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Immunology

Induction of phagocyte cytochrome *b* heavy chain gene expression by interferon γ

(macrophage/NADPH oxidase/neutrophil/chronic granulomatous disease)

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ABSTRACT **Phagocytic cells, such as macrophages and polymorphonuclear leukocytes, produce a "respiratory burst" in which oxygen is reduced to superoxide and other active oxygen species responsible for many of the microbicidal, tumoricidal, and inflammatory activities of these cells. Interferon γ has been shown to augment phagocyte superoxide production, but the molecular mechanisms underlying this effect have remained unknown. Recently a key component of the oxidase, phagocyte cytochrome *b*, has been characterized as a heterodimer of a 91-kDa glycoprotein and a 22-kDa polypeptide. The present studies examined the effects of human recombinant interferon γ on the expression of the genes for these components of the cytochrome *b*. *In vitro* treatment with interferon γ substantially increases the level of phagocyte cytochrome *b* heavy chain gene transcripts in normal polymorphonuclear leukocytes, normal monocyte-derived macrophages, and the monocytic leukemia cell line THP-1. Light chain gene transcripts are less affected. In monocyte-derived macrophages and THP-1 cells, the enhanced expression of the heavy chain gene appears in large part attributable to increased rates of transcription. Treatment of monocyte-derived macrophages with human recombinant interferon α (a down-regulator of the respiratory burst) decreased the heavy chain transcript levels; interferon β produced no detectable change. These findings demonstrate the responsiveness of one essential component of the phagocyte oxidase system to activation by interferon γ and provide a rationale for its use to augment phagocytic function in chronic granulomatous disease.**

Phagocytic cells, such as macrophages and polymorphonuclear leukocytes (PMN), contain a membrane-associated NADPH oxidase (1) that generates the reactive oxygen intermediates responsible for microbicidal, tumoricidal, and inflammatory activities (2, 3). This enzyme system is dormant in resting cells but is rapidly activated upon exposure to a variety of particulate and soluble stimuli (4). The level of NADPH oxidase activity achieved after activation is subject to modulation by prior treatment of phagocytes with priming agents, including cytokines (5, 6) and bacterial lipopolysaccharide (7). Incubation of cultured macrophages with interferon γ (IFN-γ) enhances superoxide production and raises the apparent substrate affinity of the NADPH oxidase (8, 9). Berton and coworkers have demonstrated as well that IFN-γ increases superoxide production in PMN (6, 10).

Until recently the essential proteins of the phagocyte oxidase have been largely uncharacterized. Molecular genetic and biochemical approaches have converged to establish an unusual phagocyte cytochrome *b* (11) as a critical component. The cytochrome *b* is a heterodimer of a 90-kDa glycoprotein and a 22-kDa polypeptide (12, 13). The gene encoding the heavy chain (14) was cloned by identification of the genetic locus responsible for X chromosome-linked chronic granulomatous disease (X-CGD), a hereditary disorder of NADPH oxidase activity (15, 16). Absence or abnormality of the 90-kDa heavy chain appears sufficient to severely impair NADPH oxidase activity, resulting in the chronic granulomatous disease phenotype (14, 17–19). The cytochrome *b* light chain cDNA was recently isolated by immunoscreening (20). In cultured cells the subunit genes appear to be independently regulated: the heavy chain gene is expressed only in differentiated phagocytes, yet that for the light chain is constitutively expressed in a variety of cell lineages (20).

The molecular mechanisms underlying the augmentation of phagocyte superoxide production by IFN-γ are unknown. In the studies described here we have examined the interferon responsiveness of the cytochrome *b* subunit genes. *In vitro* incubation with recombinant human IFN-γ substantially increases the level of heavy chain, but not light chain, RNA transcripts in normal PMN, normal monocyte-derived macrophages, and the promonocytic leukemia cell line THP-1. In the latter two cell types, the increased abundance of heavy chain RNA is largely attributable to increased rates of transcription.

