Arginine Transport via Cationic Amino Acid Transporter 2 Plays a Critical Regulatory Role in Classical or Alternative Activation of Macrophages

Andrée Yeramian, Lorena Martin, Neus Serrat, Luis Arpa, Concepció Soler, Joan Bertran, Carol McLeod, Manuel Palacín, Manuel Modolell, Jorge Lloberas and Antonio Celada

*J Immunol* 2006; 176:5918-5924; [http://www.jimmunol.org/content/176/10/5918](http://www.jimmunol.org/content/176/10/5918)

**References**

This article cites 49 articles, 21 of which you can access for free at: [http://www.jimmunol.org/content/176/10/5918.full#ref-list-1](http://www.jimmunol.org/content/176/10/5918.full#ref-list-1)

**Subscriptions**

Information about subscribing to *The Journal of Immunology* is online at: [http://jimmunol.org/subscriptions](http://jimmunol.org/subscriptions)

**Permissions**

Submit copyright permission requests at: [http://www.aai.org/ji/copyright.html](http://www.aai.org/ji/copyright.html)

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: [http://jimmunol.org/cgi/alerts/etoc](http://jimmunol.org/cgi/alerts/etoc)
Arginine Transport via Cationic Amino Acid Transporter 2 Plays a Critical Regulatory Role in Classical or Alternative Activation of Macrophages

Andrée Yeramian,* Lorena Martín, † Neus Serrat, * Luis Arpa,* Concepción Soler,* Joan Bertran, ‡ Carol McLeod, § Manuel Palacín, † Manuel Modolell, § Jorge Lloberas,* and Antonio Celada2*

Arginine is processed by macrophages in response to the cytokines to which these cells are exposed. Th1-type cytokines induce NO synthase 2, which metabolizes arginine into nitrites, while the Th2-type cytokines produce arginase, which converts arginine into polyamines and proline. Activation of bone marrow-derived macrophages by these two types of cytokines increases l-arginine transport only through the y+ system. Analysis of the expression of the genes involved in this system showed that Slc7A1, encoding cationic amino acid transporters (CAT), is constitutively expressed and is not modified by activating agents, while Slc7A2, encoding CAT2, is induced during both classical and alternative activation. Macrophages from Slc7A2 knockout mice showed a decrease in l-arginine transport in response to the two kinds of cytokines. However, while NO synthase 2 and arginase expression were unmodified in these cells, the catabolism of arginine was impaired by both pathways, producing smaller amounts of nitrites and also of polyamines and proline. In addition, the induction of Slc7A2 expression was independent of the arginine available and of the enzymes that metabolize it. In conclusion, the increased arginine transport mediated by activators is strongly regulated by CAT2 expression, which could limit the function of macrophages.

The Journal of Immunology, 2006, 176: 5918–5924.

To perform their function, macrophages must be activated either by Th1-type cytokines, such as IFN-γ, which is called classical activation or M1, or by Th2-type cytokines, such as IL-4, IL-10, IL-13, etc., referred to as alternative activation or M2 (1). Activation induces biochemical and morphological modifications in the macrophages that allow them to perform their functional activity (2). Activation blocks the proliferation of these cells (3). The two types of activation are characterized by the way in which arginine is processed. Th1-type cytokines induce NO synthase (NOS)2,3 causing the production of NO, while Th2-type cytokines produce arginase, which metabolizes arginine in polyamines and triggers the urea pathway (4, 5).

Cell activation requires arginine for the synthesis of proteins, for the production of NO via classical activation, and for the production of polyamines and proline through alternative activation (1, 5). The extracellular milieu is the main source of arginine. The functional significance of arginine transporters was demonstrated in macrophages, in which NOS2-mediated NO synthesis largely depends on the extracellular supply of l-arginine (6–8). Arginine crosses the plasma membrane through several transport systems. Depending on the cell type, a number of transport activities may be induced (9). The SLC7 family of transporters is divided into two subgroups, the cationic amino acid (the cationic amino acid transporter (CAT) family, SLC7A1–4) and the glycoprotein-associated amino acid transporters (the gaAT family, SLC7A5–11), also called L chains or catalytic chains of the hetero(d)meric amino acid transporters. The CAT family includes four members, CAT-1 to CAT-4, whose corresponding genes are SLC7A1 to SLC7A4. The first three members transport cationic l-amino acids, while the function of CAT-4 is not known. The hetero(d)meric amino acid transporter family comprises seven proteins, whose genes are SLC7A5 to SLC7A11; however, only y+LAT2 (SLC7A6), y+LAT1 (SLC7A7), and b0++ AT (SLC7A9) transport cationic amino acids (9). In addition, the B0++ AT transporter (SLC6A14) belonging to the SLC6 family, also transports arginine, but in a sodium- and chloride-dependent fashion (10).

