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Genetics

Gene targeting of X chromosome-linked chronic granulomatous disease locus in a human myeloid leukemia cell line and rescue by expression of recombinant gp91^{phox}

(NADPH oxidase/superoxide/respiratory burst/cytochrome *b*/neutrophil)

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ABSTRACT **The X chromosome-linked chronic granulomatous disease (X-CGD) locus, which encodes the gp91^{phox} subunit of the phagocyte respiratory-burst oxidase cytochrome *b*, was disrupted by homologous recombination in the PLB-985 human myeloid cell line to develop an *in vitro* model of X-CGD. Superoxide formation was absent in targeted cells after differentiation to granulocytes but was rescued by stable transfection and expression of wild-type gp91^{phox} cDNA. The targeted cell line should be useful in experiments aimed at defining functional regions within gp91^{phox} by expression of mutant gp91^{phox} cDNAs, complementing studies of naturally occurring mutations in X-CGD. In addition, the mutant line provides a model system in which to establish an experimental basis for the treatment of X-CGD patients with gene replacement therapy. Rescued clones containing even modest amounts of recombinant gp91^{phox} had respiratory-burst activity comparable to the wild-type PLB-985 line, suggesting that functional correction of X-CGD neutrophils may not require high-level expression of gp91^{phox}.**

Chronic granulomatous disease (CGD) is an inherited disorder in which phagocytic leukocytes lack an intact NADPH oxidase (respiratory-burst oxidase) (1). This membrane-associated enzyme complex generates large quantities of superoxide (O_2^-) during the respiratory burst by catalyzing the transfer of electrons from NADPH to oxygen. O_2^- and its derivatives (e.g., H_2O_2 and HOCl) are essential for normal microbicidal activity, and patients with CGD develop recurrent and often life-threatening bacterial and fungal infections. CGD can result from mutations in any one of four genes encoding phagocyte-specific proteins that are part of the active respiratory-burst oxidase complex (1). Two-thirds of the cases are due to mutations in the X chromosome-linked gene for gp91^{phox} (where phox stands for *ph*agocyte *ox*idase), a 91-kDa membrane glycoprotein that is the larger subunit of a cytochrome *b* heterodimer (2). The gene for the 22-kDa cytochrome *b* subunit, p22^{phox}, is the site of mutations in a rare autosomal recessive form of CGD (3). Other autosomal recessive subgroups of CGD are associated with the genetic deficiency of either of two cytosolic oxidase components, p47^{phox} or p67^{phox} (1, 4).

The respiratory-burst oxidase is dormant in resting cells, but upon activation by opsonized bacteria or other stimuli, is rapidly assembled from cytosolic and membrane components. The phagocyte cytochrome *b*, which has an unusually low midpoint potential (−245 mV), resides in the membrane compartment and is believed to act as the terminal electron carrier in the reduction of oxygen to O_2^- (1, 2). The cytochrome contains two heme groups per heterodimer, with heme centers in both subunits (5–7). Recent evidence suggests that gp91^{phox} also bears an NADPH-binding site and flavin group, indicating that the cytochrome may function as the sole redox center in the oxidase (8, 9). The functions of the cytosolic components p47^{phox} and p67^{phox}, which migrate to the membrane upon oxidase activation (10, 11), are as yet unknown. Two cytosolic GTP-binding proteins, Rac1 and Rac2, augment, and are perhaps required for, oxidase activity (12, 13). Another small GTP-binding protein, Rap1A, copurifies with the cytochrome *b* (14) and may also regulate oxidase activity under some conditions (15, 16).

The topologic and functional domains of the cytochrome *b* remain largely uncharacterized. The gp91^{phox} and p22^{phox} polypeptides, each of which contains multiple hydrophobic regions (2), are closely associated and can be separated only under extreme conditions (5). Heterodimer formation appears to be important for normal intracellular stability of each cytochrome subunit, and the genetic deficiency of one subunit is associated with a marked reduction in the level of its partner (3, 17). Mutations identified in the gp91^{phox} and p22^{phox} genes in CGD are heterogenous and largely consist of point insertions, substitutions, or deletions within exons or splice junctions (1). Most are associated with very reduced or absent levels of cytochrome *b*. A few rare patients have normal levels of a dysfunctional cytochrome, associated with missense mutations or in-frame deletions within the intracytoplasmic C-terminal domains of either gp91^{phox} (18, 19) or p22^{phox} (20).

