In Vitro Regulation of Human Phagocyte Cytochrome b Heavy and Light Chain Gene Expression by Bacterial Lipopolysaccharide and Recombinant Human Cytokines*

(Received for publication, November 26, 1990)

Peter E. Newburger,§ Qun Dai, and Constance Whitney

From the Departments of Pediatrics and Molecular Genetics/Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts 01655

We examined the effects of bacterial lipopolysaccharide and several recombinant human cytokines (tumor necrosis factor α and granulocyte-, macrophage-, and granulocyte-macrophage colony-stimulating factors) on the expression of the genes for the phagocyte cytochrome b, an essential component of the superoxide-generating oxidase. In vitro treatment with lipopolysaccharide, tumor necrosis factor α, or macrophage- or granulocyte-macrophage colony-stimulating factors increased the levels of transcripts for the cytochrome b heavy chain (gp91phox) 9- to 22-fold and transcripts for the light chain (p22phox) 2- to 5-fold in cultured human monocyte-derived macrophages. The same agents, except for macrophage colony-stimulating factor, induced the expression of the cytochrome b heavy chain gene 2- to 12-fold and light chain gene 2- to 6-fold in human granulocytes. The expression of the cytochrome b heavy and light chain genes was coordinated in both macrophages and neutrophils with regard to stimulus specificity and dose-response pattern. The time course for induction of the two genes was parallel in both cell types for all stimuli. The macrophage response to lipopolysaccharide occurred at least in part at the transcriptional level. These results show that a variety of physiological regulators modulate the coordinated expression of the cytochrome b genes.

Phagocytes, such as macrophages and granulocytes, contain a membrane-associated NADPH oxidase that produces the reactive oxygen intermediates responsible for microbicidal, tumoricidal, and inflammatory activities (1–3). Investigation of the role of gene expression in the regulation of NADPH oxidase activity has been made possible by the recent molecular cloning of the genes encoding the phagocyte cytochrome b, a critical component of the oxidase complex (4, 5). The genes for the 91-kDa heavy chain glycoprotein (also termed gp91phox) and the 22-kDa light chain polypeptide (also termed p22phox) of the cytochrome b heterodimer are the sites of mutations responsible for, respectively, the X-linked and one of the autosomal forms of chronic granulomatous disease, a hereditary disorder of NADPH oxidase activity (6).

Prior studies of cytochrome b gene regulation in cultured cells have indicated that each subunit gene is independently controlled. In studies examining tissue specificity of expression, the heavy chain gene was expressed only in differentiated phagocytes (4, 5), yet that for the light chain was constitutively expressed in a variety of cell lineages (5). We have also previously examined the induction of oxidase gene expression in phagocytes activated with interferon-γ (IFN-γ) (7). In primary human granulocyte- and monocyte-derived macrophage cultures and in human and murine macrophage cell lines, we observed up to 3-fold rises in heavy chain steady state transcript levels, but no detectable change in the apparently constitutive expression of the cytochrome b light chain gene.

In phagocytes, the level of NADPH oxidase activity achieved after cell activation is modulated by prior treatment with priming agents, including bacterial lipopolysaccharides (LPS) and a variety of cytokines (8–11). LPS has been shown to influence or modify a wide variety of functions of phagocytes, including emigration, phagocytosis, oxidative metabolism, tumor cytotoxicity, and microbicidal activity (8, 11). LPS "primed" granulocytes to generate increased amounts of superoxide upon subsequent exposure to a variety of stimuli (11). Besides its direct actions, LPS also induces macrophage production of cytokines, primarily tumor necrosis factor α (TNF-α), which mediates additional biological responses (12).

The colony-stimulating factors (CSFs) are a family of glycoprotein cytokines that regulate proliferation and differentiation of hematopoietic progenitor cells and can functionally activate mature cells as well (10, 13, 14). The CSFs function both directly and through the induction of other, often locally acting, cytokines (13). Non-CSF cytokines, such as interferons and TNF-α, also affect neutrophil and macrophage functions and act synergistically with each other or with CSFs (3, 12, 15).

