Granule Localization of Glutaminase in Human Neutrophils and the Consequence of Glutamine Utilization for Neutrophil Activity*

Received for publication, August 27, 2003, and in revised form, January 8, 2004
Published, JBC Papers in Press, January 13, 2004, DOI 10.1074/jbc.M309520200

Linda Castell‡‡, Caroline Vance‡‡, Rachel Abbott‡‡, Javier Marquez‡‡, and Paul Eggleton**

From the †Department of Biochemistry and the ‡Medical Research Council Immunochemistry Unit, South Parks Road, University of Oxford OX1 3QU, United Kingdom, the **Institute of Biomedical and Clinical Science, Peninsula Medical School, Heavitree Road, Exeter EX1 2LU, United Kingdom, and the ‡‡Molecular Biology and Biochemistry Department, University of Malaga, Malaga E-29071, Spain

The provision of glutamine in vivo has been observed to reduce to normal levels the neutrophilia observed after exhaustive exercise and to decrease the neutrophil chemoattractant, interleukin-8. Thus, the role for glutamine in the regulation of inflammatory mediators of human neutrophil activation was investigated. The study sought to establish whether glutamine supplementation in vitro affects neutrophil function at rest and whether glutaminase, the major enzyme that metabolizes glutamine, is present in human polymorphonuclear neutrophils (PMNs). During in vitro studies, the addition of 2 mM glutamine increased the respiratory burst of human PMN stimulated with both phorbol myristate acetate (PMA) and formyl-methionyl-leucyl-phenylalanine. These observations were made using a highly sensitive, real time chemiluminescent probe, Pholasin®. Glutamine alone did not stimulate the release of reactive oxygen species. In a novel finding using glutaminase-specific antibodies in combination with flow cytometry and confocal microscopy, glutaminase was shown to be present on the surface of human PMN. Subcellular fractionation revealed that the enzyme was enriched in the secondary granules and could be released into cell culture medium upon stimulation with PMA. In conclusion, human PMN appeared to utilize glutamine and possess the appropriate glutaminase enzyme for metabolizing glutamine. This may depress some pro-inflammatory factors that occur during prolonged, exhaustive exercise.

Human polymorphonuclear neutrophils (PMNs)† are a major class of the nonspecific immune response against infections. This is due, in part, to their ability to produce toxic forms of reactive oxygen species (ROS), including superoxide anions (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals (OH$^-$).

The generation of ROS requires the cytosolic NADPH oxidase proteins to form a complex with several of the membrane-bound parts of the oxidases. Once assembled, direct stimulation of cells results in the release of O$_2^-$ into the extracellular compartment or into phagosomes. In turn, activated PMN synthesizes and release a number of pro-inflammatory cytokines, including tumor necrosis factor (TNF), IL-1, IL-6, and IL-8 (1, 2). Recent work has shown that glutamine supplementation in vitro enhances both phagocytosis and ROS in isolated PMN (3) and, in vivo, suppresses IL-8 production by PMN (4). This has important consequences, as glutamine appears to exert a regulatory influence on inflammatory processes by PMN responding to inflammatory and infectious stimuli. For example, PMNs taken from patients with burns (5) or after surgery (6) have been shown to have improved bactericidal activity in vitro when glutamine is added to the culture medium for incubation.

Glutamine is an important substrate for some key cells of the immune system, such as macrophages and lymphocytes (7, 8). It acts as a nitrogen donor for purine and pyrimidine nucleotide synthesis for new DNA synthesis and for mRNA repair. Although classified as non-essential, recent evidence suggests that glutamine is conditionally essential when it becomes rapidly depleted in the blood in stressful situations. Plasma glutamine concentrations are substantially decreased by clinical trauma such as in major surgery by 37% (9) or after prolonged, exhaustive exercise by 20–25% (10). The provision of glutamine or glutamine precursors to endurance athletes has resulted in a decreased self-reported incidence of illness, particularly for upper respiratory tract infections (URT1), in four studies (see Castell, 2003; Ref. 4). Increasing evidence, in vitro and in vivo, suggests that PMN may benefit from exogenous glutamine, which repletes the decrease in the blood concentration observed after stress.

The energy substrate for PMN has traditionally been thought to be glucose. However, it is possible that PMN could use glutamine, particularly in cases such as severe infection in which glucose is restricted. Evidence for a direct effect of glutamine on neutrophil function has been demonstrated in rats, where it was shown that isolated PMN utilized glutamine at a rate of 12.8 nmol min$^{-1}$ mg$^{-1}$ protein (11) in the absence of glucose. The same workers further confirmed the presence of phosphate-dependent glutaminase (GA) in rat PMN using Western blots and immunocytochemistry methods. To date, the presence of GA in human PMN has not been demonstrated, despite several attempts to do so.

