Gene Expression Profiling Provides Insight into the Pathophysiology of Chronic Granulomatous Disease

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Gene Expression Profiling Provides Insight into the Pathophysiology of Chronic Granulomatous Disease

Scott D. Kobayashi,* Jovanka M. Voyich,* Kevin R. Braughton,* Adeline R. Whitney,* William M. Nauseef,†‡ Harry L. Malech,‡ and Frank R. DeLeo**

Human polymorphonuclear leukocytes (PMNs or neutrophils) kill invading microorganisms with reactive oxygen species (ROS) and cytotoxic granule components. PMNs from individuals with X-linked chronic granulomatous disease (XCGD) do not produce ROS, thereby rendering these individuals more susceptible to infection. In addition, XCGD patients develop tissue granulomas that obstruct vital organs, the mechanism(s) for which are unknown. To gain insight into the molecular processes that contribute to the pathophysiology of XCGD, including formation of granulomas, we compared global gene expression in PMNs from XCGD patients and healthy control individuals. Genes encoding mediators of inflammation and host defense, including CD11c, CD14, CD54, FcyR1, FcoR, CD120b, TLR5, II-4R, CCR1, p47phox, p40phox, II-8, CXCL1, Nramp1, and calgranulins A and B, were up-regulated constitutively in unstimulated XCGD patient PMNs. By comparing transcript levels in normal and XCGD PMNs after phagocytosis, we discovered 206 genes whose expression changed in the presence and the absence of ROS, respectively. Notably, altered Bcl2-associated X protein synthesis accompanied defective neutrophil apoptosis in XCGD patients. We hypothesize that granuloma formation in XCGD patients reflects both increased proinflammatory activity and defective PMN apoptosis, and we conclude that ROS contribute directly or indirectly to the resolution of the inflammatory response by influencing PMN gene transcription. The Journal of Immunology, 2004, 172: 636–643.

Materials and Methods

Materials

Sterile water and 0.9% sodium chloride (both Irrigation, USP) were purchased from Baxter Healthcare (Deerfield, IL). Dextran T-500 and Ficoll-Paque Plus were obtained from Amersham Pharmacia Biotech (Piscataway, NJ). Rabbit Ab specific for human serum albumin was purchased from ICN Biomedicals (Costa Mesa, CA). RPMI 1640 medium was purchased from Invitrogen (Carlsbad, CA). Latex beads (2.0 μm in diameter) were purchased from Polysciences (Warrington, PA). All reagents used for TaqMan real-time PCR were obtained from PE Applied Biosystems (Foster City, CA). Unless specified, all other reagents were obtained from Sigma-Aldrich (St. Louis, MO).

XCGD patient information

Eight patients (14–28 years of age) diagnosed clinically with XCGD participated in the microarray (six patients, XCGD1–XCGD6) or TaqMan real-time RT-PCR studies (two patients, XCGD7 and XCGD8) of PMN gene expression. In addition to clinical presentation, XCGD was confirmed by immunoblot analysis to verify the absence of gp91phox, and assays that measure superoxide-generating capacity in PMA-activated PMNs (e.g., oxidation of dihydrorhodamine 123 to rhodamine 123, reduction of ferricytochrome c or of nitro blue tetrazolium to formazan). Specific mutations for XCGD patients (where known) are as follows: XCGD7, g to t mutation at the 1 splice site at beginning of intron VII, resulting in skipping of exon 7 and no protein; and XCGD8, g264a splice site mutation at the end of exon 3, resulting in no protein. None of the XCGD patients participating in this study had acute illness or infection at the time blood was drawn for the experiments.

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Address correspondence and reprint requests to Dr. Frank R. DeLeo, Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 903 South 4th Street, Hamilton, MT 59840. E-mail address: fdeleo@niaid.nih.gov

Abbreviations used in this paper: PMN, polymorphonuclear leukocyte; BAX, Bcl2-associated X protein; CDG, chronic granulomatous disease; iBAX, inducible BAX; ROS, reactive oxygen species; XCGD, X-linked chronic granulomatous disease.

