Restitution of Superoxide Generation in Autosomal Cytochrome-negative Chronic Granulomatous Disease ($A22^0$ CGD)-derived B Lymphocyte Cell Lines by Transfection with $p22^{phox}$ cDNA

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Summary

The respiratory burst oxidase of phagocytes and B lymphocytes is a multicomponent enzyme that catalyzes the one-electron reduction of oxygen by NADPH. It is responsible for the O_2^- production that occurs when these cells are exposed to phorbol 12-myristate 13-acetate or physiologic stimuli, such as phagocytosis in phagocytes or cross-linking of surface immunoglobulin in B lymphocytes. The activity of this enzyme is greatly diminished or absent in patients with chronic granulomatous disease (CGD), an inherited disorder characterized by a severe defect in host defense against bacteria and fungi. In every CGD patient studied so far, an abnormality has been found in a gene encoding one of the four components of the respiratory burst oxidase: the membrane proteins $p22^{phox}$ or $gp91^{phox}$ which together form the cytochrome b558 protein, or the cytosolic proteins $p47^{phox}$ or $p67^{phox}$. Autosomal recessive cytochrome-negative CGD ($A22^0$ CGD) is associated with mutations in the gene coding for $p22^{phox}$. We report here that the capacity for O_2^- production and cytochrome b558 protein expression were restored to Epstein-Barr virus-transformed B lymphocytes from two $A22^0$ CGD patients by transfection with an expression plasmid containing a $p22^{phox}$ cDNA. No detectable O_2^- was generated by untransfected $p22^{phox}$-deficient lymphocytes. The genetic reconstitution of the respiratory burst in $A22^0$ CGD B lymphocytes by transfer of the wild-type $p22^{phox}$ cDNA represents a further step towards somatic gene therapy for this subgroup of $A22^0$ CGD. This system will also be useful for expression of genetically engineered mutant $p22^{phox}$ proteins in intact cells, facilitating the structure-function analysis of cytochrome b558.

The O_2^--forming NADPH oxidase of phagocytes (1) and B lymphocytes (2–4), also called the respiratory burst oxidase, is a multicomponent, membrane-bound enzyme that catalyzes the one-electron reduction of oxygen to O_2^- (1): $2\ O_2 + NADPH \rightarrow 2\ O_2^- + NADP^+ + H^+$. In resting phagocytes and B lymphocytes, the enzyme is not active, but acquires catalytic activity when the cells are stimulated by appropriate agents such as the protein kinase C activator PMA, or physiologically by phagocytosis (1) in case of phagocytes, and by cross-linking of surface Ig in B lymphocytes (3).

The respiratory burst oxidase plays a critical role in the generation of a complex group of reactive oxidants, including free radicals and oxidized halogens. In phagocytes, these oxidants serve as potent microbicidal agents (5). The role of O_2^- formation in B lymphocytes (2–4) which are nonphagocytic, noncytotoxic cells, is not yet defined. Oxidase activity in B lymphocytes is about 10% of that seen in phagocytes, as determined by O_2^- generation and by complementation assays in a cell-free oxidase-activating system (2–4, 6).

In the resting cell, oxidase components are distributed between the cytosol and the plasma membrane, but upon activation, the cytosolic components move to the plasma membrane to assemble the active enzyme (7, 8). Oxidase components that are always found in the membrane include a

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respiratory burst oxidase–specific cytochrome, cytochrome b558, and an associated Ras-related low molecular weight G protein, rap1A (also designated Krev-1) (9–11). The cytochrome, postulated to be the terminal electron carrier in the electron pathway from NADPH to oxygen, is an oligomeric heme- and flavin-containing glycoprotein composed of the polypeptides $p22^{phox}$ and $gp91^{phox}$ (9, 12–14). rap1A has been postulated as an oxidase component since it copurifies with cytochrome b558 (11), however, its function within the oxidase complex has not yet been elucidated. The cytosolic oxidase components include the proteins $p47^{phox}$ and $p67^{phox}$, both of which have been unequivocally established as essential elements of the oxidase (15) and one or both of the cytosolic GTP-binding proteins rac1 or rac2, each of which has been reported to support oxidase activity in a cell-free system (16, 17). During the cell activation process, all of these proteins migrate from the cytoplasm to the membrane.

