Human alveolar macrophages (A-MΦ) and macrophages (MΦ) generated from human monocytes under the influence of granulocyte-macrophage colony-stimulating factors (GM-MΦ) express high levels of catalase activity and are highly resistant to H$_2$O$_2$. In contrast, MΦ generated from monocytes by macrophage colony-stimulating factors (M-MΦ) express low catalase activity and are about 50-fold more sensitive to H$_2$O$_2$ than GM-MΦ or A-MΦ. Both A-MΦ and GM-MΦ but not M-MΦ can induce catalase expression in both protein and mRNA levels when stimulated with H$_2$O$_2$ or zymosan. M-MΦ but not GM-MΦ produce a large amount of H$_2$O$_2$ in response to zymosan or heat-killed Staphylococcus aureus. These findings indicate that GM-MΦ and A-MΦ but not M-MΦ are strong scavengers of H$_2$O$_2$ via the high basal level of catalase activity and a marked ability of catalase induction and that catalase activity of MΦ is regulated by colony-stimulating factors during differentiation.

Human alveolar macrophages (A-MΦ)$^1$ can survive for a long duration (1–4) to exposure to not only chemical pollutants and exogenous oxidants but also inflammatory mediators and endogenously generated reactive oxygen species (ROS) and play important roles in phagocytosis-mediated host defense against microbial infection via the airway (5, 6). Superoxide dismutase, catalase, and glutathione are the main cellular ROS-degrading enzyme systems; superoxide dismutase converts superoxide radical (O$_2^-$) into H$_2$O$_2$, which is metabolized by catalase and glutathione peroxidase. Previous studies indicated that these enzymes are abundant in A-MΦ (7–9). However, the mechanism to maintain high antioxidant activities in A-MΦ has not been understood because of its heterogeneity and lack of availability to study.

* This study was supported in part by grants from the Japan Health Science Foundation and the Ministry of Health and Welfare of Japan (to K.S.A.). 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.

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$^2$ The abbreviations used are: A-MΦ, alveolar macrophages; MΦ(s), macrophage(s); CSF(s), colony-stimulating factor(s); GM-CSF, granulocyte-macrophage colony-stimulating factor; GM-MΦ, GM-CSF-induced macrophages; M-CSF, macrophage colony-stimulating factor; M-MΦ(s), M-CSF-induced macrophages; ROS, reactive oxygen species; HEC, human erythrocyte catalase; HIV-I, human immunodeficiency virus, type I.
Role of Catalase in H$_2$O$_2$ Resistance of Human Macrophages

Schering-Plough Japan (Osaka, Japan) and Morinaga Milk Industry Co., Ltd. (Tokyo, Japan), respectively.

Preparation and Culture of Macrophages—Peripheral blood mononuclear cells were obtained from venous blood drawn from normal healthy volunteers as described previously (12, 13). Briefly, peripheral blood mononuclear cells were isolated by centrifugation on a Ficoll-Metrizate density gradient (Lymphoprep; Nycomed, Oslo, Norway) and then placed into monocyte-isolating plates (MSP plates; Japan Immunoresearch Laboratories, Co., Ltd., Takasaki, Japan) for 2 h at 37 °C in a humidified 5% CO$_2$ atmosphere (CO$_2$ incubator). More than 97% of the recovered cells were judged to be monocytes based on morphology, non-adherence and trypan blue dye exclusion (26). Briefly, cultures were incubated in plastic dishes for 3 d at 37 °C in a CO$_2$ incubator, and non-adherent cells were removed by repeated washing.

Assessment of Cell Number and Viability—Cell viability was assessed by trypan blue dye exclusion. The number of adherent monocytes was determined by the method described previously by Nakagawara and Nathan (26). Briefly, cultures were incubated in plastic dishes for 20 min at 4 °C and then precipitated with isopropanol and ethanol. Ammonium form-isoamylalcohol (49:1), followed by centrifugation at 10,000 rpm for 20 min and resuspension with 0.5% sarcosyl, and 0.1 mol/liter 2-mercaptoethanol. After transfer to a cell-free control wells at 0 and 60 min, respectively, and S is the amount of triplicate cultures ± S.D.

