Vol. 160, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1989 Pages 354-361

NUCLEOSIDE TRANSPORT IN ACTIVATED MACROPHAGES

Franco BUSOLO*, Luciano CONVENTI, Carlo GATTI°, Daniela MONTI°, Leonarda TROIANO° and Claudio FRANCESCHI°

Institute of Microbiology, University of Padua, Italy

°Institute of General Pathology, University of Modena, Italy

Received February 28, 1989

The study of [^{3}H]-uridine uptake by mouse peritoneal macrophages showed that this is an active, temperature- and protein synthesis-dependent phenomenon, which is early altered when are exposed to a variety of stimuli. Murine recombinant interferon-γ, a stimulus able to activate macrophage and to induce the production of tumor necrosis factor-α, within few hours markedly increased [^{3}H]-uridine uptake by mouse macrophage. Other stimuli devoid of activation capacity, such as inert phagocytable latex beads, did not affect this phenomenon, which appeared to be related to macrophage activation. The increase in [^{3}H]-uridine uptake may be an useful phenomenon in studing the early biochemical events associated with macrophage activation.

Macrophage activation is a critical step in the acquisition of the cytotoxic activity toward several targets, including tumoral cells. This phenomenon may be induced by several stimuli ranging from whole microorganisms to soluble substances, such as lymphomonokines (1,2). In a previous paper we showed that phagocytosis of heat-killed Candida albicans caused a reduction of [^{3}H]-uridine uptake from culture medium (3). These data suggested that the inhibition of [^{3}H]-uridine uptake can be used to study the metabolic events related to the phagocytosis of microorganisms. This phenomenon has been further investigated

*To whom correspondence should be addressed.

ABBREVIATIONS

pMΦ = peritoneal macrophage(s); rIFN-γ = recombinant interferon-γ; LPS = lipopolysaccharide; TNF-α = tumor necrosis factor-α.

0006-291X/89 $1.50

and in this paper we present data suggesting that [^{3}H]-uridine uptake is an active, temperature- and protein synthesis-dependent phenomenon which is early altered following pMϕ stimulation. The pMϕ activation and acquisition of cytotoxic activity appear to be correlated to an increase of [^{3}H]-uridine uptake, while the treatment of pMϕ with inert particles or with complex stimuli, such as C.albicans, are ineffective or inhibitory, respectively.

MATERIALS AND METHODS

Animals. SAVO-NMRI female (22-25 g.) mice were obtained from the Charles River (Italy).

Microorganism. Candida albicans strain PD 119, from the collection of the Institute of Microbiology of Padua University, was grown in Sabouraud broth (Difco Lab., Detroit, MI). After a 24 hours incubation at 37°C it was washed twice with phosphate buffer saline (PBS), pH 7.2, and killed by heating at 80°C for 30 min.

Macrophages collection and culture. pMϕ were collected in complete medium containing heparin (20 U/ml) by harvesting peritoneal exudate cells from mice injected 4 days before with 1 ml of sterile 10% peptone (Difco) solution and cultured as previously described (3).

Reagents. Cycloheximide, actinomycin D and latex polystirene beads (3 µm of diameter) were obtained from Sigma Chemical Co. (St. Louis, MO). Solvent, PBS, media and supplements contained less than 1 pg endotoxin/ml as demonstrated by Limulus amebocyte assay (Sigma). The recombinant murine interferon (rIFN-γ) with a specific activity of 10^3 U/ml was from Genentech Inc. (San Francisco, CA).

[^{3}H]-uridine uptake assay. In some experiments pMϕ were prelabelled for 8 hours, from 2nd to 10th by plating, with 0.1µCi/ml L-[U-^{14}C]leucine (342 mCi/mmol; Amersham, Buckinghamshire, England) in leucine-free medium. After 24 hours by plating and two washings, the appropriate stimuli (C.albicans, beads or rIFN-γ) in medium with 10% fetal calf serum (FCS) or without FCS containing 1µCi/ml of [5-^{3}H] uridine (28-30 Ci/mmol; Amersham) were added. For simplicity, this was considered the starting point (time 0). At various times (1, 2, 3 and 4 hours), the medium was harvested and pMϕ were washed twice and lysed with 1% sodium dodecyl sulfate (SDS, Sigma). Lysates were placed in 20 ml scintillation vials containing 10 ml of Instagel (Packard, Downers Groce, IL) and radioactivity was measured by liquid scintillation counter (LKB 1214,Bromma, Sweden). The results are expressed according to the following index :

$$\text{uptake index} = \frac{[^3\text{H}]/[^{14}\text{C}] \text{ cpm ratio in stimulated pM}\phi}{[^3\text{H}]/[^{14}\text{C}] \text{ cpm ratio in control pM}\phi}$$

