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Research Article

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Recombinant Interferon Gamma Augments Phagocyte Superoxide Production and X-Chronic Granulomatous Disease Gene Expression in X-Linked Variant Chronic Granulomatous Disease

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Abstract

We examined the potential of interferon gamma (IFN-gamma) to ameliorate the physiologic defect of chronic granulomatous disease (CGD) by studying its effects on CGD phagocyte superoxide generation, NADPH oxidase kinetics, cytochrome *b*₅₅₉ content, and expression of X-CGD (the gene for the X-linked disease). Granulocytes and macrophages from three patients in two kindreds with "variant" X-linked CGD (i.e., with very low, but detectable, baseline superoxide-generating activity) responded to IFN-gamma with enhanced nitroblue tetrazolium reduction and two- to eightfold increases in superoxide generation. IFN-gamma did not augment the respiratory burst activity of phagocytes from patients with "classic" CGD (i.e., no detectable baseline superoxide generation) or autosomal variant CGD. Incubation of a responding patient's granulocytes with IFN-gamma nearly doubled the maximal velocity for the NADPH oxidase, but did not change its abnormal Michaelis constant. Although the interferon-treated CGD granulocytes produced superoxide at a rate 40% of normal, the cytochrome *b* spectrum remained undetectable. IFN-gamma treatment of cultured monocytes from an IFN-gamma-responsive CGD patient increased the steady state level of RNA transcripts from the X-CGD gene from barely detectable up to ~ 5% of normal.

Introduction

Phagocytic cells, such as macrophages and polymorphonuclear leukocytes (PMN), produce reactive oxygen intermediates with microbicidal, tumoricidal and inflammatory effects (1, 2). The initial step in their formation is the one-electron reduction of oxygen to superoxide, catalyzed by a membrane-associated NADPH oxidase. The enzyme system is inactive in resting cells, but is rapidly activated upon exposure to a variety of particulate and soluble stimuli that act through several alternative pathways of transmembrane signal trans-

duction (3-5). Regulation of NADPH oxidase activity includes not only activation, but also processes of priming (6) and deactivation (7).

In chronic granulomatous disease (CGD),¹ phagocytes fail to produce superoxide and its oxygen metabolites (8, 9). Thus, PMN and macrophages from these patients ingest particles and degranulate normally, but fail to kill microorganisms (10). This defect in host defense results in recurrent, and sometimes fatal, purulent infections beginning early in childhood (11). The disease exhibits heterogeneity of inheritance, with the majority of cases being X-linked and a minority, autosomal recessive (12). In most (13), but not all (14), X-linked kindreds, patients' phagocytes lack spectrally detectable cytochrome *b*₅₅₉, a heme-containing protein that has been proposed as a critical terminal component of the oxidase system (15, 16). Recently, molecular cloning of the X-CGD gene (the gene for the X-linked disease) and its cDNA has been accomplished (17). The protein product of the X-CGD gene is predicted to be a 58-kd basic polypeptide that contains several potential glycosylation sites and bears no homology to known proteins, including cytochromes. Antibodies raised to a synthetic peptide derived from the predicted sequence assign the *in vivo* gene product as a glycosylated 90-kd membrane protein (18). This species forms part of the neutrophil cytochrome *b*₅₅₉ heterodimer, which consists of the 90-kd glycoprotein and a 22-kd nonglycosylated polypeptide (19). Both components are absent from PMN of patients with X-linked CGD (20). In contrast, PMN from most patients with the autosomal recessive disease contain normal cytochrome *b*₅₅₉, but lack a soluble activation factor (21) and fail to phosphorylate a protein with an apparent molecular weight of 44,000 (22). The molecular basis for this form of the disease remains unknown.

In addition to these "classic" forms of CGD, several "variants" have been reported, all with decreased but detectable neutrophil O₂⁻ production (23-28). Most of these patients presented later in life, with milder clinical disease, than classic CGD patients. Their neutrophils showed altered NADPH oxidase kinetics, which consists of an elevated Michaelis constant (K_m^{app} , reflecting decreased affinity for NADPH) with (24) or without (23, 25, 26) a decreased maximum velocity (V_{max}). In most of the X-linked cases (23, 24, 28) cytochrome *b*₅₅₉ was undetectable, but in one of the X-linked (27) and the autosomal recessive cases (25) the cytochrome content was normal. Most likely, these variants represent the currently detectable end of a spectrum of X-CGD gene expression, analogous to the range of beta thalassemia or hemophilia A.