Strenuous exercise stimulates leukocytosis and neutrophilia (12–14) and the release of immature PMN from bone marrow (15, 16). However, there are conflicting reports upon the effects of exercise on ROS release by PMN (17). Significant increases have been observed in the plasma concentration of IL-8 (~2-
fold) immediately after and 1.5 h after a marathon (18) and in cell IL-8 production after a rowing ergo test (19). This suggests enhanced post-exercise activation of PMN. Significant decreases in post-exercise neutrophilia (−1.75-fold) and cell IL-8 production (−3.5-fold) have been observed after a marathon in runners taking glutamine compared with a placebo (20). Since then, three further studies in our laboratory, a reduction in IL-8 associated with glutamine feeding after exercise has been observed (see Castell, 2003; Ref. 4). Clinical studies (21, 22) established that IL-8 production was reduced in both surgical patients and post-1000 m g for 45 min. The layer of PMN was removed using a Pasteur pipette and placed with an equal volume of hypertonic glutamine-enriched parenetal nutrition. IL-8 is a potent chemotaxitant that perpetuates the inflammatory response and attracts PMN to the site of tissue damage.

The present study was set up to establish whether glutamine supplementation affects human neutrophil respiratory burst via in vitro incubation of blood with/without glutamine and to determine whether human PMN utilizes glutamine via the presence of GA, using both immunochemical techniques and confocal microscopy.

MATERIALS AND METHODS

Subjects and Blood Samples—Ethical permission for the studies was obtained from the Central Oxford Research Ethics Committee (COREC). Resting blood samples were taken from healthy controls recruited from colleagues. Neutrophil function was measured with a novel chemiluminescence assay as described below.

Isolation of PMN—Isolated whole cells and subcellular neutrophil fractions were assayed for the presence of glutaminase. Neutrophils were isolated as described previously (23). Briefly, 5 ml of anticoagulated (potassium EDTA, 1.5 mg/ml) blood was layered on top of 4 ml of Phosphat-buffered saline with calcium and magnesium (PBS2+) for 30 min at 37 °C, and the response was measured until the reaction had passed (5 min with fMLP and 30 min with PMA) using a microplate luminometer (Anthos Lucy 1).

Production of Anti-GST-LGA347–602 Antibodies—An EcoRI-XhoI fragment containing nucleotides 1054–1909 of the human liver-type GA (LGA) cloned from ZR-75 breast cancer cells (26) was PCR-amplified and ligated in-frame in the EcoRI/XhoI site of expression vector pGEX-6P-1 (Amersham Biosciences). This fragment, encoding for the amino acids 347–602 of the C-terminal half of human LGA, was expressed in Escherichia coli as a GST fusion protein (GST-GA347–602). Protein expression and affinity purification were performed as previously (26) using a glutathione-Sepharose affinity column. The purified recombinant protein was used for hyperimmunization of New Zealand White rabbits, and polyclonal antibodies were generated as described elsewhere (27).

SDS-PAGE and Western Blot of Glutaminase—Approximately 40 μg of neutrophil membrane sub-fractions, prepared by nitrogen cavitation as described previously (25), were suspended in sample buffer (62 mmol/liter Tris-Cl, pH 6.8, 2% SDS, 10% glycerol, and 0.01% bromophenol blue with 5% 2-mercaptoethanol), incubated for 5 min at 100 °C, and then applied to lanes of a 12% polyacrylamide mini-gel; electrophoresis was performed in Tris-buffered saline (TBS) at a constant current of 60 mA for 2 h. Molecular weight standards (Bio-Rad) were run simultaneously. The gel was stained with Coomassie Blue and dried. A parallel SDS-PAGE gel was run as above, and the separated proteins were transferred directly by semi-dry blotting onto polyvinyl difluoride (PVDF) transfer membrane for 90 min at a constant current of 0.8 mA. After saturation of the nonspecific sites with 5% nonfat dry milk, the membranes were overlayed with goat-anti-rabbit anti-human glutaminase antibody prepared as described below. The blot was then washed in 20 ml Tris-HCI, pH 7.5, and 0.14 ml NaCl containing 0.4% TWEEN 20 (TBS-Tween) and then incubated for 1 h in an anti-rabbit peroxidase-conjugated IgG antibody diluted 1:1000 in TBS-Tween; the immunoblot was exposed to an ECL immunoblotting detection kit (Amersham Biosciences) for 1 s to check for any signal, and then the membrane was exposed to x-ray film for 5 min.