The present studies examine the regulatory effects of LPS, TNF-α, and granulocyte-, macrophage-, and granulocyte-macrophage colony-stimulating factors (G-CSF, GM-CSF respectively) upon the expression of the genes encoding the phagocyte cytochrome b. These agents, which are known to potentiate NADPH oxidase activity, also regulate expression of both cytochrome b genes. Several induce changes of much greater magnitude than those observed previously with interferon-γ and reveal the important qualitative difference that, at these levels of induction, the light chain gene no longer

* This work was supported by United States Public Health Service Grant CA38325. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed. Tel.: 508-856-4225; Fax: 508-856-4287.

§ Present address: Dept. of Pathology, New York University Medical Center, New York, NY 10016.

The abbreviations used are: IFN-γ, interferon-γ; CSF, colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α.
appears to be constitutive in expression. Rather, it is inducible and shows patterns of stimulus specificity, dose, and temporal response that generally parallel those of the heavy chain gene.

**MATERIALS AND METHODS**

Monocyte-derived macrophages and granulocytes were prepared and cultured under endotoxin-free(<10 pg/ml) conditions; all reagents were tested by Limulus amebocyte lysate assay (Whittaker Bioproducts). Monocytes from normal volunteers (including blood bank pheresis donors) were isolated from fresh peripheral blood or from plateletpheresis concentrates prepared by discontinuous gradients of buffy coat or by centrifugation from platelet pheresis residues (the latter obtained from the Blood Component Laboratory, Dana-Farber Cancer Institute, Boston). Monocytes were purified by Ficoll-Hypaque centrifugation and adherence to polystyrene tissue culture wells with serum-free RPMI 1640 medium supplemented with insulin, 5 μg/ml transferrin, 5 μg/ml; and sodium selenite, 5 ng/ml (7). After 24-h culture, the monocyte-derived macrophages were incubated with LPS or cytokines as described below.

Granulocytes were isolated from fresh peripheral blood of normal volunteers as described previously (16), suspended at 2–4 X 10^6/ml in 100 ml RPMI 1640 medium with 5% heat-inactivated autologous serum in 250 ml of polycarbonate flasks, and incubated with the indicated agents at 37 °C for various time periods in a rotary shaker. Granulocyte preparations contained less than 0.1% monocytes by nonspecific esterase staining; granulocyte RNA blots were also hybridized with probes (see below) to rule out monocyte contamination that could have been enriched during the RNA extraction process.

Macrophages or granulocytes were cultured with LPS (lipopolysaccharide B, *Escherichia coli* 026:B6 (Difco)) at various concentrations and with or without the following purified recombinant human cytokines: GM-CSF, M-CSF, or TNF-α; respective specific activities were 6.7 X 10^4 units/mg protein, 8 X 10^4 units/mg protein, and 2.99 X 10^5 units/mg protein. Recombinant human G-CSF was used in two forms; cells were incubated either with purified protein (2 X 10^4 units/mg) or with Chinese hamster ovary cell conditioned medium (assayed to yield half-maximal colony formation at a final dilution of 1:30,000 and used at a final dilution ratio of 1:10,000). G-CSF (conditioned medium), M-CSF, and GM-CSF were provided by Genentech, Inc.; TNF-α and IFN-γ were provided by Genentech, Inc.; G-CSF (purified protein) was purchased from Amgen, Inc. All working solutions contained <10 pg/ml of endotoxin.

In preliminary dose-response experiments (not shown), TNF-α dose-dependently increased production of superoxide in monocytes and PMN, with a plateau above 1000 units/ml; GM-CSF doses from 1 to 1000 units/ml produced parallel increasing responses in monocytes and PMN, with a plateau above 100 units/ml; M-CSF doses from 1 to 1000 units/ml produced increasing responses in monocytes only, with a plateau above 1000 units/ml; and G-CSF doses from 1 to 1000 units/ml increased superoxide production in granulocytes but had no effect on cytochrome b gene expression. Antisera were provided by Genentech (anti-TNF and anti-interferon-γ) and Genetics Institute (anti-G-CSF, anti-M-CSF, and anti-GM-CSF). Each was used at a concentration sufficient to neutralize maximal stimulatory concentrations of the target cytokine, as determined by the providers and verified in preliminary experiments.

