Low Environmental pH Is Responsible for the Induction of Nitric-oxide Synthase in Macrophages

EVIDENCE FOR INVOLVEMENT OF NUCLEAR FACTOR-κB ACTIVATION*

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Agnès Bellocq‡, Sidonie Suberville‡, Carole Philippe‡, France Bertrand‡, Joëlle Perez‡, Bruno Fouqueray‡, Gisèle Cherqui§, and Laurent Baud¶

From the §INSERM U. 64, Hôpital Tenon, 75020 Paris and ¶INSERM U. 402, Hôpital Saint-Antoine, 75012 Paris, France

Stimulation of macrophages with endotoxin and/or cytokines is responsible for the expression of the inducible isofrom of nitric oxide synthase (iNOS). Because macrophages are exposed to low pH within the microenvironment of inflammatory lesions, the potential role of acidic pH as an additional regulator of iNOS was investigated. Substitution of the culture medium of rat peritoneal macrophages at pH 7.4 with medium at pH 7.0 up-regulated iNOS activity, as reflected by a 2.5-fold increase in nitrite accumulation. The increase in iNOS activity was associated with a similar increase in iNOS mRNA expression that reflected an increase in iNOS mRNA synthesis rather than stability. Low environmental pH-induced iNOS gene transcription involved the activation of nuclear factor-κB (NF-κB) transcription factor since exposure of macrophages to low environmental pH both increased NF-κB binding activity in the nucleus and enhanced NF-κB-driven reporter gene expression. In addition, treatment of macrophages with pyrrolidine dithiocarbamate or n-acetyl-leucinyl-leucinyl-norleucinal, two drugs preventing NF-κB translocation to the nucleus, canceled low pH-induced nitrite accumulation. The overall mechanism required the synthesis of tumor necrosis factor α (TNFα). Indeed, 1) elevated TNFα bioactivity was observed in the medium of macrophages exposed to pH 7.0, and 2) incubation of macrophages with a neutralizing anti-TNFα antibody impaired both NF-κB activation and nitrite accumulation in response to acid challenge. In summary, exposure of macrophages to acidic microenvironment in inflammatory lesions leads to the up-regulation of iNOS activity through the activation of NF-κB.

Acidosis is a hallmark of both ischemia and inflammation processes. The decrease of pH in tissue ischemia is secondary to the release of H+ during ATP hydrolysis and to the accumulation of CO2 (1). The acidic environment in inflammatory lesions and abscesses (2) is due to increased metabolic acid generation during cell activation. This originates primarily from the hexose monophosphate shunt, by the dissociation of hydrated CO2 (3).

In most cases, acidosis occurs along with nitric oxide (NO)1 generation. In ischemia, NO generation is due in one part to the acidification and reduction of the large pool of nitrite present within the tissue (4). In inflammatory processes, macrophage exposure to bacterial lipopolysaccharide (LPS) or cytokines such as tumor necrosis factor α (TNFα) and interferon-γ (IFN-γ) causes the expression of the inducible isofrom of NO synthase (NOS II or iNOS) that is responsible for high output production of NO (5). The expression of iNOS is regulated mainly at the transcriptional level. Analyses of the murine iNOS promoter have shown the presence of numerous consensus sequences for the binding of transcription factors (6, 7), of which nuclear factor-κB (NF-κB) (8), interferon regulatory factor-1 (IRF-1) (9), and signal transducer and activator of transcription (STAT) 1a (10) are functionally important for iNOS induction. NF-κB is composed of a p50/p65 (or p50/RelA) heterodimer that is retained in the cytoplasm of macrophages by its binding to the inhibitory protein IκBα (11). Macrophage exposure to LPS or TNFα results in the rapid phosphorylation of IκBα and its degradation by the proteasome, allowing NF-κB to translocate to the nucleus and promote iNOS gene transcription.

Whether an acidic environment beside LPS and cytokines contributes to the regulation of iNOS in inflammatory processes has not been investigated. Thus, the experiments reported here were performed to investigate if a low environmental pH was able to activate iNOS in macrophages and, if so, to examine the signal transduction pathway involved. Our results provide the first evidence that, indeed, limited acidification of macrophage environment is sufficient to induce the expression of iNOS gene and the synthesis of NO. This effect is due to increased translocation of NF-κB to the nucleus. Additionally, our data show that an amplification loop involving TNFα production and NF-κB activation is required for this process.