Defective activity of the respiratory burst oxidase is the cause of an inherited disorder known as chronic granulomatous disease (CGD)[1] (18). In this condition, phagocytes produce little or no O_2^-, and as a consequence, affected patients suffer from recurrent life-threatening infections. In all CGD patients studied to date, the abnormality in oxidase activity has been attributed to a defect in one of the four components: the α ($p22^{phox}$) or β ($gp91^{phox}$) subunit of cytochrome b558, $p47^{phox}$, or $p67^{phox}$. All of these oxidase components have been cloned (19–22). However, only the cytosolic proteins $p47^{phox}$ and $p67^{phox}$ have so far been expressed as functional proteins in bacterial fusion constructs and in the baculovirus expression system (21, 23, 24). Recombinant $p47^{phox}$ and $p67^{phox}$ have been shown to restore oxidase activity to $p47^{phox}$- or $p67^{phox}$-deficient cytoplasm in the cell-free, oxidase-activating system (23, 24), and transfection of an expression vector directing $p47^{phox}$ synthesis has been shown to restore oxidase activity to $p47^{phox}$-deficient B lymphocyte lines (25, 26, 27).

Partially purified human neutrophil cytochrome b558 has been reported to reconstitute the defective oxidase activity of cytochrome-deficient CGD neutrophils in a cell-free oxidase activation system (28). However, attempts at expression of recombinant cytochrome b558 or its subunits in COS cells (29) and baculovirus expression systems (30) have met with limited success, and functionally active, recombinant cytochrome has thus far not been obtained. Cytochrome b558 is a structurally unusual cytochrome and shares only modest partial homology with other cytochromes. Furthermore, in CGD patients with mutations in one cytochrome subunit, the level of the unaffected subunit protein is generally greatly reduced (20, 31), suggesting that the single subunits are not stable in the absence of heterodimer formation. Mutations of the $p22^{phox}$ gene have been identified in patients with autosomal, cytochrome-negative ($A22^0$) CGD (29), and mutations of the gene for $gp91^{phox}$ are found in patients with X-linked cytochrome-negative ($X91^0$) CGD (19). However, the causal nature of the genetic defects in these subgroups of CGD has not been shown formally because of a lack of an expression system for generation of functionally active recombinant $p22^{phox}$ and $gp91^{phox}$. Structure-function studies of the cytochrome b558 have also been hampered by the lack of a suitable system for expression of recombinant derivatives of $p22^{phox}$ and $gp91^{phox}$, as altered by site-directed mutagenesis.

In this paper, we report the reconstitution of oxidase activity in intact autosomal cytochrome–negative CGD ($A22^0$ CGD)-derived B lymphocyte cell lines by stable transfection with an expression vector containing a cDNA that encodes $p22^{phox}$.

Materials and Methods

Autosomal Cytochrome-negative CGD ($A22^0$ CGD) Patients. CGD patients were diagnosed as autosomal cytochrome-negative ($A22^0$) CGD according to established clinical and laboratory criteria (32). The p22 gene of patient $A22^0$:1 was sequenced and exhibits a nucleotide change (C-382 → A) predicting a nonconservative amino acid change (Ser-118 → Arg) in the mature $p22^{phox}$ protein (29). Patient $A22^0$:2 was also characterized previously as autosomal recessive cytochrome negative CGD (33), and is unrelated to patient $A22^0$:1.

Cell Lines and Medium. EBV-transformed B cell lines from $A22^0$ CGD patients were established by transformation of PBMC with EBV. Briefly, after obtaining informed consent, mononuclear cells were isolated from samples of venous blood and cultured with supernatant of the B95-8 marmoset B cell line containing EBV virions, using RPMI 1640 medium supplemented with 20% heat-inactivated FCS (Hyclone Laboratories, Logan, UT), the antibiotics penicillin, streptomycin, and fungizone, and buffered to pH 7.2. Cells were kept in a humidified incubator at 37°C gassed with ambient air plus 5% CO_2. Continuously growing EBV-transformed cell lines were obtained after 5–6 wk and were carried further in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, the antibiotics penicillin, streptomycin, and ciprofloxacin, and buffered with 20 mM Hepes to pH 7.2 (3). Viability of EBV-transformed cell lines was routinely >90% by trypan blue exclusion test. EBV-transformed B lymphoblast lines from patients with X-linked CGD ($X91^0$-CGD B cell lines) and from normal individuals (wild-type EBV B cell lines) were derived in an analogous fashion.