Total cell RNA was extracted from cells by the guanidine HCl method (17) and analyzed by Northern blots performed according to standard procedures (18) or slot blots, prepared according to the instructions of Schleicher & Schuell for their Minifold II apparatus. Hybridization probes were full-length cDNAs for the human cytochrome *b* heavy chain and light chains. Procedures for sequential cycles of prehybridization, hybridization, washes, and filter stripping were performed as described by Gatti *et al.* (19). Equal loading of lanes was demonstrated by examination of gels after ethidium bromide staining and by rehybridization with either a full-length cDNA for human phosphoglycerate kinase (20) or a 5.8-kilobase HindIII restriction fragment of rat ribosomal DNA (21). Absence of mouse RNA contamination of the granulocyte extracts was confirmed by lack of hybridization with *c-sis* cDNA (22) (obtained from Dr. D. Crawford) or CD33 cDNA (obtained from Dr. A. Ezechowitz).

Nuclear run-on transcription assays were performed by minor modifications of previously published methods (23). Briefly, nuclei were isolated by lysis, in 0.1% Nonidet P-40, of monocyte-derived macrophages that had been incubated 3 h with or without LPS, 10 ng/ml. Freshly prepared nuclei were incubated 30 min at 30 °C in a reaction mixture containing [35S]UTP (250 μCi, 3000 Ci/mmmol) in buffer modified from Greenberg *et al.* (23) by addition of 0.8 mM MnCl₂. Newly synthesized RNA was prepared by extraction in guanidine thiocyanate and hot phenol and then ethanol precipitation. Equal amounts of incorporated label from each group (1–2 X 10⁶ cpm) were then hybridized to saturating amounts of nonlabeled cDNA probes, immobilized on filters by slot blotting.

Band densitometry was performed by computer image analysis of the integrated optical density of autoradiograph bands, using ImageMeasure software (Microscience, Inc.) on an Epson Equinox III+ computer. Ratios of band density were calculated for RNA from variously treated cells versus untreated cells; when lanes of Northern blots showed mildly unequal loading (1–22% differences in hybridization of the RNA probe), band densities were normalized to the rRNA signals prior to ratio calculation.

**RESULTS**

We first examined the effect of LPS on the expression of the genes for phagocyte cytochrome *b* in monocyte-derived macrophages and granulocytes. Fig. 1 presents autoradiographs of cellular RNA hybridized with complementary DNA probes to the phagocyte cytochrome *b* heavy and light chains. The rises in signal level indicate that incubation with LPS resulted in dose-dependent increases in amounts of cytochrome *b* heavy and light chain mRNA transcripts. The changes were detectable at an LPS concentration of 50 pg/ml and maximal at 1000 pg/ml for macrophages (upper panel) and at 250 pg/ml for granulocytes (lower panel). Higher concentrations of 5–25 ng/ml produced no further enhancement in macrophages (not shown). Quantitation of the responses by densitometry of the bands from seven experiments, including those illustrated, showed that maximally stimulatory doses of LPS increased the steady state levels of cytochrome *b* heavy chain transcripts 10-fold in macrophages and 3-fold in granulocytes.

**MACROPHAGES**

<table>
<thead>
<tr>
<th>LPS (pg/ml)</th>
<th>HEAVY CHAIN</th>
<th>LIGHT CHAIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa 0</td>
<td>50</td>
<td>250</td>
</tr>
</tbody>
</table>

**GRANULOCYTES**

<table>
<thead>
<tr>
<th>LPS (pg/ml)</th>
<th>HEAVY CHAIN</th>
<th>LIGHT CHAIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa 0</td>
<td>50</td>
<td>250</td>
</tr>
</tbody>
</table>

**FIG. 1.** Dose-dependent induction of phagocyte cytochrome *b* heavy and light gene mRNA in monocyte-derived macrophages and granulocytes by LPS. Northern blot analysis was performed on RNA extracted from macrophages after incubation for 48 h or from previously published experiments with granulocytes (23). Bands were sequentially hybridized with cDNA probes for the human phagocyte cytochrome *b* heavy chain and light chains as indicated.
2.4-fold in granulocytes (Table I). Light chain transcript levels rose 3.5-fold in macrophages and 2.6-fold in granulocytes.