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Patients XCGD1–XCGD6 were chosen for the microarray study in part because they were not on IFN-γ therapy, and no patient had IFN-γ discontinued for the purpose of conducting this study. Patients were not receiving IFN-γ either because of a personal choice on the part of the patient or because the patient had experienced unacceptable side effects, such as intractable headaches or malaise not controlled by nonsteroidal anti-inflammatory medications. All these patients, with the exception of XCGD1 (not taking prednisone) and the other five XCGD patients (taking prednisone). Additionally, patients were taking oral and/or i.v. antibacterial, antifungal, or viral medications at the time of the studies as follows: XCGD1, trimethoprim-sulfamethoxazole, meropenem, and posaconazole; XCGD2, vancomycin and moxifloxacin; XCGD3, azithromycin, voriconazole, levofloxacin, and trimethoprim-sulfamethoxazole; XCGD4, trimethoprim-sulfamethoxazole and itraconazole; XCGD5, itraconazole, levofloxacin, and azithromycin; and XCGD6, linezolid, voriconazole, acyclovir, and cefixime. Although we cannot exclude the possibility that these medications alter gene expression in neutrophils, recent reports suggest that any such effects in human cells would be limited (19–21). Furthermore, the overall expression pattern of individual genes was relatively similar among all XCGD patients (Figs. 1 and 2), a finding incompatible with the idea that changes in transcript levels are due to the diverse combinations of patient medications. Analyses of genes expressed by phagocytosing human neutrophils is important to note that any effects of antibacterial, antifungal, or antiviral medications would be present in both unstimulated and stimulated PMNs. Therefore, it is unlikely that there would be significant net differences in gene expression (due to prophylactic therapies) between unstimulated and stimulated PMNs.

The XCGD patients who participated in the confirmation studies of PMN gene expression by TaqMan real-time PCR, XCGD7 and XCGD8, were undergoing IFN-γ therapy and taking trimethoprim-sulfamethoxazole at the time of the studies. In addition, patient XCGD8 was taking voriconazole. The decision to use patients undergoing IFN-γ therapy (XCGD7 and XCGD8) for the confirmation of microarray studies was entirely coincidental and based on limited patient availability. As any effects of IFN-γ would be present in both unstimulated and stimulated PMNs in patients XCGD7 and XCGD8, it is unlikely that there would be differences in gene expression (due to IFN-γ) between unstimulated and stimulated PMNs. Notably, there was strong positive confirmation of microarray results by TaqMan real-time RT-PCR (see below), indicating that treatment of patients XCGD7 and XCGD8 with IFN-γ did not significantly alter the patterns of PMN gene expression induced by phagocytosis.

Isolation of human PMNs

PMNs were isolated from heparinized venous blood (22) of healthy individuals or patients with XCGD in accordance with a protocol approved by the institutional review boards for human subjects at the National Institute of Allergy and Infectious Diseases and University of Iowa. All studies were conducted in accordance with the Declaration of Helsinki principles. PMNs were isolated as previously described (13, 14). Briefly, PMNs were isolated from 1 ml of whole blood by gradient centrifugation. The leukocyte-containing supernatant was centrifuged at 670 × g for 10 min and resuspended in 35 ml of 0.9% sodium chloride. The leukocyte-rich saline suspension was underlayered with 10 ml of Ficoll-Paque Plus (1.077 g/L; Amersham Pharmacia Biotech) and centrifuged at ambient temperature for 25 min at 350 × g to separate PMNs from PBMCs. PBMCs were removed by aspiration, and erythrocytes were lysed with water (Irrigation USP, Baxter Healthcare) for 15–30 s, followed by immediate mixing with 1.7% sodium chloride. Purified PMNs were centrifuged at 350 × g, resuspended in RPMI 1640 (Invitrogen), PMNs were glass filtered with a 0.22-μm filter (Millipore), and enumerated by microscopy. The purity of the PMN preparations and cell viability were confirmed by flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA). Cell preparations contained 99% PMNs, and all reagents used contained <25.0 pg/ml endotoxin.