METHODS

Monocytes, isolated as previously described (21) from platelet pheresis residues (obtained from the Blood Component Laboratory, Dana–Farber Cancer Institute, Boston), were cultured in polystyrene tissue culture wells or flasks as previously described (22, 23) in serum-free RPMI medium supplemented with insulin at 5 μg/ml, transferrin at 5 μg/ml, and sodium selenate at 5 ng/ml (ITS Premix; Collaborative Research, Waltham, MA). After 24-hr culture, the medium was changed and the cells were further incubated in the absence or presence [at 100 units (U)/ml] of recombinant human IFN-γ (Genentech, South San Francisco, CA), specific activity approximately 2×10^7 U/mg of protein, referenced to the National Institutes of Health IFN standard Gg23901530. PMN were isolated from fresh peripheral blood of normal volunteers as previously described (24) and incubated for 90 min with or without IFN-γ at 50 U/ml in a gyratory rotator as described (6). In preliminary experiments, IFN-γ concentrations of 0.1, 10, 100, and 300 U/ml were tested; optimal stimulation of superoxide production occurred at 100 U/ml.

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Abbreviations: PMN, polymorphonuclear leukocytes; IFN, interferon; X-CGD, X chromosome-linked chronic granulomatous disease; U, unit(s); PMA, phorbol 12-myristate 13-acetate.

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In one set of experiments, cells were incubated for 24 hr without IFN or with IFN-α or IFN-β (obtained from P. Trown, Hoffmann–La Roche) at 1000 U/ml. The dose of IFN-α and -β was based on previous studies of their effects on mannose receptor (25).

All media and reagents for isolation and culture of monocytes, monocyte-derived macrophages, and PMN contained <25 ng of endotoxin per ml by limulus amoebocyte lysate assay (Sigma); IFN-γ preparations contained <1 ng of endotoxin per 10^7 U of IFN-γ.

THP-1 cells were cultured as previously described (26), then incubated for 48 hr with IFN-γ at 100 U/ml, phorbol 12-myristate 13-acetate (PMA) (Sigma) at 160 nM, or no addition prior to harvesting of RNA or nuclei. HL-60 human myeloid leukemia cells were cultured as previously described (27) and incubated with or without 60 mM dimethylformamide for 6 days prior to harvesting.

At various times after addition of IFN, cells were harvested and RNA was extracted in guanidine hydrochloride as previously described (28). RNA was analyzed by "northern" blots, prepared as previously described (29), or slot blots, prepared according to the instructions of Schleicher & Schuell, for their Minifold II apparatus. Hybridization (29) was performed with cDNA labeled by oligonucleotide priming (30).

For nuclear run-off experiments, nuclei were prepared from monocyte-derived macrophages or THP-1 cells after 48 hr of incubation in medium with or without IFN-γ at 100 U/ml. After 1 hr of incubation with a reaction mixture (31) containing [α-^{32}P]UTP (3000 Ci/mmol; 10 μCi/μl; 1 Ci = 37 GBq), newly synthesized RNA was extracted and specific sequences were detected by hybridization to saturating amounts of nonlabeled cDNA probes, immobilized on filters by slot blotting. In most experiments, total run-off RNA (5–10 $\times$ 10^6 cpm from IFN-γ-treated cell nuclei, 2–5 $\times$ 10^6 cpm from untreated controls) was used for hybridization with each filter.

Probes for the "northern" blots and nuclear run-offs included full-length human phagocyte cytochrome *b* heavy (14) and light (20) chain cDNAs, full-length human complement protein factor B cDNA (32) (obtained from S. B. Dowton and H. R. Colten, Washington University School of Medicine, Saint Louis, MO), full-length human phosphoglycerate kinase cDNA (33), and plasmid pBR322. Densitometry was performed on a Helena Laboratories QuickScan densitometer and areas under the curves were determined by the weight of cut-out chart paper.