To further investigate the regulation of cytochrome b gene expression, we examined the effects of cytokines selected from those known to affect superoxide-generating activity in mature phagocytes: TNF-α and G-,- M-, and GM-CSF. Fig. 2 illustrates the effects of the tested cytokines on the expression of the phagocyte cytochrome b genes in monocyte-derived macrophages and granulocytes. Quantitative ratios of transcript levels in treated relative to control cells, calculated from densitometry of autoradiographs from multiple replications of these experiments, are presented in Table I. The dose of each agent was that which produced maximal effects in preliminary experiments measuring both formyl peptide-induced superoxide generation and cytochrome b heavy chain gene expression (see “Materials and Methods”). The duration of exposure to each agent in the illustrated experiment was determined by time course studies described below.

Heavy chain expression in macrophages increased more dramatically in response to M-CSF, with lesser responses to GM-CSF and TNF-α; there was no detectable change with G-CSF treatment. Light chain expression rose in parallel but with lesser magnitudes of change. Granulocytes showed rises in heavy chain expression in response to G-CSF, despite biological activity of the G-CSF from both sources to stimulate granulocyte colony growth and to enhance superoxide generation (not shown). Predictably, M-CSF had no effect on expression of the cytochrome b genes in granulocytes.

In order to compare these results with our previous findings with IFN-γ (7), we repeated those experiments using current laboratory protocols. Dose-response experiments (using fresh IFN-γ from a different lot from the prior studies) showed optimal cytochrome b heavy chain gene induction at 10 units/ml (not shown) rather than the previously observed maximum at 100 units/ml (7). As shown in Fig. 3, both macrophages and granulocytes responded to IFN-γ incubation with increases in cytochrome b heavy chain expression, approximately 12- and 4-fold, respectively. In the present study, unlike the previous, we were also able to detect a 3-fold rise in heavy chain mRNA in macrophages.

### Table I

**Induction of phagocyte cytochrome b heavy and light gene mRNA in human monocyte-derived macrophages and granulocytes by LPS and cytokines**

Macrophages and granulocytes were prepared, incubated with the indicated agents, total cell RNA was extracted, and northern blots were analyzed as described under "Materials and Methods." Macrophages were incubated 6 h with LPS 1000 pg/ml or 48 h with recombinant human cytokines TNF-α, 1000 units/ml; G-CSF, 100 units/ml (or conditioned medium 1:10,000 dilution); M-CSF, 100 units/ml; or GM-CSF 100 units/ml. Granulocytes were incubated 30 min (light chain measurements) or 180 min (heavy chain) with LPS, 250 pg/ml; 180 min with TNF-α, 1000 units/ml; or 90 min with M-, GM-, or G-CSF at the same concentrations as for macrophages.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Heavy chain</th>
<th>Light chain</th>
<th>Heavy chain</th>
<th>Light chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>10 (4.9–15)</td>
<td>2.8 (2.7–3.0)</td>
<td>3.5 (1.5–8.1)</td>
<td>2.6 (1.4–4.2)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>8.7 (3.5–23)</td>
<td>2.1 (1.1–3.2)</td>
<td>6.5 (3.4–12)</td>
<td>1.8 (1.2–2.7)</td>
</tr>
<tr>
<td>G-CSF</td>
<td>1.5 (0.7–1.8)</td>
<td>1.0 (0.9–1.0)</td>
<td>0.9 (0.8–1.1)</td>
<td>1.0 (0.9–1.0)</td>
</tr>
<tr>
<td>M-CSF</td>
<td>22 (3.2–47)</td>
<td>4.7 (1.8–9.6)</td>
<td>1.1 (0.9–1.4)</td>
<td>1.1</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>12 (4.2–18)</td>
<td>4.5 (1.8–7.2)</td>
<td>12 (2.4–36)</td>
<td>6.2 (1.7–14)</td>
</tr>
</tbody>
</table>

**Notes:**
- Steady state mRNA levels relative to untreated controls.
- Data are expressed as the mean and (range) of 3 to 7 experiments except as noted.
- Single determination.

