	—
3. D.H.††	M	6.7±4.3	11.5±2.2	N	X91 ⁻	gp91-phox	38 (1)	16
4. T.C.††	M	5.0±3.7	13.1±2.6	N	X91 ⁻	gp91-phox	41 (1)	—
5. S.G.§§	F	0	65.9	0	A67 ⁰	p67-phox	64 (2)	16
6. C.G.§§	F	0	59.4	0	A67 ⁰	p67-phox	0 (2)	16
7. M.D.	F	0	62.1	0	A67 ⁰	p67-phox	63 (2)	—
8. N.S.	F	0	52.7	0	A67 ⁰	p67-phox	ND	15
9. L.C.	F	0	72.5	0	A67 ⁰	p67-phox	28 (1)	—

* Normal range = 149.2±33.8 SD (*n* = 14) using TPA (200 ng/ml) as stimulus.

† Normal range = 73.0±30.9 SD (*n* = 42) pmol/10⁷ neutrophils.

‡ 0, undetected; N, normal.

|| CGD type: In this nomenclature, the first letter represents the mode of inheritance (X-linked [X] or autosomal [A]), while the number indicates the *phox* component that is genetically affected. The superscript symbols indicate whether the level of protein component affected is undetectable (⁰), diminished (⁻), or normal (⁺) as measured by immunoblot analysis.

¶ Translocation of p47-*phox* to the cytoskeleton is defined as the extent of incorporation of immunoreactive p47-*phox* into the Triton X-100 (0.75%) insoluble fraction of neutrophils treated with 320 nM TPA for 5 min, using an adaptation of a method by Nauseef et al. (20). Numbers in parentheses refer to the number of individual determinations (each with a different blood sample) performed for each patient (21a).

** Cytochrome b is nonfunctional in patients 1 and 2. Patient 1 has a Pro-415 → His substitution in gp91-*phox*, while Patient 2 has an Arg-54 → Ser nonconservative amino acid substitution.

†† Patients 3 and 4 are maternal first cousins.

§§ Patients 5 and 6 are sisters.

||| Data are means±SD.

patients (i.e., <15% of control H⁺ conductance; reference 12), which completely lack the cytochrome b component of the oxidase. From these data, we can conclude that the magnitude of the conductive H⁺ flux is not directly proportional to the quantity of cytochrome b in the cell.

Neutrophils from A67⁰ patients lack p67-*phox* yet contain normal levels of all other oxidase components (1). Five such patients were tested in this study (patients 5–9 in Table I). As reported earlier (1), though the A67⁰ patients express normal

levels of cytochrome b, they failed to secrete superoxide (Table I). Consistent with the absence of oxidase activity, these neutrophils showed a severely depressed level of metabolic acid production (5.7% of control; Table II). In terms of oxidase activity and metabolic acid production, these cells therefore resemble the A47⁰ and X91⁰ neutrophils studied previously (12). Unlike these mutants, however, the A67⁰ neutrophils displayed sizable stimulation of the H⁺ conductance. The rate of alkalinization in patients 5–9 averaged 0.047±0.004 pH/min, corre-