RESULTS

IFN-γ Response in Macrophages and PMN. To determine whether IFN-γ treatment of phagocytes increases the expression of genes encoding critical components of the oxidase, we examined its effect on the levels of phagocyte cytochrome *b* gene transcripts in human monocyte-derived macrophages and PMN. The blots in Fig. 1 show that mRNA containing the phagocyte cytochrome *b* heavy chain gene sequence increased dramatically in both cell types after incubation with IFN-γ. Densitometric scanning of the illustrated autoradiographs and of those from three repeated experiments showed the increase to average 5-fold and 3-fold compared to control macrophages and PMN, respectively. Reprobing the blots with a cDNA for the light chain gene showed a visually indistinguishable difference in transcript levels (not shown); densitometry revealed only a 1.1- to 1.5-fold increase with IFN-γ treatment. Reprobing with a cDNA for the constitutively expressed phosphoglycerate kinase gene (33) demonstrated the lanes to be equally loaded (data not shown).

Because of the dramatic change in heavy chain mRNA at 24 hr of incubation, we examined the time course of the response in greater detail. The slot blot presented in Fig. 2

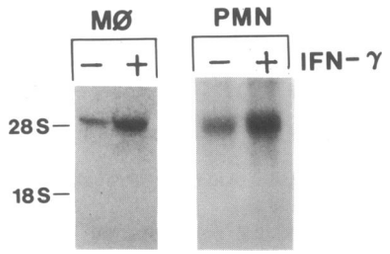

FIG. 1. Effect of IFN-γ on phagocyte cytochrome *b* heavy chain gene mRNA levels in cultured human monocyte-derived macrophages (Mϕ) and PMN. The autoradiograph demonstrates hybridization of a labeled phagocyte cytochrome *b* heavy chain cDNA probe to RNA from cells incubated for 24 hr (macrophages) or 90 minutes (PMN) with (+) or without (−) IFN-γ as indicated and analyzed by blotting after electrophoresis. Each lane contains 5 μg (macrophages) or 40 μg (PMN) of total cellular RNA. Positions of rRNAs are indicated on the left.

shows a high level of phagocyte cytochrome *b* heavy chain transcripts in fresh peripheral blood monocytes, with a marked fall-off during the initial 24 hr in culture, corresponding to the known decrease in superoxide generation in monocytes cultured *in vitro* (5). Addition of IFN-γ at "0 hr" (24 hr after plating the monocytes) produces an increase in heavy chain transcripts detectable after 9 hr of incubation and maximal at 24–48 hr. By 72 hr of IFN-γ treatment (96 hr of culture) transcript levels decrease, approaching those of untreated cells.

IFN-γ Response of Heavy Chain Gene Transcription. The observed rise in phagocyte cytochrome *b* heavy chain mRNA could be the consequence of transcriptional regulation by the lymphokine, increased mRNA stability, or a combination of these and other factors. To determine the contribution of transcriptional control to the change in the steady-state level of phagocyte cytochrome *b* heavy chain mRNA, we measured transcription rates by nuclear run-off assays. Fig. 3 presents the results of one of three such experiments, with

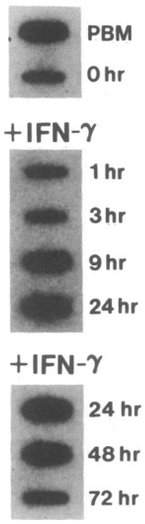

FIG. 2. Time course of the effect of IFN-γ on phagocyte cytochrome *b* heavy chain gene transcript levels in cultured human monocyte-derived macrophages. The slot blot autoradiograph shows the amount of hybridization of a labeled phagocyte cytochrome *b* heavy chain cDNA probe to RNA from peripheral blood monocytes (PBM) and monocyte-derived macrophages incubated for the indicated time periods with IFN-γ, starting 24 hr after plating of the monocytes ("0 hr" of incubation with IFN-γ). The bottom panel represents RNA from a separate experiment, applied to the same filter for hybridization. Each slot contained 5 μg of total cellular RNA, extracted and analyzed as described in the text.