In some experiments [^{14}C]-leucine prelabelled pMϕ monolayers were treated for 4 hours with 1, 10 or 50 µg/ml cycloheximide before time 0. After two washings, the medium was replaced with

medium containing 1 μCi/ml [^{3}H]-uridine and cycloheximide. The subsequent steps were the same as above described.

Cytotoxic activity determination. Supernatants from stimulated pMϕ were tested on L929 target cells for the presence of cytotoxic activity referable to TNF-*α*, according to the method described by Nedwin et al.(4). The amount of lysed L929 target cells was assessed either by inspecting the cristalviolet stained wells of the microplate or by calculating the dye uptake using an automatic micro-E.L.I.S.A. reader (Dynatech Lab. Inc., Alexandria, VA).

RESULTS

Fig. 1 shows the kinetics of [^{3}H]-uridine uptake in monolayers of peptone-elicited mouse pMϕ in medium with or without FCS. In both conditions, [^{3}H]-uridine was rapidly incorporated into pMϕ. [^{3}H]-uridine uptake was optimal in pMϕ 24 and 48 hours after

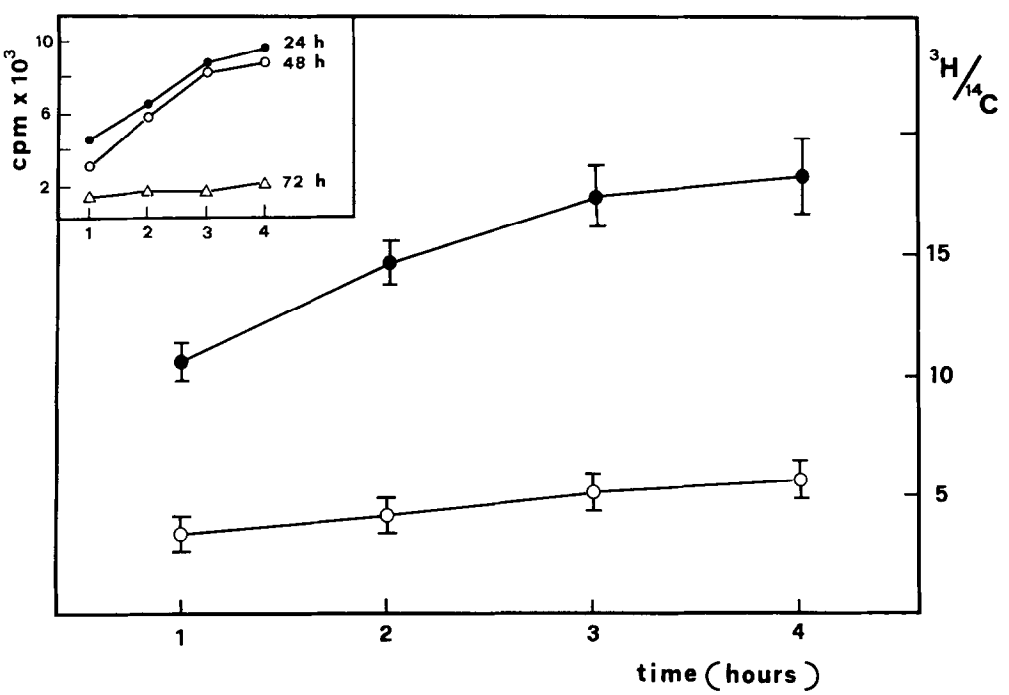

Fig. 1 Influence of fetal calf serum on [^{3}H]-uridine uptake by pMϕ. Monolayers were prelabelled with [^{14}C]-leucine and pulsed with [^{3}H]-uridine as described in Materials and Methods. Radioactivity was measured at the indicated times and results are expressed as [^{3}H]/[^{14}C] cpm ratio. Values represent means ± SD of duplicate determinations of 5 separate experiments. (○) = medium with 10% FCS; (●) = medium without FCS. In the box, the [^{3}H]-uridine uptake as a function of the time after pMϕ plating is reported. In this case pMϕ prelabelling with [^{14}C]-leucine was omitted and [^{3}H]-uridine pulse was performed, using medium with 10% fetal calf serum, at day 1, 2 and 3 after plating.