Flow Cytometry and Confocal Microscopy—Detection of GA on the surface of human PMN was achieved using both flow cytometry and a Bio-Rad Radiance 2000 Confocal Laser Scanning Microscope (Zeiss Axiovert 135M) and an Image Analysis work station. PMNs were incubated with 1:50 diluted rabbit anti-human glutaminase for 30 min, and, after three washes in PBS−+, the cells were incubated with 1:50 goat-anti-rabbit-FITC-conjugated IgG for 30 min at 4 °C and then washed three times in PBS−+. In the flow cytometry studies, the PMNs were stimulated with either 0.16 μg (100 ng/ml) PMA or 0.1 μM fMLP or left in phosphate-buffered saline with calcium and magnesium (PBS−+) for 30 min at 37 °C before probing with test antibodies. The cells were then either fixed in 2% (v/v) paraformaldehyde and analyzed on a BD Biosciences FACScan or spun onto microscope slides at 500 rpm in a Cytospin centrifuge (Shandon Southern Instruments), and cover slips were mounted over the slides using an anti-quinone reagent Citifluor (Citifluor UK Chemical Lab, Canterbury, United Kingdom) and sealed with nail polish. The cells were viewed on the Zeiss microscope fitted with a confocal argon-krypton mixed gas laser. Images were taken serially from the top to the bottom of each cell using a z plane motorized sub-stage. The appropriate excitation and emission filters for FITC were employed. Several data sets were collected for each experiment.

Statistical Analysis—Results were compared using non-parametric analysis with Wilcoxon paired t tests.

RESULTS

Real Time Oxidative Response of Stimulated Leukocytes in the Presence of Glutamine—Whole blood leukocytes from healthy control individuals stimulated with PMA (final concentration 0.16 μg) and fMLP (final concentration 1 μg) were incubated with the highly sensitive real time chemiluminescence probe Pholasin® to examine ROS release in the presence and absence of glutamine. Four of six samples gave an increased response (39.2%) to fMLP when incubated with glutamine compared with no glutamine. In the fMLP experiments there were two individuals who did not respond to the addition of glutamine. However, the response was not diminished whether or not glutamine was present. In a separate group,
Glutaminase in Human Neutrophils

A. Neutrophil response to PMA

B. Neutrophil response to fMLP

**FIG. 1.** Effect of glutamine on real time release of ROS from whole blood human leukocytes stimulated with PMA and fMLP and detected by Pholasin-dependent chemiluminescence. Aliquots of diluted whole blood (1:200) were incubated for 30 min at 37 °C before incubation with Pholasin® (0.5 μg/well) before the addition of PMA (0.16 μM) (A) or fMLP (1 μM) (B). The effect of glutamine (solid line) on ROS release was monitored for up to 30 min for PMA or 5 min for fMLP at 12 s intervals. Results are shown for single experiments performed in duplicate on four different healthy individuals. ALU, actual light units.

Thus, it was next decided to examine neutrophil subfractions, which had been prepared previously by nitrogen cavitation into four main fractions, namely primary granules (α), secondary granules (β), endoplasmic and plasma membrane-enriched fraction (γ), and cell supernatant (γ). The protein profiles of these four fractions are shown in Fig 4a, and the immunoblot of these protein fractions (Fig 4b) reveal that a protein 65–70 kDa was detected in the secondary granules but not in any of the other purified subcellular fractions.

**DISCUSSION**

The fact that lymphocytes and macrophages use high rates of glutamine has been known for a number of years (7, 8). More recent observations have provided evidence that glutamine is also utilized by rat PMN (28). Blannin et al. (42) observed no uptake of glutamine by human neutrophils stimulated in vitro with lipopolysaccharide. More recently (43), Healy et al. reported glutamine utilization by human neutrophils (apparent $K_m$ 1.29 mM). The ability of glutamine metabolism to enhance the bacterial function of PMN has been investigated in burn patients (5) and postoperative individuals (3). The molecular mechanism of the production of enhanced reactive oxygen intermediates by glutamine is only just being characterized. Recent data suggest that glutamine can increase the expression of some of the proteins that together form the NADPH oxidase components, which, in turn, regulate the production of the superoxide anion (29). In particular, glutamine appears to increase the expression of the cytosolic protein gp91phox as well as the membrane-associated components and NADPH oxidases p22phox and p47phox. Moreover, blocking glutamine metabolism by inhibiting GA in PMN causes a significant decrease in superoxide production. Therefore, the presence and subcellular location of glutamine appears to be important for glutamine-dependent superoxide production by PMN.