This work was presented in part at the 28th annual meeting of the American Society of Hematology, San Francisco, CA, December 1986, and published as an abstract (1986. *Blood*. 68:82. [Abstr.]

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1. Abbreviations used in this paper: CGD, chronic granulomatous disease; IFN-alpha, -beta, -gamma, interferons alpha, beta, gamma; K_m^{app} , Michaelis constant; NBT, nitroblue tetrazolium.

Previous work in our laboratory (Newburger, P. E., S. H. Orkin, and R. A. B. Ezekowitz. Unpublished data) has shown an increase in steady state levels of RNA derived from the X-CGD gene in normal cultured monocyte-derived macrophages incubated with interferon gamma (IFN-gamma). Also, the difference in superoxide production and substrate affinity between the NADPH oxidases of CGD variant and normal neutrophils is similar to that between the NADPH oxidases of resting and IFN-gamma-activated cultured macrophages (29, 30). Further, Berton et al. have recently demonstrated the ability of IFN-gamma to enhance respiratory burst activity in neutrophils as well (31). We therefore explored the potential of IFN-gamma to ameliorate the physiologic defect of CGD by studying its effects on CGD phagocyte superoxide generation, NADPH oxidase kinetics, cytochrome *b*₅₅₉ content, and X-CGD gene expression.

Methods

Media and reagents. RPMI 1640 was obtained from Grand Island Biological Co., Grand Island, NY; cytochrome *c*, phorbol myristate acetate (PMA), nitroblue tetrazolium (NBT), superoxide dismutase, Triton X-100, Triton X-114, and zymosan, from Sigma Chemical Co., St. Louis, MO; ficoll 400 and dextran 500 from Pharmacia Fine Chemicals, Piscataway, NJ; and sodium hypaque, 50%, from Winthrop Laboratories, New York, NY.

Recombinant human INF-alpha, -beta, and -gamma were obtained from Genentech, Inc., San Francisco, CA, and from Dr. P. Trown, Hoffmann-La Roche, Inc., Nutley, NJ; all contained < 2 EU endotoxin/mg interferon (< 1 EU/10⁷ U interferon) as determined by limulus amoebocyte lysate assay. Interferon stock solutions of 10⁶ and 10² U/μl in RPMI 1640 were stored at -80°C and 4°C, respectively, and the latter diluted to final concentrations immediately before use.

Cells. Peripheral venous blood in acid-citrate-dextrose anticoagulant was obtained from CGD patients, normal volunteer donors, and the granulocyte and mononuclear cell fractions isolated by dextran sedimentation and Ficoll-Hypaque centrifugation (32). Procedures and consent forms were approved by the University of Massachusetts Medical School and The Children's Hospital Committees on the Protection of Human Subjects in Research.

Clinical and biochemical descriptions of variant CGD patients RaJ, ReJ, and RQ have recently been published (28); RaJ and ReJ are brothers, with granulocytes showing virtually identical biochemical characteristics. Patient BS has variant CGD (based on uniformly trace-positive granulocytes on NBT slide tests and 5% normal rate of PMA-stimulated superoxide production) and probable autosomal recessive inheritance (based on normal granulocyte cytochrome *b* content and normal parental NBT slide tests and quantitative superoxide production). Patients MD, TB, JW, and JM have classic X-linked CGD, based on absent NBT reduction and superoxide generation, undetectable cytochrome *b*, and maternal NBT slide tests showing a mosaic population of normal and inactive granulocytes. Patient LN has classic autosomal recessive CGD based on absent NBT reduction and superoxide generation, normal granulocyte cytochrome *b*, and normal maternal NBT slide tests.

Monocytes were further purified by adherence to polystyrene tissue culture wells (Falcon Div., Becton-Dickinson & Co., Oxnard, CA) at 37°C in RPMI 1640 medium in the absence of serum for 1 h. The nonadherent cells, mainly lymphocytes, were then washed off, leaving adherent cells that were 90–95% nonspecific esterase positive. Cells were either assayed at that time or cultivated further in 10% heat-inactivated autologous serum with or without 100 U/ml of recombinant human IFN-gamma or 1,000 U/ml each of recombinant human IFN-alpha and -beta.