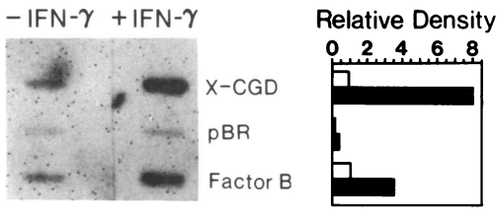

FIG. 3. Effect of IFN-γ on transcription rates of cultured human monocyte-derived macrophages, measured by nuclear run-off assay. The autoradiograph shows the amount of RNA transcribed *in vitro* by nuclei from cells incubated with or without IFN-γ as indicated. The filter, with each slot containing the indicated bound cDNA, was hybridized with total run-off RNA from equal numbers (2×10^8) of nuclei, which were isolated from cells incubated either with or without IFN-γ, and which incorporated 8×10^6 and 4×10^6 cpm of [^{32}P]uridylate, respectively. The graph on the right indicates the relative density of the autoradiographic exposure for each slot, measured by absorbance densitometry, with phagocyte cytochrome *b* heavy chain without IFN-γ assigned the value of 1.0. Filled bars represent incubation with IFN-γ. The labels in the center indicate the gene products assayed: phagocyte cytochrome *b* heavy chain (X-CGD), pBR322, plasmid control for nonspecific binding (pBR), and properdin factor B.

the autoradiograph of newly synthesized mRNA transcripts to the indicated genes on the left and a graphic representation of densitometry readings (from a shorter exposure of the same filter) on the right. The amount of phagocyte cytochrome *b* heavy chain message produced during *in vitro* incubation of monocyte-derived macrophages in the three experiments was 5- to 8-fold higher in nuclei isolated from cells incubated for 24 hr in IFN-γ than in those from control cells. The increase was greater than that of the known IFN-γ-responsive gene for properdin factor B (32, 34). IFN-γ also appears to induce a generalized increase in transcription, seen as the moderate (1.5- to 2.5-fold) rise in transcription of the phosphoglycerate kinase gene (33) and 1.5- to 2-fold greater [^{32}P]uridylate incorporation by nuclei from the treated cells (data not shown). However, the magnitude of the increase in phagocyte cytochrome *b* heavy chain and factor B transcription was much greater than that for the other genes, and the difference was readily evident whether the cDNA-loaded filters were hybridized with total run-off RNA (as in Fig. 3) or with amounts equalized for incorporated [^{32}P]uridylate. The very low rate of transcription by PMN precluded evaluation by nuclear run-off procedures.

Effects of IFN-α and IFN-β. In contrast to IFN-γ, IFN-α and IFN-β have suppressive or insignificant effects (respectively) on macrophage superoxide-generating activity (22, 35). As shown in Fig. 4, incubation of monocyte-derived macrophages with IFN-α for 24 hr under conditions identical to those used for IFN-γ resulted in a decrease in phagocyte cytochrome *b* heavy chain transcript, and IFN-β produced no detectable change.

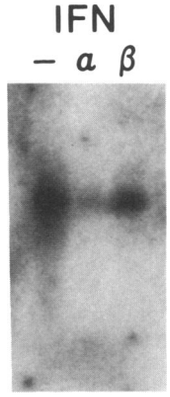

FIG. 4. Effect of IFN-α and IFN-β on amounts of phagocyte cytochrome *b* heavy chain gene transcript in cultured human monocyte-derived macrophages. Cells were incubated for 24 hr without IFN (left lane) or with IFN-α (center lane) or IFN-β (right lane), each at 1000 U/ml prior to extraction of RNA, electrophoresis, and blot analysis. Each lane contained 10 μg of total cellular RNA.