Macrophages require arginine to elaborate gene products when they become activated, e.g., IFN-γ induces the expression of >300 genes (11). Consequently, to meet their metabolic demands, macrophages require the uptake of exogenous arginine; this process may therefore be a key regulatory step for physiological responses in these cells. In this study, we investigated how classical and alternative activation affects arginine transport activity. To this end, we used bone marrow-derived macrophages, which are non-transformed cells that respond to both Th1- and Th2-type activating stimuli. We showed that macrophage activation leads to an increase in arginine transport. For both types of activation, the only gene to show increased expression was Slc7A2, the product of

1 Macrophage Biology Group, Institute of Biomedical Research, Barcelona Science Park, and Department of Biochemistry and Molecular Biology, University of Barcelona and Institute of Biomedical Research, Barcelona Science Park, Barcelona, Spain; 2 Cancer Center and Department of Medicine, University of California, San Diego, CA 92093; and 3 Max Planck Institute for Immunobiology, Freiburg, Germany

Received for publication September 7, 2005. Accepted for publication February 27, 2006.

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.

1 This work was supported by grants from the Ministerio de Ciencia y Tecnología BFU2004-05725/BMC (to A.C.) and SAF03-08940-C02-01 (to M.P.), and by the European Commission Grant EUINDAT LSHM-CT-2003-502852 (to M.P.), and by the European Commission Grant EUGINDAT LSHM-CT-2003-502852 (to M.P.). C.S. and J.B. are researchers from the Programa Ramón y Cajal of the Spanish Ministry of Science and Technology. L.M. and L.A. are recipients of fellowships from the Comisión Interdepartamental de Recerca i Innovació Tecnològica (Generalitat de Catalunya). A.Y. is recipient of a fellowship from the Ministerio de Asuntos Exteriores, Madrid. 2 Address correspondence and reprint requests to Dr. Antonio Celada, Macrophage Biology Group, Institute of Biomedical Research, Barcelona Science Park, Josep Samitier 1-5, E-08028 Barcelona, Spain. E-mail address: acelada@ub.edu

3 Abbreviations used in this paper: NOS, NO synthase; CAT, cationic amino acid transporter; KO, knockout; nor-NOHA, N-hydroxy-nor-l-arginine.
which is a limiting factor that regulates the catabolism of arginine and the production of NO and polyamines.

Materials and Methods
Reagents
LPS and recombinant cytokines were purchased from Sigma-Aldrich. N'-hydroxy-nor-arginine (nor-NOHA) was purchased from Bachem. In several experiments, the results obtained with commercial LPS were compared with highly purified LPS from Salmonella abortus equi, donated by C. Galanos (Max Planck Institute, Freiburg, Germany) (12), and no differences were found. All other chemicals were of the highest purity grade available and were purchased from Sigma-Aldrich. Deionized water that had been further purified with a Millipore Milli-Q system was used.

Cell culture
Bone marrow-derived macrophages as well as peritoneal macrophages were isolated from 6-wk-old BALB/c mice (Charles River Laboratories), as previously described (13). Bone marrow-derived macrophages were cultured in plastic tissue culture dishes (150 mm) in 40 ml of DMEM containing 20% FBS (Sigma-Aldrich) and 30% L cell-conditioned medium as a source of M-CSF. Penicillin and streptomycin were added. Cells were incubated at 37°C in a humidified 5% CO2 atmosphere. After 7 days of culture, macrophages were obtained as a homogenous population of adherent cells (>99% Mac-1+). To render cells quiescent, at 80% confluency, cells were deprived of L cell-conditioned medium for 16–18 h before treatment. Macrophages from knockout (KO) mice and the corresponding wild-type controls were isolated under the same conditions. The KO for Slc7A2 has been reported (14), while the NO2 KO mice were purchased from The Jackson Laboratory. Peritoneal exudate cells were harvested by lavage from mice that had been injected i.p. with 3 ml of 10% protease peptide (Difco Laboratories) 3 days earlier. Macrophage monolayers were prepared by seeding the exudate cell suspension into flat-bottom tissue culture plates. The cells were adhered for 2 h at 37°C, and the plates were washed vigorously to remove nonadherent cells. Animal experiments were performed in accordance with institutional and government guidelines (University of Barcelona).