Granulocytes were further purified by hypotonic lysis of erythrocytes (32). Granulocytes were cultivated in Krebs-Ringer phosphate

buffer with 5 mM glucose (32) with or without 100 U/ml of IFN-gamma for 40 min to 4 h at 37°C in either polystyrene tissue culture wells (for superoxide assays) or polypropylene tubes (for superoxide assays, NADPH oxidase kinetics, and cytochrome *b* assays). The results for superoxide assays were identical for each vessel; when cultured in polystyrene, both adherent and nonadherent cells were used in subsequent assays.

Assays. For NBT-reduction assays, granulocytes or macrophages were cultured as above, but on sterile glass coverslips, then incubated for 15 min at 37°C in Krebs-Ringer phosphate buffer with 5 mM glucose containing NBT and either PMA (100 ng/ml) or opsonized zymosan (10 μg/ml) as previously described (33). Two investigators examined the coverslips by phase microscopy, and scored NBT reduction in each experimental group on a semiquantitative five-point scale (0 to +++++). Scoring was confirmed by a third independent observer, familiar with the NBT method and blinded to the experimental design.

Superoxide generation was measured by a discontinuous assay of superoxide dismutase-inhibitable ferricytochrome *c* reduction, as previously described (33) except for the use of PMA (100 ng/ml) or serum-opsonized zymosan (1 mg/ml) as stimulants.

Granulocyte NADPH oxidase activity and kinetics were measured as NADPH-dependent superoxide dismutase-inhibitable cytochrome *c* reduction at 28°C in particulate fractions from PMA-stimulated neutrophils (34–36). The NADPH concentration was varied from 0.1 to 5 mM and the data analyzed by the method of Lineweaver and Burk (37). Before PMA stimulation, the PMN were incubated (as above) for 3 h at 37°C with or without IFN-gamma 500 U/ml.

Cytochrome *b*₅₅₉ content was measured in whole granulocytes by anaerobic-aerobic and dithionite-aerobic difference spectroscopy of cell suspensions in phosphate-buffered saline with and without 0.2% (vol/vol) Triton X-100, as previously described (35). Cytochrome *b*₅₅₉ was also extracted and concentrated from cell sonicates with Triton X-114 (38) and then spectrophotometrically assayed as above.

Monocyte-derived macrophages for messenger RNA (mRNA) analysis were cultured in plastic tissue culture wells, as previously described (33), with or without IFN-gamma 100 U/ml. At day 8 of culture, cells were scraped directly into 6 M guanidine HCl, and RNA was extracted by ethanol precipitation from guanidine HCl as described by Ginsburg et al. (39). Parallel wells were assayed for NBT reduction to confirm the interferon effect for each experiment. Total cellular RNA was examined by Northern blot analysis (40), using randomly primed (36, 41) complementary DNA probes for sequences from X-CGD (17) and constitutively expressed genes for phosphoglycerate kinase (42).

Results

NBT reduction. The histochemical NBT test semiquantitatively measures respiratory burst activity in individual cells (33, 35). As shown in Table I, PMN and macrophages from patients with variant CGD, both X-linked and autosomal recessive, responded with NBT reduction that was detectable, but below normal. The cells from the X-linked variant CGD patients showed augmented responses after incubation with IFN-gamma. In contrast, phagocytes from the classic CGD patients, which showed no detectable NBT reduction, and from the autosomal variant did not increase NBT reduction after IFN-gamma treatment. Normal macrophages showed the expected augmentation. Normal granulocytes did not change their response (perhaps because it was already maximal in this assay system). Cell-by-cell analysis showed that the populations of cells responded uniformly, i.e., no subpopulations of high- or nonresponders were present.

Similar responses were observed with opsonized zymosan as the phagocytic cell stimulus. Fig. 1 presents photomicro-

Table I. PMA-stimulated NBT Reduction by CGD and Normal Phagocytes

	Patient	PMN		Macrophages	
		-IFN γ	+IFN γ	-IFN γ	+IFN γ
X-linked CGD					
Variant	RaJ	+	+++	+	+++
	ReJ	+	+++	+	+++
	RQ	+	++	+	++
Classic	TB	0	0	0	0
	MD	0	0	0	0
	JM	0	0	0	0
	JW	0	0	ND	ND
Autosomal CGD					
Variant	BS	+	+	+	+
Classic	LN	0	0	0	0
Normal		++++	++++	++	++++