**Fig. 2.** Induction of phagocyte cytochrome b heavy and light chain gene mRNA in monocyte-derived macrophages and granulocytes by recombinant human cytokines. Upper panel, Northern blot analysis was performed on RNA extracted from macrophages after incubation for 48 h with G-CSF, 100 units/ml; M-CSF, 100 units/ml; or TNF-α, 1000 units/ml. Lower panel, Northern blot analysis was performed on RNA extracted from granulocytes after incubation for 180 min with TNF-α, 1000 units/ml, or 90 min with M-, GM-, or G-CSF at the same concentrations as for macrophages. Cytokine doses were those producing maximal induction of gene cytochrome b expression in preliminary dose-response experiments (see “Materials and Methods”). Filters from representative experiments were sequentially hybridized with cDNA probes for the human phagocyte cytochrome b heavy chain and light chains as indicated. HeLa RNA represents a negative control for heavy chain expression in macrophages; the light chain gene is weakly expressed in these cells (5).

**Fig. 3.** Induction of phagocyte cytochrome b heavy and light chain gene mRNA in monocyte-derived macrophages and granulocytes by IFN-γ. Northern blot analysis was performed on RNA extracted from macrophages (left panel) and granulocytes (right panel) after incubation for 24 and 1.5 h, respectively, with IFN-γ, 10 units/ml. Filters from representative experiments were sequentially hybridized with cDNA probes for the human phagocyte cytochrome b heavy chain and light chains as indicated.

in macrophage light chain transcripts. As before, there was no consistently measurable response in granulocyte light chain expression.

The time course of the macrophage and granulocyte responses to LPS are shown in Fig. 4. In macrophages (upper panel), the steady state level of cytochrome b heavy chain
transcript rises rapidly, reaching a peak at 6 h of incubation and then declining gradually back nearly to base line by 72 h. The initial rise (shown in the lower panel) is detectable at 0.5 h and continues steadily up to the 6 h point. The temporal pattern of response for cytochrome b light chain transcripts was parallel to that of the heavy chain in both long (middle panel) and short term (not shown) experiments. In the granulocyte response to LPS (Fig. 4, lower panel), the steady state level of cytochrome b heavy chain transcript initially falls slightly (a consistent finding in three separate experiments) and then rises to the maximum observed at 180 min. Longer incubations were not possible due to loss of cell viability and appearance of RNA degradation. In marked contrast to the heavy chain response, the light chain transcripts rise to maximum at 30 min and return to base-line levels of expression by 180 min. This highly reproducible discoordinate pattern was the only occurrence we observed of nonparallel responses of the heavy and light chain genes.

The time courses for the macrophage responses to TNF-α, M-CSF, and GM-CSF (Fig. 5) show that these cytokines induced peak cytochrome b gene expression at 16 h of incubation, with little or no drop by 24 h. The experiment illustrated in the figure shows slight declines in expression from 16 to 24 h, but in multiple repeat experiments the difference was inconsistent or expression was equal at the two time points. The temporal pattern of response for the heavy and light chain genes was parallel, with the magnitude of change in heavy chain greater than that for light chain transcripts. The lower panel of Fig. 5 demonstrates the levels of heavy chain transcript declining, but still above base line, at 72–96 h. Again, light chain expression followed in parallel (not shown). Because M-CSF prolongs both the competence and survival of monocyte-derived macrophages in culture (24, 25), we examined later time points and found a persistent response at day 6 of incubation, with return to base-line levels of cytochrome gene expression at day 8 (not shown).

The time courses of granulocyte responses to TNF-α and GM-CSF are shown in Fig. 6. The steady state levels of cytochrome b heavy and light chain mRNAs fell slightly after a 30-min incubation with TNF-α and then rose well above base line by 90 min and continued to increase at 180-min incubation. Longer exposure to the cytokine was not possible due to loss of viability and markedly decreased RNA recovery. In contrast, the response to GM-CSF was much more rapid, reaching a peak at 30–90 min and falling off to base line by a 180-min incubation. As in macrophages, the temporal pattern of response for the heavy and light chain genes was parallel, with the magnitude of change in heavy chain greater than that for light chain transcripts.

To test whether the observed effects occurred by direct stimulation or through secondary induction of other cytokines.

