X-Linked Chronic Granulomatous Disease: Correction of NADPH Oxidase Defect by Retrovirus-Mediated Expression of gp91-phox

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Chronic granulomatous disease (CGD) is an inherited immunodeficiency resulting from the inability of an individual's phagocytes to produce superoxide anions because of defective NADPH oxidase. The disease may be treated by bone marrow transplantation and as such is a candidate for somatic gene therapy. Two thirds of patients have defects in an X-linked gene (X-CGD) encoding gp91-phox, the large subunit of the membrane cytochrome b$_{245}$ component of NADPH oxidase. Epstein-Barr virus–transformed B-cell lines from patients with CGD provide a model system for the disease. We have used retrovirus-mediated expression of gp91-phox to reconstitute functionally NADPH oxidase activity in B-cell lines from three unrelated patients with X-CGD. The protein is glycosylated and membrane associated, and the reconstituted oxidase is appropriately activated via protein kinase C. The kinetics of superoxide production by such reconstituted cells is similar to that of normal B-cell lines. These data show the potential of gene therapy for this disease.

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From Chronic Granulomatous disease (CGD) is a rare inherited immunodeficiency resulting in recurrent bacterial and fungal infections. The disease is caused by defects in NADPH oxidase, a multicomponent system responsible for the reduction of molecular oxygen to superoxide (O$_2^-$). The oxidase is functional principally in neutrophils and phagocytes. O$_2^-$ is produced within the phagocytic vacuole where, along with its derivatives hydrogen peroxide (H$_2$O$_2$) and hypochlorite, it is involved in bacterial cell killing. NADPH oxidase consists of four specific protein components, and a nonspecific G-protein of the rac family. There are two membrane proteins, p22-phox and gp91-phox, that together form a b-type cytochrome with a midpoint potential of $-245$ mV, termed cytochrome b$_{245}$ or b$_{556}$, and two cytosolic proteins, p47-phox and p67-phox. The cytochrome, in addition to binding the heme moiety, appears to be responsible for binding the substrate NADPH and the cofactor flavin adenine dinucleotide (FAD). CGD resulting from genetic defects in each of these specific components has been described. Because the majority (two thirds) of cases of CGD are caused by defects in an X-linked gene (X-CGD) encoding gp91-phox, whereas the other forms of CGD are autosomal recessive (A-CGD), most of which are caused by defects in the p47-phox gene. Isolation of the cDNA for the X-CGD gene and its identification as that for the large subunit of cytochrome b$_{245}$ was the first success of the positional cloning approach for human genetic disease.

Because CGD affects only the hematopoietic system and bone marrow (BM) transplantation can offer a satisfactory cure, the disease is a candidate for treatment by somatic gene therapy. As a first step, we have used retrovirus-mediated gene transfer of the X-CGD cDNA to reconstitute NADPH oxidase activity in cells possessing the X-linked defect. Although there is no evidence for B-cell involvement in the pathogenesis of CGD and B cells do not undergo phagocytosis, they do express functional NADPH oxidase. We and others have previously shown that components of the oxidase are expressed in Epstein-Barr virus (EBV)-transformed B-cell lines from normal individuals and that the oxidase is functional in terms of O$_2^-$ production, although at low levels compared with neutrophils. Furthermore, B-cell lines derived from patients with CGD display the disease phenotype in that they lack the ability to produce O$_2^-$ Therefore patient-derived B-cell lines are appropriate targets for gene transfer to assay NADPH oxidase function and as an in vitro model of gene therapy for this disease. EBV-transformed B-cell lines established from patients with leukocyte adhesion deficiency (LAD) have proven to be useful reagents in similar experiments for correction of the leukocyte function antigen (LFA)-1–mediated aggregation defect by retrovirus-mediated CD18 expression. Recent experiments have also been successful in correcting p47-phox deficiency in B-cell lines derived from patients with A-CGD.