EXPERIMENTAL PROCEDURES

Cell Isolation and Culture—Resident macrophages were obtained from male Sprague-Dawley rats by peritoneal lavage with MEM (Life Technologies, Inc., Cergy Pontoise, France). Peritoneal cells were washed by centrifugation and then resuspended in MEM containing 2 mm l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin and supplemented with 10% FCS. Cells were plated either in 24-well culture plates (Costar, Cambridge, MA) (106 cells in 0.5 ml/well) or in 60-mm Petri dishes (Nunc, Roskilde, Denmark) (107 cells in 5 ml). After

3-phosphate dehydrogenase; HMA, 5-(N,N-hexamethylene) amiloride; iNOS, inducible NO synthase; NF-κB, nuclear factor-κB; n-acetyl-leucinyl-leucinyl-norleucinal; PDTC, pyrrolidine dithiocarbamate; TNFα, tumor necrosis factor α; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; RT-PCR, reverse transcription-polymerase chain reaction; STAT, signal transducer and activator of transcription; IFN, interferon; LPS, lipopolysaccharide; BCECF, 2’7’-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; MEM, minimum Eagle’s medium; IRF-1, interferon regulatory factor-1; DRB, 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole.

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‡ To whom correspondence should be addressed: INSERM U.64, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France. Tel.: 33 1 40 30 79 51; Fax: 33 1 40 30 20 89; E-mail: laurent.baud@tnn.ap-hop-paris.fr.

§ The abbreviations used are: NO, nitric oxide; AP-1, activator protein 1; EMSA, electrophoretic mobility shift assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMA, 5-(N,N-hexamethylene) amiloride; iNOS, inducible NO synthase; NF-κB, nuclear factor-κB; n-acetyl-leucinyl-leucinyl-norleucinal; PDTC, pyrrolidine dithiocarbamate; TNFα, tumor necrosis factor α; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; RT-PCR, reverse transcription-polymerase chain reaction; STAT, signal transducer and activator of transcription; IFN, interferon; LPS, lipopolysaccharide; BCECF, 2’7’-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; MEM, minimum Eagle’s medium; IRF-1, interferon regulatory factor-1; DRB, 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole.

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a 2-h incubation at 37 °C in a 5% CO2, 95% air atmosphere, the non-adherent cells were removed by aspiration, and the macrophage monolayers were then overlaid with MEM supplemented with 1% FCS and adjusted by addition of HCl to pH ranging between 7.4 and 6.8. The pH of the culture medium was checked just before use. In addition, and when culture with cell media was used, 10 mM sodium pyruvate, 1 mM Na2EDTA, 10 μM aminolevulinic acid (N-hexamethylene) amiloride (HMA, LC Services, Woburn, MA), monensin, actinomycin D, 5,6-dichloro-1-b-D-ribofuranosyl benzimidazole (DRB), pyrrolidine dithiocarbamate (PDTC), or murine leukemia virus-reverse transcriptase (200 units/mL) were added to the culture medium. 

Electroretinographic Mobility Shift Assay (EMSA)—Nuclear extracts were prepared from adherent macrophages by the method previously described (17) and were stored at −80 °C until analysis. Protein was determined using the Bio-Rad reagent according to the manufacturer’s instructions. Where indicated, nuclear extracts were incubated for 30 min at 4 °C with polyclonal antibodies against p50 or RelA subunits of NF-κB (Santa Cruz, CA), before the binding assay and assayed for nitrite concentration. Adherent cells were used for nuclear or RNA extraction.

RAW 264.7 cells (American Type Culture Collection) were grown in DMEM (Life Technologies, Inc.) supplemented with 10% FCS and antibiotics at 37 °C in a 5% CO2, 95% air atmosphere.