Quantitative RT-PCR analysis
Cells were washed twice with cold PBS, and total RNA was extracted with the acidic guanidinium thiocyanate-phenol-chloroform method, as described (15). RNA was treated with DNase (Ambion) to eliminate DNA contamination. For cDNA synthesis, 1 μg of RNA and TaqMan reverse-transcriptase reagents (including Multiscribe reverse transcriptase and random hexamers) were used, following the manufacturer’s instructions (Applied Biosystems). The primers used to amplify mouse were: for Nos2, GCCACCAAAATGGCACAA and CGTACCGGATGAGCTGTGA ATT; for arginase 1, AACACGCAGTGGTTTAAAC and GGTIT TCACTGTGGCGCATC; for Slc7A1, CTTGGGACGTGCAAATGACG and TGATCGGACGTGAAATGCG; for Slc7A2, GTGAAGAAGTT CGAGATCCCA and CGTTAAACGCTGCA; and for Slc7A4, GGCCTCCCTGTGCACCTTCA and TAGCAAGGACACGGAAC GGA. Real-time monitoring of PCR amplification of cDNAs was done using the TaqMan Universal master mix (Applied Biosystems) in an ABI Prism 7700 sequence Detection System (Applied Biosystems). Relative quantification of gene expression was performed using β-actin, as described in the ‘TaqMan users’ manual (GCCACACCTTTCAAT GACGCTG and CTGGTCGAAAGTCTAGGACACA). The threshold cycle was defined as the cycle number at which the fluorescence corresponding to the amplified PCR product is detected. The PCR arbitrary units of each gene were defined as the mRNA levels normalized to the β actin expression level in each sample. The RT-PCR analysis was controlled by sequencing the amplification products. In addition, we included a sample without RNA in each reaction.

Transport measurements
Cells were plated in 6-well plates 1–2 days before the transport assay (106 cells/well) and treated as indicated in the figures. To measure t-arginine uptake, cells were washed three times in preheated (37°C) uptake solution (10 mM HEPES, 5.4 mM KCl, 1.2 mM MgSO4, 7 mM H2O2, 2.8 mM CaCl2, 1 mM KH2PO4, and 137 mM NaCl (pH 7.4)). They were then incubated with 0.5 ml of uptake solution containing 50 μM l-arginine (5 μCi/ml) in the presence or absence of l-leucine (5 mM) or t-arginine (5 mM) for 1 min. Uptake was stopped by removing the uptake solution and washing cells with 2 ml of ice-cold stop solution (10 mM HEPES, 10 mM Tris, and 137 mM NaCl (pH 7.4) with 10 mM nonradioactive t-arginine) three times. After the third wash, cells were lysed in 200 μl of 0.1% SDS and 100 mM NaOH, and 100 μl was used to measure the radioactivity associated with the cells. Values obtained in the presence of 5 mM l-arginine as competitor were always below 10% of the total transport and were subtracted to estimate 1 activity.

Catabolism of l-arginine in macrophages
Macrophages were incubated with a number of cytokines in a microplate (105 cells/well). After 24 h, cells were washed and incubated for 2 or 6 h at 37°C in 0.1 ml of arginine-free DMEM containing 2% FCS and 0.1 μCi of l-(U-14C) arginine (Amersham Biosciences). Cells were subsequently lysed by two freeze-thaw cycles. The remaining arginine and synthesis of metabolic products were evaluated by TLC. To identify the spots, 10 μl of a solution containing 25 mg/ml arginine, ornithine, and spermidine was added to the cell lysates. A total of 20 μl of the samples was spotted onto TLC plates (Cromatoplates TLC 20 x 20 cm; Silica Gel 60 F254; Merck) and dried for 1 h at 42°C. Plates were developed in the solvent system chloroform/methanol/ammonium hydroxide/water 0.5/4.5/2/0.1 (v/v) and dried. Spots were developed with ninhydrin (Spray Solution; Merck) by heating at 120°C for 5 min and scanned into scintillation tubes containing 6 ml of EcoscintA (National Diagnostics). Radioactivity was determined by scintillation counting (Beckman Instruments), and the values for each compound were expressed as percentage of the total radioactivity measured in triplicate cultures ± SD.

