Effect of Oxygen on Induction of the Cystine Transporter by Bacterial Lipopolysaccharide in Mouse Peritoneal Macrophages*

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Amino acid transport in mouse peritoneal macrophages is mediated by several membrane carriers with different substrate specificity and sensitivity to environmental stimuli. We reported previously that transport activities of cystine and arginine in the macrophages were induced markedly by low concentrations of bacterial lipopolysaccharide (LPS). It is known that a variety of macrophage functions are affected by ambient oxygen tension. In this study, we have investigated the effects of oxygen on the induction of amino acid transport activity by LPS and found that the induction of cystine, but not arginine, transport activity was dependent on the ambient oxygen tension. When the macrophages were cultured with 2% O2 in the presence of 1 ng/ml LPS, induction of cystine transport activity was reduced by ~70% compared with cells cultured under normoxic conditions. In macrophages, transport of cystine is mediated by a Na+-independent anionic amino acid transporter named system xc−. System xc− is composed of two protein components, xCT and 4F2hc, and the expression of xCT was closely correlated with system xc− activity. A putative NF-κB binding site was found in the 5'-flanking region of the xCT gene, but the enhanced expression of xCT by LPS and oxygen was not mediated by NF-κB binding. An increase in intracellular GSH in macrophages paralleled induction of xCT, but not γ-glutamylcysteine synthetase. These results suggest the importance of system xc− in antioxidant defense in macrophages exposed to LPS and oxidative stress.

Transport of amino acids across the plasma membrane of mammalian cells is mediated by several systems with different substrate specificity (1). We have previously characterized anionic, neutral, and cationic amino acid transport systems in mouse peritoneal macrophages, which are known to be activated by various factors such as bacterial lipopolysaccharide (LPS) and cytokines (2–4). Macrophages exhibit cytotoxic functions including antigen presentation and microbicidal or tumoricidal activity. The major anionic amino acid transport system in macrophages is system xc−, which mediates the exchange of an anionic form of cystine and glutamate across the plasma membrane (2). The transport system for neutral amino acids in macrophages seems to be unique, transporting most neutral substrates such as serine, alanine, and leucine via a Na+-dependent mechanism (3). For cationic amino acids, a system γ+-like cationic amino acid transport system mediates the uptake of arginine, lysine, and ornithine, although the system in macrophages has slightly different characteristics from those of the typical system γ+ (4).

We have found that a very low concentration of LPS markedly enhances the activities of cystine and arginine transport in mouse peritoneal macrophages (5, 6). The enhanced uptake of cystine results from the induction of system xc− activity and increased influx of arginine is because of the induction of the typical system γ+ activity. The induction of cystine transport may contribute to the maintenance of intracellular GSH levels because cystine taken up by the cells is reduced to cysteine, a rate-limiting precursor for GSH synthesis (7). In contrast, the induction of the arginine transport activity is a key event for production of nitric oxide involved in microbicidal and tumoricidal processes in activated macrophages (8, 9). A CDNA encoding a protein with properties consistent with system γ+ in LPS-activated mouse macrophages has been identified and named mCAT-2B (10). Recently, we reported that system xc− is composed of two proteins and have cloned cDNAs for these proteins, namely 4F2hc (heavy chain of the surface antigen 4F2, also named CD98) and xCT (11). Coexpression of the ubiquitous transmembrane protein 4F2hc with xCT (and cDNAs of some other amino acid transporters) has been shown to induce amino acid transport activity in Xenopus oocytes (12–15).

Recent studies have shown that in macrophages, ambient oxygen tension alters the morphology, expression of cell surface markers, viability, phagocytosis, metabolic activity, and release of cytokines (16). It is likely that antioxidant systems such as intracellular GSH are affected by ambient oxygen tension and/or oxidative stress in these cells activated by LPS. In the present study, we have investigated the effects of oxygen on the induction of cystine and arginine transport activity in the macrophages caused by treatment with LPS. We have established that induction of cystine transport by LPS is dependent on ambient oxygen levels, whereas LPS-induced activation of arginine transport via system γ+ was unaffected.