MATERIALS AND METHODS

Cloning the X-CGD cDNA. Neutrophils and erythrocytes from human peripheral blood (PB) were sedimented through Ficoll-Paque. The erythrocytes were selectively lysed by treatment with isotonic ammonium chloride at room temperature, and the neutrophils were used for the preparation of total cellular RNA by lysis in 4 mol/L guanidine isothiocyanate and centrifugation through 5.7 mol/L cesium chloride. The RNA was reverse transcribed using Moloney-murine leukemia virus (MLV) reverse transcriptase and oligo(dT)$_{24-18}$ as primer. Polymerase chain reaction (PCR) with Taq DNA polymerase was performed (30 cycles of 1 minute at 94°C, 2 minutes at 50°C, 3 minutes at 72°C) using primers spanning the coding sequence and designed to introduce BamHI cloning sites at the termini. The primers were designed from the published cDNA sequence, correcting for the erroneous inclusion of an additional nucleotide within the start codon, to amplify between positions 1 and 1776. The forward primer was 5'-AGAGGATCCCCCTCTCTCTCGGCCACCATGCGG: position 1 of the published sequence is italicized and the start codon is in lower case letters. The reverse primer was 5'-CGCGGGATCCGGACATTTGGGCCGACACCC: the nucleotide complementary to position 1776 is italicized. The 1.8-kb product was isolated after agarose gel electrophoresis and digested...
Fig 1. (A) Schematic representation of the provirus in cells infected with the recombinant retrovirus MBcgd.SP. The positions of KpnI (K), BamHI (B), HindIII (H), and Clal (C) restriction sites are indicated. The LTRs are hybrids equivalent to that of MPSV and the X-CGD cDNA is a 1.8-kb BamHI fragment. There are two transcriptional units, one controlled by the 5' LTR for expression of the cDNA and one controlled by the SV40 promoter for expression of PuroR. (B) Southern blot analysis of KpnI-digested DNA using the X-CGD cDNA probe. Several fragments resulting from the presence of endogenous X-CGD gene sequences were detected. A 4.5-kb fragment (arrowed) indicates the presence of the provirus. 1 copy controls: normal B-cell DNA with added pMB.SP or pMBcgd.SP DNA equivalent to single copy per diploid genome. HeLa, DD: DNA samples of HeLa cells or the CGD B-cell line from patient D.D., non-transduced (control) or transduced with the recombinant retroviruses MB.SP or MBcgd.SP. (C) An equivalent blot to that in (B), using the PuroR probe. The provirus size is 2.7 kb for MB.SP and 4.5 kb for MBcgd.SP.

with BamHI before cloning in the plasmid vector pSP72 (Promega, Madison, WI). Nucleotide sequence analysis of individual clones showed a high rate of misincorporation during the PCR reaction, and so a hybrid of two clones was generated. This clone differs from the published sequence at position 1472, changing the codon TCC to TCT, both being frequently used to encode serine. As a further verification of this clone, it was analyzed by in vitro transcription with T7 RNA polymerase, followed by in vitro translation using a rabbit reticulocyte lysate: a single product of 55 Kd, corresponding in size to the protein core of gp91-phox, was obtained by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

Production of recombinant retroviruses. A 1-kb EcoRI/Clal fragment containing the simian vacuolating virus (SV40) promoter and the puromycin acetyl transferase gene (PuroR) was removed from the plasmid pBabePuro and inserted between the EcoRI and Clal sites upstream of the 3' long terminal repeat (LTR) in pMPSV. The latter plasmid, a basic retroviral construct derived from myeloproliferative sarcoma virus (MPSV), was obtained by deletion of sequences between the two Clal sites of the plasmid pM5Gneo. A 1.5-kb BamHI/SalI fragment containing the SV40.PuroR cassette and the majority of the U3 region of the MPSV LTR was used to replace the equivalent fragment of pBabePuro and generating the plasmid pMB.SP. The latter construct thus includes a 5' MLV LTR and a hybrid 3' LTR containing MPSV sequences as far as the SstI site between the CCAAT and TATA box promoter elements (position 8229/-35 of the MLV provirus), which is expected to be functionally equivalent to the MPSV LTR. Between the LTRs are 900 bp of MLV sequence encompassing the packaging sequence but with a mutated splice donor site and gag start codon as in pBabePuro, the 1-kb
Fig 2. Western blot analysis of gp91-phox expression. (A) Cellular membrane proteins (5 μg for HL60 cells, 50 μg for others) were analyzed by Western blotting with the MoAb 48. A signal of heterogeneous high molecular weight indicates gp91-phox expression. HL60: DMSO-induced HL60 cells. Normal: uninfected B-cell line. PT: B-cell line from patient P.T. HeLa, DD, PT: HeLa cells and the B-cell lines from patients D.D. and P.T. transduced with the recombinant retroviruses MB.SP or MBcgd.SP. The positions of protein size markers (Kd) are indicated. (B) Membrane preparations from a normal B-cell line, and from HeLa cells and B-cell lines from patients D.D. and P.T. transduced with MBcgd.SP were digested with PNGase F before Western blot analysis as above. Controls were treated equivalently but without the addition of enzyme. Additionally, normal B cells were treated with tunicamycin before membrane preparation. The positions of protein size markers (Kd) are indicated. The gp91-phox core protein has an apparent molecular weight of 55 Kd.