PMN phagocytosis and gene expression analysis

Phagocytosis experiments were performed as described by Kobayashi et al. (13, 14). Briefly, PMNs (106) were centrifuged on ice with or without foli and C3bi-coated latex beads (8 × 107) in wells of a 12-well tissue culture plate ( precoated with 20% normal human serum) and centrifuged at 350 × g for 8 min at 4°C to synchronize phagocytosis. For the purpose of these studies, activated or stimulated PMNs are defined as those that have been stimulated by phagocytosis of IgG- and C3bi-coated latex beads. After centrifugation, plates were incubated at 37°C in a CO2 incubator for up to 6 h. At the indicated times, tissue culture medium was aspirated from the plate, and PMNs were lysed directly with RLT buffer (Qiagen, Valencia, CA). Purification of PMN RNA and subsequent preparation of labeled cRNA target (12 μg) were performed as previously described (13, 14). Labeling of samples, hybridization of cRNA with Hu95Av2 oligonucleotide arrays (Affymetrix, Santa Clara, CA), and scanning were performed according to standard Affymetrix protocols (http://www.affymetrix.com/pdf/expression_manual.pdf) as described previously (13–15). Inasmuch as the control and activated cells were each placed on ice before the phagocytosis experiments, it is unlikely that there would be significant net differences in gene expression due to cold shock. Experiments were performed with blood from four healthy donors at all time points and from four to six XCGD patients at the indicated time points using a separate oligonucleotide array for each donor. Gene expression in healthy control and XCGD individuals was always compared at the same time points after phagocytosis.

Data were analyzed with Microarray Suite, version 5.0 (MAS5) and GeneSpring version 5.0 (Silicon Genetics, Redwood City, CA) as described previously (13–15). We judged genes to be differentially expressed in unstimulated control and XCGD cells only when 1) the gene was identified as present by MAS5 (Affymetrix) in all samples studied; 2) the difference in expression between normal and XCGD samples was ≥2-fold; and 3) the extent of difference in expression was statistically significant (p ≤ 0.05, by Student’s t test).

To confirm phagocytosis experiments were performed with blood from four healthy donors at all time points and from four to six XCGD patients at the indicated time points using a separate oligonucleotide array for each donor. Gene expression in healthy control and XCGD individuals was always compared at the same time points after phagocytosis.

PMN gene expression induced by phagocytosis

PMN phagocytosis and gene expression analysis

Phagocytosis experiments and RNA preparation for TaqMan analysis were performed under conditions identical with those used for the microarray analysis. TaqMan analysis of samples from three healthy control individuals and two patients with XCGD were performed with an ABI 7700 thermocycler (PE Applied Biosystems) as previously described (13, 14). The expression of M14G after treatment with 100 μM ZnCl2 was measured in PMNs from three individuals at the indicated times. The correlation between TaqMan real-time RT-PCR and microarray analysis was 87.5%. That is, in 87.5% of the TaqMan real-time RT-PCR assays (21 of 24 TaqMan real-time RT-PCR assays [21 of 24 TaqMan microarray comparisons]) in which gene expression changed at least 1.5-fold in the healthy control or patient samples, genes changed at least 1.5-fold by microarray analysis, or they were similarly unchanged (not at least 1.5-fold induced or repressed) in either the healthy control or XCGD patient samples.

Flow cytometric analysis of apoptosis and expression of Bcl-2-associated X protein (BAX) and metallothionein

PMN apoptosis after phagocytosis was measured with flow cytometry (13, 14) using a modified TUNEL assay (Apo-BRDU Apoptosis Detection Kit; BD Biosciences) as described by the manufacturer. Alternatively, cells were stained with annexin V-FITC (Annexin V FITC Apoptosis Detection Kit II; BD Biosciences) as described by the manufacturer and were fixed overnight in Cytofix/Cytoperm (BD Biosciences) before staining for BAX.