The human PLB-985 myelomonoblastic cell line has been described earlier (34). To obtain granulocyte-like forms, PLB-985 cells were exposed to 60 mM dimethylformamide for 6 d. The levels of cytochrome b558 in these induced cells is about 50% of that seen in normal peripheral blood neutrophils (our unpublished observation).

Construction of Expression Vector pEBOp22^{phox}. The expression vector pEBOp22^{phox} was constructed by inserting a full-length $p22^{phox}$ cDNA into the episomal expression vector pEBOpLPP. pEBOpLPP, a 10.6-kb plasmid constructed from portions of the four plasmids pcDV, pL, pUC19, and EBOpSVNeo (35) was from R. Margolskee (Roche Research Institute, Nutley, NJ) and F. V. Chisari (The Scripps Research Institute). The vector contains the following functional units: a pBR322 origin, a β-lactamase-gene, an EBNA-1 gene driven by a SV40 late promoter, a gene encoding hygromycin phosphotransferase driven by an SV40 early promoter, an SV40 late-region intervening sequence, the SV40 origin of replication oriP, and a polylinker region containing unique sites 5′-SacI-HindIII-

[1] *Abbreviations used in this paper*: CGD, chronic granulomatous disease; NBT, nitroblue tetrazolium.

XbaI-SalI-NotI-KpnI-3′ the insert of which is driven by a second SV40 early promoter.

A full-length clone of $p22^{phox}$ cDNA (20) was subcloned into the EcoRI site of pGEM9 (Promega, Madison, WI). The 700-bp insert containing $p22^{phox}$ cDNA was then directionally cloned in the sense orientation into the HindIII-SstI site of pEBOpLPP. The final construct, pEBOp22^{phox}, was amplified in *Escherichia coli* DH5α and purified by alkaline lysis and cesium chloride banding for transfection of cell lines. Where not otherwise indicated, molecular genetic manipulations were performed as described by Sambrook et al. (36).

Transfection and Selection. pEBOp22^{phox} plasmid DNA was introduced into $A22^0$ CGD B lymphocyte cell lines by electroporation (37). For this, cell lines were kept in logarithmic growth phase by splitting them 1:2 for three consecutive days. Before electroporation, cells were transferred into serum-free RPMI 1640 medium supplemented with 1 mM glucose and 0.1 mM dithiothreitol to give a final density of 20×10^6/ml. 500 μl cell suspension (10^7 cells) were mixed gently with 10 μg plasmid DNA in an electroporation cuvette (0.4 cm electrode distance, BioRad Laboratories, Cambridge, MA) and immediately subjected to electroporation at 200 V and 960 μF using a gene pulser (BioRad Laboratories). These manipulations were done at ambient temperature under sterile conditions. Transfection efficiency was about 5% using β-galactosidase as reporter gene. Immediately after the electroporation pulse, cells were transferred to 10 ml of complete culture medium. After 48 h of culture, selection of transfectants was started by adding hygromycin at 50 μg/ml. Selection of transfectants was complete after 4 wk of culture in hygromycin; selected cells were maintained at 25 μg/ml hygromycin. Viability of transfectant lines was 80–90% as assessed by trypan blue exclusion.

Isolation and Analysis of RNA. Total RNA was isolated from B cell lines by the acid guanidinium thiocyanate method (38). Northern blot analysis with ^{32}P-labeled cDNA probes was performed essentially as previously described (29). Briefly, samples were electrophoresed on denaturing formaldehyde-agarose gels, transferred to nylon membranes (Magnagraph; Micron Separations, Westboro, MA), hybridized with radiolabeled probes using conditions suggested by the manufacturer, and washed under high stringency conditions. The $p22^{phox}$ probe was a full-length cDNA (20). The human β-actin cDNA was obtained from C. Srivastava (Indiana State University School of Medicine, Indianapolis, IN).

Immunoblot Analysis of Protein Expression. Triton X-100 extracts of whole cells (19) and of cytosolic and membrane fractions isolated from sonicated cells (8) were prepared for immunoblot analysis as described (19). Ab binding was detected by a chemiluminescent method using a horseradish peroxidase–conjugated second Ab (Amersham Corp., Arlington Heights, IL). The $p22^{phox}$ Ab was affinity purified from rabbits immunized with a synthetic peptide derived from $p22^{phox}$ sequence (39). The $gp91^{phox}$ Ab was a mouse mAb raised to purified cytochrome b (31), kindly provided by Dr. Dirk Roos (The Netherlands Red Cross Central Laboratory, Amsterdam, The Netherlands).