Nitrite production and arginase activity
NO was measured as nitrite using the Griess reagent (16). Culture supernatant was mixed with 100 μl of 1% sulfanilamide, 0.1% N-(1-naphthyl)-ethyl-enediamine dihydrochloride, and 2.5% H3PO4. Absorbance was measured at 540 nm in a microplate reader (Molecular Devices). Arginase activity was measured in cell lysates, as previously described (16), but with slight modifications. Briefly, cells were lysed with 100 μl of 0.1% Triton X-100. After 30 min on a shaker, 100 μl of 25 mM Tris-HCl was added. We then added 10 μl of 10 mM MnCl2 to 100 μl of this lysate, and the enzyme was activated by heating for 10 min at 56°C. Arginine hydrolysis was conducted by incubating the lysate with 100 μl of 0.5 M L-arginine (pH 9.7) at 37°C for 15–120 min. The reaction was stopped by adding 0.5 ml of 1% sulfanilamide, 0.1% 25 mM Tris-HCl, and 0.25% N-(1-naphthyl)-ethylenediamine dihydrochloride, and 2.5% H3PO4. Absorbance was measured at 540 nm after addition of 40 ml of α-isonitrosopropiophenone (dissolved in 100% ethanol), followed by heating at 95°C for 30 min. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the formation of 1 μmol of urea per minute.

Statistical analysis
To calculate the statistical differences between the control and treated samples, we used the Student’s paired t test. Values of p < 0.05 or lower were considered significant.

Results
M1 and M2 activation catabolizes arginine differently
In our experiments, we used bone marrow-derived macrophages, a homogeneous population of primary and quiescent cells. Treatment of these cells with several cytokines causes a series of modifications that allow them to develop their functional activities (3). To study M1 activation, we used IFN-γ or LPS, while IL-4 and IL-10 were used to examine M2 activation. Although these two types of activation regulate distinct sets of genes (17), both require arginine, either for NO production in classical activation or as a substrate for arginase induced during alternative activation. Only molecules from M1 activation induced nitrite production, while only cytokines involved in M2 activation stimulated arginase activity (Fig. 1, A and B). IFN-γ and LPS, but not IL-4 or IL-10, led to NO2 expression. In contrast, Th2-type but not Th1-type cytokines induced arginase (Fig. 1, A and B). To determine the metabolism of arginine, we incubated activated macrophages with radiolabeled substrate and then separated the distinct products by TLC. When macrophages were incubated with Th2-type cytokines, an increased amount of ornithine and spermidine was produced as a result of the catabolic activity of arginase (Fig. 1C). However, when these cells were treated with IFN-γ or LPS, the
amount of citrulline increased, indicating the processing of arginine by NOS2. Therefore, the reagents used in this study determined macrophage activation via the M1 or M2 pathway. No production of NOS2 was observed when cells were incubated with IL-4 or IL-4 + IL-10 (Fig. 1A). However, an increased amount of citrulline was produced (Fig. 1C). This was probably mediated through the urea cycle, which is active in macrophages (18). Through the action of carbamoylphosphate and ornithinecarbamyltransferase, ornithine may be converted into citrulline.

**M1 and M2 activation increases arginine transport through the y<sup>+</sup> system, thereby inducing Slc7A2 expression**

Because arginine is required for macrophage metabolism, in this study we examined whether M1 and M2 activation modulates the transport of this amino acid. In a dose-dependent manner, both LPS and IL-4 produced an increase in arginine transport (Fig. 1B). In a dose-dependent manner, both LPS and IFN-γ produced an increase in arginine transport (Fig. 1D). In a dose-dependent manner, both LPS and IFN-γ + LPS-treated samples and IL-4 + IL-4 + IL-10-treated samples. C. Macrophages were incubated for 2 h or 6 h with radiolabeled arginine. The products were then separated by TLC. The results are indicated as percentage of the arginine added at the beginning of the assay. The values shown correspond to the mean ± SD of three independent experiments. There is a significant difference for ornithine and spermine production between macrophage controls and those treated with IL-4 and IL-4 + IL-10 (p < 0.01). Also, a significant difference was found for citrulline between the controls and cells treated with IFN-γ or IFN-γ + LPS (p < 0.01). D. Macrophages were incubated for 24 h with the activators indicated, and arginine uptake was then measured. There is a significant difference between the control values and the values after LPS treatment with 50 and 100 ng/ml or after 5 and 10 U/ml LPS (p < 0.01). Results are representative of three independent experiments. E. Macrophages were incubated for 24 h in the presence of IFN-γ (500 U/ml) and LPS (100 ng/ml) or IL-4 (10 U/ml) and IL-10 (10 U/ml). Then, 50 μM l-arginine was added and uptake was measured in the absence (□) or in the presence of 5 mM l-leucine (■) under linear conditions (1-min incubation). There is a significant difference in arginine transport before and after treatment with LPS, IFN-γ + LPS, or IL-4 + IL-10 (p < 0.01). Results are representative of three independent experiments.
IFN-γ and also by IL-4 and IL-4 + IL-10. Slc7A3 was not detected. In addition, the expression of the genes of the y⁺ system was measured in arginine-free medium; however, no differences in induction by the distinct cytokines were observed in relation to the controls (Fig. 2).