**Fig. 4. Time course of the macrophage and granulocyte responses to LPS.** Northern blot analysis of cytochrome b heavy and light chain transcript levels was performed on RNA extracted from monocyte-derived macrophages incubated with LPS at 1000 pg/ml (upper panels) or from peripheral blood granulocytes incubated with LPS at 250 pg/ml (lower panel) for the indicated time periods. Filters from representative experiments were sequentially hybridized with cDNA probes for the human phagocyte cytochrome b heavy chain and light chains as indicated.

**Fig. 5. Time courses of macrophage responses to TNF-α, M-CSF, and GM-CSF.** Northern blot analysis was performed on RNA extracted from macrophages after incubation for the indicated time periods with each cytokine at doses as in Fig. 3. Filters from representative experiments for each cytokine were sequentially hybridized with cDNA probes for the human phagocyte cytochrome b heavy and light chains as indicated.
from the target or accessory cells (12, 13, 26, 27), monocyte-derived macrophages were incubated with several of the agents in the presence of neutralizing antibodies to other cytokines. Incubation of macrophages with LPS plus polyclonal anti-TNF and anti-IFN-γ antisera with M-CSF or GM-CSF plus antibodies to each other or with M-CSF plus antibodies to TNF-α did not inhibit the induced rises in heavy chain expression (not shown). Thus, these agents probably act directly, although we cannot rule out autocrine or paracrine stimulation by secondary cytokines inaccessible to the antibodies or by other cytokines not tested or neutralized. Secondary secretion of cytokines by granulocytes (28) could not be tested because the large volumes of incubation required to accommodate the 10⁶ cells per assay group precluded the achievement of adequate antibody titers.

In preliminary investigations of the mechanisms of up-regulation of the cytochrome b genes, nuclear run-on assays showed increased rates of transcription of the heavy and light chain genes in macrophages responding to LPS. In the example shown in Fig. 7, nuclei from macrophages incubated 3 h in LPS showed 2-fold rises in newly synthesized mRNA for both heavy and light chain transcripts. These responses were similar to that of the known LPS-responsive TNF-α gene (12, 29) in the same experiment.

**DISCUSSION**

These studies show that in vitro treatment with bacterial LPS, TNF-α, or M- or GM-CSF increased the levels of cytochrome b heavy chain and, to a lesser extent, light chain transcripts in cultured human monocyte-derived macrophages. The same agents, except for M-CSF, also induced the expression of cytochrome b heavy chain gene in human granulocytes. The nuclear run-on assays indicate that the mechanism of up-regulation is at least in part transcriptional in macrophages responding to LPS. Furthermore, ongoing studies will determine whether mRNA stabilization also plays a role and whether the same mechanisms are involved in the responses to other inducing agents.

These results indicate that enhancement of expression of genes for an essential component of the phagocyte NADPH oxidase occurs in response to agents that modulate superoxide generation in these cells and imply a role for cytochrome b gene regulation in the processes of macrophage activation and neutrophil priming. Similar ratios of heavy and light chain expression, and correlations with respiratory burst capacity, have also been observed with TNF-α and IFN-γ-induced maturation of the HL-60 human myeloid leukemia cell line and with IFN-γ treatment of peripheral blood monocytes (30).

In particular, the present studies demonstrate the coordinated expression of the cytochrome b heavy and light chain genes in both macrophages and neutrophils with regard to stimulus specificity, dose response, and (with one significant exception) temporal pattern. This finding stands in important contrast to prior data showing marked disparities in the tissue specificity of expression of the two genes (5). In the original description of cytochrome b gene regulation in cultured cells, the subunit genes appeared to be independently controlled; the heavy chain gene was expressed only in differentiated phagocytes, yet that for the light chain was constitutively expressed in a variety of cell lineages (5). The present studies show that, in phagocytes, the two genes generally demonstrate coordinated regulation. However, the observed difference in the temporal pattern of heavy and light chain expression in response to LPS in granulocytes reveals that regulation of the genes is not invariably parallel.