Here we sought to develop a cultured cell model of X chromosome-linked CGD (X-CGD) to serve as a system in which to express recombinant forms of gp91^{phox}. The X chromosome-linked gp91^{phox} gene was disrupted by homologous recombination in a human myeloid leukemia cell line, PLB-985. PLB-985 cells are bipotential and can differentiate into either granulocytic or monocytic forms. Targeted clones derived from the parental line, which is XO, are devoid of respiratory-burst activity after induction to granulocytic forms. Stable transfection of wild-type gp91^{phox} cDNA restores the capacity of targeted cells to generate O_2^- after granulocytic differentiation.

METHODS

Plasmids. To prepare a gene-targeting construct, a 4.0-kb *Hin*dIII–*Spe* I fragment of the human gp91^{phox} gene contain-

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Abbreviations: CGD, chronic granulomatous disease; X-CGD, X chromosome-linked CGD; DMF, dimethylformamide; EF-1α, elongation factor 1α; NBT, nitro blue tetrazolium.

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ing exons 2 and 3 was cloned into Bluescript II KS (Stratagene), and a *Pst* I site within exon 3 was changed to a *Bam*HI site. A PGK-promoter-hygromycin B phosphotransferase cassette (21) (kindly provided by R. Mortenson, Harvard Medical School) was then cloned into exon 3 as a *Bam*HI–*Bgl* II fragment. The targeting construct was liberated from the plasmid backbone by digestion with *Cla* I and *Not* I prior to electroporation. For expression of gp91^{phox} cDNAs, we constructed a vector, pEF-PGKneo, that contains the human elongation factor 1α (EF-1α) promoter region from pEF-BOS (22) and a simian virus 40 polyadenylylation signal linked to a separate neomycin-resistance cassette, PGKneo (21). A full-length wild-type gp91^{phox} cDNA, extending from 12 nt upstream of the initiator ATG to a *Sac* I site in the 3′ untranslated region, was inserted downstream from the EF-1α promotor in pEF-PGKneo. We also prepared a second gp91^{phox} pEF-PGKneo construct using a mutant cDNA derived from a patient with normal levels of a nonfunctional cytochrome and a P415H missense mutation due to a C → A transversion at nt 1256 (18). The pEF-PGKneo plasmids were linearized with *Kpn* I prior to electroporation. Preparation and other manipulations of plasmid DNA were by standard methods (23).

Cells and Differentiation Conditions. PLB-985 cells (24) were obtained from P. Newburger (University of Massachusetts) and grown in RPMI 1640 medium supplemented with 2 mM glutamine, 10% (vol/vol) fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml), in a humidified incubator at 37°C under an atmosphere of 5% CO_2/95% air. For granulocytic differentiation, logarithmically growing cells at a density of 1–5 × 10^5 cells per ml were exposed to 0.5% dimethylformamide (DMF) for 5–6 days (25); under these conditions, ≈80% of wild-type or rescued targeted PLB-985 cells acquired respiratory-burst activity, as assayed by the nitro blue tetrazolium (NBT) test (see below). Peripheral blood neutrophils were isolated by Ficoll/Paque and dextran sedimentation as described (26). The amount of cytochrome *b* in induced wild-type PLB-985 cells was 25–50% relative to that seen in peripheral blood neutrophils, as assessed on immunoblots (see below).

Transfection and Selection of PLB-985 Cells. Between 1 and 2 × 10^7 PLB-985 cells in logarithmic growth were electroporated on ice in 0.7 ml of Hepes-buffered saline with 25–50 μg of linearized plasmid DNA plus 75 μg of salmon sperm DNA as carrier at 230 V and 960 mF in a Gene Pulser unit (Bio-Rad). After 24 h in normal growth medium (except supplemented with 20% fetal calf serum), viable cells (typically 20–50% of starting number) were plated at a density of 5 × 10^4 cells per ml in 96-well microtiter plates in normal growth medium with antibiotics for selection of transfected cells [hygromycin B (Calbiochem) at 350 μg/ml or G418 (GIBCO) at 1.5 mg/ml]. After 2–3 weeks in selection, individual clones were expanded for further analysis. Subclones of each of the three gp91^{phox}-targeted clones were isolated by limiting dilution; one such targeted subclone, 3-3, was used for subsequent electroporations with plasmids containing gp91^{phox} cDNAs. Clones transfected with pEF-PGKneo constructs were maintained in G418 (0.8 mg/ml) after initial selection. We have observed no effect of either a hygromycin B phosphotransferase or a neomycin-resistance transgene on the rate of acquisition of respiratory-burst activity in PLB-985 clones (unpublished observations).