EXPERIMENTAL PROCEDURES

Materials—L-[1-14C]Cystine, L-[1-14C]arginine, and L-[1-14C]serine were obtained from PerkinElmer Life Sciences. Thioglycolate broth (Brewer’s
formula) and Bacto LPS (Salmonella typhosa 0901) were from Difco Laboratories, Detroit, MI. Fetal bovine serum was obtained from Bio-Whittaker, Walkersville, MD, and the lot which contains less than 0.3 ng/ml endotoxin was used. Monomobromobimane was purchased from Molecular Probes, Inc., OR.

Macrophage Culture—Macrophages were collected by peritoneal lavage from female C57BL/6 mice weighing 20–25 g who had previously received 4 days prior an intraperitoneal injection of 2 ml of 4% thiglycolate broth. The lavage medium was RPMI 1640 containing 10 units/ml heparin. The cells were washed twice with RPMI 1640 and plated at 1 × 10⁵ cells/ml diameter culture dish containing RPMI 1640, 20% fetal bovine serum, 50 units/ml penicillin, and 50 μg/ml streptomycin. Cells were incubated at 37 °C in 5% CO₂, 95% air, and then incubated in 0.5 ml of the warmed uptake medium at 37 °C for specified time periods. The uptake medium was PBSG with a labeled amino acid (0.1 μCi/0.5 ml). Uptake was terminated by rapidly rinsing the culture dishes three times with ice-cold phosphate-buffered saline, and radioactivity associated with cell extracts was determined as described previously (17). Amino acid uptake was determined under conditions approaching initial rates of uptake, i.e. measuring uptakes for cystine, serine, and arginine at 120, 30, and 15 s, respectively. For each of the amino acids, uptake increased linearly during the specified incubation interval.

Determination of Intracellular GSH Levels and Eflux of GSH—Intracellular GSH was extracted with 5% trichloroacetic acid and then treated with ether to remove the acid. The GSH content in the aqueous layer was measured using an enzymatic method described previously, which is based on the catalytic action of GSH in the reduction of 5,5'-dithiobis(2-nitrobenzoic acid) by the GSH reductase system (18). The GSH extracted from the cells was mostly reduced GSH, and the content of the oxidized form, GSSG, was negligible throughout the experiments in this study. The efflux of GSH was measured as follows. The cells were rinsed three times with PBSG and incubated with 0.5 ml of PBSG at 37 °C for 1 h. Then GSH in the PBSG was quantified by the method described above.

Measurement of Intracellular Cystine—The cystine content in the cells was determined by the method of Cotgreave and Molèdes (19) with a slight modification (20). The cells were rinsed three times with PBSG and incubated in the dark at room temperature for 10 min with 100 μl of 8 mM monobromobimane in 50 mM N-ethylmorpholine, pH 8 and 100 μl of 50 mM phosphate-buffered saline containing 0.01% CaCl₂, 0.01% MgCl₂-6H₂O, and 0.1% glucose. Then 10 μl of 100% trichloroacetic acid was added. The protein precipitate was removed by centrifugation at 1500× g at 4 °C for 15 min and products were electrophoresed on a steel column (300 mm) following the manufacturer’s protocol.

Isolation of Nuclear Extracts and Electrophoretic Mobility Shift Assay—The nuclear extracts were prepared by a modified procedure based on the method of Monick et al. (21). Briefly, cells (5 × 10⁵) were washed with phosphate-buffered saline and collected by centrifugation. The cell pellets were resuspended in 0.2 ml of lysis buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 2 mM MgCl₂, 0.1 mM EDTA) and incubated for 15 min on ice. The cells were vortexed for 30 s after addition of Nonidet P-40 (0.6%), then centrifuged, and the nuclear pellet was resuspended in 20 μl of extraction buffer (50 mM HEPES, pH 7.9, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol). After a 20-min incubation on ice, followed by a 5-min centrifugation, nuclear proteins were recovered from the supernatant. Electrophoretic mobility shift assays were performed as described previously using a 32P-labeled oligonucleotide corresponding to the consensus NF-κB DNA-binding site (22). The DNA probes (20,000 cpm) were incubated with 3 μg of poly(dI-dC), and 5% glycerol) in a final volume of 20 μl. The reactions were carried out at 37 °C for 15 min and products were electrophoresed on native 6% polyacrylamide gels. The gels were analyzed by autoradiography.