In the present study, the results obtained supported the notion that glutamine can enhance oxidative burst by human PMN in the presence of phorbol esters and formyl tripeptide stimulants. These data were achieved using a real time in vitro assay of oxidative burst. The supplementation of whole blood leukocytes with 2 mM glutamine not only led to a greater production of superoxide but also increased the initial rate of activation. GA activity has been reported in rat PMN (28), and numerous
studies have detected GA in human tumor cells (30, 31), where GA is an essential requirement for cell growth.

In vitro data from oxidative burst measurements in culture medium, with or without glutamine used to incubate whole blood samples from marathon runners, suggested a role for glutamine and glutaminase in neutrophil metabolism during inflammation. However, during inflammation, phagosomes only fuse with the extracellular matrix at the cytosolic and membrane components, and the NADPH oxidase pathway translocates to form the complex upon alteration. Therefore, if glutamine plays a role in \( \mathrm{O}_2^- \) production, one might expect to find GA in a similar location. The present results indicate that GA is present on the surface of cells and is released into the medium upon PMA, but not fMLP, stimulation. In the present investigation, more precise information was obtained on the localization of GA by employing confocal microscopy. By gen-
erating 0.5 μM optical sections through individual PMN, it was observed that GA was distributed 0–3.5 μM into the cell surface. PMNs are ruffled cells, and the GA appeared to be predominantly localized in pseudo lamellipodia at or near the plasma membrane.

In an initial study, little or no evidence of the presence of GA in human PMN was found using three different methods, namely freeze-thaw, homogenization, and nebulization. None of these techniques was apparently able to break open the granules. Consequently, to determine in more detail the subcellular source of glutamine in human PMN, other techniques were employed. Nitrogen cavitation and discontinuous Percoll density gradient centrifugation provided a simple and rapid means of separating azurophil granules (α), specific granules (β), and plasma membrane (γ) vesicles, as well as a cytoplasmic fraction. GA was detected in the secondary granules but, surprisingly, not in the plasma membrane fractions. Our data suggest that, in intact PMN, GA is secreted from the secondary granules and binds to the cell surface. Following PMA but not fMLP stimulation, the GA is removed from the membrane fractions and can be found in the extracellular culture medium. It seems that, as granules fuse with the plasma membrane at the sites of developing phagosomes, the NADPH oxidase and GA enzymes would be in close proximity to the plasma membrane in response to stimulation. This would bring them into close contact with their target substrates. Interestingly, a number of groups have recently identified isoforms of glutaminase in human tumor cell lines at the mRNA and protein level (31) and in mammalian brain cells (32). In the present study, treatment of human PMN with PMA, a degranulating agent, resulted in the release of glutaminase from the cell surface into cell culture media. However, three bands were recognized by the polyclonal anti-glutaminase antibody. This is consistent with the results of previous investigators that revealed limited proteolytic digestion of isoforms of GA (33). When intact secondary granules were subsequently examined by Western blotting, a single non-proteolytic glutaminase band of ~65 kDa was observed and compared favorably with that found for rat neutrophil GA, which is ~65 kDa (11).

Somewhat surprisingly, although a marked increase in glutaminase expression in human neutrophils on the cell surface occurred following stimulation with PMA, there was no change in expression after stimulation with fMLP. Both fMLP and PMA are well known proinflammatory agonists that activate protein kinase C (PKC) by different cellular pathways. fMLP activates PKC indirectly via interactions with surface fMLP receptors and G-protein activation and subsequent inositol phosphate hydrolysis. The phorbol ester PMA, on the other hand, passes directly through the cell membrane and mimics diacylglycerol, a natural ligand and activator of protein kinase C. Both of these agonists can activate the release of various enzymes and inflammatory mediators into the extracellular fluid of stimulated cells. In this study, the source of glutaminase appears to be located in the secondary granules of leukocytes and expressed on the cell surface, where it is available for glutamine metabolism. The origin of the PMA-induced change observed might be distal to the stage of PKC activation in the process that results in glutaminase-containing vesicles moving toward and fusing with the plasma membrane. Differences in PKC activity and translocation have been reported previously (34). Alternatively, PMA can activate intracellular enzymes other than PKC, which may explain the release of glutaminase by PMA as compared to fMLP (35). Further studies will be necessary to elucidate the cause of this difference.