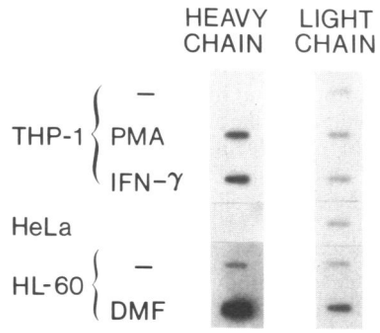

FIG. 5. Levels of heavy and light chain gene transcripts in THP-1 cells incubated in the absence or presence of IFN-γ or PMA for 48 hr. Control HeLa and HL-60 cells were incubated in medium or (for HL-60 cells) medium containing 60 mM dimethylformamide (DMF). Aliquots (5 μg) of RNA from the indicated cells were extracted and hybridized in parallel to the heavy (left column) and light (right column) chain cytochrome *b* probes.

IFN-γ Response in Monocytic Leukemia Cell Line THP-1. The monocytic leukemia cell line THP-1 serves as an *in vitro* model for monocyte differentiation and activation. Fig. 5 presents slot blots of RNA extracted from THP-1 cells in their native state and after 48 hr of incubation with PMA or IFN-γ. Both agents increased the steady-state level of mRNA transcripts for the heavy chain gene. Hybridization to the heavy chain cDNA probe rose 13- and 17-fold in response to PMA and IFN-γ, respectively. This change slightly exceeds the 11-fold increase in HL-60 myeloid leukemia cells induced by dimethylformamide to differentiate to granulocytes. HeLa cells, the negative control, contain no detectable heavy chain transcripts (14). Corresponding enhancement of light chain gene expression was only 1.7- and 1.9-fold, respectively; the light chain transcript level rose 2.9-fold in induced HL-60 cells. HeLa cells, although not phagocytes, contained nearly as much light chain mRNA as the THP-1 and HL-60 cells (20).

As in macrophages derived from peripheral blood monocytes, there was a corresponding increase in transcription of the heavy chain gene. Fig. 6 illustrates the results of a nuclear run-off experiment in which IFN-γ treatment produced a 14-fold increase in heavy chain gene transcription. Also as in monocyte-derived macrophages, this change exceeded both the response of the factor B gene and the generalized increase in transcription.

DISCUSSION

IFN-γ is the major lymphokine that activates human macrophages and PMN to increased levels of oxidative metabolism

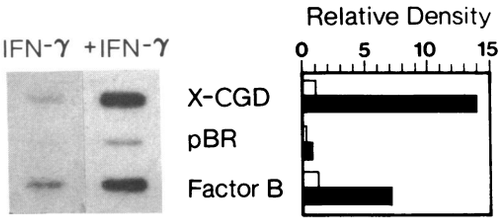

FIG. 6. Effect of IFN-γ on transcription rates of THP-1 cells, measured by nuclear run-off assay. Representations and procedures are as described for Fig. 3. Equal numbers (2×10^8) of nuclei were isolated from cells incubated with or without IFN-γ and incorporated 9×10^6 and 3×10^6 cpm of [^{32}P]uridylate, respectively.

and antimicrobial activity (6, 8, 36). It enhances the microbicidal, tumoricidal, and inflammatory activities of these cells in mice and humans *in vitro* and *in vivo* (5, 37-40). However, the molecular mechanisms underlying these effects have remained unknown. The present studies utilized recently developed molecular probes (14, 20) to examine the IFN responsiveness of the genes for the phagocyte cytochrome *b*, a key component of the respiratory burst oxidase (1, 11).

The findings demonstrate the IFN-γ substantially increase the level of phagocyte cytochrome *b* heavy chain gene transcripts in human monocyte-derived macrophages, PMN, and monocytic leukemia cell line THP-1. The 12- to 24-hr time course for the induction of heavy chain gene expression is within the range reported for other IFN-γ-responsive genes (41, 42). The steady-state level of heavy chain mRNA roughly paralleled the previously described changes in respiratory burst and NADPH oxidase activity, not only for up-regulation by IFN-γ treatment but also for down-regulation by IFN-α treatment and by placement of monocytes in culture (2).