Analysis of DNA and RNA. DNA was prepared from PLB-985 clones and subjected to Southern blot analysis by standard methods (23), using Magnagraph membranes (Micron Separations, Westboro, MA) and Quickhyb (Stratagene) for blot hybridizations. The acid phenol method (27) was used to isolate total cellular RNA. Northern blot analysis was performed essentially as described (18), except that Magnagraph nylon membranes were used, under conditions suggested by the manufacturer. Double-stranded probes were labeled by random priming and included full-length gp91^{phox}, p22^{phox} (25), and p47^{phox} cDNAs (28), the cDNA for human β-actin (kindly provided by C. Srivastava, Indiana University School of Medicine), and fragments derived from the human gp91^{phox} gene: an ≈200-bp *Hin*dIII fragment containing exon 1, an ≈800-bp *Eco*RI fragment in intron 1, and an ≈500-bp *Spe* I fragment in intron 3. The preparation of cDNA from total cellular RNA using reverse transcriptase, amplification of gp91^{phox} sequences using PCR, and DNA sequencing was as described (18).

Immunoblot Analysis. Immunoblot analysis of Triton X-100 extracts of PLB-985 cells was performed as described (17), except that binding of primary antibodies was detected by a chemiluminescent method with a horseradish-peroxidase-conjugated second antibody (Amersham). Affinity-purified p47^{phox} and p67^{phox} (both kindly provided by J. Curnutte, The Scripps Institute) and p22^{phox} antibodies have been described (20). The gp91^{phox} antibody was a mouse monoclonal antibody raised to purified cytochrome *b* (29), kindly provided by D. Roos (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service).

Assay of O_2^- Formation. The NBT test (26) was modified for use in microtiter wells containing 0.5–2.5 × 10^5 PLB-985 cells in 150 μl of standard growth medium with or without 0.5% DMF (for granulocytic differentiation). A saturated NBT solution (50 μl) freshly prepared in RPMI 1640 medium was added to each well, along with phorbol 12-myristate 13-acetate (Sigma) to a final concentration of 100 ng/ml to activate the respiratory burst. After incubation for 30–60 min in a 37°VC tissue culture incubator, deposition of purple formazan granules (from the reduction of NBT by O_2^-) in and around individual cells was scored visually with the aid of a phase-contrast microscope. For quantitative measurement of O_2^- production, the continuous cytochrome *c* reduction assay was used as adapted for a kinetic microplate reader (30). The assay was performed at 37°C on phorbol 12-myristate 13-acetate-stimulated whole cells, using a Thermomax microplate reader and associated SOFTMAX Version 2.02 software (Molecular Devices) for the Macintosh. Virtually all of the cytochrome *c* reduction by PLB-985 cells was sensitive to superoxide dismutase.

RESULTS

Homologous Recombination in the gp91^{phox} Gene in PLB-985 Cells. PLB-985 cells are a diploid human myelomonoblastic cell line derived from a female with relapsed myeloid leukemia (24). Although the line was XX when originally isolated (24), the PLB-985 cells used in this study are XO (unpublished data). To disrupt the X chromosome-linked gp91^{phox} gene, we prepared a targeting construct using a 4.0-kb human gp91^{phox} genomic fragment by inserting a cassette for hygromycin B phosphotransferase expression into the third exon (Fig. 1*A*). The targeting construct was introduced into PLB-985 cells by electroporation. Clones surviving selection in hygromycin were induced to differentiate into granulocytic forms with DMF for 5–6 days and screened for respiratory-burst activity using the NBT assay. Out of 726 hygromycin-resistant clones examined, 3 clones failed to reduce NBT. Southern blot analysis of the gp91^{phox} locus using probes flanking the targeting sequences demonstrated that all 3 NBT-negative clones displayed the expected pattern of hybridization for homologous recombinants (Fig. 1*B*). Hybridization with internal probes also indicated that the targeted allele had the predicted structure and contained a single hygromycin B phosphotransferase cassette (data not shown).