**Determination of Intracellular pH in Macrophages**—Macrophages at 106 cells/ml were washed twice with MEM and loaded with the ace
toxyethyl ester of 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF, Sigma) at 10 μM by incubation at 37 °C for 10 min. Cells were washed twice, and the cell suspension was immediately analyzed in a Perkin-Elmer model LS-5 spectrofluorometer for the fluorescence of BCECF. The sample was alternately excited at 450 and 500 nm, and the emitted fluorescence was measured at 530 nm using an 8-nm slit. For experimental procedures, the cells were permeablized with 0.05% (v/v) Triton X-100, and aliquots of the suspension were titrated with 1N HCl and 1 N NaOH to establish the fluorescence at known pH values. Calibration curves of fluorescence versus pH were obtained as described previously (12).

**Determination of iNOS Activity**—Inducible NOS activity was assayed indirectly by measuring nitrite production. Nitrite was measured by a colorimetric assay based on the Griess reaction, as described previously (12).

**Determination of TNFα Activity**—The concentration of bioactive TNFα was measured by the luciferase reporter gene (a gift from Dr. A. Israel, Institut Pasteur, Paris, France), using the DEAE-dextran procedure (6, 20). Briefly, after cells were washed with Tris-buffered saline solution, 10 μg of plasmid was added per 106 cells in 1 ml of warm Tris-buffered saline solution containing 250 μg of DEAE-dextran. The cells were incubated at 37 °C for 1 h followed by a 2-min wash with 10% MeSO at room temperature. The cells were washed twice, plated in 35-mm plates at 2.5 × 104 cells/ml in 2 ml of DMEM supplemented with 10% FCS at 37 °C in 5% CO2. Twenty-four h later, the medium was replaced with DMEM supplemented with 1% FCS and adjusted at different pH, and the cells were incubated for additional 2 h. The cells were then washed, exposed for 15 min to chilled lysis buffer (25 mM Tris, pH 7.8, 10 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol, 1% Triton X-100, and 15% glycerol), and scraped. The lysates were centrifuged at 13,000 × g for 10 min. Luciferase activity was assayed in lysis buffer containing 50 mM ATP and 100 μM luciferin, using a Lumat LB 9507 luminometer (Berthold Bad Wildbad, Germany) (17).

**Transient Transfection of RAW 264.7 Cells and Luciferase Assay**—RAW 264.7 cells were transfected with Igea3-conaluc, a reporter plasmid that contains three copies of the immunglobulin κ chain enhancer κB site upstream of the minimal conalbumin promoter fused to the luciferase reporter gene (a generous gift from Dr. A. Israel, Institut Pasteur, Paris, France), using the DEAE-dextran procedure (6, 20). Briefly, after cells were washed with Tris-buffered saline solution, 10 μg of plasmid was added per 106 cells in 1 ml of warm Tris-buffered saline solution containing 250 μg of DEAE-dextran. The cells were incubated at 37 °C for 1 h followed by a 2-min wash with 10% MeSO at room temperature. The cells were washed twice, plated in 35-mm plates at 2.5 × 104 cells/ml in 2 ml of DMEM supplemented with 10% FCS at 37 °C in 5% CO2. Twenty-four h later, the medium was replaced with DMEM supplemented with 1% FCS and adjusted at different pH, and the cells were incubated for additional 2 h. The cells were then washed, exposed for 15 min to chilled lysis buffer (25 mM Tris, pH 7.8, 10 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol, 1% Triton X-100, and 15% glycerol), and scraped. The lysates were centrifuged at 13,000 × g for 10 min. Luciferase activity was assayed in lysis buffer containing 50 mM ATP and 100 μM luciferin, using a Lumat LB 9507 luminometer (Berthold Bad Wildbad, Germany) (17).

**Determination of TNFα Production**—The concentration of bioactive TNFα in the culture medium of macrophages was measured by a L-929 fibroblast lytic assay, as described previously (21). Results were expressed as percent cytotoxicity.

**Statistics**—Results are presented as the mean ± S.E. Statistical significance (p < 0.05) was determined by Student’s test.