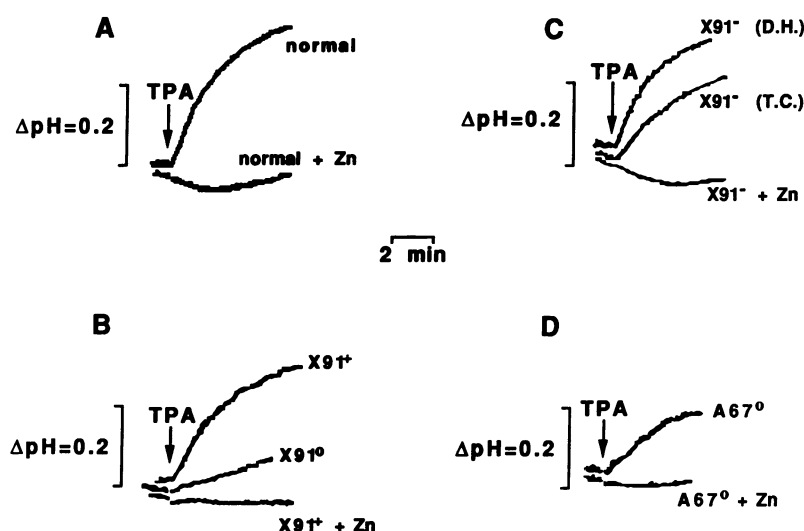


Figure 1. Determinations of H⁺ conductance in TPA-activated neutrophils. Human neutrophils from normal (A) or CGD patients (B–D) were isolated from whole blood and pH_i was measured fluorimetrically using BCECF as described in the text. Neutrophils (2 × 10⁶) were sedimented and resuspended in K⁺ medium containing 3 μM DPI. Valinomycin (VAL; 1 μM) and bafilomycin A₁ (BAF; 100 nM) were added to the cell suspension 1–5 min before stimulation with 100 nM TPA (arrow). See text and references 11 and 12 for details. The CGD type is indicated for the individual panels, using the nomenclature described in the text. Two different X91⁻ patients, identified by their initials, are shown in C. The lower trace in each panel included 50 μM ZnCl₂ from the outset to block the H⁺ conductance. Temperature was 37°C. The time scale applies to all traces. The slight acidification in normal cells treated with Zn²⁺ (A) likely reflects incomplete inhibition of the metabolic burst by DPI.

Table II. Determinations of Metabolic Acidification and H⁺ Conductance Activity in Activated Neutrophils

Patient	Sex	CGD type*	Mutant oxidase gene	Metabolic acidification (ΔpH/min)	H ⁺ conductance (ΔpH/min)	H ⁺ conductance (% of control)
1. R.C. [‡]	M	X91 ⁺	gp91- <i>phox</i>	ND	0.102	71
2. T.J. [‡]	M	X91 ⁺	gp91- <i>phox</i>	0	0.132	92
Mean					0.117	81
3. D.H. [§]	M	X91 ⁻	gp91- <i>phox</i>	0	0.141	98
4. T.C. [§]	M	X91 ⁻	gp91- <i>phox</i>	0.010	0.058	40
Mean					0.100	70
5. S.G.	F	A67 ⁰	p67- <i>phox</i>	0.004	0.037	26
6. C.G.	F	A67 ⁰	p67- <i>phox</i>	0.009	0.058	40
7. M.D.	F	A67 ⁰	p67- <i>phox</i>	0	0.043	30
8. N.S.	F	A67 ⁰	p67- <i>phox</i>	0	0.051	35
9. L.C.	F	A67 ⁰	p67- <i>phox</i>	ND	0.044	31
Mean±SE				0.003±0.002	0.047±0.004	32±2
Control		Normal		0.053±0.017	0.144±0.021	ND

* CGD type: In this nomenclature, the first letter represents the mode of inheritance (X-linked [X] or autosomal [A]) while the number indicates the *phox* component which is genetically affected. The superscript symbols indicate whether the level of protein component affected is undetectable (⁰), diminished (⁻), or normal (⁺) as measured by immunoblot analysis.

[‡] Cytochrome b is nonfunctional in patients 1 and 2. Patient 1 has a Pro-415 → His substitution in gp91-*phox*. Patient 2 has an Arg-54 → Ser nonconservative amino acid substitution.

[§] Patients 3 and 4 are maternal first cousins.

^{||} Patients 5 and 6 are sisters.

sponding to ~ 33% of the control response (Fig. 1 D and Table II). These data indicate that expression of p67-*phox* is not an absolute requirement for activation of the H⁺ conductance, although the presence of this subunit potentiates the conductive activity.