Staining for intracellular BAX (mAbs 2D2 and 6A7; Sigma-Aldrich) and metallothionein (mAbs E9, DAKO, Carpinteria, CA) in PMNs from
healthy control donors and patients with XCGD was performed at the indicated times after phagocytosis (BAX staining) or after treatment with 100 μM ZnCl₂ (metallothionein staining). Metallothionein is a metal toxicity-responsive protein that is induced by ZnCl₂ in many cell types (23). Briefly, PMNs were fixed overnight with Cytofix/Cytoperm (BD Biosciences), washed twice with Perm/Wash buffer, and stained with 20 μg/ml anti-BAX, anti-metallothionein, or IgG1 isotype control mAb (BD Biosciences) for 30 min on ice. Cells were washed twice and incubated for 30

<table>
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<tr>
<th>Gene Name</th>
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<tr>
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<td>TGFB-inducible early growth response (TIEG)</td>
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**FIGURE 1.** Normal patterns of gene expression (up-regulated genes) are altered in activated PMNs from patients with XCGD. Differential gene transcription was measured with oligonucleotide microarrays after phagocytosis in human PMNs from four healthy control donors or four to six XCGD patients as indicated. The fold change for each individual is indicated by the scale bar. Time after PMN activation is indicated to the left of the boxes, which indicate the magnitude of change for each differentially expressed gene. The number of XCGD patients used at each time is as follows: PMN gene expression at 1.5 h included patients XCGD1, -2, -3, and -5; at 3 h, patients XCGD1, -2, -4, -5, and -6; and at 6 h, all XCGD patients were included. Gene expression was measured in the samples from four healthy control individuals at each time point. NC, No change.
min on ice with PE-conjugated donkey Fab’ fragments specific for mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). After two washes, samples were analyzed using a FACSCalibur flow cytometer (BD Biosciences), and a single gate was used to eliminate debris. In some experiments cells were stained with anti-myeloperoxidase (mAb 5B8, BD Biosciences) or IgG1 isotype mAb conjugated with FITC.

Statistics
Statistics were determined using Student’s t test with SigmaStat version 2.0 (SPSS, Chicago, IL) or Microsoft Excel 2002 (Microsoft, Bellevue, WA) unless indicated otherwise.

Results
Microarray analysis of neutrophils from XCGD patients reveals increased proinflammatory capacity and down-regulation of anti-inflammation-signaling mediators

To identify constitutive differences in gene expression that underlie chronic inflammation in XCGD patients, we compared transcript levels in unstimulated PMNs from XCGD patients and healthy control individuals (Table I, and supplemental Table II on the Journal of Immunology web site, which contains the complete set of microarray data for these experiments). Notably, we found that 19 genes encoding proteins that directly mediate host defense or facilitate the inflammatory response, including IL-8, CXCL1, CD14, Toll-like receptor 5, CD11c, CD54, CD64, CD89, CCR1, calgranulins A and B, and Nramp1, were significantly up-regulated in PMNs from XCGD patients (Table I). Further, genes encoding p47phox and p40phox were up-regulated in these patients (Table I). These findings suggest that proinflammatory capacity in XCGD patients is up-regulated due to the absence of gp91phox - ROS-dependent regulatory mechanisms and/or the long term influence of recurrent infection. Increased proinflammatory capacity in XCGD patient cells was not due to prophylaxis with an immunomodulatory agent, as none of the patients participating in the microarray studies was receiving IFN-γ therapy (see Materials and Methods). Genes encoding proteins involved in TGF-β signaling, TGF-β receptor 2, SMA- and MAD-related protein 2, and SMA- and MAD-related protein 4, were down-regulated in unstimulated PMNs from XCGD patients (Tables I and II). Inasmuch as TGF-β plays a prominent role in suppressing inflammatory responses of phagocytic cells during ingestion of apoptotic cells (24, 25), down-regulation of genes involved in TGF-β signaling in XCGD patient PMNs may contribute to pathologic sequelae of chronic granulomatous disease.

Normal gene expression patterns are altered in activated neutrophils from XCGD patients