**RESULTS**

Macrophages Exposed to Low Environmental pH Demonstrate Increased iNOS Activity—Substitution of the medium of adherent macrophages with media at different pH modified iNOS activity as reflected by changes in nitrite accumulation; as the environmental pH became more acidic, more nitrite was detected in the culture media (Fig. 1). Its amount plateaued at pH 6.9–7.0. Because modification of the microenvironmental pH generally induces parallel modification of the steady-state intracellular pH (22), involvement of intracellular acidification.
in this process was tested. First, intracellular pH decreased from 7.04 ± 0.03 to 6.77 ± 0.01 after lowering environmental pH from 7.4 to 6.9–7.0, as measured fluorimetrically with the H+ indicator dye BCECF. Second, pharmacological control of the Na+/H+ antiport, which exchanges extracellular Na+ for intracellular H+, caused measurable changes in NO synthesis. As indicated in Fig. 2, the addition of the amiloride analog HMA, a Na+/H+ exchange inhibitor which decreases intracellular pH (23), caused a dose-dependent increase in nitrite accumulation. Conversely, the addition of monensin, a Na+ ionophore mimicking the effects of the antiporter activation (24), caused a dose-dependent decrease in nitrite accumulation. These results suggested that intracellular acidification was sufficient to trigger NO synthesis.

Inducible NOS is susceptible to control mainly at the transcriptional level (6, 7). Thus DRB, a specific RNA polymerase II inhibitor, was used to determine to what extent nitrite accumulation in response to low environmental pH was due to increased transcription of the iNOS gene. Pretreatment of macrophages with this drug reduced nitrite accumulation in the macrophage culture medium adjusted at pH 7.4 and blunted the response to acid challenge (Fig. 3A). To confirm the involvement of increased expression of the iNOS gene, semi-quantitative RT-PCR was first performed. A detectable expression of the iNOS mRNA was always observed in control macrophages, as described previously (25) (Fig. 3B). Exposure of cells to low environmental pH determined a marked increase in this mRNA expression. The amount of iNOS mRNA quantified by densitometry and expressed as iNOS/GAPDH ratio was 0.76 ± 0.11 and 1.75 ± 0.08 for macrophages exposed to culture medium at pH 7.4 and 7.0, respectively (p < 0.01, n = 3). For further verification, quantitative RT-PCR was performed (Fig. 3C). The amount of iNOS mRNA was sharply increased in cells exposed to acidic pH as judged by the difference in the number of amplification cycles necessary to generate similar amounts of PCR products. Next, iNOS mRNA stability was assessed in actinomycin D experiments. After exposure to media at different pH for 1 h, macrophages were treated with actinomycin D to inhibit further transcription. At various times after the addition of actinomycin D, total RNA was isolated and examined by enzyme-linked immunosorbent assay-PCR (Fig. 3C). The decay of iNOS mRNA, which was revealed by a shift to the right of OD curves, was enhanced in cells exposed to acidic pH. Thus low environmental pH increased iNOS mRNA expression by up-regulating its synthesis rather than its stability.

Low pH Enhances iNOS Expression through NF-κB Activation

**Low Environmental pH Response Is Due to the Activation of NF-κB**—The 5′-flanking region of mouse iNOS gene contains numerous consensus sequences for the binding of transcription factors, including NF-κB, IRF-1, STAT 1α, and AP-1 (6, 7). Therefore, it is possible that low environmental pH stimulates iNOS gene transcription by modulating the activity of some of these factors. To test the above hypothesis, the effect of extracellular pH on the DNA binding activity of these transcription factors was measured by EMSA. Nuclear extracts from control macrophages cultured at neutral environmental pH exhibited weak DNA binding activity to the NF-κB site-containing oligonucleotide (Fig. 4A). As the environmental pH became more acidic, more binding activity of NF-κB was detected. At pH 7.0, NF-κB activation was similar to that promoted by LPS challenge. By contrast, acidic pH did not affect DNA binding activity of IRF-1, STAT 1α, or AP-1, whereas LPS was stimulatory (Fig. 4B).

The specificity of low pH-induced bands was assessed in competition experiments. These bands disappeared in the presence of the unlabeled oligonucleotide containing the NF-κB binding sites (Fig. 4A). We further characterized the two NF-κB-DNA complexes by using antibodies directed against the p50 and RelA NF-κB subunits (Fig. 4A). Incubation of macrophage nuclear extracts with the anti-p50 antibody reduced the density of both bands with a concomitant supershift suggesting the presence of p50 in the two complexes. The anti-RelA antibody suppressed the upper band suggesting the presence of RelA. Thus we identified the lower and upper complexes as the p50 homodimer and the p50/RelA heterodimer, respectively.