Measurement of Catalase Activity—Intracellular and extracellular catalase activity was measured according to the method described by Aebi (27). Briefly, MΦs were cultured in the phenol red-free medium (Life Technologies, Inc.) supplemented with the indicated concentrations of M-CSF or GM-CSF. Culture supernatants were harvested at 48 h for the measurement of extracellular catalase. To measure the intracellular catalase, cell lysates were prepared with a specific lysis buffer (10 mmol/liter EDTA, 2% Triton-X, 0.05% deoxycholic acid in phosphate-buffered saline, pH 7.4) and then diluted with 50 mol/liter phosphate buffer (pH 7.0). 2 ml of the diluted sample was dispensed into a quartz tube, followed by 1 ml of 30 mol/liter H$_2$O$_2$ in phosphate buffer (pH 7.0). Catalase activity was measured by the consumption of H$_2$O$_2$ at 240 nm in a spectrophotometer (Graphrecord UV-240; Shimadzu Co., Kyoto, Japan) at 20 °C. The slope was converted into catalase activity units based on the standard curve of purified human erythrocyte catalase (HEC, 5 × 10$^4$ units/mg. Lot. number 643793; Calbiochem-Novabiochem). The activity is shown as milliunits/ml per well (2.5 × 10$^5$ cells) or units per mg of protein using a protein assay kit (Bio-Rad Laboratories, Hercules, CA).

Isolation of RNA and Northern Blot Analysis—Isolation of total RNA and Northern blot analysis were performed as described previously (13). Briefly, cells were lysed with denaturing solution containing 4 mol/liter guanidine thiocyanate, 25 mol/liter sodium citrate (pH 7.0), 0.5% sarcosyl, and 0.1 mol/liter 2-mercaptoethanol. After transfer to a polypropylene tube, total RNA was extracted by sequential addition of 2 mol/liter sodium acetate (pH 4.0), water-saturated phenol, and chloroform-isomylalcohol (49:1), followed by centrifugation at 10,000 rpm for 20 min at 4 °C and then precipitated with isopropanol and ethanol. Total RNA (10 μg/lane) were size-fractionated by electrophoresis after denaturation with 6% (v/v) deionized glyoxal and 50% (v/v) dimethyl sulfoxide and then transferred to a nylon membrane (Pall BioSupport, East Hills, NY). After cross-linking the membrane under UV irradiation and boiling in 80 mol/liter Tris-Cl (pH 8.0) for 5 min, the membrane was prehybridized in prehybridization buffer (50% formamide, 5× sodium phosphate) with 5.5 mmol/liter glucose) was dispensed into the quartz cuvette, followed by 1 ml of 30 mol/liter H$_2$O$_2$ in phosphate buffer (pH 7.0). Catalase activity was measured by the consumption of H$_2$O$_2$. In contrast, more than 90% of GM-MΦs were viable even in 0.1 mmol/liter H$_2$O$_2$. In contrast, more than 90% of GM-MΦs were viable even.
when treated with 10 mmol/liter H$_2$O$_2$. Thus, GM-Mφ were about 50-fold more resistant to H$_2$O$_2$ than M-Mφ. A-Mφ also showed a strong resistance to H$_2$O$_2$, and the level of resistance was similar to that of GM-Mφ (Fig. 1).

Intracellular and Extracellular Catalase Activities and Catalase Gene Expression in Monocyte-derived Mφs and A-Mφ—Because GM-Mφ and A-Mφ were markedly more resistant to H$_2$O$_2$ than M-Mφ, we examined the levels of cell-associated and extracellular catalase activity that catalyze H$_2$O$_2$ to H$_2$O in these Mφs. Extracellular catalase activity in the culture supernatants obtained from GM-Mφ incubated for 48 h was about 4-fold higher than that from M-Mφ (about 160 and 40 milliunits/ml/well in GM-Mφ and M-Mφ, respectively) (Fig. 2A).