In the macrophages and THP-1 cells, treatment with the lymphokine enhances the transcription of the heavy chain gene. The magnitude of the change in transcription rate approaches that of the rise in steady-state mRNA, suggesting that transcriptional control may account for a large part of the IFN effect on heavy chain gene expression in these cells. IFN-γ induction of some proteins, such as class I histocompatibility antigens (43), has been reported to operate through similar IFN-responsive transcription mechanisms; but expression of other genes appears to be augmented at least in part by mRNA stabilization (41, 44).

In THP-1 cells, the increase in heavy chain gene expression appears to be independent of morphologic differentiation, since the steady-state mRNA level increased with exposure both to PMA (which induces maturation) and to IFN-γ (which does not) (45). However, such findings in a proliferating leukemia cell line may not be directly applicable to normal mononuclear phagocytes *in vitro* or *in vivo*.

In contrast to the heavy chain gene, the light chain gene showed little or no response to IFN-γ treatment. This finding is consistent with previous observations, which suggested independent regulation of the two phagocyte cytochrome *b* components (20). In view of the near-constitutive expression of light chain mRNA and the low level or absence of light chain protein in cells that do not express heavy chain RNA (20), assembly of the heterodimeric cytochrome *b* may be determined in large part through availability of heavy chain protein. In this manner induction of just one subunit by IFN-γ may effect an overall increase in cytochrome *b* in phagocytic cells.

IFN-γ treatment of macrophages produces a decrease in the K_m of the NADPH oxidase without much change in the V_{max} of the enzyme complex (8, 9). An increase in phagocyte cytochrome *b* heavy chain gene expression, and hence production of the cytochrome b_{559} heavy chain component of the oxidase, might be expected to affect the quantity of enzyme and hence the V_{max}. However, the multicomponent enzyme system may not be perfectly amenable to Lineweaver–Burk or other such manipulation of the Michaelis–Menton equation. Alternatively, a change in the availability of the cytochrome *b* heavy chain component, and thus in the balance of the heavy and light chains, might affect the K_m of the enzyme complex. For example, in PMN from several variant X-CGD patients, a K_m defect appears to be associated with a marked reduction in heavy chain gene expression (23).

PMN are usually considered transcriptionally inactive and unresponsive, perhaps because of their highly condensed nuclei and low RNA content. The observed increase in the steady-state level of phagocyte cytochrome *b* heavy chain transcripts in IFN-γ-treated PMN indicates that these terminally differentiated cells are capable of a response to IFN involving modulation of either transcription or mRNA stability. Cassatella *et al.* (10) reported that the effect of IFN-γ on PMN superoxide generation could be blocked by actinomycin D, suggesting a transcriptional control mechanism for at least some components of the PMN response. Other evidence for gene regulation (by unknown control mechanisms) in PMN includes the findings that the level of c-*fos* oncogene transcripts rises upon stimulation of PMN by chemotactic oligopeptides (46) and that heat shock proteins, which are generally subject to transcriptional control, are inducible by heat treatment of PMN (47). Regulation of mRNA levels for a gene responsible for phagocytic function, such as phagocytic cytochrome *b* heavy chain, has not previously been directly demonstrated in mature granulocytes.

The transcriptional activation of heavy chain gene expression by IFN-γ provides an experimental basis for attempts to correct the functional deficiency of phagocytes from X-CGD patients by lymphokine administration. In accord with this concept we have previously observed that IFN-γ treatment *in vitro* partially corrects the oxidase defect in the phagocytes of some patients with X-CGD (23). More recently, a pilot clinical trial demonstrated improvement of superoxide production; cytochrome *b* mRNA, protein, and spectral levels; and bacterial killing in several X-CGD patients after *in vivo* administration of the IFN-γ (48).