Our results to date indicate that, in basal conditions, CAT1 mediates the transport of arginine through the y⁺ system, while CAT2 is the main L-arginine transporter for activated macrophages. To confirm these data, we used macrophages from mice with disrupted Slc7A2 (14). In basal conditions, without stimulation, the amount of arginine transported was similar in macrophages from Slc7A2 KO mice and controls (Fig. 3A). However, while classical or alternative activators produced an increase in arginine transport in controls, no increase in cells from Slc7A2 KO mice was observed (Fig. 3A). When analyzed in more detail, the increased transport activity corresponded to the y⁺ system (data not shown). Slc7A1 expression was not modified in Slc7A2 KO macrophages or in controls when they were treated with activators, and Slc7A3 was not expressed in this model (data not shown). These results confirm that macrophage activation induces Slc7A2 expression, which is responsible for increased arginine transport.

In the absence of Slc7A2, macrophage activation is limited

We next addressed the functional consequences of Slc7A2 disruption on macrophages. M1 activation produced NO2, and M2 activation induced arginase 1 in Slc7A2 KO and control cells at similar levels (Fig. 3B). Comparable results were found when we measured the expression of protein by Western blot and when we used peritoneal macrophages (data not shown). When macrophages were incubated with activating agents, Slc7A2 KO macrophages showed a reduced catabolism of arginine (Fig. 4A). Also, the amounts of ornithine, spermine, and citrulline produced by these cells were decreased in relation to controls (Fig. 4B).

On the basis of the decreased catabolism of arginine in macrophages from Slc7A2 KO mice, we next studied the functional activity of these cells. Although the amounts of NOS2 were similar to those of controls, a significant decrease in NO production was detected in both bone marrow-derived and peritoneal macrophages (Fig. 4B). As the measurement of NO production is made with the entire cell, we conclude that the low amount of NO produced is due to the lower amount of intracellular arginine available. The enzymatic assay for arginase activity requires the rupture of the cell and the addition of external substrate. Under these conditions, arginase activity in Slc7A2 KO macrophages was similar to the controls. However, because the metabolic conversion of arginine into ornithine and polyamines is impaired in the entire cell, these data show that the amount of arginine available is the limiting factor for arginase activity. Therefore, CAT2 limits the activation of macrophages by Th1- and Th2-type cytokines.

Arginine transport is not regulated by the levels of NOS2 or arginase

We next studied whether the induction of the enzymes that catabolize arginine produces an increase in the transport of this amino acid. To explore the role of arginase 1, we used the specific inhibitor nor-NOHA, which blocks arginase activity by inhibiting the degradation of arginine into ornithine, but does not block the production of NO after IFN-γ treatment. Treatment with nor-NOHA did not affect the transport of arginine induced by the IL-4 + IL-10 or LPS + IFN-γ treatments (Fig. 5A). As expected, the induction of Slc7A2 expression in macrophages via classical and
alternative activation was not modified by incubation with nor-NOHA (Fig. 5B).

To determine the role of NOS2, we used macrophages from NOS2 KO mice. As in the case of arginase, the elimination of NOS2 did not modify the increase in arginine transport induced by M1 and M2 activators (Fig. 5C). No significant difference in Slc7A2 expression was observed when compared NOS2 KO macrophages with the controls (Fig. 5D).