Culture supernatants obtained from A-Mφ also contained high extracellular catalase activity (about 160 milliunits/ml/well), and the level was similar to that of GM-Mφ (Fig. 2A). In accordance with the findings of the enzyme activity, protein levels of extracellular catalase in GM-Mφ and A-Mφ cultures were about 4-fold higher than that in M-Mφ cultures by Western blot analysis using anti-HEC antibody (Fig. 2B). Similarly, catalase activity in M-Mφ lysates at 24 h was about 1 units/mg protein, whereas those in GM-Mφ and A-Mφ-lysates were about 5 units/mg protein. (Fig. 3A). In agreement with the enzyme activity, protein levels of catalase among these Mφ lysates were significantly different; catalase protein levels in GM-Mφ and A-Mφ lysates were higher than that in M-Mφ lysate, and the difference was about 5-fold (Fig. 3B). As the above findings suggest that expression of catalase gene is quite different between M-Mφ and GM-Mφ or A-Mφ, we examined the levels of catalase mRNA among these Mφs at 24 h after their cultivation by Northern blot analysis. Catalase mRNA in GM-Mφ was about 5-fold higher than that in M-Mφ, which was similar to that in A-Mφ (Fig. 3C).

Oxidant Stress- or Microbial Stimulant-induced Catalase Gene Expression Is High in Both GM-Mφ and A-Mφ but Low in M-Mφ—Although there is a significant difference in basal levels of catalase activity between M-Mφ and GM-Mφ or A-Mφ, the findings cannot fully explain their distinct susceptibility to H$_2$O$_2$; the difference in catalase activity was 4–5-fold, whereas the difference in sensitivity to H$_2$O$_2$ was about 50-fold. We therefore examined whether oxidant stress triggers the augmented expression of catalase gene in monocyte-derived Mφs. When these Mφs were treated for 3 h with 0.1 mmol/liter H$_2$O$_2$, catalase mRNA in GM-Mφ was augmented up to about 3-fold, whereas that in M-Mφ did not change significantly (Fig. 4A). Next we examined whether zymosan stimulation induces catalase gene activation in these Mφs. When Mφs were stimulated for 3 h with 0.1 mg/ml zymosan, catalase mRNA in GM-Mφ, but not in M-Mφ, also increased about 3-fold (Fig. 4A).

To confirm that catalase protein was synthesized by induction of the catalase gene via oxidant stress or microbial stim-
Treated with H2O2 or zymosan, and the induction level was also observed in A-M. Oxidant stress or microbial stimulant-mediated catalase induction in both gene and protein levels was also observed in M-M. Catalase protein was not observed in lysates of M-M. Agents—As demonstrated above, GM-M has a marked ability to induce catalase expression in response to oxidant stress. The precise mechanism, however, remains unknown.

In contrast to GM-MF and A-MF, M-MF are sensitive to exogenous H2O2 up to about 50-fold. In accordance with the susceptibility to H2O2, M-MF express lower levels of basal catalase activity and lack the ability to induce catalase gene expression in response to H2O2. M-MF also produced a large amount of H2O2 compared with GM-MF in response to microbial stimulants (see Fig. 5 and Ref. 24). These findings suggest the possibility that M-F induced by M-CSF support oxidant-induced inflammation or H2O2-mediated bacterial activity.

In agreement with the present findings, M-CSF augments anticyclophilin activity of fluconazole in the mouse M-F mediated by H2O2 production (31, 32).