Chemiluminescence Monitoring of Peroxide Generation by B Cells. Cells were transferred from culture medium into phenol red–free MEM (GIBCO BRL, Gaithersburg, MD), supplemented with BSA (100 μg/ml) and buffered with 5 mM Hepes, pH 7.2, to give a final cell density of 5×10^6/ml. Luminol (100 μM final concentration) and horseradish peroxidase (10 U/ml) were added for luminescent detection of hydrogen peroxide (40). A 200-μl cell suspension, containing 10^6 cells, was placed into the wells of a white opaque microtiter plate (Dynatech Laboratories Inc., Chantilly, VA). After adding stimuli, chemiluminescence was recorded as 4-h integrals or kinetically by repeated recordings of 15- or 30-min integrals by single photon imaging in a camera luminometer (Argus 100; Hamamatsu Photonic Systems Corp., Bridgewater, NJ) thermostatted to 37°C as described earlier (41).

Nitroblue Tetrazolium (NBT) Reduction. NBT reduction was used to estimate the frequency of superoxide-generating cells as described earlier (4). Briefly, cells at a density of 2×10^6/ml in RPMI 1640 medium containing 10% FCS were stimulated at 37°C with PMA (100 ng/ml) in the presence of 1 mg/ml NBT.

The reaction was stopped by the addition of an equal volume of ice-cold 2% paraformaldehyde and the frequency of NBT-positive (blue) cells determined by light microscopy.

Reagents. NBT, Luminol, glucose, dithiothreitol, PMA, horseradish peroxidase, and superoxide dismutase were purchased from Sigma Chemical Co. (St. Louis, MO). Protein A–bearing staphylococci (Pansorbin) and hygromycin were obtained from Calbiochem-Novabiochem Corp. (La Jolla, CA).

Results

Northern Blot Analysis of $p22^{phox}$. To verify the effectiveness of transcription from the plasmid pEBOp22^{phox} transfected into B cell lines, Northern blots were performed using a $p22^{phox}$ probe. Normal wild-type EBV B cells contained $p22^{phox}$ mRNA of the expected size (0.9 kb), which was barely detectable in both $A22^0$ CGD–derived EBV B cell lines. In contrast, after transfection with pEBOp22^{phox}, both $A22^0$ CGD–derived EBV B cell lines contained an abundant 1.6-kb plasmid-derived $p22^{phox}$ mRNA species in addition to the endogenous 0.9-kb transcript (Fig. 1).

Immunoblot Analysis of Cytochrome b558 Subunit Proteins. Next, cellular expression of the $p22^{phox}$ cytochrome subunit was investigated by immunoblotting. Granulocyte-induced PLB-985 cells, wild-type EBV B cell lines and, to a much lesser extent, $X91^0$ CGD–derived EBV B cell lines expressed $p22^{phox}$ immunoreactive protein. In contrast, $p22^{phox}$ immunoreactive protein was undetectable in both parental $A22^0$ CGD lines. However, correlating with the abundant production of plasmid-derived $p22^{phox}$ mRNA, $A22^0$ CGD lines transfected with EBO-$p22^{phox}$ contained readily detectable $p22^{phox}$ protein, at levels equal to or even exceeding those seen in wild-type EBV B cell lines (Fig. 2, *top*). The $p22^{phox}$ in the wild-type B cell line and the $A22^0$ line transfected with EBO-$p22^{phox}$ was localized to the membrane fraction (Fig. 2, *top*), as expected.

It has been noted previously that $A22^0$ CGD neutrophils also lack the glycosylated large cytochrome subunit, $gp91^{phox}$, apparently because of a lack of stabilizing interaction with $p22^{phox}$ (29, 42). It was therefore of interest to determine expression of $gp91^{phox}$ in $A22^0$ CGD–derived B cell lines before and after correction of $p22^{phox}$ expression by transfection. Using a mAb raised to purified cytochrome b (31), the $gp91^{phox}$ detected in wild-type B cell lines appeared as a diffuse band ranging from about 55 to 95 kD, in contrast to its appearance as a band centered at about 90–110 kD in granulocyte-induced PLB-985 cells (Fig. 2, *bottom*) and peripheral blood neutrophils (19). This finding may reflect differences in glycosylation between B cells and myeloid cells. $A22^0$ CGD–derived B cell lines contained a membrane-