Groups were assigned values on a 0 to ++++ scale on the basis of the scoring on NBT slide tests of 100 cells per group by two readers, as described in Methods. IFN γ , IFN-gamma.

graphs of NBT slides of PMN (top, *a-d*) and macrophages (bottom, *e-h*) from X-linked variant CGD patient RaJ (left; *a, c, e, g*) and a normal control (right; *b, d, f, h*). IFN-gamma incubation dramatically increases the amount of NBT reduction, seen as dark formazan associated with ingested zymosan particles, in the CGD PMN and macrophages. Its effect is also evident in normal macrophages but not PMN, which are intensely strained even without the interferon.

The effect of IFN-gamma was first observed on granulocytes after 40 min exposure and was maximal at 2½ to 4 h. Thereafter, cell viability began to fall off. The earliest detectable response of monocyte-derived macrophages to IFN-gamma was after 36 h of incubation, with maximal responses reached by 48–72 h and then maintained for 8 d in culture. Macrophages cultivated with IFN-gamma displayed other interferon effects, including enhanced spreading and decreased mannose receptor activity, compared with untreated controls (data not shown).

Incubation of PMN and macrophages with IFN-alpha and -beta produced no augmentation of NBT reduction (data not shown).

Superoxide production. We next quantitatively examined superoxide generation by a spectrophotometric assay of superoxide dismutase-inhibitable cytochrome *c* reduction. Table II shows that superoxide production followed the same pattern as NBT reduction: IFN-gamma augmented the responses in X-linked variant, but not classic, CGD phagocytes, and similarly affected normal macrophages. The proportional increases in superoxide generation were at least two- to threefold for PMN and two- to eightfold for macrophages; the exact relative change could not be determined because baseline responses were undetectable for most of the CGD cells. Similar results were obtained with opsonized zymosan as the stimulus (data not shown).

Normal PMN did not show enhanced superoxide generation in response to IFN-gamma, again probably due to the maximum stimulation provided by PMA and opsonized zy-

mosan. Berton et al. detected such augmentation only with suboptimal stimuli such as chemotactic peptide or very low dose PMA (31). They, too, noted a loss of the IFN-gamma effect with higher doses of PMA.

In the absence of a respiratory burst stimulus, there was no detectable superoxide production by PMN or macrophages, whether incubated with or without IFN-gamma. That is, as previously observed (43), IFN-gamma does not by itself activate the superoxide-generating system.

IFN-alpha and -beta did not augment superoxide responses in any of the cells tested (data not shown).

NADPH oxidase kinetics. To elucidate the mechanism for the increase in superoxide production induced by IFN-gamma, we examined its effect on the kinetics of granulocyte NADPH oxidase. Only patients RaJ and ReJ could donate enough blood and had shown sufficient baseline granulocyte superoxide-generating activity to provide the cell numbers and unenhanced enzyme activity necessary for kinetic studies. As shown in Fig. 2, Lineweaver-Burk analysis revealed linear relationships ($r > 0.96$ by simple linear regression) between the reciprocals of the rate of superoxide production by particulate fraction from PMA-stimulated granulocytes and the reciprocals of NADPH concentrations. Table III presents the calculated V_{max} and K_m^{app} of the CGD and normal oxidases. As previously reported (28), the V_{max} for NADPH oxidase from the variant CGD granulocytes was < 10% that from normal cells. Incubation for 4 h in IFN-gamma nearly doubled the maximal velocity for the CGD oxidase, but did not affect the already high velocity of the normal enzyme (a response pattern parallel to that described above for intact cells). IFN-gamma only slightly lowered the abnormal K_m^{app} of the CGD enzyme.

Although the interferon-treated CGD granulocytes produced superoxide at a rate 40% of normal, cytochrome *b*₅₅₉ remained spectrophotometrically undetectable. As illustrated in Fig. 3, the characteristic cytochrome *b* difference spectrum of intact granulocytes was not observed either with or without IFN-gamma, in an assay capable of detecting the spectrum at an intensity 5% that of normal cells. Addition of Triton X-100 (0.02% final concentration) enhanced the 474 nm (myeloperoxidase) peak, but did not reveal any cytochrome *b*, nor did extraction with Triton X-114 (data not shown). Anaerobic-aerobic difference spectra showed similar baseline drift but no absorption peaks (data not shown). Quantitative measurement of cytochrome *b* from the absorption spectra revealed no detectable content (i.e., < 10 pg/mg protein) in PMN from patients RaJ, ReJ, and RQ either with or without IFN-gamma treatment. Normal PMN contained 105 and 97 pg/mg protein with and without IFN-gamma, respectively.