In the present study, GM-MF and A-MF, but not M-MF, have a marked ability to induce catalase gene expression by exposure of low levels of H2O2 and zymosan, which can augment their protection against oxidant-rich environments. Analysis of the 5'-flanking region of the catalase gene in human hepatoma cells and bronchoepithelial cells demonstrated that several transcriptional regulation sites in response to oxidant stress exist in the promoter region (33, 34). These cells, however, express low levels of catalase activity, and hyperoxia fails to augment catalase transcript but induces transactivation of the heat shock protein 70 gene to support their survival (35–38). These findings suggest that catalase activity is a semiautomated microassay.

**DISCUSSION**

We showed in the present study that GM-MF and A-MF are highly resistant to H2O2 via the high basol level of catalase activity and a marked ability to express catalase in response to H2O2. About 1–10 mmol/liter H2O2, similar to levels found on expiration in the adult respiratory distress syndrome (29, 30), did not induce cell death of GM-MF and A-MF. A strong antioxidant mechanism of human A-MF supported by high catalase activity may help them to be long survivors in an oxidant-rich environment and contribute to lung homeostasis.

**Fig. 4.** Induction ability of the catalase gene and protein in CSF-induced monocyte-derived M-F and A-MF by exogenously added H2O2 or zymosan. M-MF and GM-MF were cultured in the medium containing M-CSF or GM-CSF, and A-MF were cultured in the medium without CSF supplemented with or without 0.1 mmol/liter H2O2 or 0.1 mg/ml zymosan. mRNA levels (10 μg/ lane) of the catalase gene at 3 h (A) or the protein levels (25 μg protein/lane) at 24 h (B) were examined as indicated in the legend for Fig. 3. kb, kilobase pair.

**Fig. 5.** Stimulant-induced H2O2 release in M-MF and GM-MF. The assay mixture containing 1 mg/ml zymosan or 1 mg/ml heat-inactivated S. aureus was dispensed into the wells of M-MF and GM-MF. The cellular release of H2O2 was recorded for 60 min at 37 °C using a semiautomated microassay.
Catalase plays a critical role in the development of ROS scavenging ability via catalase activity in human A-MΦ.

We demonstrated that GM-ΜΦ are resistant to H₂O₂ and a weak producer of H₂O₂ by bacterial and fungal stimuli via high catalase activity. In contrast, M-MΦ produce and release a large amount of H₂O₂ because of their low catalase activity. We previously reported that M-ΜΦ has a great capacity to produce HIV-I_PAR whereas GM-ΜΦ inhibits HIV-I_PAR replication (12). Numerous studies have shown that ROS, including H₂O₂, trigger HIV-I replication via NF-κB transactivation in the HIV-I long terminal repeat promoter region (43, 44). Furthermore, a critical role of H₂O₂ in NF-κB-mediated HIV-I replication was confirmed by reduction of HIV-I replication with the scavengers, including catalase in human monocytic/MΦ lineage cells (45, 46). In some studies, exposure to bacterial products rendered MΦ highly susceptible to T lymphocyte-tropic HIV-I via production of endogenous ROS and proinflammatory cytokines (47). These findings suggest that the difference in catalase activity between M-MΦ and GM-MΦ is a critical factor in the determination of their susceptibility to HIV-I replication.

In summary, we present evidence that catalase contributes to protect human tissue MΦ from oxidant-induced cell death and control their respiratory burst, and the activity is regulated at both the protein and mRNA levels by CSF during their differentiation. GM-CSF but not M-CSF plays a critical role in the induction of a strong antioxidant mechanism. The comparison of GM-MΦ and A-MΦ with M-MΦ in response to ROS helps clarify the self-defense mechanism of ΜΦ against oxidant stress in vivo.

Acknowledgments—We thank Dr. K. Onozaki Faculty of Pharmaceutical Science, Nagoya City University, Nagoya, Japan) for the human catalase cDNA probe. We also thank Professor S. Gordon (Sir William Dunn School of Pathology, University of Oxford) for critical comments and for additional help on the manuscript.

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