RESULTS

The activity of cystine transport was measured in mouse peritoneal macrophages cultured under various oxygen tensions in the absence or presence of LPS (Fig. 1). Cystine transport activity was induced by LPS in an oxygen tension-dependent manner. However, hyperoxia (50% O₂) significantly reduced the activity of cystine transport. To establish whether other amino acid transporters responded in a similar manner, we investigated the effect of oxygen tension on arginine and serine transport activity (Fig. 2). Although arginine transport was induced markedly by the low concentrations of LPS, as reported previously (6), the induction of transport was unaffected by hyperoxia. Neither LPS nor oxygen tension had any effect on the activity of serine transport.

The dose-dependent expression of xCT and 4F2hc mRNAs by LPS was investigated in macrophages cultured under 20% O₂ (normoxia) and 2% O₂ (hypoxia). As shown in Fig. 3A, under normoxia, expression of xCT was not detected in the cells in the absence of LPS, but three xCT transcripts (~12, 3.5, and 2.5 kilobases) from cells treated with 1 ng/ml LPS were observed. These multiple bands may represent alternative splicing, alternative polyadenylation sites, or a combination of both (11). The expression of these transcripts was significantly decreased in cells treated with 1000 ng/ml LPS. These results are consistent with the previous report that the induction of the activity of xCT was maximal in cells treated with 1 ng/ml LPS. The expression of xCT in cells treated with 1 ng/ml LPS under hypoxia was lower than that in the cells treated with the same amount of LPS under normoxia. The expression of xCT mRNA in the cells treated with 1000 ng/ml LPS was barely detected. Fig. 3B shows the time-dependent expression of xCT and 4F2hc mRNAs in cells cultured with...
1 ng/ml LPS under hypoxia and normoxia. The expression of xCT mRNA was not detected in the freshly isolated macrophages, but expression increased gradually, reaching maximal levels after 8–12 h of culture under normoxia. Expression of xCT mRNA in cells cultured under hypoxia was increased over a similar time course, although expression was significantly lower than that in cells cultured under normoxia. The expression of 4F2hc also increased during culture with LPS, but changes in oxygen tension had negligible effects on expression. These results suggest that the decreased activity of cystine transport in the cells cultured with LPS under hypoxia is caused in part by the transcriptional regulation of xCT mRNA expression.

We have isolated the xCT gene from the mouse genome library and analyzed the sequence of 5'-flanking region. As shown in Fig. 4, there are several putative AP-1 binding sites and a putative NF-κB binding site. We have investigated the effects of oxygen on the binding activity of NF-κB by LPS. The binding activity of NF-κB remained relatively low in the macrophages treated with 1 ng/ml LPS, and a significant increase was observed when the cells were treated with 1000 ng/ml LPS (Fig. 5). Oxygen did not affect the NF-κB binding activity. The pattern of the NF-κB binding was inconsistent with those of xCT mRNA, suggesting that the induction of xCT mRNA by LPS and oxygen is not mediated by NF-κB.

We have previously demonstrated that intracellular GSH levels are significantly increased in the macrophages cultured with LPS (5). It is known that γ-glutamylcysteine synthetase (γ-GCS) catalyzes the rate-limiting step in de novo GSH synthesis. We have investigated the effect of LPS and oxygen on the expression of γ-GCS in these cells. Fig. 6 shows the expression of mRNAs for γ-GCS and xCT in the cells cultured with diethyl maleate or LPS under normoxic or hypoxic conditions.

**Fig. 2.** Effects of oxygen on the uptake of arginine (A) and serine (B) by macrophages incubated with LPS. Macrophages were incubated under hypoxia (2% O₂) for 1 h, and the medium was replaced by fresh medium with (filled bars) or without (open bars) 1 ng/ml LPS. The cells were further incubated under hypoxia (2% O₂) or normoxia (20% O₂) for 11 h, and the rates of uptake of 0.05 mM L-[¹⁴C]arginine and L-[¹⁴C]serine were measured. Each point represents the mean ± S.D. (n = 4–6).