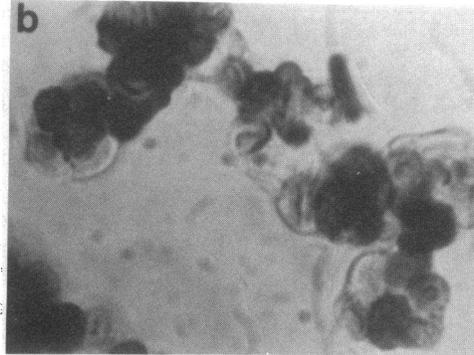
X-CGD gene transcripts. As shown in Fig. 4, IFN-gamma treatment of cultured monocyte-derived macrophages from variant patient RaJ increased the steady state level of RNA transcripts from the X-CGD gene ~ 5- to 10-fold. The amount of hybridization to X-CGD probe (upper bands) on Northern blot rose from a barely detectable level (right panel, right lanes) up to a prominent band (right panel, left lanes) ~ 5% the density of the signal detected in RNA from normal IFN-gamma-treated macrophages (right panel). Simultaneous hybridization of the nitrocellulose filter with a probe for phosphoglycerate kinase (42) (lower bands) demonstrated nearly equal expression of that constitutively expressed gene in all lanes. Additional hybridization bands below the primary, ~ 5-kilobase (kb); X-CGD signal are consistently observed

CGD

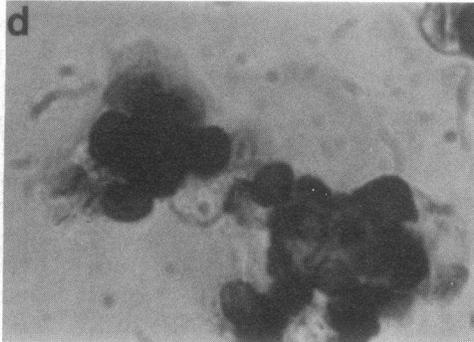
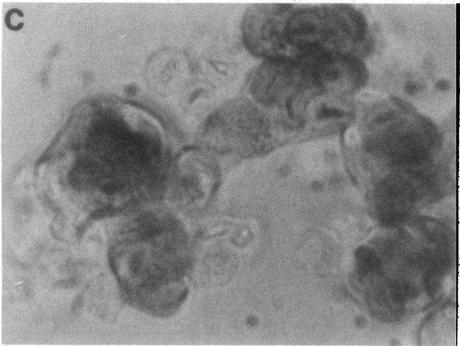
CONTROL

PMN

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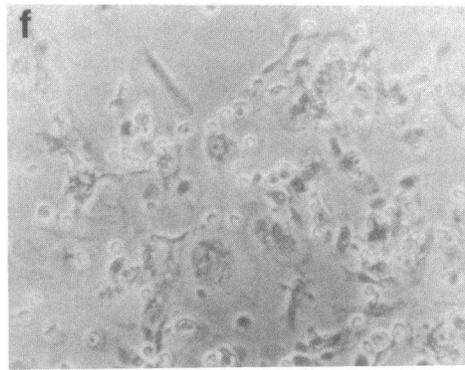
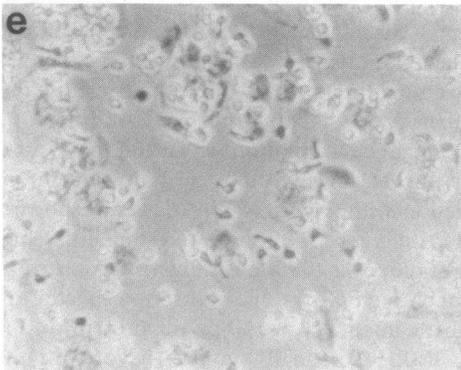