Our findings establish the IFN responsiveness of the gene for one critical component of the respiratory burst oxidase, the heavy chain of the phagocytic cytochrome *b*. The induction of heavy chain gene transcription by IFN-γ demonstrates that gene regulation may play a role in the functional activation of phagocytes by a physiologic mediator. We do not suggest, however, that all the physiologic effects of IFN-γ on superoxide production, particularly in normal phagocytes, are attributable to modulation of heavy chain gene expression. IFN-γ can, and probably does, induce many other changes that serve to augment oxidase activity and ultimately contribute to the net physiologic effect. The studies reported here represent a first step in dissecting the regulation of specific components of the oxidase in response to lymphokine treatment.

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1. Babior, B. M. (1987) *Trends Biochem. Sci.* **12,** 241–245.
2. Nathan, C. F. (1985) *Mononuclear Phagocytes: Characteristics, Physiology, and Function* (Nijhoff, Dordrecht, The Netherlands), p. 411.
3. Babior, B. M. (1984) *Blood* **64,** 959–966.
4. McPhail, L. C., Clayton, C. C. & Snyderman, R. (1984) *J. Biol. Chem.* **259,** 5768–5775.
5. Nathan, C. F. & Tsunawaki, S. (1986) *Ciba Found. Symp.* **118,** 211–230.
6. Berton, G., Zeni, L., Cassatella, N. A. & Rossi, F. (1986) *Biochem. Biophys. Res. Commun.* **138,** 1276–1282.
7. Guthrie, L., McPhail, L., Henson, P. & Johnston, R. B., Jr. (1984) *J. Exp. Med.* **160,** 1656–1671.
8. Tsunawaki, S. & Nathan, C. F. (1984) *J. Biol. Chem.* **259,** 4305–4312.
9. Cassatella, M. A., Della Bianca, V., Berton, G. & Rossi, F. (1985) *Biochem. Biophys. Res. Commun.* **132,** 908–914.
10. Cassatella, M. A., Cappelli, R., Della Bianca, V., Grzeskowiak, M., Dusi, S. & Berton, G. (1988) *Immunology* **63,** 499–506.