plating, as shown in the box of Fig.1. In order to assess cell viability and to reduce the variability of [^{3}H]-uridine uptake due to different number of cells in the monolayers, cells were prelabelled with [^{14}C]-leucine. Thus, the results are expressed as [^{3}H]/[^{14}C] cpm ratio to indicate the [^{3}H]-uridine uptake per viable pMϕ. Furthermore, the assessment of pMϕ-associated [^{14}C]-radioactivity along the experiments allowed us to evaluate possible toxic effects of the different stimuli used in the present study. As expected, when the radioactive nucleoside was added to the serum-free medium the amount of its incorporation was higher as compared to that of pMϕ in medium containing 10% of serum. In order to take into account this variable, the majority of subsequent experiments were performed in both conditions. To verify if [^{3}H]-uridine uptake was an active phenomenon , the influence of temperature was studied (Tab.1). Cell-associated [^{3}H]-uridine was constant at 0°C and increased considerably with time at 37°C. Moreover, this phenomenon appeared to be protein synthesis-dependent as

TABLE 1. Influence of temperature on ^{3}H-uridine uptake by mouse pMϕ

time(min)	incubation temperature	
	0°C	37°C
0	1.1	0.71
5	1.04	1.35
10	0.99	1.9
15	0.71	3.2
20	0.93	3.9

Experiments were performed as described in Materials and Methods but with some modifications: (i) the reduced time of exposure to [^{3}H]-uridine and (ii) pMϕ monolayers were kept either at 0°C floating on ice-bath or at 37°C immediately before the [^{3}H]-uridine pulse. Values given (mean of two experiments) are expressed as [^{3}H]-uridine/[^{14}C]-leucine cpm ratio as described in Materials and Methods.

TABLE 2. Effect of cycloheximide treatment on [^{3}H]-uridine uptake by pMϕ

cycloheximide (ug/ml)	% inhibition of [^{3}H]-uptake after different hours of exposure				cytotoxicity[b]
	5[a]	6	7	8	
1	2	5	11	26	14
10	7	15	18	46	45
50	27	43	59	76	60

[a] 5, 6, 7 and 8 refer to the different periods of exposure to cycloheximide, i.e. 4 hours of treatment before time 0 plus 1, 2, 3, or 4 hours after the addition of [^{3}H]-uridine, as indicated in Materials and Methods.

[b] The cytotoxicity of cycloheximide was measured as percent decrease in [^{14}C]-radioactivity associated to pMϕ.

suggested by experiments in which cycloheximide was used (Tab.2). As in previous experiments we observed an inhibition of [^{3}H]-uridine uptake when pMϕ were exposed to phagocytable microorganisms, we asked whether a similar phenomenon could be observed when pMϕ were exposed to others stimuli with different capacity to activate Mϕ and to induce their cytotoxic function. Fig.2 shows that, as expected, killed C.albicans was able to inhibit [^{3}H]-uridine uptake. This phenomenon was present either in absence or in presence of FCS. On the contrary, no inhibition of [^{3}H]-uridine uptake was observed when pMϕ were exposed to inert particles, such as polystirene latex beads. When pMϕ were exposed to a well known macrophage activator, i.e. murine rIFN-γ, a consistent increase of [^{3}H]-uridine uptake was observed, but only when pMϕ were stimulated in serum-deprived medium. The capacity of these stimuli to activate pMϕ and to induce TNF-α production is reported in Fig.2. In our experimental conditions, i.e. 4 hours of treatment with different stimuli, a consistent production of TNF-α was observed only with rIFN-γ.