SV40.Puro8 cassette, and 135 bp from MPSV preceding the 3’ LTR. The X-CGD cDNA (a 1.8-kb BamHI fragment) was inserted at the BamHI site of pMB.SP, generating the plasmid pMBcgd.SP. Two ecotropic packaging cells23 were transfected with 20 μg pMBcgd.SP DNA by calcium phosphate coprecipitation followed by glycerol shock and subsequently selected in 2 μg/mL puromycin. GP + envAm-12 amphotropic packaging cells24 were infected with the ecotropic virus and similarly selected. The resultant colonies were pooled to generate a polyclonal population of producer cells that yielded a titer of approximately 10⁸ puromycin-resistant colonies per milliliter when assayed on 3T3 and HeLa cells. Producer cells of the construct pMB.SP were similarly made for use as a control. The recombinant retroviruses were designated MBcgd.SP and MB.SP, respectively. Infected 3T3 and HeLa cells were selected in 6 and 1 μg/mL puromycin, respectively.

X-CGD patient B-cell lines. The EBV-transformed B-cell lines used in this study were derived from three male patients with X-CGD (patients D.D., L.B., and P.T.) and one female patient with A-CGD (patient R.H.). Patients L.B. and P.T. have differing deletions of the gp91-phox gene (that for P.T. extends from within the first 500 bp of the 5’ end of the gene to beyond the 3’ end, whereas that for L.B. covers the whole gene), and the defect for D.D. is not caused by detectable deletion25 (unpublished results). Patient R.H. is defective in p22-phox expression (C. Casimir, personal communication, March 1992, and our own unpublished results) and so provides a control for the specificity of the functional correction achieved in the X-CGD cell lines. All four patient cell lines lack membrane-associated gp91-phox and are defective in O₂ production (see below).

Generation of transduced B-cell lines. EBV-transformed CGD B-cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS). The day before establishing the coculture, the target cells were placed in 20% FCS. 2 × 10⁶ B cells were cocultured with a 90-mm plate of subconfluent virus-producing cells in 10 mL RPMI with 20% FCS and 2 μg/mL polybrene for 2 days. The nonadhering B cells were removed and maintained without selection for 1 to 2 weeks to allow for expansion. Adherent cells were removed by replating. Infected B cells were subjected to an initial period of selection in 0.4 μg/mL puromycin, allowed to expand, and resel ected in 1.0 μg/mL for at least 2 weeks. Southern blotting confirmed the presence of proviral DNA at single copy or greater per diploid genome.

PCR was applied to DNA from transduced B-cell lines derived from the two patients with endogenous X-CGD gene deletions (L.B. and P.T.). Primers specific for a 480-bp region of the 3’ untranslated portion of the gene not present within the cDNA and known to be deleted in both patients24 (and our own unpublished observations) were used to confirm the absence of such sequences in the reconstituted cell cultures, eliminating the possibility of artifactual results caused by contamination with normal cells. Primers specific for the gene for mannan-binding protein were used as a control in duplex PCR and yielded a 320-bp product in each case.

Southern blot analysis. DNA samples (10 μg) were digested with KpnI, fractionated by agarose gel electrophoresis and transferred to Hybond-N (Amersham, Buckinghamshire, IL). Filters were hybridized with DNA probes (either the 1.8-kb BamHI X-CGD cDNA or the 660-bp HindIII/ClaI fragment encoding Puro8) labeled to high specific activity with [α-32P]-dCTP by random priming.