**Fig. 3.** Changes in the expression of xCT and 4F2hc mRNAs in macrophages cultured with LPS. A, macrophages were incubated under hypoxia (2% O₂) for 1 h, and the medium was replaced by fresh medium containing 0, 1, or 1000 ng/ml LPS. The cells were further incubated for 7 h under hypoxia (2% O₂) or normoxia (20% O₂), and total RNA was isolated. B, macrophages were incubated under hypoxia (2% O₂) for 1 h, and the medium was replaced by fresh medium containing 1 ng/ml LPS. The cells were further incubated under hypoxia or normoxia with 1 ng/ml LPS for the specified time intervals (3, 7, 11, and 23 h), and total RNA was isolated. Ten μg each of total RNA were loaded per lane. The hybridization was performed with 32P-labeled cDNAs of mouse xCT, 4F2hc, and β-actin.

**Fig. 4.** Sequence of the 5’-flanking region of the mouse xCT gene. The clone containing the exon 1 and 5’-flanking region of the xCT gene was isolated, sequenced, and the transcription initiation site was determined as described under “Experimental Procedures.” The sequence of exon 1 is represented in capital letters. The putative NF-κB binding site is boxed. Putative AP-1 binding sites and a TATA-like box are represented by underlines and a double underline, respectively. The AP-1 site indicated by an asterisk partially overlaps the electrophile response element.
Dependence of Cystine Transport on Oxygen Tension

**Fig. 5.** Activation of NF-κB in macrophages cultured with LPS under hypoxia and normoxia. Macrophages were incubated under hypoxia (2% O₂) for 1 h, and the medium was replaced by fresh medium containing 0, 1, or 1000 ng/ml LPS. The cells were further incubated for 1 h under hypoxia or normoxia (20% O₂), and the nuclear extracts were prepared. The electrophoretic mobility shift assay was performed as described under “Experimental Procedures.”

**Fig. 6.** Effects of diethyl maleate and LPS on the expression of γ-GCS and xCT mRNAs in macrophages incubated under hypoxic and normoxic conditions. Macrophages were incubated under hypoxia (2% O₂) for 1 h, and the medium was replaced by fresh medium (Cont) in the presence of 100 μM diethyl maleate (DEM), or 1 ng/ml LPS (LPS). The cells were further incubated under hypoxia or normoxia (20% O₂) for 7 h, and total RNA was isolated. Ten μg each of total RNA was loaded per lane. The hybridization was performed with 32P-labeled cDNAs (mouse γ-GCS, xCT, 4F2hc, and β-actin).

Diethyl maleate is an electrophilic agent, which reacts with GSH enzymatically or nonenzymatically. The expression of γ-GCS mRNA was induced markedly by diethyl maleate, but unaffected by LPS. As shown in Fig. 7, the intracellular GSH level in LPS-challenged cells cultured under hypoxia for 12 h was significantly lower than the level in cells cultured under normoxia. These results are consistent with the results that the cystine transport activity in the cells treated with LPS is decreased under hypoxia (Fig. 1). In comparison with GSH levels in cells treated without LPS under normoxia, the GSH level was 1.8-fold higher in cells treated with LPS. By contrast, the GSH level in cells cultured under normoxia for 12 h with diethyl maleate, which induces both mRNAs for system x⁻⁻⁻⁻ and γ-GCS (Fig. 6), were increased 2.4-fold.