To test the hypothesis that ROS influence (directly or indirectly) patterns of gene transcription in human neutrophils, we next compared changes in gene expression in activated PMNs from healthy control individuals and XCGD patients (Figs. 1 and 2, and supplemental Table III on the Journal of Immunology web site). We chose receptor-mediated phagocytosis as a model system for PMN activation based on the relevance of this process in patients with recurrent infection, i.e., in XCGD. Moreover, previous studies indicate that phagocytosis induces global changes in PMN gene expression (13–15). Fifty-two genes that were differentially transcribed in activated PMNs from XCGD patients remained unchanged in neutrophils from control individuals (Figs. 1 and 2,
and Table III). By comparison, 154 genes that were up- or down-regulated in activated PMNs from healthy control individuals were not changed significantly in cells from XCGD patients or were changed to a lesser extent (Figs. 1 and 2, and Table III). Inasmuch as the signature biochemical defect in cells from patients with XCGD is inability to produce superoxide, changes in neutrophil gene expression between healthy control individuals and XCGD patients was compared with expression in cells from four healthy control individuals. For all genes except CD11c (ITGAX), ρ = 0.04 for changes in PMN gene expression from XCGD patients vs healthy control cells (by Student’s t test). The common name of the protein encoded by the gene precedes the gene abbreviation (in parentheses). "A" Data represented by two separate probe sets on the array. "B" Data represented by three separate probe sets on the array.

Recent studies by Cramer et al. (28) underscore the importance of transcription factors in phagocyte-mediated inflammation. However, relatively little is known about how transcription factors contribute to chronic inflammation. After phagocytosis, 32 genes encoding transcription factors or proteins that bind RNA or DNA were differentially regulated in normal PMNs and not in the cells from XCGD patients (Figs. 1 and 2, and Table III). For example, genes encoding SFRS protein kinase 1 (SRPK1), lipoma HMGIC fusion partner-like 2 (LHFPL2), CBF1-interacting corepressor (CIR), oligodendrocyte lineage transcription factor 2 (RACK17), zinc finger protein 147, and zinc finger protein 254 were up-regulated only in normal cells (Fig. 1). Although specific functions have not been ascribed to some of these genes (e.g., LHFPL2 and ZNF254), it is known that SRPK1 is associated with U1-snRNP in apoptotic cells and is activated early during apoptosis to phosphorylated sequence 2), CFDP1, WTAP, and EGFL5 were differentially expressed in cells from patients with XCGD, but not in normal neutrophils (Figs. 1 and 2, and Table III). There is no
Intracellular expression of metallothionein was determined by three separate individuals, and each TaqMan experiment was assayed in triplicate after treatment with 100 μM zinc chloride. Metallothionein was detected with an mAb that recognizes metallothionein isoforms I and II (αMT). Isotype, IgG1 isotype control Ab. Results are representative of two or three experiments.

reported function for these genes in human PMNs, although cold shock domain protein 2 may repress the GM-CSF promoter (30), and breast carcinoma amplified sequence 2 is probably associated with the spliceosome complex (31). Further studies are necessary to determine whether these genes contribute, directly or indirectly, to the pathophysiology of XCGD or chronic inflammation in the patients.

There were significant changes in the expression of 11 genes involved in apoptosis in normal PMNs not observed in cells from XCGD patients (Figs. 1 and 2, and Table III). Notably, genes encoding modulator of apoptosis-1 (MOAP1) and BAX were significantly up-regulated in normal PMNs, but not in cells from patients with XCGD (Fig. 1, green arrow). The finding that BAX was up-regulated only in normal human PMNs after phagocytosis suggests that the protein is regulated at least indirectly by ROS. As it is possible that the induction of BAX contributes to a difference in PMN apoptosis between healthy control individuals and XCGD patients, we tested the hypothesis that up-regulation of BAX accompanies PMN apoptosis (below).

We identified 13 apoptosis and cell fate-related genes that were differentially expressed in PMNs from XCGD patients, but were not significantly changed in normal cells (Figs. 1 and 2). Genes encoding four distinct isoforms of metallothionein (MT1X, MT1G, MT1F, and MT3) were significantly up-regulated in PMNs from patients with XCGD and not in cells from healthy control individuals (Fig. 1). We observed that MT1X, MT1G, and MT1F were not expressed in resting human PMNs (data not shown). However, metallothioneins are induced by many factors, including hypoxia, in other cell types (32, 33), and notably, metallothioneins protect against apoptosis (34, 35). Although we obtained insufficient numbers of cells to test whether metallothionein protein was up-regulated in neutrophils from patients with XCGD, we found that up-regulation of metallothionein correlated closely with induction of MT1G in normal human PMNs (Fig. 3).