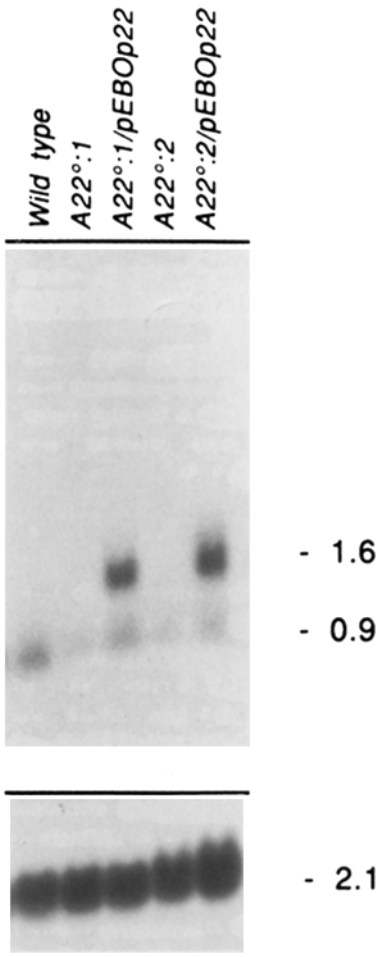

Figure 1. Northern blot of total cellular RNA derived from control and transfected B lymphoblast lines. RNA was isolated from a wild-type B cell line and B cell lines derived from A22^0 CGD patients 1 and 2 before and after transfection with the pEBOp22 expression plasmid. After denaturing gel electrophoresis, RNAs were transferred to a nylon membrane and hybridized with a ^{32}P-labeled full-length cDNA probe for p22^{phox} (*top*), and subsequently with a labeled cDNA probe for actin (*bottom*). The marks note the positions of the endogenous p22^{phox} (*0.9* kb) and actin (*2.1* kb) transcripts, as well as the p22^{phox} transcript derived from the pEBOp22 construct (*1.6* kb).

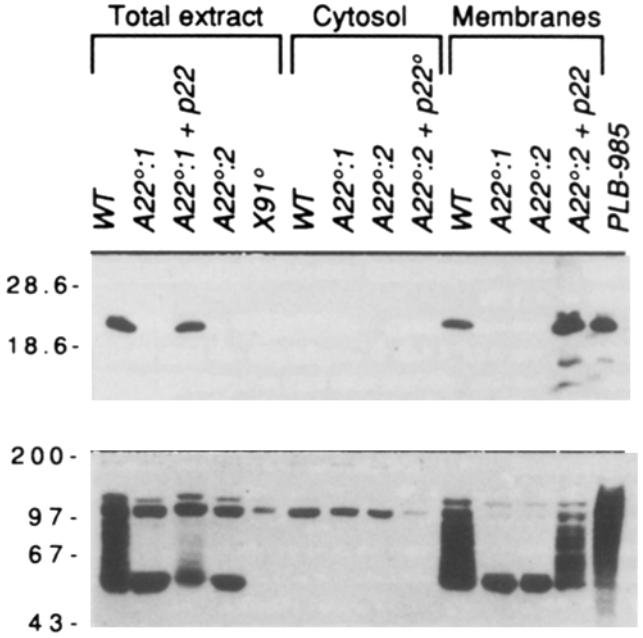

Figure 2. Immunoblot of protein extracts isolated from myeloid and B lymphoblast cell lines. B cell lines and granulocyte-induced PLB-985 were solubilized and analyzed for expression of p22^{phox} (*top*) or gp91^{phox} (*bottom*) using Abs raised to these cytochrome subunits. Membrane and cytosolic fractions were also analyzed for some of the B cell lines. Each lane of whole cell extract (*Total extract*) was loaded with 25 μg of protein, except for granulocyte-induced PLB-985 cells which contained only 5 μg of protein. 15 and 10 μg were loaded for extracts prepared from cytosol and membrane fractions, respectively. Cell lines analyzed were a B cell line from a normal individual (*WT*), B cell lines from the 2 A22^0 CGD patients before (*A22^0:1* and *A22^0:2*) and after transfection with the pEBOp22 expression plasmid (*A22^0:1 + p22* and *A22^0:2 + p22*), a B cell line from a patient with cytochrome-negative X-linked CGD (*X91^0*), and the myeloid PLB-985 cell line, induced to differentiate into granulocytic forms with dimethylformamide.