Western blot analysis of gp91-phox expression. Cellular membranes were prepared by hypotonic lysis.26 Cells were lysed in 10 mmol/L TRIS-HCl, pH 7.4, 2 mmol/L MgCl₂, 1 mmol/L CaCl₂, and 2 mmol/L phenylmethylsulfonyl fluoride (PMSF) at 4°C, and the nuclei were pelleted at 500g. The supernatant was centrifuged at
FUNCTIONAL CORRECTION OF X-CGD

Fig 3. Chemiluminescence assays for production of $\mathbf{O}_2^-$.

(A) Normal B cells ($10^6$) and nontransduced X-CGD B cells from patient P.T. (i) or 2.5 X $10^6$ X-CGD B cells from patients P.T. (ii) or D.D. (iii) transduced with MBcgd.SP or MB.SP, using the luminol chemiluminescence assay and stimulated with 200 ng/ml PMA. Because of the nature of this assay, the response is not a true reflection of the kinetics of $\mathbf{O}_2^-$ production.

(B) Normal B cells ($10^6$) (i) or $10^7$ X-CGD B cells from patients P.T. (ii) or D.D. (iii) transduced with MBcgd.SP, using the lucigenin chemiluminescence assay and stimulated with 200 ng/ml PMA (a-c) or PMA-ME (d). This assay is more quantitative and indicative of the response kinetics than that in (A). The inhibitors superoxide dismutase (b) or Tiron (c) were used to verify the specificity of the response. A second normal B-cell line showed a similar response to that illustrated.

NADPH oxidase functional assays. Cells were resuspended in 1 mL Hanks' balanced salt solution (HBSS) containing either 13 μmol/L luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) with 10 U/mL horseradish peroxidase or 170 μmol/L lucigenin (N,N-dimethyl-9,9'-biacridinium dinitrate) and assayed at 37°C as described previously. Chemiluminescence readings were taken at 1-minute intervals. The cells were stimulated with phorbol myristate acetate (PMA) (final concentration 20 or 200 ng/mL) or the methyl ether derivative (PMA-ME). Specificity was shown using 1 mmol/L Tiron (4,5-dihydroxy-1,3-benzene-disulfonic acid), a free-radical scavenger, or 30 U/mL superoxide dismutase, as discussed in the text.
Table 1. Summary of Functional Data Obtained by Luminol Chemiluminescence Assays of O₂⁻ Production by All Transduced Cell Lines Used in This Study

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Defect</th>
<th>Treatment</th>
<th>Stimulus</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>X-CGD</td>
<td>MBcgd.SP</td>
<td>PMA</td>
<td>Peak 37 mV</td>
</tr>
<tr>
<td></td>
<td>(Deletion)</td>
<td>transduced</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>PMA-ME</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Nontransduced</td>
<td>PMA-ME</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>LB</td>
<td>X-CGD</td>
<td>MBcgd.SP</td>
<td>PMA</td>
<td>Peak 23 mV</td>
</tr>
<tr>
<td></td>
<td>(Deletion)</td>
<td>transduced</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>PMA-ME</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Nontransduced</td>
<td>PMA-ME</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>DD</td>
<td>X-CGD</td>
<td>MBcgd.SP</td>
<td>PMA</td>
<td>Peak 20 mV</td>
</tr>
<tr>
<td></td>
<td>(Deletion)</td>
<td>transduced</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>PMA-ME</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Nontransduced</td>
<td>PMA-ME</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>RH</td>
<td>A-CGD</td>
<td>MBcgd.SP</td>
<td>PMA</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>transduced</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nontransduced</td>
<td>PMA</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>HeLa</td>
<td>MBcgd.SP</td>
<td>PMA-ME</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Normal</td>
<td>MBcgd.SP</td>
<td>PMA-ME</td>
<td></td>
<td>None</td>
</tr>
</tbody>
</table>

As indicated in the legend to Fig. 3A, 2.5 × 10⁶ cells were assayed; stimulating with 200 ng/mL PMA or PMA-ME. The response indicated is that of one typical experiment. Control puromycin-resistant transduced cells and nontransduced cells did not give a response above background. For comparison, the response from the normal cell line used in this study (10⁶ cells assayed) is included.