Confirmation of differentially expressed genes by TaqMan real-time RT-PCR

We next used TaqMan real-time RT-PCR to verify changes in expression detected by microarray analysis (Fig. 4). We selected 12 genes representative of the entire microarray dataset for confirmation (Fig. 4). These genes were assigned to five different functional categories and included several key genes encoding proteins that participate in apoptosis and/or survival. There was strong correlation (87.5%) between the TaqMan and microarray gene expression data (Fig. 4), consistent with previous studies (13–15).

BAX expression is altered in activated PMNs from XCGD patients

BAX plays a key role in the execution of apoptosis in eukaryotic cells (36) and probably contributes to neutrophil-mediated inflammation (16). We measured BAX in human PMNs to determine whether changes in the gene encoding BAX were reflected directly by increased protein (Fig. 5). PMNs constitutively expressed BAX (Fig. 5A, arrowheads). However, there was an increase in BAX (inducible BAX (iBAX)) protein over time that was accelerated significantly after phagocytosis (iBAX was 20.1 ± 7.3% in unstimulated PMNs vs 43.3 ± 5.0% in activated cells at 9 h, p < 0.0002; Fig. 5B). Importantly, cells expressing iBAX had a concomitant increase in surface expression of Annexin-V (Fig. 5C, arrows), a marker for programmed cell death (Fig. 5C). The observation that there was heterogeneous induction of BAX after phagocytosis may be a reflection of neutrophil populations that differ slightly in age, as emigration of neutrophils from the bone marrow is a continuous and thus asynchronous process. Differences in BAX expression and subsequent apoptosis due to cell maturity could play an important role in modulating host defense in conditions such as sepsis, where there is an efflux of immature neutrophils from bone marrow (37–39). Although PMNs from patients with XCGD constitutively expressed BAX, induction of

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**FIGURE 3.** Increased expression of metallothionein 1G (MT1G) correlates with up-regulation of metallothionein protein. A, Induction of MT1G in human PMNs. Expression of the metallothionein 1G (MT1G) gene was determined by TaqMan real-time PCR at 0, 4.5, 9, and 20 h after treatment with 100 μM zinc chloride. Results shown are representative of data from three separate individuals, and each TaqMan experiment was assayed in triplicate. B, Up-regulation of metallothionein in human PMNs. Intracellular expression of metallothionein was determined by flow cytometry after treatment with 100 μM zinc chloride. Metallothionein was detected with an mAb that recognizes metallothionein isoforms I and II (αMT). Isotype, lgG1 isotype control Ab. Results are representative of two or three experiments.

**FIGURE 4.** Confirmation of microarray data by TaqMan real-time RT-PCR. TaqMan confirmation of microarray results. Genes (n = 12) identified as differentially expressed by oligonucleotide microarrays were selected for confirmation by TaqMan real-time PCR after phagocytosis. TaqMan data represent the mean fold change in gene expression from three healthy control donors (red) and two XCGD patients (green). Microarray data are the mean fold change in four healthy control donors (black) and six XCGD patients (blue). TaqMan data for GPNMB was scaled down by a factor of 3 to fit on the graph.
BAX in these patients was not accelerated significantly by phagocytosis (6.8 ± 6.4% induced BAX in CGD PMNs (p = 0.3 vs unstimulated cells) compared with 21.7 ± 8.0% induced BAX in PMNs from normal individuals (p = 0.006 vs unstimulated cells); Fig. 5D). Taken together, these findings suggest that ROS play a role in regulating BAX synthesis in human PMNs and are consistent with studies demonstrating that BAX expression is increased during neutrophil apoptosis induced by phagocytosis of Mycobacterium tuberculosis (40).

Altered gene expression in neutrophils from XCGD patients is accompanied by defective apoptosis after phagocytosis

We next investigated apoptosis in PMNs from the six XCGD patients used in the microarray studies to determine whether global changes in patient gene expression, including diminished transcription of BAX, reflected altered cell fate. Compared with cells from healthy control donors, apoptosis in PMNs from XCGD patients was reduced significantly after phagocytosis (e.g., 36.5 ± 9.4 vs 12.2 ± 5.2% at 6 h for neutrophils from control and XCGD donors, respectively; Fig. 6). Furthermore, there was no significant difference in apoptosis between unstimulated and stimulated PMNs from XCGD patients (e.g., 11.4 ± 3.1 vs 12.2 ± 5.2% at 6 h for unstimulated and stimulated cells, respectively; Fig. 6). These findings suggest that ROS produced during phagocytosis by normal PMNs accelerate apoptosis and are consistent with studies by Coxon et al. (41). Taken together, the data provide strong evidence that altered gene expression in PMNs from XCGD patients underlies defective apoptosis.