associated immunoreactive protein of about 55 kD, which was absent in an X91^0 B cell line, that may represent unglycosylated gp91^{phox} (Fig. 2, *bottom*). After transfection with EBO-p22^{phox}, both A22^0 CGD–derived B cell lines contained forms of gp91^{phox} ranging from about 55 to 100 kD, now resembling the wild-type B cells (Fig. 2, *bottom*). All of the EBV B cell lines examined had a prominent band of about 100 Kd (Fig. 2, *bottom*), which localized to the cytosolic fraction in sonicated cells. As this species was also present in a cell line derived from a patient with X91^0 CGD (Fig. 2, *bottom*), it is likely to represent a cross-reactive protein.

Transfection with pEBOp22^{phox} Restores Oxidase Activity. Before transfection with p22^{phox}, both A22^0 CGD–derived B cell lines failed to generate chemiluminescence and to reduce NBT in response to stimulation with either phorbol ester PMA or the surface Ig cross-linking reagent, protein A (Table 1 and Fig. 3). This inability to perform an oxidative burst is in line with previous reports on the missing oxidase activity of CGD-derived, EBV-transformed B cell lines (2). In contrast, the transfectants A22^0:1/pEBOp22 and A22^0:2/pEBOp22 clearly possessed oxidase activity (Fig. 3 and Table 1). This was evident both in the NBT reduction assay which is sensitive to superoxide, and in the chemiluminescence assay which detects hydrogen peroxide. The frequency of NBT-positive cells among PMA-stimulated A22^0:2/pEBOp22 was about double that of PMA-stimulated A22^0:1/pEBOp22 cells, and in parallel, A22^0:2/pEBOp22 cells exhibited higher chemiluminescence than A22^0:1/pEBOp22. Interestingly, in addition to phorbol ester PMA, pEBOp22-transfected A22^0 CGD–derived B cell lines also responded with oxidase activity in response to cross-linking of surface Ig by protein A (Fig. 3) which represents a more physiologic and receptor-mediated stimulus of NADPH oxidase in normal EBV B cell lines (3). The relative level of oxidase activity of the A22^0 pEBOp22 transfectants (as measured both by NBT reduction and chemiluminescence) was within the range of oxidase activity seen in a panel of normal

Table 1. *NBT Reduction by A22⁰ CGD B Cell Lines before and after Transfection with pEBOp22^{phox}*

Cell line	Additions	Incubation time	NBT-positive cells
		min	%
A22⁰:1	–	30	0.2*
	–	60	1.06
A22⁰:1/pEBOp22	–	30	1.75
	–	60	8.08
	SOD‡	60	2.2
	Boiled SOD§	60	7.94
	HSA‖	60	8.2
A22⁰:2	–	30	0.3
	–	60	2.5
A22⁰:2/pEBOp22	–	30	3.9
	–	60	23.1
	SOD	60	3.2
	Boiled SOD	60	21.2
	HSA	60	22.2

* 10^6 cells in 500 μl RPMI/10% FCS with 1 mg/ml NBT were stimulated with 100 ng/ml PMA and incubated at 37°C. The reaction was stopped with ice-cold 2% paraformaldehyde/PBS and the frequency of NBT-positive (blue) cells determined by light microscopy from a minimum of 500 cells counted.
‡ Superoxide dismutase, 100 μg/ml.
§ SOD boiled for 15 min, 100 μg/ml.
‖ Human serum albumin, 100 μg/ml.

EBV B cell lines (data not shown). Transfection of wild-type, oxidase-competent EBV B cell lines with pEBOp22^{phox} had no effect on oxidase activity (data not shown).

Discussion

Mutations in the p22^{phox} gene, which codes for the small subunit of cytochrome b558, have previously been identified in several patients with autosomal cytochrome-negative CGD (29). However, in the absence of an expression and assay system for natural or mutant cytochrome b558, it could not be formally demonstrated that the missense variants result in a defective p22^{phox} cytochrome subunit. Here, we made use of the fact that the NADPH oxidase of phagocytes is also present in B lymphocytes (2–4) and that EBV-transformed B lymphocyte cell lines generated from CGD patients share the genetic defects apparent in the patients' phagocytes (2). Transfection of A22⁰ CGD–derived B cell lines with an expression vector containing a wild-type p22^{phox} cDNA led to production of recombinant p22^{phox} protein, "rescue" of the large subunit of cytochrome b558, and most importantly, to restoration of the capacity to generate an oxidative burst in response to defined stimuli. Restitution of p22^{phox} expression and oxidase activity was observed in cell lines derived from two unrelated A22⁰ CGD patients. These data represent the first formal demonstration that the respiratory burst in CGD patients with p22^{phox} gene defects can be reconstituted by expression of wild-type p22^{phox}. These results also underscore the importance of cytochrome b558 expression for intact oxidase function. We (25) and other authors (26, 27) have previously used similar approaches to functionally correct genetic defects in EBV-transformed B cell lines from p47^{phox}-deficient patients. The ability to achieve genetic