It is of importance to determine whether the intracellular cysteine, which is a rate-limiting substrate for GSH synthesis, increases in response to induction of cystine transport activity. We investigated the intracellular cysteine levels in the cells cultured with or without LPS under hypoxia or normoxia (Fig. 8). The intracellular cysteine paralleled the activity of system x⁻⁻⁻⁻, supporting the hypothesis that the induction of system x⁻⁻⁻⁻ is responsible for increased GSH. It might be possible that LPS and/or oxygen inhibit the efflux of GSH from the cells, and this inhibition accounts for increased intracellular GSH. However, as shown in Fig. 9, the rate of efflux of GSH was higher in the cells cultured with LPS than that in the cells cultured without LPS. The higher rate of the efflux probably reflects the higher intracellular GSH level, because the efflux of GSH is carrier-mediated and depends on the intracellular GSH levels (23). Thus, it is unlikely that the GSH efflux system is affected by LPS and/or oxygen. Taken together, the present findings indicate that the elevation in intracellular GSH levels caused by LPS is dependent on the induction of xCT but not γ-GCS. By contrast, the increased intracellular GSH levels caused by diethyl maleate are due to an induction of both xCT and γ-GCS. It is worth noting that GSH levels decreased significantly in the cells treated with diethyl maleate for 4 h. This initial depletion of intracellular GSH levels seems to be the result of an enzymatic reaction between GSH and diethyl maleate catalyzed by glutathione S-transferase (24).

**DISCUSSION**

In a variety of types of cells in culture, the intracellular GSH level is regulated by the activity of system x⁻⁻⁻⁻ (7). γ-GCS is known to be the rate-limiting enzyme in GSH synthesis and also regulates GSH level. The enzyme is induced by various stress agents including the electrophilic agent, diethyl maleate (25). This agent also induces the activity of system x⁻⁻⁻⁻ (5). Therefore, the increase in intracellular GSH level caused by diethyl maleate is most likely caused by the induction of both γ-GCS and system x⁻⁻⁻⁻. In the present study, we have demonstrated that LPS and oxygen strongly induce the expression of xCT mRNA without significantly altering γ-GCS mRNA levels. The intracellular cysteine level was increased concomitantly with the increase in cystine transport activity. The increase in the intracellular GSH level by LPS reflects the increase in intracellular cysteine. The increase in GSH level in the macrophages exposed to LPS is mainly dependent on the induced activity of system x⁻⁻⁻⁻, not the activity of γ-GCS.
We have identified several AP-1 binding sites in the 5'-flanking region of the xCT gene. Several AP-1 sites also exist in the 5'-flanking region of the human 4F2hc gene (28). These sites may be involved in the regulation of the expression of xCT and 4F2hc. However, induction of system x<sub>C</sub> activity by phorbol myristate acetate is very low in comparison with LPS under normoxia (5), suggesting only a limited role for AP-1 in the stimulation of xCT mRNA transcription by LPS. We have also found that there is a putative NF-κB binding site in the 5'-flanking region. NF-κB plays a central role in the regulation of many genes involved in cellular defense mechanisms and expression of cytokines. Reactive oxygen species have been proposed as the intermediate second messengers involved in the activation of NF-κB by TNF-α and IL-1 (29). Recent studies have demonstrated that the LPS signal is mediated by the Toll-like receptor 4 in the presence of CD14 and activates NF-κB in macrophages (30). Perhaps NF-κB is one of the candidate transcription factors involved in the induction of xCT mRNA by LPS and oxygen. However, in the presence of LPS, the activation of NF-κB derived from the macrophages cultured under hypoxia seemed to be similar to that derived from the cells cultured under normoxia (Fig. 5). These results are inconsistent with those of the uptake of cysteine. In addition, NF-κB was hardly activated by 1 ng/ml LPS, whereas induction of system x<sub>C</sub> activity was maximally activated by 1 ng/ml (5). In contrast, NF-κB was potently activated by 1000 ng/ml LPS, nevertheless expression of xCT in cells treated with 1000 ng/ml LPS was significantly lower than that in the cells treated with 1 ng/ml LPS. These results suggest that other signaling pathway(s) exist downstream of the signal transduction involved in the induction of xCT mRNA by LPS and oxygen, although it is not clear at present which transcription factor is involved in the induction of xCT mRNA by LPS and oxygen.