RESULTS

Retroviral transduction of X-CGD patient B cell lines. Complementary DNA encompassing the entire coding region of the X-CGD gene was cloned as a 1.8-kb BamHI fragment after reverse transcription and PCR amplification of total RNA obtained from human neutrophils. The cDNA was inserted into a retroviral construct designed to direct expression with the tissue specificity of the LTR of the MPSV, which is active in cells of hematopoietic origin. The vector also expresses the gene for puromycin acetyl transferase (encoding resistance to puromycin) from the SV40 early promoter to allow for selection of retrovirus-infected cells. Plasmid DNA was introduced into the packaging cell line 293LP, which cuts the provirus within each LTR, and analyzed by Southern blotting for the presence of both X-CGD and puromycin-resistance coding sequences (Fig 1B,C). This indicated that both recombinant viruses were transmitted without rearrangement and were present at single copy or greater per diploid genome in puromycin-resistant cell populations. Southern blot analysis of DNA digested with BamHI confirmed that the cDNA was intact (data not shown). Messenger RNA from the B-cell lines was analyzed by Northern blot hybridization with the X-CGD cDNA probe. A 4.5-kb LTR-derived transcript was detected in patient cells transduced with MBcgd.SP at a level comparable with that of the endogenous transcript detected in a normal B-cell line (data not shown).

Analysis of gp91-phox expression. To examine expression of gp91-phox in the transduced B-cell lines, cellular membrane fractions were prepared by hypotonic lysis and were analyzed by SDS-PAGE and Western blotting with the MoAb 48.27, which is specific for gp91-phox. Expression was detected in membranes from DMSO-induced HL60 cells (at a level comparable with that in neutrophil membranes; data not shown) and from a normal B-cell line but not from any of the nontransduced patient cell lines. The presence of this protein was also shown in the membrane fraction of both HeLa and B cells transduced with the virus MBcgd.SP but not with the control virus MB.SP (Fig 2A). In all cases, as for normal B cells and HL60 cells, the antigen was of heterogeneous molecular weight because of variable glycosylation of the protein core.

Treatment of membrane preparations from normal and transduced patient B cells with peptide-N-glycosidase F to remove N-linked sugars resulted in loss of the higher molecular weight material and the appearance in each case of the 55-Kd core protein (Fig 2B). The core protein was also detected in membrane preparations from normal B cells treated with tunicamycin to inhibit N-linked glycosylation. The stronger signal obtained with the deglycosylated protein may be due to the reduction in heterogeneity, better transfer, or a higher affinity of the antibody for the core protein compared with the glycosylated form.

Glycosylation was less heterogeneous in the transduced HeLa cells than in normal or transduced patient B cell lines. Similar variations have been reported for different phagocytic cell types for which the protein core size was identical. Expression of gp91-phox in the transduced HeLa and patient B cell lines was approximately 10% of that in the normal B-cell line.

Functional reconstitution of NADPH oxidase. Restoration of NADPH oxidase function in the transduced X-CGD B-cell lines expressing gp91-phox was shown using the sensitive luminol-based chemiluminescence assay for H₂O₂ formed by dismutation of O₂⁻. As for normal B-cell lines, O₂⁻ production by these reconstituted cells was dependent on their stimulation with phorbol myristate acetate (PMA), whereas the nontransduced CGD B cells and those transduced with the control virus did not produce O₂⁻ (Fig 3A). The response was dose dependent for PMA and was abrogated in the presence of a free-radical scavenger, as previously described for normal B-cell lines. There was no response when the reconstituted cells were stimulated with PMA-ME.
PMA-ME, the methyl ether derivative of PMA, which is not a protein kinase C agonist. HeLa cells transduced with the virus MBcgd.SP and expressing gp91-phox did not produce O₂⁻, presumably because not all the other NADPH oxidase components are expressed in nonhematopoietic cells. The A-CGD B-cell line from patient R.H., defective in p22-phox expression, was not functionally reconstituted by transduction with MBcgd.SP, confirming the specificity of correction by complementation with gp91-phox in only those cells for which all other NADPH oxidase components are present. Table 1 summarizes these functional data for all the cell lines used in this study. Equivalent reconstitution of the cell line derived from patient P.T. was achieved in two separate experiments.