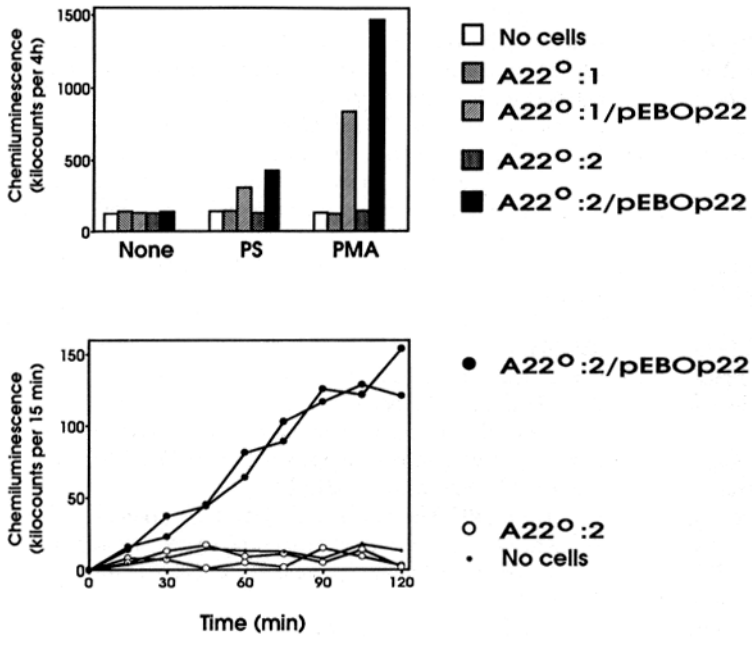

Figure 3. Chemiluminescence of A22⁰ CGD B cell lines before and after transfection with pEBOp22^{phox}. (*Top*) Integrated chemiluminescence. 10^6 cells in 250 μl phenol red–free MEM were placed in wells of a white opaque microtiter plate in the presence of 100 μM Luminol and 10 μg/ml horseradish peroxidase. After addition of stimuli (Pansorbin [*PS*], 1 mg/ml; *PMA*, 100 ng/ml), chemiluminescence was accumulated for 4 h simultaneously from all samples by single photon imaging. (*No cells*) Mean chemiluminescence accumulated over four empty wells = camera background. Addition of catalase (50 μg/ml), but not of irrelevant protein (human serum albumin) abolished these chemiluminescence responses. One representative experiment of five is shown, and data are means of triplicates with SD <15%. (*Bottom*) Kinetic chemiluminescence measurement. Native A22⁰:2 and pEBOp22^{phox}-transfected A22⁰:2/pEBOp22 cells were each set up in duplicates as in the upper panel. After stimulation with PMA (100 ng/ml), chemiluminescence was repeatedly accumulated over 15-min intervals. Data points represent measurements from individual wells.

reconstitution of CGD B cell lines is a useful in vitro model in which to develop an experimental framework for somatic gene therapy of this life-threatening disease.

Although the cDNAs for the $p22^{phox}$ and $gp91^{phox}$ subunits of cytochrome b558 have been isolated some years ago (19, 20), the functional organization of this heterodimer has been largely uncharacterized. The $p22^{phox}$-deficient B cell lines derived from $A22^0$ CGD patients provide a useful whole cell model for expression of recombinant $p22^{phox}$ and for evaluation of the effects of specific modifications in $p22^{phox}$ as introduced by site-directed mutagenesis. Superoxide and derived reactive oxygen species not only serve useful microbicidal functions, but can also cause serious tissue damage. The excessive generation of phagocyte-derived oxidants has been implicated in the pathogenesis of a wide variety of inflammatory conditions (5, 43). Definition of critical functional domains within cytochrome b558 may aid in the rational design of antiinflammatory agents that specifically interfere with superoxide formation.

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