11. Cross, A. R., Higson, F. K., Jones, O. T. G., Harper, A. M. & Segal, A. W. (1982) *Biochem. J.* **204,** 479–485.
12. Parkos, C. A., Allen, R. A., Cochrane, C. G. & Jesaitis, A. J. (1987) *J. Clin. Invest.* **80,** 732–742.
13. Segal, A. W. (1987) *Nature (London)* **326,** 88–91.
14. Royer-Pokora, B., Kunkel, L. M., Monaco, A. P., Goff, S. C., Newburger, P. E., Baehner, R. L., Cole, F. S., Curnutte, J. T. & Orkin, S. H. (1986) *Nature (London)* **322,** 32–38.
15. Baehner, R. L. & Nathan, D. G. (1967) *Science* **155,** 835–836.
16. Curnutte, J. T. (1988) *Hematology/Oncology Clinics of North America*, ed. Curnutte, J. T. (Saunders, Philadelphia), Vol. 2, pp. 241–252.
17. Segal, A. W., Jones, O. T. G., Webster, D. & Allison, A. C. (1978) *Lancet* **ii,** 446–449.
18. Segal, A. W., Cross, A. R., Garcia, R. C., Borregaard, N., Valerius, N. H., Soothill, J. F. & Jones, O. T. G. (1983) *N. Engl. J. Med.* **308,** 245–251.
19. Dinauer, M. C., Orkin, S. H., Brown, R., Jesaitis, A. J. & Parkos, C. A. (1987) *Nature (London)* **327,** 717–720.
20. Parkos, C. A., Dinauer, M. C., Walker, L. E., Allen, R. A., Jesaitis, A. J. & Orkin, S. H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3319–3323.
21. Mentzer, S. J., Guyre, P. M., Burakoff, S. J. & Faller, D. V. (1986) *Cell. Immunol.* **101,** 312–319.
22. Ezekowitz, R. A. B., Sim, R. B., MacPherson, G. G. & Gordon, S. (1985) *J. Clin. Invest.* **76,** 2368–2376.
23. Ezekowitz, R. A. B., Orkin, S. H. & Newburger, P. E. (1987) *J. Clin. Invest.* **80,** 1009–1016.
24. Haslett, C., Guthrie, L., Kopaniak, M., Johnston, R. B., Jr., & Henson, P. (1985) *Am. J. Pathol.* **119,** 101–110.
25. Ezekowitz, R. A. B., Hill, M. & Gordon, S. (1986) *Biochem. Biophys. Res. Commun.* **136,** 737–744.
26. Tsuchiya, S., Yamabe, M., Yamaguchi, Y., Kobayashi, Y., Konno, T. & Tada, K. (1980) *Int. J. Cancer* **26,** 171–176.
27. Newburger, P. E., Speier, C., Borregaard, N., Walsh, C. E., Whitin, J. C. & Simons, E. R. (1984) *J. Biol. Chem.* **259,** 3771–3776.
28. Ginsburg, D., Handin, R. I., Bonthron, D. T., Donlon, T. A., Bruns, G. A. P., Latt, S. A. & Orkin, S. H. (1985) *Science* **228,** 1401–1406.
29. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 199–203.
30. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137,** 226.
31. Linial, M., Gunderson, N. & Groudine, M. (1985) *Science* **230,** 1126–1132.
32. Woods, D. E., Markham, A. F., Ricker, A. T., Goldberger, G. & Colten, H. R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5661–5665.
33. Michelson, A. M., Markham, A. F. & Orkin, S. H. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 472–476.
34. Strunk, R., Cole, F. S., Perlmutter, D. H. & Colten, H. R. (1985) *J. Biol. Chem.* **260,** 15280–15285.
35. Szuro-Sudol, A., Murray, H. W. & Nathan, C. F. (1983) *J. Immunol.* **131,** 384–387.
36. Nathan, C. F., Murray, H. W., Wiebe, M. E. & Rubin, B. Y. (1983) *J. Exp. Med.* **158,** 670–689.
37. Nathan, C. F., Kaplan, G., Levis, W., Nursat, A., Wetmer, M., Sherwin, S., Job, C. K., Horowitz, C. R., Steinman, R. M. & Cohn, Z. A. (1986) *N. Engl. J. Med.* **315,** 6–11.
38. Nathan, C. F., Horowitz, C. R., de la Harpe, J., Vadhan-Raj, S., Sherwin, S. A., Oettgen, H. F. & Krown, S. E. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8686–8690.
39. Murray, H. W., Scavuzzo, D., Jacobs, J. L., Kaplan, M. H., Libby, D. M., Schindler, J. & Roberts, R. B. (1987) *J. Immunol.* **138,** 2457–2462.
40. Clark, I. A., Hunt, N. H., Butcher, G. A. & Cowden, W. B. (1987) *J. Immunol.* **139,** 3493–3496.
41. Freidman, R. L., Manly, S. P., McMahon, M., Kerr, I. M. & Stark, G. R. (1984) *Cell* **38,** 745–755.
42. Weil, J., Epstein, C. J., Epstein, L. B., Sedmak, J. J., Sabran, J. L. & Grossberg, S. E. (1983) *Nature (London)* **301,** 437–439.
43. Israel, A., Kimura, A., Fournier, A., Fellous, M. & Kourilsky, P. (1986) *Nature (London)* **322,** 743–746.
44. Chen, E., Karr, R. W., Frost, J. P., Gonwa, T. A. & Ginder, G. D. (1986) *Mol. Cell. Biol.* **6,** 1698–1705.
45. Mehta, K. & Lopez-Berestein, G. (1986) *Cancer Res.* **46,** 1388–1394.
46. Itami, M., Kuroki, T. & Nose, K. (1987) *FEBS Lett.* **222,** 289–292.
47. Eid, N. S., Kravath, R. E. & Lanks, K. W. (1987) *J. Exp. Med.* **165,** 1448–1452.
48. Ezekowitz, R. A. B. E., Dinauer, M. C., Jaffe, H. S., Orkin, S. H. & Newburger, P. E. (1988) *N. Engl. J. Med.*, in press.