ifn γ



M ϕ

none



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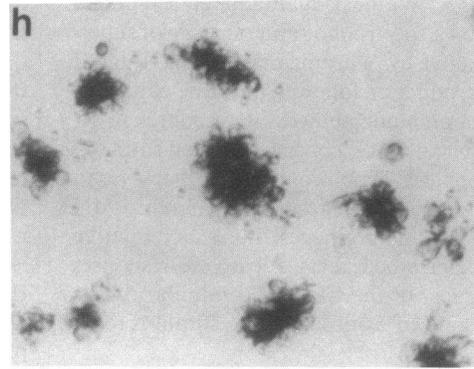
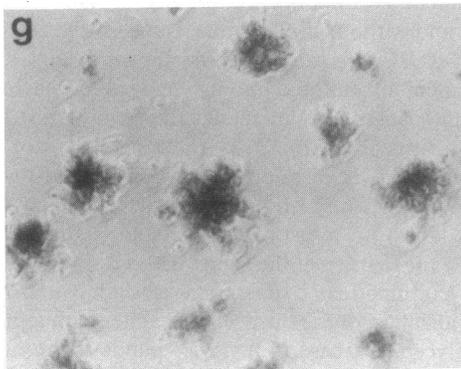


Table II. PMA-stimulated Superoxide Production by CGD and Normal Phagocytes

Patient	PMN		Macrophages		
	-IFN γ	+IFN γ	-IFN γ	+IFN γ	
	<i>nmol O₂/5 min per 10⁶ cells</i>				
X-linked CGD					
Variant	RaJ	1.7±0.4*	3.8±0.3	ND	ND
	ReJ	3.3±0.6*	8.2±1.0	<0.7	5.3
	RQ	<0.7	1.5	<0.7	1.6
Classic	TB	<0.7	<0.7	<0.7	<0.7
	MD	<0.7	<0.7	<0.7	<0.7
	JW	<0.7	<0.7	<0.7	<0.7
Autosomal CGD					
Classic	LN	<0.7	<0.7	<0.7	<0.7
Normal		17.8±2.1*	19±3.8	7.0±1.1*	13.1±1.3

Superoxide generation was measured by an assay of superoxide dismutase-inhibitable cytochrome *c* reduction, as described in Methods. Results represent the means of duplicate determinations, except where expressed as means±SEM for triplicate (ReJ's PMN) or quadruplicate (normal PMN and macrophages). IFN γ , IFN-gamma. * $P < 0.05$ by paired *t* test.

with the X-CGD probe and may represent mRNAs with alternative patterns of splicing.

Discussion

The respiratory burst plays a major role in the antitumor and microbicidal activity of phagocytes (1, 2). IFN-gamma, a product of T cells and natural killer cells, enhances the respiratory burst capacity in both human and murine macrophages (44); nonimmune interferons and some products of tumor cells have the opposite effect (45, 46). In this study we show that the respiratory burst in phagocytes from three patients with X-linked CGD is enhanced by the addition of IFN-gamma. The low, but detectable, level of phagocyte superoxide-generating activity in these patients, thus classified as CGD variants, probably represents the upper end of a spectrum of oxidase activity in the disease.

Single cell analysis of PMN revealed a homogeneous increase in NBT reduction rather than a responding subpopulation. No response to IFN-gamma was observed in phagocytes from patients with no detectable baseline respiratory burst activity (so-called classic X-linked CGD) or from patients with autosomal recessive CGD, with or without baseline respiratory burst activity. The effect of IFN-gamma required at least 40–60 min incubation for PMN and 36 h for macrophages. Quantitative measurement of superoxide generation confirmed the findings of the NBT reduction assay, and indicated the IFN-gamma-induced augmentation to be two- to threefold in PMN and two- to eightfold in macrophages.

The mechanism by which IFN-gamma augments superoxide generation by normal and X-CGD phagocytes is not known. Analysis of the kinetics of superoxide generation by