By Northern blot analysis, the level of the ≈4.7-kb gp91^{phox} mRNA in wild-type PLB-985 cells increased substantially

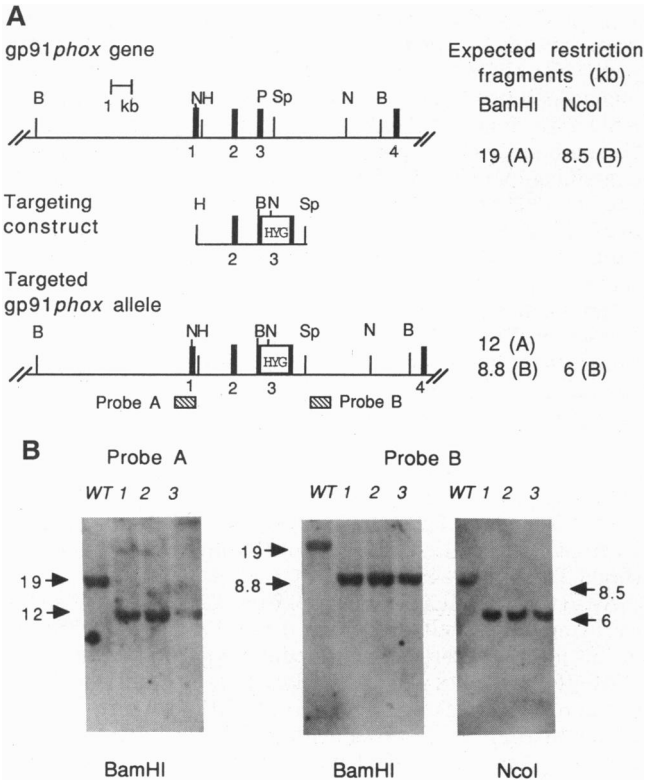

FIG. 1. Homologous recombination at the gp91^{phox} locus in human PLB-985 myeloid leukemia cells. (*A*) Structure of the targeting vector and partial restriction map of the human gp91^{phox} locus before and after targeted integration. Solid rectangles represent exons 1 through 4. B, N, H, P, and Sp represent cleavage sites for *Bam*HI, *Nco* I, *Hin*dIII, *Pst* I, and *Spe* I, respectively. HYG represents the cassette for hygromycin B phosphotransferase expression that was inserted into exon 3 in the same transcriptional orientation. Probes A and B and expected lengths of restriction fragments recognized by these probes are indicated. (*B*) Southern blot analysis of PLB-985 lines. DNA (7.5 μg) from wild-type cells (lanes WT) and targeted clones 1–3 (lanes 1–3) was digested with *Bam*HI or *Nco* I and subjected to Southern blot analysis using probe A or B, as indicated. The sizes of the hybridizing fragments are indicated in kilobases.

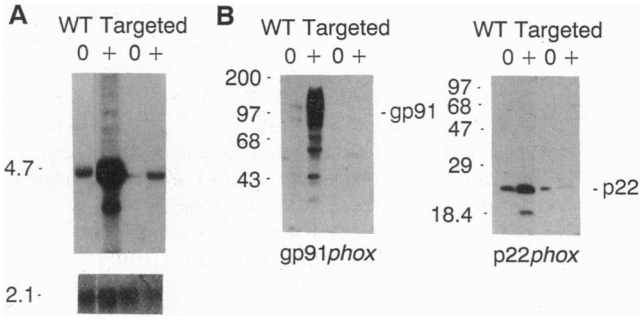

FIG. 2. Expression from mutant gp91^{phox} locus in targeted PLB-985 cells. RNA and protein was isolated from wild-type PLB-985 cells (lanes WT) and targeted clones that had undergone homologous recombination at the gp91^{phox} locus (lanes Targeted). Both undifferentiated cells (lanes 0) and cells induced to differentiate as granulocytes with DMF (lanes +) were analyzed. Results are shown for one of the three targeted clones isolated (clone 3 in Fig. 1) and were identical to those obtained for the other two clones. (*A*) Northern blot analysis. RNAs (10 μg per lane) were probed with a radiolabeled gp91^{phox} cDNA (upper blot) and then with a radiolabeled actin cDNA (lower blot). Sizes of the gp91^{phox} and actin transcripts are indicated in kilobases. (*B*) Immunoblot analysis. Wild-type and targeted cells were solubilized and analyzed for expression of gp91^{phox} or p22^{phox} by using specific antibodies, as indicated. Each lane was loaded with 25 μg of protein. The glycosylated gp91^{phox} subunit migrates as a broad band, ranging from ≈90 to 130 kDa in PLB-985 cells.