Discussion

Earlier results had shown that activation of the H⁺ conductance during neutrophil stimulation is abnormal in classical X-linked (in which cytochrome b is absent, termed X91⁰) and in p47-*phox* deficient (A47⁰) CGD patients (12). These observations are compatible with two working models: (a) that the NADPH oxidase itself undertakes H⁺ translocation, or (b) that assembly of the oxidase may be required for activation of a separate conductive entity. The data presented here are more easily reconciled with the latter interpretation. In addition, the present findings indicate that while assembly of certain oxidase components appears to be required for the conductance change, a functionally competent oxidase is not essential. The rationale leading to these conclusions is presented below.

Production of reduced oxygen metabolites is undetectable in X91⁺ and A67⁰ CGD neutrophils (Table I), yet activation of the conductance in these cells reached 81 and 32% of the control rate, respectively (Table I). Clearly, oxidation of NADPH and/or generation of superoxide are not essential requirements to elicit the increased H⁺ permeability. In accordance with this interpretation, the conductance can be activated in normal neutrophils treated with DPI, a potent inhibitor of the flavoprotein component of the oxidase (24).

Observations made with the X91⁻ variant form of CGD suggest, though do not prove, that the NADPH oxidase does not itself provide the pathway for H⁺ permeation. In these cells, which express only ~ 16–18% as much cytochrome as

normal neutrophils, stimulation resulted in substantial elevation of H⁺ permeability (70% of control). It is noteworthy that, in our assay, counterion conductance is increased by addition of valinomycin and is, therefore, not the factor limiting the rate of H⁺ permeation. Hence, at constant driving force, the magnitude of the H⁺ fluxes is determined by the abundance of the H⁺ conductive pathway. The discrepancy between the expression level of the cytochrome in X91⁻ cells and the degree of stimulation of the conductance would, therefore, suggest that the oxidase does not mediate the observed H⁺ fluxes. However, this conclusion does not consider the possibility that in normal cells only a subpopulation of the oxidase molecules may be engaged in H⁺ transport, nor does it take into account possible differences in subcellular localization of the oxidase in normal vs X91⁻ neutrophils. Therefore, conclusive differentiation between the oxidase and the H⁺ transport pathway must await more direct evidence.

A tentative model for the stimulation of the H⁺ conductance can be suggested based on our current knowledge of the assembly and activation of the NADPH oxidase. Stimulation of the cells leads to rapid phosphorylation of the cytosolic p47-*phox* subunit of the oxidase, followed by its association with the cytoskeleton and binding to the plasmalemmal cytochrome b, where it undergoes further phosphorylation (2, 4–6, 19, 20, 25). These events appear to trigger the subsequent interaction of p67-*phox* (which is constitutively present in the cytoskeleton; reference 26) with the membrane and/or cytochrome b, leading to activation of the oxidase. While not absolutely required for binding of p47-*phox* to the cytochrome, association of p67-*phox* appears to stabilize the multisubunit complex. This is indicated by the reduced fraction of p47-*phox* that is recovered with the cytoskeleton after stimulation of A67⁰ cells (see Tables I and III). We propose that correct assembly of the oxidase is required for optimal activation of the H⁺ conduc-

tance. Our accumulated data are generally consistent with this notion. First, though cytochrome b levels are normal in A47⁰ patients (Tables I and III), the conductance cannot be activated due to the absence of p47-*phox* (Table III and reference 12). Similarly, the conductance is not stimulated in X91⁰ CGD neutrophils that completely lack cytochrome b (Table III and reference 12). The mutant cytochrome expressed by X91⁺ patients is unable to catalyze the reduction of oxygen, but supports assembly, estimated as translocation of p47-*phox* to the cytoskeleton, in near normal fashion (Tables I and III; ref 25). In accordance with the model, activation of the conductance is also close to normal in these cells (Tables II and III). In X91⁻ patients, a functional cytochrome moiety is expressed, but total cytochrome b protein levels are only 16–18% of normal. Nevertheless, ~40% of p47-*phox* does associate with the cytoskeleton in these cells (Tables I and III; see ref. 21a). In this case, moderate levels of translocation are associated with ~70% stimulation of the conductance (Tables II and III). While the degree of conductance stimulation would appear to be disproportionately large, the qualitative relationship between H⁺ transport and oxidase assembly is nevertheless upheld.