Discussion

Arginine is one of the metabolic and signaling functions required for protein synthesis by macrophages. In addition, it is the key amino acid involved in both M1 and M2 activation (1, 5). Macrophages require exogenous arginine to meet their metabolic demands. Therefore, the transport of this amino acid across the plasma membrane is an essential regulatory first step during activation of the macrophage, which synthesizes a large number of proteins (11). In addition, these cells catabolize arginine to NO by NOS2 induced by Th1-type cytokines or to ornithine, proline, and polyamines by arginase induced by the Th2-type cytokines (1, 5). Moreover, these cells constitute a unique culture model, which, unlike peritoneal or alveolar macrophages, is not previously activated and which responds to Th1- and Th2-type cytokines (2). Because the two types of macrophage activation require extracellular arginine, we studied the transport systems and their regulation in this model. Under basal conditions, y′-mediated L-arginine transport is due only to CAT1, and after activation, the increase in transport is due to CAT2. Interestingly, although CAT1 is functional, the absence of CAT2 caused a decrease in NO production and also in the production of polyamines and proline. This observation indicates that arginine transported by CAT1 does not fulfill the requirements of the machinery responsible for macrophage activation.

Our results disagree partially with those described by Nicholson et al. (14), who reported the absence of NO production in Slc7A2−/− macrophages after activation. In these macrophages, the arginine content was unchanged in relation to controls. This finding indicates that two defined pools of L-arginine may be present in these cells, only one of which is accessible for NOS2, while the other covers the CAT1-dependent metabolic requirements of the cell. Nicholson et al. (14) proposed that CAT2 and NOS2 coassociate. However, no physical interaction was demonstrated. The disagreement between these and our results may be attributable to technical differences in experimental design. In fact, in our case, NO production was determined after incubating macrophages for 19 h in the presence of cytokines, while in the study performed by Nicholson et al. (14), macrophages were incubated with cytokines and then washed and incubated in fresh cytokine-free medium for an additional 24 h, after which NO production was measured. Therefore, under these conditions, in the absence of cytokines, the levels of NOS2 decline (34) and NO production also decreases. However, we cannot exclude other explanations for the discrepancy, such as differences in culture conditions or the measurement method used. However, the background of KO animals

FIGURE 5. Arginine uptake and Slc7A2 induction are independent of arginase or NOS2 activity. A, Macrophages were incubated for 2 h with or without nor-NOHA (50 μM), and the activators indicated were then added and macrophages were incubated for 24 h. Arginine uptake was then measured. B, Slc7A1, Slc7A2, and Slc7A3 expression was determined by real-time PCR under the same conditions as A. For arginine transport and gene expression, no significant differences were found between cells treated or not with nor-NOHA. C, Macrophages from NOS2 KO mice and wild-type controls were incubated for 24 h with the activators indicated. Arginine uptake was then measured. D, Slc7A1, Slc7A2, and Slc7A3 expression was determined by real-time PCR under the same conditions as C. For arginine transport and gene expression, no significant differences were found between cells from NOS2 KO mice and the controls. Results are representative of three independent experiments.
(C57BL/6 or BALB/c) does not explain these discrepancies because similar results were found when we used the two strains (data not shown). It should be noted that for these authors, the production of NO in fibroblasts and astrocytes is not completely dependent on CAT2 (35, 36).

Recently, we reported that the transport system of nucleosides in macrophages differs when cells proliferate or when they become activated by either IFN-γ or LPS (37, 38). This finding indicates distinct pools for different activities. When we studied the effect of M-CSF on arginine transport, no increase was observed in relation to the controls and no expression of Slc7A2 was detected (39). This observation shows that the arginine transport systems used in macrophages differ when these cells are activated or when they proliferate. Therefore, the regulation of Slc7A2 is a limiting factor for the activation of macrophages by restricting the supply of the substrate to the appropriate machinery that triggers M1 or M2 activation.

Regarding the control of arginine transport by NOS2, there are contradictory data in the literature. Although some studies report that the LPS-induced NOS2 expression regulates arginine transport (27, 40), in other cases, the regulation of this transport is independent of NOS2 activity (30). These differences may be related to the distinct populations of macrophages used in the studies, as well as to the methods applied to inhibit NOS2 activity. Using macrophages from NOS2 KO animals, we found that M1 and M2 activators induce Slc7A2 expression, which is responsible for the increase in L-arginine transport. Furthermore, no modifications of Slc7A2 expression or transport induced by activators were detected when we blocked arginase activity, thus demonstrating that the activators’ control over Slc7A2 is independent of the induction of either NOS2 or arginase activity. However, several signal transduction pathways may be common for the regulation of NOS2 and Slc7A2 genes, such as NF-kB (41).