Reconstituted cells also showed a response when assayed using the less sensitive lucigenin-based chemiluminescence assay, which responds to O₂⁻ directly. As for normal B-cell lines, this response was PMA dependent and was significantly reduced in the presence of a free radical scavenger or superoxide dismutase (Fig 3B). This latter assay is more quantitative than the luminol-based assay allowing an estimate of the level of reconstitution of O₂⁻ production in the B-cell line from patient P.T. of approximately 10% compared with the normal B-cell line used in this study (comparable responses were obtained with 10² and 10⁶ cells, respectively). The level of reconstitution achieved for the B-cell line from patient D.D. was somewhat less, although protein levels were similar. This may simply reflect the variation observed between B-cell lines, which may be caused by heterogeneity in cytosolic or activation components of NADPH oxidase and is unlikely to be caused by the nondeletion nature of the defect in this patient, because the level of reconstitution for the cell line from patient L.B., with a deletion defect, was similar.

The level of functional reconstitution of X-CGD B-cell lines is in general agreement with their relative levels of expression of gp91-phox (see above). Because the cytosolic components of NADPH oxidase are present in B-cell lines at similar levels to neutrophils, it is likely that cytochrome b₂₄₅ is the rate-limiting component in these cells, which is consistent with these findings. The time course of the lucigenin-based response by the reconstituted cells was similar to that of normal B cells, indicating the correct regulation of O₂⁻ production by the reconstituted NADPH oxidase.

DISCUSSION

CGD patient-derived EBV-transformed B-cell lines are valuable reagents for establishing the feasibility of expression of NADPH oxidase components functionally to correct the CGD defect. Such cell lines from patients with defective p47-phox have recently been used as targets for reconstitution using retrovirus-mediated gene transfer or transfection of EBV-based episomal vectors. However, expression of gp91-phox, defects of which comprise the most common, X-linked form of CGD, has not been so readily achieved. The experiments described here show that B-cell lines derived from patients with X-CGD that are unable to produce O₂⁻ because of a lack of gp91-phox can be functionally reconstituted by retrovirus-mediated expression of this component. The protein is correctly membrane-associated and glycosylated. Its association with p22-phox and incorporation of heme to form a functional cytochrome that appropriately interacts with the cytosolic components p47-phox and p67-phox is inferred from the reconstitution of NADPH oxidase activity. The functionally reconstituted oxidase in these cells is appropriately regulated by protein kinase C, with the magnitude of their response being comparable with the level of protein expression. Reconstituted cells were functionally stable over at least 2 months in culture, with or without continued selection in puromycin. Although the level of reconstitution is modest, these data are important in their demonstration that retrovirus-mediated expression of gp91-phox is feasible. Further to the work described here we have constructed a high-titer retrovirus for expression of gp91-phox that does not additionally encode antibiotic selection. Viral splice donor and acceptor sites are used to splice the mRNA and so to increase its translational efficiency. Preliminary data indicate that similar levels of reconstitution of NADPH oxidase activity can be achieved with this vector.

We have shown that defects in the gp91-phox component of NADPH oxidase can be corrected after introduction and expression of the X-CGD cDNA indicating the feasibility of the treatment of X-CGD by somatic gene therapy techniques. This could offer a significant advantage over current clinical management, for which there is still a 50% mortality rate by the age of 20 years. Because NADPH oxidase is required in neutrophils and phagocytes, such an approach requires introduction of the cDNA into patient BM stem cells and hence into the myeloid cell lineage. Recent studies using stromal cell support have enhanced the efficiency with which this can be attained by retroviruses using long-term BM culture in vitro. The use of autologous stromal cell support during the transduction of patient BM cell populations selected for expression of the CD34 antigen and thus enriched for the reconstituting stem cell compartment may enable significant efficiencies of gene transfer to be achieved.

That we have reconstituted function in cell lines from three unrelated patients, each of which has a different molecular defect in the gene, suggests the general applicability of a gene therapy approach for patients with X-CGD once the problems discussed above have been surmounted. The data presented here indicate that X-CGD will be a suitable disease for treatment by gene therapy.

ACKNOWLEDGMENT

We thank T. Hunt for provision of the rabbit reticulocyte lysate; J. Morganstern and W. Ostertag for the plasmids pBabePuro and pMSGneo, respectively; R. Mulligan and A. Bank for the packaging cell lines $\Psi_2$ and GP + $\textit{envAm}$-12, respectively; A.J. Verhoeven for the MoAb 48; and C. Casimir for the EBV-transformed B-cell line from patient R.H.

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