with granulocytic differentiation (Fig. 2*A*). The gp91^{phox} transcript derived from the targeted allele exhibited a similar pattern, albeit at greatly reduced levels. The PCR was used to amplify the 5′ portion of the gp91^{phox} transcript in cDNA prepared from targeted cells. DNA sequencing showed that gp91^{phox} transcript lacked exon 3 (data not shown), which had been disrupted in the targeted gp91^{phox} locus by the hygromycin B phosphotransferase cassette. This produces an in-frame deletion of nt 154–264 (numbered according to ref. 2) and predicts the absence of aa 48–84. This portion of gp91^{phox} includes a very hydrophobic region that may be an intramembranous domain.

The expression of p47^{phox} mRNA in the targeted PLB-985 clones was normal, as assessed by Northern blot hybridization, with very low levels in uninduced cells that increased markedly with granulocytic differentiation (data not shown). The abundance of the p22^{phox} cytochrome subunit mRNA, which is expressed at similar levels in both uninduced and differentiated cells, was also normal in the targeted clones (data not shown).

The level of gp91^{phox} detected in immunoblots of wild-type PLB-985 cell extracts paralleled the level of RNA, with a marked increase upon granulocytic differentiation (Fig. 2*B*). No gp91^{phox} protein was detected in uninduced targeted cells, but a faint band of ≈56 kDa was consistently seen after differentiation, which was not further characterized. This species perhaps represents unglycosylated mutant protein. Very little p22^{phox} protein was detected in uninduced and induced targeted cells, consistent with the lack of wild-type gp91^{phox} available for heterodimer formation. The induction of the p47^{phox} and p67^{phox} oxidase proteins with granulocytic differentiation of targeted cells was normal by immunoblot analysis (data not shown).

Rescue of Respiratory Burst Activity by Expression of Recombinant gp91^{phox}. We next sought to rescue respiratory-burst oxidase activity in targeted clones by stable transfection of gp91^{phox} cDNA. The expression vector pEF-PGKneo was used in these experiments, in which expression of the cDNA of interest is driven by the powerful human EF-1α promoter. The vector also contains a separate neomycin-resistance cassette. Two different gp91^{phox} constructs were prepared, containing either (*i*) wild-type gp91^{phox} cDNA or (*ii*) as a control a cDNA encoding a nonfunctional form of gp91^{phox} with a Pro-415 → His substitution (P415H) (18). The P415H mutation was identified in a patient with X-CGD whose neutrophils contain normal levels of cytochrome *b* but lack a respiratory burst (6, 18, 31).

Linearized expression constructs containing either the wild-type gp91^{phox} or gp91^{phox}(P415H) cDNA were electroporated into a targeted PLB-985 line and G418-resistant clones selected, each of which harbored a single copy of the transgene by Southern blot analysis (data not shown). Northern blot hybridization showed that the abundance of the transgene-derived gp91^{phox} mRNA in both uninduced and DMF-induced cells was comparable to the high level of gp91^{phox} expression seen in DMF-induced wild-type cells (Fig. 3*A*). The abundance of the EF-1α promoter-derived gp91^{phox} transcript appeared to increase somewhat with granulocytic differentiation.

Transgene-derived gp91^{phox} protein was present in both uninduced and DMF-induced PLB-985 cells (Fig. 3*B*). The relative amount of recombinant protein detected by immunoblot analysis was less than might be predicted from the level of mRNA seen in Northern blots. Expression of recombinant gp91^{phox} in different clones was 5–20% of the level present in DMF-induced wild-type PLB-985 cells, based on