The catabolism of arginine by macrophages through NOS2 or arginase generates several crucial products for immune regulation. For example, NOS2 produces NO, while arginase gives proline and polyamines, thereby inducing the synthesis of fibrinogen, which plays a role in inflammation (42). However, in some cases, the degradation products of arginine have a beneficial effect on intracellular microbes. For example, polyamines are required for the intracellular growth of Leishmania (24). Also, Mycobacterium enhances arginase transport in infected macrophages and acquires the metabolites necessary for bacterial growth (43). Furthermore, polyamines modulate the functional activities of macrophages (44). For example, spermine inhibits proinflammatory cytokine synthesis (45–48) and has been reported to down-regulate arginase transport and NOS2 expression in rat alveolar macrophages (49).

Several of the genes involved in the control of growth or amino acid metabolism are regulated by amino acid availability (50). In acid metabolism are regulated by amino acid availability (50). In acid metabolism are regulated by amino acid availability (50). (44). For example, spermine inhibits proinflammatory cytokine synthesis (45–48) and has been reported to down-regulate arginase transport and NOS2 expression in rat alveolar macrophages (49).

Several of the genes involved in the control of growth or amino acid metabolism are regulated by amino acid availability (50). In acid metabolism are regulated by amino acid availability (50). (44). For example, spermine inhibits proinflammatory cytokine synthesis (45–48) and has been reported to down-regulate arginase transport and NOS2 expression in rat alveolar macrophages (49).

According to the appropriate machinery that triggers M1 or M2 activation. According to the appropriate machinery that triggers M1 or M2 activation. According to the appropriate machinery that triggers M1 or M2 activation. According to the appropriate machinery that triggers M1 or M2 activation. According to the appropriate machinery that triggers M1 or M2 activation. According to the appropriate machinery that triggers M1 or M2 activation. According to the appropriate machinery that triggers M1 or M2 activation. According to the appropriate machinery that triggers M1 or M2 activation.}

Acknowledgments

We thank Tanya Yates for editing the manuscript.
26. Kakuda, D. K., M. J. Sweet, C. L. MacLeod, D. A. Hume, and D. Markovich. 1999. CAT2-mediated L-arginine transport and nitric oxide production in activated macrophages. *Biochem. J.* 340: 549–553.

27. Venketaraman, V., M. T. Talaeu, Y. K. Dayaram, M. A. Peteroy-Kelly, W. Bu, and N. D. Connell. 2003. Nitric oxide regulation of L-arginine uptake in murine and human macrophages. *Tuberculosis* 83: 311–318.

28. Messeri Dreissig, M. D., R. Hammermann, J. Mossner, M. Gothert, and K. Racke. 2000. In rat alveolar macrophages lipopolysaccharides exert divergent effects on the transport of the cationic amino acids L-arginine and L-ornithine. *Naunyn-Schmiedeberg’s Arch. Pharmacol.* 361: 621–628.

29. Bogle, R. G., A. R. Baydoun, J. D. Pearson, S. Moncada, and G. E. Mann. 1992. L-Arginine transport is increased in macrophages generating nitric oxide. *Biochem. J.* 284: 15–18.

30. Baydoun, A. R., R. G. Bogle, J. D. Pearson, and G. E. Mann. 1993. Selective inhibition by dexamethasone of induction of NO synthase, but not of induction of L-arginine transport, in activated murine macrophage J774 cells. *Br. J. Pharmacol.* 110: 1401–1406.

31. Peteroy-Kelly, M. A., V. Venketaraman, and N. D. Connell. 2001. Effects of *Mycobacterium bovis* BCG infection on regulation of L-arginine uptake and synthesis of reactive nitrogen intermediates in J774.1 murine macrophages. *Infect. Immun.* 69: 5823–5831.

32. Caivano, M. 1998. Role of MAP kinase cascades in inducing argininate transporters and nitric oxide synthetase in RAW264 macrophages. *FEBS Lett.* 429: 249–253.

33. Rotoli, B. M., O. Bussolati, R. Sala, A. Barilli, E. Talarico, G. C. Gazzola, and V. Dall’Asta. 2004. IBN-γ stimulates arginine transport through system y⁺ L in human monocytes. *FEBS Lett.* 571: 177–181.

34. Xaus, J., M. Comalada, A. F. Valledor, J. Lloberas, F. Lopez-Soriano, J. M. Arguilles, C. Bogdan, and A. Celada. 2000. LPS induces apoptosis in macrophages mostly through the autocrine production of TNF-α. *Blood* 95: 3823–3831.

35. Nicholson, B., C. K. Manner, and C. L. MacLeod. 2002. Cat2L-arginine transporter-deficient fibroblasts can sustain nitric oxide production. *Nitric Oxide* 7: 236–243.