There is an increase in the plasma concentration of neutrophil granule contents after strenuous exercise (36). Large numbers of PMN are produced by bone marrow on a daily basis in healthy humans (37). It is possible that this rapid turnover relates to constant immunosurveillance as the PMN pass through the capillary bed. In these circumstances, glutamine utilization might play an important role in maintaining these cells in a constant state of "awareness." PMN also contain large reserves of the endogenous antioxidant glutathione (38), for which glutamine is a precursor.
Glutaminase in Human Neutrophils

The fact that in vivo glutamine feeding has affected cell production of IL-8 in several in vitro studies is clearly important. IL-8 acts as a potent chemotaxtractant for PMNs and induces them to leave the blood stream and migrate into damaged or infected tissue. IL-8 also activates NADPH oxidase and nitric oxide synthase, which, in turn, causes the release of ROS from PMN granules.

Results from recent studies suggest that glutamine has a modulating effect on IL-8 cytokine production in various cell types during activation by inflammatory mediators (4, 21, 22, 39). The signaling of this neutrophil chemotaxtractant might be diminished because of the effect of additional glutamine in the blood upon circulating mature or immature PMN. Increased numbers of the latter are recruited during the leukocytosis that occurs in response to strenuous exercise. However, glutamine supplementation has been shown to help reduce the number of circulating PMNs to near normal levels compared with a placebo 16 h after a race (20). The mechanism of glutamine-mediated regulation of IL-8 production by peripheral blood mononucleocytes is not yet known. It could reflect changes in cell receptor signaling, transcription, and/or translation expression by modulatory anti-inflammatory cytokines such as IL-10 (39).

In conclusion, the present study suggests that glutamine supplementation might enhance the respiratory burst of human PMN to specific inflammatory stimuli in vitro. Moreover, the novel finding has been made that there is a sub-cellular source of GA, the major enzyme responsible for glutamine metabolism, in the secretory granules of human PMN. This is likely to contribute to the modulation of immune function of PMN in clinical and exercise-induced stress.

Acknowledgments—We are grateful to Dr Jan Knight of Knight Scientific Limited, Plymouth, United Kingdom for advice and help with the microplate Pholasin® assay and to LabTech for the loan of the Lucy Anthos microplate luminometer.