Studies using transcription factor Nrf2-knockout mice have revealed that expression of phase II enzymes is regulated by Nrf2, whose activity is induced by oxidative stress and electrophilic agents (31). Transcription factor Nrf2 is thought to bind to the electrophile response element (EpRE) in the 5'-flanking region of these genes and to enhance the activity of transcription. The gene for γ-GCS contains the EpRE in its 5'-flanking region, and this region is important for inducible expression of γ-GCS by the electrophilic agent (32). We have found that the mouse xCT gene also contains an EpRE-like sequence in its 5'-flanking region (Fig. 4), suggesting that its expression is regulated by the electrophilic agent in a manner similar to that documented for γ-GCS. Recently, we have shown that in peritoneal macrophages from Nrf2-deficient mice, LPS still potently induces the activity of system x<sub>C</sub>, whereas diethyl maleate does not (33). This result indicates that the signal initiated by LPS is transduced by a different mechanism from EpRE-mediated transcription. Gene expression of xCT seems to be an interesting model system for study of signal transduction from LPS, because the expression is regulated by a pathophysiologic concentration of LPS and is oxygen-sensitive.

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REFERENCES
1. Christensen, H. N. (1990) Physiologist. Rev. 70, 43–77
2. Watanabe, H. and Bannai, S. (1987) J. Exp. Med. 165, 628–640
3. Sato, H., Watanabe, H., Ishii, T., and Bannai, S. (1987) J. Biol. Chem. 262, 13015–13019
4. Sato, H., Ishii, T., Sugita, Y., and Bannai, S. (1991) Biochim. Biophys. Acta 1069, 46–52
5. Sato, H., Fujii, K., Sagara, J., and Bannai, S. (1995) Biochem. J. 310, 547–551
6. Sato, H., Fujii, M., and Bannai, S. (1992) J. Leukoc. Biol. 52, 161–164
7. Bannai, S. and Tateishi, N. (1986) J. Membrane Biol. 89, 1–8
8. Bogle, R. G., Baydoun, A. R., Pearson, J. D., and Mann, G. E. (1992) Biochem. J. 284, 15–18

FIG. 8. Intracellular cysteine levels in macrophages incubated with LPS under hypoxic and normoxic conditions. Macrophages were incubated under hypoxia (2% O<sub>2</sub>) for 1 h, and the medium was replaced by fresh medium (Cont) containing 1 ng/ml LPS (LPS). The cells were further incubated under hypoxia (2% O<sub>2</sub>, open bars) or normoxia (20% O<sub>2</sub>, striped bars) for 11 h, and intracellular cysteine levels were measured as described under “Experimental Procedures.” Each point represents the mean ± S.D. (n = 4–6).

FIG. 9. Effect of LPS on GSH efflux in macrophages incubated under hypoxic and normoxic conditions. Macrophages were incubated under hypoxia (2% O<sub>2</sub>) for 1 h, and the medium was replaced by fresh medium (Cont) containing 1 ng/ml LPS (LPS). The cells were further incubated under hypoxia (2% O<sub>2</sub>, open bars) or normoxia (20% O<sub>2</sub>, striped bars) for 11 h, and efflux of GSH was measured as described under “Experimental Procedures.” Each point represents the mean ± S.D. (n = 4–6).

The results of the present study indicate that the induced activity of system x<sub>C</sub> by LPS is further enhanced by oxygen. It is likely that the activity of system x<sub>C</sub> is relatively low in unstimulated macrophages in vivo, because average oxygen tension in the peritoneum is ~5% (26). Once these cells are activated by endotoxins, the activity of system x<sub>C</sub> is induced, and oxygen or reactive oxygen species probably enhances the induction. Because GSH antagonizes the oxygen toxicity or oxidative stress, it is likely that GSH turns over rapidly in the macrophages exposed to endotoxins and oxidative stress in a pathological state such as inflammation. The response of system x<sub>C</sub> activity to LPS and oxygen may be advantageous to the macrophages, which are elicited into the inflammatory regions.