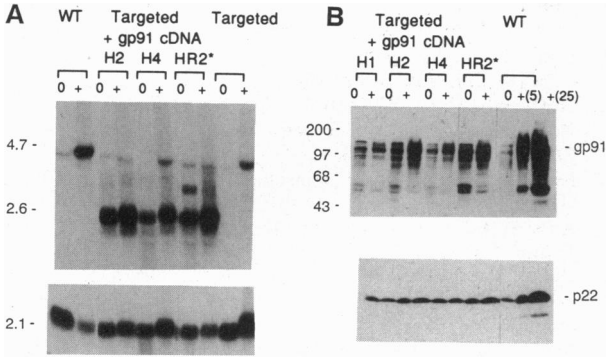

FIG. 3. Expression of gp91^{phox} in targeted PLB-985 cells containing a gp91^{phox} transgene. RNA and protein were isolated from targeted PLB-985 cells that had been transfected with a construct for gp91^{phox} cDNA expression [clones H1, H2, and H4, wild type; clone HR2*, (P415H) mutant] and from wild-type PLB-985 cells (WT) and an untransfected targeted line (Targeted). Undifferentiated cells (lanes 0) and cells induced to differentiate as granulocytes with DMF (lanes +) were analyzed. (*A*) Northern blot analysis. RNAs (10 μg per lane) were probed with a radiolabeled gp91^{phox} cDNA (upper blot) and then with a radiolabeled actin cDNA (lower blot). The sizes of the endogenous (4.7 kb) and transgene-derived (2.6 kb) gp91^{phox} transcripts and actin transcript are indicated in kilobases. A ≈3.4-kb gp91^{phox} transcript in clone HR2* presumably results from incomplete splicing of the EF-1α gene intron. Northern blot hybridization of gp91^{phox} in clone H1 (data not shown) resembled that seen in clones H2 and H4. (*B*) Immunoblot analysis. Wild-type and targeted cells harboring a gp91^{phox} cDNA transgene were solubilized and analyzed for expression of gp91^{phox} or p22^{phox} by using specific antibodies, as indicated. Each lane was loaded with 25 μg of protein, except for a lane of DMF-induced wild-type cells, which was loaded with 5 μg of protein, as noted, to facilitate comparison with gp91^{phox} levels in transfected cells. Positions of molecular mass standards are indicated in kilodaltons.

serial dilution analysis in immunoblots (data not shown). Transgene-derived gp91^{phox} increased during granulocytic differentiation (Fig. 3*B*), which could reflect the increase seen in the level of EF-1α-derived message (Fig. 3*A*) and/or other factors affecting protein translation or stability. Expression of mutant gp91^{phox}(P415H) was similar to the wild-type recombinant protein (Fig. 3*B*). The presence of recombinant gp91^{phox} led to a marked increase in the amount of p22^{phox} in targeted cells, as expected from previous observations indicating that coexpression of both cytochrome subunits is important for intracellular stability of each polypeptide (17, 25).

We next examined respiratory-burst oxidase activity in targeted clones expressing recombinant gp91^{phox} using the superoxide dismutase-inhibitable reduction of cytochrome *c* to quantitatively measure O_2^- formation. With granulocytic differentiation, wild-type PLB-985 cells are capable of generating almost as much O_2^- as peripheral blood neutrophils (Fig. 4). Uninduced targeted clones that express recombinant gp91^{phox} did not exhibit a respiratory burst (data not shown), as expected, since p47^{phox} and p67^{phox} are absent in undifferentiated cells. After granulocytic differentiation, O_2^- formation by targeted clones expressing recombinant wild-type gp91^{phox} approached levels observed in wild-type PLB-985 cells (Fig. 4 and Table 1), although the rescued clones contained less gp91^{phox} (5–20% of wild type). Targeted PLB-985 clones expressing the mutant gp91^{phox} (P415H) did not generate O_2^-, reproducing the phenotype seen in the X-CGD patients with this mutation (18, 31).

DISCUSSION

We have used gene targeting to disrupt the X-linked gene for the gp91^{phox} subunit of the respiratory-burst cytochrome *b* in

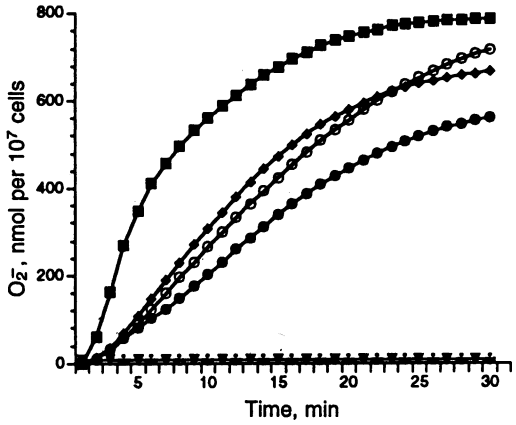

FIG. 4. O_2^- production by peripheral blood neutrophils and DMF-induced PLB-985 cell lines. O_2^- production elicited by phorbol 12-myristate 13-acetate was measured in whole cells by using the cytochrome *c* reduction assay. Results are shown for peripheral blood neutrophils (■), wild-type PLB-985 (◆), targeted PLB-985 (†), and targeted PLB-985 lines isolated after stable transfection with wild-type gp91^{phox} cDNA [H2 (●) and H4 (○)] or the mutant gp91^{phox} (P415H) cDNA [HR2* (▼)].