To test whether low extracellular pH enhanced NF-κB-driven reporter gene expression, RAW 264.7 cells were transfected transiently with (Igκ3-cona)luc, a reporter plasmid in which the activity of the minimal conalbumin promoter could be enhanced by three copies of the NF-κB response element (17). Luciferase activity from such transfected cells after exposure to media at different pH is shown in Fig. 5; low pH stimulation of luciferase activity was analogous to that of nitrite accumulation under the same conditions.

The contribution of NF-κB in low pH-induced iNOS up-regulation was investigated using two inhibitors of NF-κB activation in macrophages as follows: the thiol compound PDTC, a radical scavenger (26), and the peptide aldehyde non-LEU, a proteasome inhibitor (27). Addition of drug blunted the macrophage response to low environmental pH (Fig. 6).
Low Environmental pH-induced NF-κB Activation Requires the Autocrine Production of TNFα—Because NF-κB initiates the transcription of a variety of genes that are all involved in inflammatory processes, it is likely that low environmental pH-induced NF-κB activation amplifies the synthesis of pro-inflammatory cytokines beside that of iNOS. To address this issue, the capacity of macrophages exposed to acidic pH to release bioactive TNFα was determined. Fig. 7 shows that a decrease in environmental pH from 7.4 to 7.0 caused a 1.8-fold increase in TNFα release. PDTC blunted this response. We next evaluated whether the rise of TNFα level in the culture medium of macrophages was involved in the observed increase in nitrite accumulation. To this end, a neutralizing anti-TNFα antibody diluted at 1/100 was added to the culture medium. This concentration was sufficient to totally inactivate the TNFα released by macrophages (data not shown). Anti-TNFα both prevented the activation of NF-κB and suppressed the accumulation of nitrites (Fig. 8), indicating that an autocrine loop involving TNFα induction of NF-κB was responsible for the induction of iNOS by low environmental pH.

DISCUSSION

Our results provide evidence that low environmental pH stimulates NO synthesis by macrophages and, more specifically, up-regulates iNOS mRNA expression. These responses were observed for a range of extracellular pH values occurring in inflammatory lesions (2). The decrease in intracellular pH in macrophages exposed to low environmental pH and the increase in nitrite accumulation in the culture medium of macrophages treated by the amiloride analog HMA indicate that acidic intracellular pH is involved in triggering NO synthesis. Since NO has been shown to impair intracellular pH recovery in macrophages following acid loading (28), an amplification loop involving low pH induction of NO synthesis is likely in these cells.

The mechanisms responsible for low pH-induced NO synthesis are potentially numerous. First, changes in pH might affect the activity of the enzyme iNOS. Indeed, in vitro analysis has shown that iNOS activity was pH-dependent (29). However, the optimum activity was reached at pH 8.0, whereas reduced activity occurred at acidic pH. Second, a low environmental pH
might influence the availability of substrates such as L-arginine and cofactors necessary for NO production. However, L-arginine and its precursor, L-citrulline, are transported by pH-insensitive specific carriers (30). In fact, the main rate-limiting step for NO production by macrophages in low environmental pH is transcriptional since DRB blunted this response. The possibility that pH controls the transcription of genes has been demonstrated previously. For instance, Yamaji et al. (31) reported that exposure of epithelial cells from mouse proximal tubule to an acid environment led to transcriptional activation of immediate early genes such as c-fos and c-jun. Similarly, an acute decrease in extracellular pH from 7.4 to 6.9 caused a marked increase in the expression of mRNA for phosphoenolpyruvate carboxykinase in epithelial cells from pig proximal tubule (32).