REFERENCES
1. Cassatella, M. A. (1995) Immunol. Today 16, 21–26
2. Eggleton, P. (1999) Anticancer Res. 19, 3711–3715
3. Furukawa, S., Saito, H., Inoue, T., Matsuda, T., Fukatsu, K., Han, I., Ikeda, S., and Hidemura, A. (2000) Biochem. Biophys. Res. Commun. 263, 323–329
4. Castell, L. (2003) Sports Med. 33, 223–235
5. Ogle, C. K., Ogle, J. D., Mao, J. X., Simon, J., Noel, J. G., Li, B. G., and Alexander, J. W. (1994) J. Parenter. Enteral Nutr. 18, 128–133
6. Furukawa, S., Saito, H., Fukatsu, K., Hashiguchi, Y., Inaba, T., Lin, M. T., Inoue, T., Han, I., Matsuda, T., and Muto, T. (1997) Nutrition 13, 863–869
7. Ardawi, M. S., and Newsholme, E. A. (1985) Essays Biochem. 21, 1–44
8. Newsholme, P., and Newsholme, E. A. (1989) Biochem. J. 261, 211–218
9. Powell, H., Castell, L. M., Parry-Bilings, M., Desborough, J. P., Hall, G. M., and Newsholme, E. A. (1994) Clin. Physiol. 14, 569–580
10. Castell, L. M., Poortmans, J. R., and Newsholme, E. A. (1996) Eur. J. Appl. Physiol. Occup. Physiol. 73, 488–490
11. Curi, T. C., De Melo, M. P., De Azevedo, R. B., Zorn, T. M., and Curi, R. (1997) Am. J. Physiol. 273, C1124–C1129
12. McCarthy, D. A., and Dale, M. M. (1988) Sports Med. 6, 333–363
13. Woods, J. A., Davis, J. M., Smith, J. A., and Nieman, D. C. (1999) Med. Sci. Sports Exerc. 31, 57–66
14. Larrabee, R. C. (1992) J. Med. Res. 2, 76–82
15. Hanssen, J. B., Wilsgard, L., and Osterud, B. (1991) Eur. J. Appl. Physiol. Occup. Physiol. 62, 157–161
16. Suzuki, K., Yamada, M., Kurakake, S., Okamura, N., Yamaya, K., Liu, Q., Kudoh, S., Kowatari, K., Nakaji, S., and Sugawara, K. (2000) Eur. J. Appl. Physiol. 81, 281–287
17. Suzuki, K., Sato, H., Kikuchi, T., Abe, T., Nakaji, S., Sugawara, K., Totsuka, M., Sato, K., and Yamaya, K. (1996) J. Appl. Physiol. 81, 1213–1222
18. Nieman, D. C., Henson, D. A., Smith, L. L., Utter, A. C., Vezin, D. M., Davis, J. M., Kaminsky, D. E., and Stute, M. (2001) J. Appl. Physiol. 91, 109–114
19. Castell, L. M. (1996) The Role of Some Amino Acids in Exercise, Fatigue, and Immunosuppression. M.Sc. Thesis, University of Oxford, Oxford
20. Castell, L. M., and Newsholme, E. A. (1998) Clin. J. Physiol. Pharmacol. 76, 524–532
21. de Beaure, M. A., O’Riordan, M. G., Ross, J. A., Jodozi, L., Carter, D. C., and Fearon, K. C. (1996) Nutrition 12, 828–834
22. Eggleton, P., Wang, L., Penhallow, J., Crawford, N., and Brown, K. A. (1995) Ann. Rheum. Dis. 54, 916–923
23. Kjeldsen, L., Sengelov, H., and Borregaard, N. (1999) J. Immunol. Methods 232, 131–141
24. Wurthmeuller, U., Dewald, B., Thelen, M., Schafer, M. K., Stover, C., Whaley, K., North, J., Eggleton, P., Reid, K. B., and Schweaee, W. J. (1997) J. Immunol. 158, 4444–4451
25. Gomez-Fahre, P. M., Alejo, J. C., Del Castillo-Olivares, A., Alonso, F. J., Munoz De Castro, I., Campos, J. A., and Marquez, J. (2000) Biochem. J. 345, 365–375
26. Segura, J. A., Alejo, J. C., Gomez-Biedma, S., Munoz de Castro, I., and Marquez, J. (1995) Protein Expression Purif. 6, 343–351
27. Piton-Curi, T. C., Tresena, A. G., Tavares-Lima, W., and Curi, R. (2002) Cell Biochem. Funct. 20, 81–86
28. Piton-Curi, T. C., Leva da, A. C., Lopes, L. R., Dari, S. Q., and Curi, R. (2002) Clin. Sci. (Lond.) 103, 403–408
29. Knex, W. E., Tremblay, G. C., Spanier, B. B., and Friedell, G. H. (1967) Cancer Res. 27, 1456–1458
30. Turner, A. R., and McGivan, J. D. (2003) Biochem. J. 370, 403–408
31. Olalla, L., Gutierrez, A., Campos, J. A., Khan, Z. U., Alonso, F. J., Segura, J. A., Marquez, J., and Alejo, J. C. (2002) J. Biol. Chem. 277, 38939–38944
32. Hunt, J. F., Erwin, E., Palmer, L., Vaugham, J., Malhotra, N., Platt-Mills, T. A., and Gaston, B. (2002) Am. J. Respir. Crit. Care Med. 165, 101–107
33. Nishizuka, Y. (1984) Nature 308, 693–698
34. Searl, T. J., and Silinsky, K. M. (1998) J. Pharmacol. Exp. Ther. 285, 247–251
35. Dufaux, B., and Order, U. (1989) Int. J. Sports Med. 10, 434–438
36. Cannistra, S. A., and Griffin, J. D. (1988) Semin. Hematol. 25, 173–188
37. Voetman, A. A., Lees, J. A., and Ross, D. S. (1980) Blood 55, 741–747
38. Cofein, M., Marion, R., Ducrotte, P., and Dechelette, P. (2003) Clin. Nutr. 22, 407–413
39. Huang, Y., Li, N., Li, H., and Neu, J. (2003) Cytokine 22, 77–83
40. Lin, M. T., Saito, H., Furukawa, S., Fukusima, R., Kanikou, L. F., Lee, H. P., Chang, J. K., and Chen, W. J. (2001) J. Parenter. Enteral Nutr. 25, 346–351
41. Blazin, J. K., Walsh, N. P., Clark, A. M., and Gleeson, M. (1998) J. Physiol. (Lond.) 506, 121P
42. Healy, D. A., Watson, R. W. G., and Newsholme, P. (2002) Clin. Sci. 103, 179–189