Evidence obtained using cells from A67⁰ individuals is also generally supportive of the model. Both p47-*phox* and cytochrome b are present in normal amounts in these patients (Table I). However, the absence of p67-*phox* destabilizes the association of p47-*phox* with the oxidase complex (Tables I and III and reference 25) by a mechanism that is poorly understood. Regardless of the underlying mechanism, the reduced translocation of p47-*phox* is paralleled by partial impairment of the conductance (Tables II and III), generally consistent with the proposed hypothesis. It is noteworthy, however, that while three patients had comensurate decreases in translocation and conductance, the third patient (C.G. in Tables I and II) showed significant H⁺ conductance without detectable cytoskeletal association of p47-*phox*. It is conceivable that while association occurred in situ, the complex was very unstable in this patient, leading to dissociation during extraction with non-ionic detergent, the operational criterion used to define the translocation event.

In summary, there appears to be a close correlation between the correct assembly of the NADPH oxidase and activa-

tion of the H⁺ conductance. Interference with normal assembly, whether by absence or diminished expression of gp91-*phox*, p47-*phox*, or p67-*phox*, results in partial or complete failure of the conductance to activate. Yet, while assembly of the oxidase is important in regulating H⁺ permeability, its redox function is not required. Therefore, reactive oxygen species do not appear to be involved in activation of the H⁺ conductance, as has been suggested for other ion channels (23). Finally, the transmembrane cytochrome moiety of the oxidase appears unlikely to mediate H⁺ translocation, based on the quantitative discrepancy between the expression of gp91-*phox* and the stimulation of the conductance. While not directly involved in H⁺ translocation, the oxidase instead appears to function in a signaling capacity to activate the conductance. This relationship would ensure coordination of the H⁺ generating and H⁺ extruding processes in activated neutrophils.

Acknowledgments

We wish to thank Dr. J. A. Badwey for sharing information regarding translocation of oxidase components. We also thank Ms. J. Rae, Mr. R. Erickson, Dr. P. Heyworth, and Dr. A. Cross for their help, and Drs. H. Rosen, R. Roberts and J. Bastian for allowing us to study patients under their care.

This work was supported by grants of the Medical Research Council of Canada (to S. Grinstein) and by National Institutes of Health grants AI24838 and RR00833 (to J. T. Curnutte). A. Nanda is supported by a Studentship from the Arthritis Society of Canada. S. Grinstein is the recipient of an International Research Scholars Award from the Howard Hughes Medical Institute.

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Table III. Summary of Relationships between H⁺ Conductance and Biochemical Properties of NADPH Oxidase

CGD type	Cytochrome b level (% control)	p47- <i>phox</i> level (% control)	p47- <i>phox</i> translocation to cytoskeleton (% control)*	H ⁺ conductance (% control)
X91 ⁰	0	100	24 (7)	<15 (6)
X91 ⁻	16–18	100	40 (2)	70 (2)
X91 ⁺	100	100	76 (2)	80 (2)
A47 ⁰	100	0	Absent	<15 (3)
A67 ⁰	100	100	39 (7)	33 (5)

* Translocation of p47-*phox* to the cytoskeleton is defined as the extent of incorporation of p47-*phox* into the Triton X-100 (0.75%) insoluble fraction of neutrophils treated with 320 nM TPA for 5 min. using an adaptation of a method by Nauseef et al. (20, 21a). Data in the last two columns are presented as means of the number of individual determinations performed, indicated in parentheses. 0, undetectable.

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