a human myelomonoblastic cell line and have, thereby, created an *in vitro* model of X-CGD. Targeted clones, in which the third exon of the gp91^{phox} gene is interrupted by a cassette conferring hygromycin resistance, express a small amount of an abnormally spliced gp91^{phox} transcript lacking exon 3. In this regard, the mutant PLB-985 clones resemble neutrophils in an X-CGD patient with a splice-site mutation associated with the absence of exon 3 in the mature gp91^{phox} transcript (32). Neutrophils from this patient are devoid of respiratory-burst activity, as are the targeted PLB-985 cells after granulocytic differentiation unless rescued by stable transfection with wild-type gp91^{phox} cDNA.

The PLB-985 myeloid line appears to be a suitable system in which to generate cellular mutants using strategies based on gene targeting by homologous recombination. The frequency of homologous recombination with the gp91^{phox} locus relative to random integration of the targeting construct in PLB-985 cells (3 of 723 hygromycin-resistant clones) falls within the range reported for murine embryonic stem cells and several other immortalized cell lines (33). A construct employing both a positive and negative selectable marker (34) has also been used to disrupt the gp91^{phox} locus in the PLB-985 line (unpublished data). Although having the gene of interest located on the X chromosome is clearly an experimental advantage, inactivation of both alleles of an autosomal gene can potentially be performed using two successive

Table 1. O_2^- generation by PLB-985 cell lines after granulocytic differentiation

Cell line	O_2^- generation: V_{max}, nmol of O_2^- per 10^7 cells per min	O_2^- generation: O_2^-, nmol per 10^7 cells per 30 min	*n*
Wild type	30.8 ± 8.8	534 ± 64	4
Targeted	0	0	4
Targeted + gp91^{phox} cDNA			
Wild type			
H2	24.0 ± 5.5	486 ± 50	5
H4	25.1 ± 6.7	474 ± 160	6
Mutant (P415H) HR2	0	0	4

PLB-985 lines were induced to differentiate with 0.5% DMF for 6 days. O_2^- formation was measured by a continuous cytochrome *c* reduction assay. Data for O_2^- generation are the mean ± SD.

rounds of gene targeting (21) or increasing concentrations of the antibiotic used for positive selection (35).

The availability of a cultured myeloid line genetically deficient in gp91^{phox} should be useful for expression of mutant forms of recombinant gp91^{phox} in whole cells. The systematic structure–function analysis of gp91^{phox} will complement the study of naturally occurring mutations identified in X-CGD patients. B-cell lines established by immortalization with the Epstein–Barr virus express modest levels of cytochrome *b* as well as p47^{phox} and p67^{phox} and can generate small amounts of O_2^- (10- to 100-fold less than neutrophils) under some conditions (36, 37). Although the functional significance of respiratory-burst activity in B cells is unclear, lines derived from CGD patients can also be used to evaluate the reconstitution of the oxidase by gene transfer methods, as has been reported for p47^{phox}-deficient lines (27, 38, 39). The gp91^{phox}-deficient PLB-985 myeloid line created by gene targeting provides the advantage of performing reconstitution experiments in a myeloid-cell environment. For example, the functional analysis of mutant forms of gp91^{phox} will be facilitated by the higher levels of O_2^- formation than can be achieved in granulocyte-induced cells.

X-CGD results from a single gene defect affecting cells of the myeloid lineage and is a candidate for therapy by gene transfer into hematopoietic stem cells. The targeted PLB-985 cell lines are a model system in which to develop an experimental framework for the genetic reconstitution of the respiratory burst in patients with X-CGD. It is noteworthy that O_2^- formation in targeted clones containing a gp91^{phox} transgene approached wild-type levels, although the relative amount of recombinant gp91^{phox} in the rescued clones was ≤20% of wild type. This observation suggests that the cytochrome may not be the rate-limiting component for respiratory-burst activity and that expression of even modest amounts of recombinant gp91^{phox} in X-CGD neutrophils may improve function considerably.

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