NADPH oxidase showed that interferon treatment increases the V_{max} of the enzyme, but has little effect on the abnormally low substrate affinity (high K_m^{app}). The initial kinetic abnormality of these cells is characteristic of phagocytes that produce only low levels of reactive oxygen intermediates, such as normal resident macrophages and PMN from patients with so-called variant CGD (23–25, 27, 28). Treatment of macrophages with IFN-gamma produces an increase in superoxide production, accompanied by a decrease in the K_m^{app} without much change in the V_{max} of the oxidase (30). The different pattern of kinetic response to IFN-gamma observed in the present study suggests that in X-CGD granulocytes it increases the number of active enzyme molecules per cell, but does not render them functionally normal. This discrepancy may arise from the residual CGD defect or from the difference in cell types examined. The polypeptide encoded by the X-CGD gene (for which the transcript is induced) is the larger (90 kd) of two proteins in the cytochrome *b*₅₅₉ heterodimer (18) isolated from membranes of activated neutrophils. It thus constitutes only one component of the NADPH oxidase complex. An increase in this one part, not balanced by another component (e.g., the cytochrome heme moiety, which might not be stable in the absence of the 90-kd glycoprotein) could theoretically produce such a defect. In fact, the absence of a detectable cytochrome *b* spectrum in IFN-gamma-induced CGD cells suggests that the partial restoration of X-CGD gene transcription (and presumably of its protein product) does not lead to equivalent incorporation of heme into the cytochrome *b*₅₅₉. The finding of interferon-induced superoxide-generating activity at 40% the normal rate in these cells further implies that the resultant NADPH oxidase complex is capable of electron transport (albeit abnormally) through pathways independent of the heme

Figure 1. Effect of IFN-gamma on NBT reduction by PMN and macrophages (M ϕ) from X-linked CGD and normal subjects. PMA-stimulated NBT slides were prepared for PMN (top, a–d) and macrophages (bottom, e–h) from X-linked CGD patient RaJ (left; a, c, e, g) and a normal control (right; b, d, f, h). PMN had been incubated

for 3 h and macrophages for 4 d in the absence (a, b, e, f) or presence (c, d, g, h) of IFN-gamma. Cultured wells were photographed at magnifications of 100 for PMN (a–d) and 40 for macrophages (e–h). Dark formazan deposits indicate sites of NBT reduction.

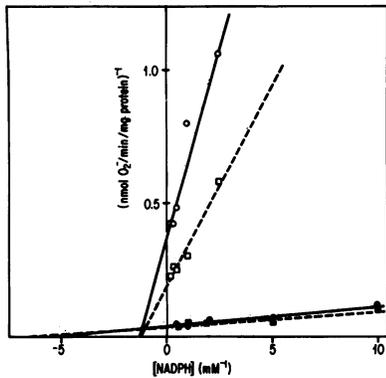


Figure 2. Lineweaver-Burk plots of NADPH oxidase activity from normal and X-linked-CGD (RaJ) granulocytes, incubated with or without IFN-gamma. The reciprocal of particulate fraction NADPH-dependent superoxide generation (*ordinate*) was plotted against the reciprocal of NADPH concentration (*abscissa*) as described in

Methods. Each point represents the mean of two experiments, each performed with triplicate determinations. (—○—) Represent oxidase from CGD cells; (---□---), oxidase from CGD cells treated with IFN-gamma; (—●—), oxidase from normal cells; and (---■---), oxidase from normal cells treated with IFN-gamma.

or for which small, undetectable amounts of heme are sufficient.

IFN-gamma treatment increased the very low steady state level of RNA transcripts from the X chromosome CGD gene (17) up to one that was ~ 5% that in normal macrophages. X-CGD gene expression also increases two- to fivefold in normal monocyte-derived macrophages incubated with IFN-gamma (Newburger, P. E., S. H. Orkin, and R. A. B. Ezekowitz. Unpublished data). This cytokine therefore appears to up-regulate expression of the X-CGD gene in both normal and CGD macrophages, either by increasing gene transcription or through posttranslational stabilization of mRNA.

However, the proportional rates of superoxide production in treated CGD cells compared with normal cells still appear to be greater than the proportional steady state levels of X-CGD transcripts. This discrepancy suggests that NADPH oxidase enzyme activity is a non-linear function of the level of X-CGD RNA and its protein product, so that small increases in functional RNA transcripts produce major changes in cell function. Alternatively (or additionally), IFN-gamma may increase

Table III. Effect of IFN-Gamma on V_{max} and K_m^{app} of NADPH Oxidase from Variant CGD and Normal PMN

	V_{max}	K_m^{app}
	nmol O_2 /min per mg protein	mM NADPH
Variant CGD		
RaJ		
-IFN-gamma	2.7	0.77
+IFN-gamma	5.0	0.56
ReJ		
-IFN-gamma	3.7	0.49
+IFN-gamma	6.8	0.57
Normal		
-IFN-gamma	27.8	0.14
+IFN-gamma	27.1	0.12

Results were calculated by Lineweaver-Burk (37) analysis of triplicate (normal), duplicate (RaJ), or single (ReJ) experiments, as described in Methods.