36. Manner, C. K., B. Nicholson, and C. L. MacLeod. 2003. CAT2 arginine transporter deficiency significantly reduces iNOS-mediated NO production in astrocytes. *J. Neurochem.* 85: 476–482.

37. Garcia-Manteiga, R. Valdes, J. Xaus, M. Comalada, F. J. Casado, M. Pastor-Anglada, A. Celada, and A. Felipe. 2001. Macrophages require different nucleoside transport systems for proliferation and activation. *FASEB J.* 15: 1979–1988.

38. Soler, C., R. Valdes, J. Garcia-Manteiga, J. Xaus, M. Comalada, F. J. Casado, M. Modolell, B. Nicholson, C. MacLeod, A. Felipe, et al. 2001. Lipopolysaccharide-induced apoptosis of macrophages determines the up-regulation of concentrative nucleoside transporters Cnt1 and Cnt2 through tumor necrosis factor-α-dependent and -independent mechanisms. *J. Biol. Chem.* 276: 30043–30049.

39. Yeramian, A., L. Martin, L. Arpa, J. Bertran, C. Soler, C. McLeod, M. Modolell, M. Palacin, J. Lloberas, and A. Celada. 2006. Macrophages require distinct arginine catalatolism and transport systems for proliferation and for activation. *Eur. J. Immunol.* In press.

40. Hamermann, R., C. Stichtnhe, E. I. Closs, H. Nawrath, and K. Racke. 2001. Inhibition of nitric oxide synthase abrogates lipopolysaccharides-induced up-regulation of L-arginine uptake in rat alveolar macrophages. *Br. J. Pharmacol.* 133: 379–386.

41. Hamermann, R. M., D. Dreissig, J. Mossner, M. Fuhrmann, L. Berrino, M. Gothert, and K. Racke. 2000. Nuclear factor-κB mediates simultaneous induction of inducible nitric-oxide synthase and up-regulation of the cationic amino acid transporter CAT-2B in rat alveolar macrophages. *Mol. Pharmacol.* 58: 1294–1302.

42. Munder, M., F. Mollinedo, J. Calafat, J. Canchado, C. Gil-Lamaignere, J. M. Puentes, C. Luckner, G. Doschko, G. Soler, K. Eichmann, et al. 2005. Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity. *Blood* 105: 2549–2556.

43. Peteroy-Kelly, M. A., V. Venketaraman, M. Talaeu, A. Seth, and N. D. Connell. 2003. Modulation of J774.1 macrophage L-arginine metabolism by intracellular *Mycobacterium bovis* BCG. *Infect. Immun.* 71: 1011–1015.

44. Zhang, M., H. Wang, and K. J. Tracey. 2000. Regulation of macrophage activation and inflammation by spermine: a new chapter in an old story. *Crit. Care Med.* 28: N60–66.

45. Zhang, M., T. Caragine, H. Wang, P. S. Cohen, G. Botchkina, K. Soda, M. Bianchi, P. Ulrich, A. Cerami, B. Sherry, and K. J. Tracey. 1997. Spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells: a counterregulatory mechanism that restrains the immune response. *J. Exp. Med.* 185: 1759–1768.

46. Hasko, G., D. G. Kuhel, A. Marton, Z. H. Nemeth, E. A. Deitch, and C. Szabo. 2000. Spermine differentially regulates the production of interleukin-12 p40 and interleukin-10 and suppresses the release of the T helper 1 cytokine interferon-γ. *Shock* 14: 144–149.

47. Kaczmarek, L., B. Kaminska, L. Messina, G. Spampinato, A. Arcidiacono, L. Malaguarnera, and A. Messina. 1992. Inhibitors of polyamine biosynthesis block tumor necrosis factor-induced activation of macrophages. *Cancer Res.* 52: 1891–1894.

48. Perez-Can, F. J., A. Franch, C. Castellote, and M. Castell. 2003. Immunomodulatory action of spermine and spermidine on NR8383 macrophage line in various culture conditions. *Cell. Immunol.* 226: 86–94.

49. Mossner, J., R. Hamermann, and K. Racke. 2001. Concomitant down-regulation of L-arginine transport and nitric oxide (NO) synthesis in rat alveolar macrophages by the polyamine spermine. *Palms Pharmacol. Ther.* 14: 297–305.

50. Falournoux, P., A. Bruhat, and C. Jousse. 2000. Amino acid regulation of gene expression. *Biochem.* J. 351: 1–12.