Transcription factors such as NF-κB (8), IRF-1 (9, 33), and STAT 1α (10) underlie the synergistic activation of the promoter of the murine iNOS gene in response to LPS and IFN-γ.
abrogated by addition of PDTC or nor-LEU is consistent with the involvement of IκB degradation. Indeed, both drugs prevent IκB inactivation as follows: PDTC, as other antioxidants, limits IκB phosphorylation and nor-LEU, as other proteasome inhibitors, inhibits IκB degradation but not phosphorylation (27). Furthermore, the results of experiments with nor-LEU suggest that low environmental pH induces NF-κB activation by promoting IκB degradation via the ubiquitin-proteasome pathway. Our study did not address the relationship between environmental pH and expression of proteins involved in this pathway. However, Bailey et al. (35) recently reported that acidosis in chronic renal failure stimulated muscle proteolysis by activating the ATP-ubiquitin-proteasome-dependent pathway. This response included increased transcription of genes encoding ubiquitin and proteasome subunits C3 and C9. Thus, further studies would be required to determine whether low environmental pH induces NF-κB activation in macrophages by these mechanisms.

In addition, a possibility to explain the in vivo effect of low pH on the transcription step is that acidosis could directly affect the binding of NF-κB subunits to DNA. However, this is unlikely. Indeed, studies by Zabel et al. (18) have demonstrated that NF-κB could form a complex with DNA within a large pH range, the highest amount of protein-DNA complex being formed at the physiological pH of 7.5.

The NF-κB transcription factor regulates the transcription of a great variety of genes that are involved in inflammatory responses. They include genes coding for cytokines such as IFN-β, interleukin-1, -2, -6, and -8, granulocyte/macrophage or granulocyte colony-stimulating factor, TNFα, as well as genes coding for acute phase response proteins and cell adhesion molecules (reviewed in Ref. 36). One could speculate that transcription of these genes might be similarly susceptible to induction by low environmental pH. Accordingly, acidosis was found to potentiate TNFα synthesis by macrophages (Fig. 7). We addressed the issue of whether low environmental pH-induced TNFα synthesis was in turn responsible for NO synthesis by using a neutralizing antibody specific for TNFα (Fig. 8). This antibody blunted both NF-κB activation and nitrite accumulation. Thus, TNFα appears to be involved in NF-κB activation which eventually leads to iNOS gene transcription.

A similar amplification loop involving TNFα induction of NF-κB has been already described. For instance, O’Connell et al. (37) demonstrated that the proliferation rate of cells derived from a Sezary lymphoma was stimulated by the autocrine production of TNFα. This response resulted from the activation of NF-κB which also led to further TNFα production.

In summary, in addition to hypoxia (38), LPS, and cytokines (5), low environmental pH causes amplification of NO synthesis in inflammatory tissues. Evidence that this up-regulation is mediated through the activation of NF-κB suggests a novel mechanism whereby the nuclear translocation of this transcription factor may be triggered. Our studies also suggest that correction of acidosis in inflammatory processes may have a therapeutic role by limiting the transcription of cytokine genes with a conserved NF-κB binding site in the promoter.