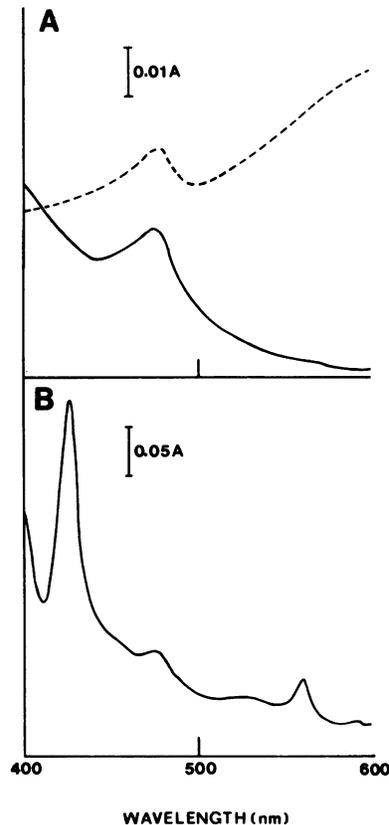


Figure 3. Dithionite difference spectra of intact granulocytes from X-linked CGD and normal subjects. (A) CGD variant (RaJ) granulocytes incubated 3 h with (solid line) or without (broken line) IFN-gamma before spectroscopy. Similar spectra with no detectable cytochrome b_{559} were obtained for PMN from patients ReJ and RQ. (B) Normal granulocytes without IFN-gamma treatment; normal cytochrome b spectrum is present. Identical spectra were obtained with IFN-gamma-treated PMN (see text for quantitation of cytochrome b_{559} contents). Bars indicate optical absorption; note the enhanced scale in spectra A, performed at fivefold greater sensitivity than that in B.

NADPH oxidase activity by posttranslational mechanisms (e.g., cell priming [6, 47]) that could increase the apparent efficacy of a partially disabled oxidase. Definition of the precise mutations in the X-CGD gene from IFN-gamma-responsive and -unresponsive patients should provide insight into the molecular basis of the cellular and clinical defects in the disorder. The range of residual phagocyte oxidase activity observed in the variant CGD patients suggests that they do not

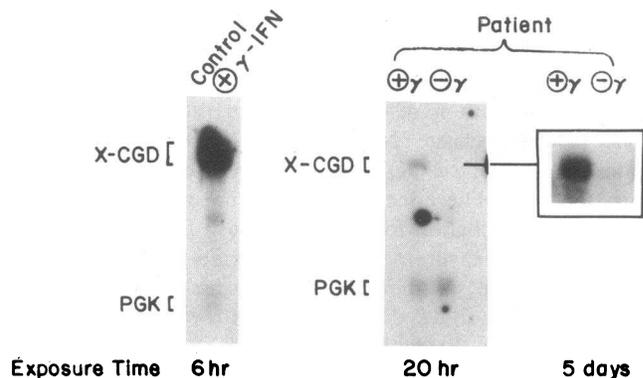


Figure 4. Expression of X-CGD and phosphoglycerate kinase gene transcripts in normal and X-linked CGD phagocytes. Total cell RNA (10 μ g) from normal macrophages (*left*), cultured with IFN-gamma, and from CGD patient ReJ's macrophages (*right*), cultured with or without IFN-gamma as indicated, was examined on Northern blot for X-CGD (11) and phosphoglycerate kinase (47) gene sequences, as described in Methods. "X-CGD" and "PGK" indicate the 5- and 2-kb bands of their respective transcripts. Exposure times for the autoradiographs were 6 h for the *left*, 20 h for the *right*, and 5 d for the *inset*.

represent a homogeneous subclassification of the disease, but rather a spectrum akin to the β^+ thalassemias.

Systemic IFN- γ therapy has been used to augment hydrogen peroxide secretion by monocytes in patients with malignancy (48). Further, local subcutaneous IFN- γ therapy can partially correct the systemic defect in monocyte peroxide production in lepromatous leprosy (49). These studies provide a precedent for systemic use of IFN- γ that may eventually be applicable to the subset of X-linked CGD patients whose cells respond in vitro. This approach would represent a unique opportunity to apply in vitro molecular findings to the pharmacologic modulation of gene expression in human disease.

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