Recently, Li et al. (27) have demonstrated that chronic exposure of bovine aortic endothelial cells to S-nitroso-N-acetylpenicillamine (NO donor) led to a concentration-dependent increase in cystine transport activity and that the increased cystine transport activity is mediated by system x<sub>C</sub>. It might be that the induction of xCT mRNA in macrophages by LPS is mediated by NO produced by the cells. However, as we demonstrated previously (9), NO production by the macrophages incubated with 1 ng/ml LPS was almost negligible. Nevertheless, the activity of system x<sub>C</sub> reaches maximum by 1 ng/ml LPS. Thus, the involvement of NO in the induction of xCT mRNA under the conditions used in the present study is eliminated.
9. Shibazaki, T., Fujiwara, M., Sato, H., Fujiwara, K., Abe, K., and Bannai, S. (1996) *Biochim. Biophys. Acta* **1311**, 156–154
10. Close, E. I., Lyons, C. R., Kelly, C., and Cunningham, J. M. (1993) *J. Biol. Chem.* **268**, 20796–20800
11. Sato, H., Tamba, M., Ishii, T., and Bannai, S. (1999) *J. Biol. Chem.* **274**, 11445–11458
12. Mastrobartolome, L., Spindler, B., Pfeiffer, R., Skelly, P. J., Loffing, J., Shoemaker, C. B., and Verrey, F. (1998) *Nature* **395**, 288–291
13. Kanai, Y., Segawa, H., Miyamoto, K., Uchino, H., Takeda, E., and Endou, H. (1998) *J. Biol. Chem.* **273**, 23629–23632
14. Torrents, D., Estevez, R., Pineda, M., Fernandez, E., Lloberas, J., Shi, Y-B., Zorzano, A., and Palacín, M. (1998) *J. Biol. Chem.* **273**, 32437–32445
15. Deves, R., and Boyd, C. A. R. (2000) *J. Membrane Biol.* **173**, 165–173
16. Lewis, J. S., Lee, J. A., Underwood, J. C. E., Harris, A. I., and Lewis, C. E. (1999) *J. Leukoc. Biol.* **66**, 889–900
17. Bannai, S., and Kitamura, E. (1980) *J. Biol. Chem.* **255**, 2372–2376
18. Tietze, F. (1969) *Anal. Biochem.* **27**, 502–522
19. Cotgreave, I. A., and Molidu, P. (1986) *J. Biochem. Biophys. Methods* **13**, 231–249
20. Sagara, J., Miura, K., and Bannai, S. (1993) *J. Neurochem.* **61**, 1667–1671
21. Mesnick, M. M., Carter, A. B., and Hunninghake, G. W. (1999) *J. Biol. Chem.* **274**, 18075–18080
22. Kaul, N., Choi, J., and Forman, H. J. (1998) *Free Radic. Biol. Med.* **24**, 202–207
23. Sagara, J., Makino, N., and Bannai, S. (1996) *J. Neurochem.* **66**, 1876–1881
24. Chasseaud, L. F. (1979) *Adv. Cancer Res.* **29**, 175–274
25. Sekhar, K. R., Long, M., Long, J., Xu, Z. Q., Summar, M. L., and Freeman, M. L. (1997) *Radiat. Res.* **147**, 592–597
26. Towell, M. E., Lysak, I., Layne, E. C., and Bessman, S. P. (1976) *J. Appl. Physiol.* **41**, 245–250
27. Li, H., Marshall, Z. M., and Whorton, A. R. (1999) *Am. J. Physiol.* **276**, C803–C811
28. Gottfried, R. M., Karpinski, B. A., Lindsten, T., Strominger, J. L., Jones, N. H., Thompson, C. B., and Leiden, J. M. (1988) *Mol. Cell. Biol.* **8**, 3809–3819
29. Schreck, R., Rieber, P., and Beuerle, P. A. (1991) *EMBO J.* **10**, 2247–2258
30. Takeuchi, O., Hoshiba, K., Kawai, T., Sanjo, H., Takeda, H., Ogawa, T., Takeda, K., and Akira, S. (1999) *Immunity* **11**, 443–451
31. Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I., Yamamoto, M., and Nabeshima, Y. (1997) *Biochem. Biophys. Res. Commun.* **236**, 313–322
32. Mulcahy, R. T., Wartman, M. A., Bailey, H. H., and Gipp, J. J. (1997) *J. Biol. Chem.* **272**, 7445–7454
33. Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T., Katoh, Y., Bannai, S., and Yamamoto, M. (2000) *J. Biol. Chem.* **275**, 16023–16029
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