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REFERENCES
1. Weinberg, J. M. (1991) Kidney Int. 39, 476–500
2. Bryant, R. E., Rashad, A. L., Mazza, J. A., and Hammond, D. (1980) J. Infect. Dis. 142, 594–601
3. Wright, J., Schwartz, J. H., Olson, R., Kosowsky, J. M., and Tauber, A. I. (1986) J. Clin. Invest. 77, 782–788
4. Zweier, J. L., Wang, P., Samouilov, A., and Kuppuram, P. (1995) Nat. Med. 1, 804–809
5. Nathan, C. (1992) FASEB J. 6, 3051–3064
6. Xie, Q.-W., Whisnant, R., and Nathan, C. (1993) J. Exp. Med. 177, 1779–1784
7. Lowenstein, C. J., Alley, E. W., Raval, P., Snowman, A. M., Snyder, S. H., Russell, S. W., and Murphy, W. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9730–9734
8. Xie, Q.-W., Kashiwabara, Y., and Nathan, C. (1994) J. Biol. Chem. 269, 4705–4708
9. Martin, E., Nathan, C., and Xie, Q.-W. (1994) J. Exp. Med. 180, 977–984
10. Gao, J., Morrison, D. C., Parmely, T. J., Russell, S. W., and Murphy, W. J. (1977) J. Biol. Chem. 272, 1226–1230
11. Grimm, S., and Bauerle, P. A. (1993) Biochem. J. 290, 297–308
12. Baud, L., Perez, J., Cherqui, G., Cragoe, E. J., Jr., and Koo, C. H. (1988) J. Cell. Physiol. 136, 355–360
13. Boutard, V., Fouqueray, B., Philippe, C., Moulinoux, J.-P., and Baud, L. (1995) J. Immunol. 155, 2077–2084
14. Liu, S., Adcock, I. M., Old, R. W., Barnes, P. J., and Evans, T. W. (1993) Biochem. Biophys. Res. Commun. 196, 1208–1213
15. Lantz, O., and Bendelac, A. (1994) J. Exp. Med. 180, 1097–1106
16. Alard, P., Lantz, O., Sebagh, M., Calvo, C. F., Senik, A., and Charpentier, B. (1993) BioTechniques 15, 730–737
17. Bertrand, F., Philippe, C., Antoine, P. J., Baud, L., Groyer, A., Capeau, J., and Cherqui, G. (1995) J. Biol. Chem. 270, 24435–24441
18. Zabel, U., Schreck, R., and Bauerle, P. A. (1991) J. Biol. Chem. 266, 252–260
19. Demea, S., Harbers, M., and Vennstrom, B. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2574–2578
20. Selden, R. F. (1996) in Current Protocols in Molecular Biology, (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds) pp. 9.2.1–9.2.6, John Wiley & Sons, Inc., New York
21. Philippe, C., Philippe, B., Fouqueray, B., Perez, J., Lebret, M., and Baud, L. (1993) Am. J. Pathol. 143, 1713–1723
22. Mollergård, P., Ou-Yang, Y., and Siesjo, B. K. (1994) Am. J. Physiol. 267, C581–C589
23. Baud, L., Perez, J., Cherqui, G., Cracoe, E. J., and Ardaillou, R. (1989) Am. J. Physiol. 257, C232–C238
24. Ghigo, D., Russolino, F., Garbarino, G., Heller, R., Turrini, F., Pescarmona, G., Cracoe, E. J., Jr., Pegoraro, L., and Bosia, A. (1988) J. Biol. Chem. 263, 19437–19446
25. Chersnow, S. E., Monnier, J., Visser, G., and Nick, H. S. (1994) Biochem. Biophys. Res. Commun. 200, 126–154
26. Schreck, R., Rieber, P., and Bauerle, P. A. (1991) EMBO J. 10, 2247–2258
27. Griscavage, J. M., Wilk, S., and Ignarro, L. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 3308–3312
28. Swallow, C. J., Grinstein, S., Sudsbury, R. A., and Rotstein, O. D. (1991) J. Exp. Med. 174, 1009–1021
29. Yaqoob, M., Edelstein, C. L., Wieder, E. D., Alkhunaizi, A. M., Gengaro, P. E., Nemenoff, R. A., and Schrier, R. W. (1996) Kidney Int. 49, 1314–1319
30. Baydoun, A. R., Bogle, R. G., Pearson, J. D., and Mann, G. E. (1994) Br. J. Pharmacol. 112, 487–492
31. Yamaji, Y., Moe, O. W., Miller, R. T., and Alpern, R. J. (1994) J. Clin. Invest. 94, 1297–1303
32. Kaiser, S., and Curthyos, N. P. (1991) J. Biol. Chem. 266, 9397–9402
33. Spink, J., and Evans, T. (1997) J. Biol. Chem. 272, 24417–24425
34. Dourdevani, A., Abramson, O., Tamir, A., Kondurtiy, A., Isakov, N., and Chainomvita, C. (1995) Kidney Int. 47, 1537–1545
35. Bailey, J. L., Wang, X., England, B. K., Price, S. R., Ding, X., and Mitch, W. E. (1996) J. Clin. Invest. 97, 1447–1453
36. Kopp, E. B., and Ghosh, S. (1995) Adv. Immunol. 58, 1–27
37. O’Connell, M. A., Cleere, R., Long, A., O’Neill, L. A. J., and Kelly, D. (1995) J. Biol. Chem. 270, 7399–7404
38. Melillo, G., Musso, T., Sica, A., Taylor, L. S., Cox, G. W., and Varesio, L. (1995) J. Exp. Med. 182, 1683–1693

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