Discussion

Several lines of evidence suggest that increased proinflammatory capacity and decreased PMN turnover in patients with XCGD contribute to the pathophysiology of XCGD, including granuloma formation. First, PMNs from XCGD patients have increased expression of proinflammatory molecules and decreased expression of genes encoding anti-inflammatory mediators compared with PMNs from healthy controls (Tables I and II, and Fig. 1). Increased proinflammatory capacity could contribute to the recruitment and activation of immune cells and thereby prolong inflammation in CGD patients. Second, a comparison of activated PMNs from XCGD

![FIGURE 5. BAX is induced during PMN apoptosis. BAX expression is induced by ROS and accompanies apoptosis in activated PMNs. A, Intracellular BAX was measured in unstimulated and stimulated (Phagocytosis) PMNs from healthy individuals as indicated. Green histograms represent cells stained with Ab specific for myeloperoxidase in the same experiment. B, Quantitation of intracellular BAX in unstimulated and stimulated (Phagocytosis) PMNs. Results are from five or six separate experiments. *, p < 0.001 vs unstimulated PMNs (by Student’s t test). C, Cells stained with Annexin V^green_ and anti-BAX Ab. Note the strong positive correlation between percentages of cells staining with annexin V and BAX. D, BAX is not induced by PMN phagocytosis in patients with CGD. The percentage of BAX induced by phagocytosis was quantitated in PMNs from five healthy individuals or in two XCGD patients (red circles) and one patient with autosomal deficiency of p22^phox_ (blue circle), *, p = 0.35 vs CGD patients. cBAX, constitutively expressed BAX.](http://www.jimmunol.org/)

![FIGURE 6. PMN apoptosis is defective in XCGD patients. Apoptosis was measured in unstimulated human PMNs (lower panels) or after phagocytosis (upper panels) in cells from healthy control donors (CTL) or XCGD patients as indicated. Results are from ≥10 healthy control donors (n = 10 at 3 and 9 h, n = 14 at 6 h) and five or six XCGD patients (n = 6 at 3 and 6 h, n = 5 at 9 h) in stimulated cells (Phagocytosis). *, p < 0.001 vs unstimulated cells; **, p = 0.04 vs CTL at 3 h or p < 0.001 vs CTL at 6 and 9 h (by Student’s t test).](http://www.jimmunol.org/)
patients and normal individuals identified genes encoding key regulators of apoptosis, transcription, inflammation, host defense, and other processes accompanying apoptosis that were differentially expressed in the absence or the presence of ROS, respectively. Among these regulators was the proapoptotic protein BAX, whose expression was not induced significantly in activated neutrophils from XCGD patients. Third, XCGD PMNs have defective apoptosis after phagocytosis that could delay resolution of inflammation during infection. Therefore, the lack of induction or repression of key cell fate-related genes in XCGD patients (e.g., BAX) and the associated defective apoptosis of XCGD PMNs has important implications for our understanding of the pathogenesis of granulomas in CGD. Notably, these granulomas form at sites of infection to which neutrophils have been recruited, and these cells predominate in granulomatous tissue and abscesses resected from patients with CGD (not shown).

We used gene expression profiling to generate a global view of how PMN-derived oxidants probably contribute to the resolution of inflammation in humans. Taken together, our findings suggest that altered PMN apoptosis after phagocytosis results in delayed resolution of inflammation, which facilitates the formation of granulomas in XCGD patients. Insight into defects in resolution of neutrophil-mediated inflammation revealed by our genomic studies of XCGD PMNs is probably applicable to other types of chronic inflammatory diseases. Importantly, these studies identified potential gene targets for prophylaxis and treatment of chronic inflammatory processes facilitated by PMNs.

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References