The magnitude of the changes in heavy chain gene expression by the inducers used in the present studies exceeded the respective 5- and 3-fold rises we reported previously in macrophages and granulocytes incubated with recombinant human IFN-γ (7). No change in levels of transcripts from the light chain gene was detected in those experiments, leading to the prior conclusion that its expression was constitutive and not subject to cytokine regulation. However, the present studies indicate that light chain responses to cytokines are generally qualitatively parallel, but quantitatively less, than those of the heavy chain gene and are thus detectable only with the more potent inducers. Our previous inference of "constitutive" expression of the cytochrome b light chain gene during the phagocyte response to IFN-γ (7) probably represented a failure to detect small changes in transcript levels. In fact, repeating the prior experiments with more a potent IFN-γ preparation, we now find a measurable light chain response in macrophages, accompanying the higher level of induction of the heavy chain observed in the present study.

In the present studies, up-regulation of cytochrome b gene expression correlated qualitatively but not quantitatively with superoxide production. That is, agents that induced rises in heavy chain transcript levels also increased the rates of phagocyte superoxide generation, as reported previously (10, 15, 31–33) and as we have confirmed with measurements of superoxide dismutase-inhibitable cytochrome c reduction (16) (not shown). However, the respiratory burst responses of macrophages to LPS, GM-CSF, M-CSF, and TNF-α do not exceed 5-fold for LPS and 2-fold for the cytokines; granulo-
cyte superoxide generation rises no more than 2-fold after incubation with LPS, GM-CSF, or TNF-α. The induction of superoxide generation may thus have a ceiling. Possibly another noninduced NADPH oxidase component (perhaps even the light chain) becomes rate-limiting beyond a certain level of heavy chain induction.

More importantly, cytochrome b gene regulation must play only one part in the processes of macrophage activation and neutrophil priming for superoxide generation. The clearest example is G-CSF, which primes neutrophils (32) yet induced no change in cytochrome b gene expression. For the other agents, as well as numerous activators not tested in this study, multiple mechanisms doubtless operate in concert. Priming of neutrophils by LPS or various cytokines has been reported to involve increases in intracellular free calcium (34), protein phosphorylation (35), and formyl peptide receptor number and affinity (36); G protein activation (37); phospholipase A2 activation; and de novo protein synthesis (38), possibly of both receptors and oxidase components. Activation of macrophages involves even more complex changes in morphology, metabolism, and function (8). Enhancement of microbial killing by activated PMNs involves even more complex changes in morphology, metabolism, and function (8).

The time courses of the responses in cytochrome b gene expression are roughly similar to those observed for neutrophil priming and macrophage activation with LPS, GM-CSF, and TNF (11, 33, 42–46). However, much more rapid neutrophil priming (5–10 min at 37 °C) has been reported with G-CSF (32), which might explain the discordance between priming and cytochrome b gene expression for that agent, and with TNF (35, 47), which appears to have both rapid and slower phases of response. Exact temporal correspondence of respiratory burst activity and cytochrome b gene expression would not be expected, both because of the time lag for protein translation and processing and (as discussed above) the multiple complex processes involved in the final physiological effects.

The granulocyte was once considered incapable of modulation of gene expression, perhaps because of its highly condensed chromatin structure. Recent studies of the c-fos, heat shock protein, and cytokine genes have shown neutrophils to be capable of rapid regulation of the levels of gene expression (28, 48, 49). The results presented here, together with our previous data on the effects of IFN-γ (7), show that the regulation of mRNA levels is a mechanism broadly utilized by physiologic regulators for the modulation of genes important to phagocytic function.

If cells from patients with chronic granulomatous disease respond similarly to GM- and M-CSF, as they do to IFN-γ (50–52), then these agents may warrant in vivo testing as supplements to, or less toxic substitutes for, IFN-γ in the treatment of the disease.

Acknowledgments—We thank Drs. Stuart Orkin and Mary Dinauer for the cytochrome b cDNA probes, Dr. Dana Crawford for c-sti eRNA, Dr. R. A. B. Ezekowitz for CD33 cDNA, and Genentech, Inc. and Genetics Institute, Inc. for generous gifts of cytokines and antisera.

REFERENCES

Phagocyte Cytochrome b Regulation by LPS and Cytokines

J. Immunol. 136, 1393–1399