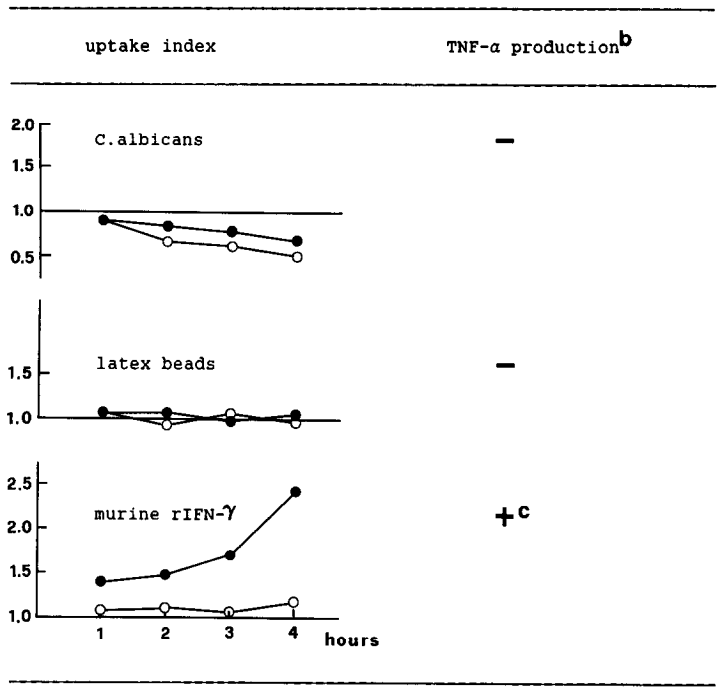

Fig. 2 Influence of various stimuli on [^{3}H]-uridine uptake by mouse pMϕ. [a] = C. albicans 40 CFU/pMϕ; rIFN-γ 10 U/ml. [b] = TNF-α production by pMϕ after 4 hours of stimulation, was titrated according to Nedwin et al. (4). [c] = approximatively 40 U/ml. (●) = stimulus in medium without FCS. (○) = stimulus in medium with 10% FCS.Each point represents the mean of 3 determinations.

DISCUSSION

The major findings of the present paper are the following: i) [^{3}H]-uridine uptake in pMϕ is an active, temperature- and protein synthesis-dependent phenomenon. Similar observations on nucleoside transport in other cell type have been reported (5); ii) an alteration of this phenomenon appears to be an early event in stimulated pMϕ and it is likely related to early alterations of RNA metabolism, as previously reported (6,7); iii) different stimuli affect differently [^{3}H]-uridine uptake, and this may be useful to dissect the metabolic events which follow various stimuli. When pMϕ were treated with a typical activating agent such as murine rIFN-γ , at the concentration able to prime pMϕ for cytolytic function (8), an increase of

nucleoside uptake was observed. Preliminary data with LPS, another activating agent, suggest that the increase of [^{3}H]-uridine uptake may be considered as a general and early phenomenon related to pMϕ activation. Instead, the ingestion of latex bead particles , per se', did not show any effect. Such inert particles, although easily phagocytosed, are unable to activate pMϕ -the so-called silent phagocytosis (9)- and did not altered [^{3}H]-uridine uptake. We confirmed that phagocytosis of C.albicans was able to inhibit [^{3}H]-uridine uptake (3). At present, it is difficult to explain this effect. However, preliminary experiments, using purified yeast cell-wall components and pMϕ from different sources, suggest that this phenomenon depends on the chemical complexity of the cell-wall of these microorganisms and on the genetic background of the pMϕ donors, in accordance with similar observations in other cell types from different species (10). In conclusion, an alteration of nucleoside transport may be considered a useful parameter in studing the early metabolic consequences of Mϕ activation. An alteration of nucleoside transport could be taken as an indirect index of the effectiveness of the transduction signal in Mϕ stimulated with different compounds .

ACKNOWLEDGMENTS

This work has been supported by M.P.I. grants (40 and 60%) to Franco Busolo and Claudio Franceschi and by C.N.R. grant n.87.01292.44 to Claudio Franceschi. Daniela Monti is recipient of a Sigma-Tau fellowship.

REFERENCES

1) Adams, D.O. and Hamilton, T.A. (1984) Ann. Rev. Immunol. 2, 283-318.
2) Hamilton, T.A. and Adams, D.O. (1987) Immunology today 8, 151-158.
3) Busolo, F., Tonellato, L., Scremin, L.,Tonin, E., Bertoloni, G. and Franceschi, C. (1986) J.Immunol. Methods 90, 235-240.

4) Nedwin, G.E., Svedersky, L.P.,Bringman, T.S., Palladino Jr., M.A. and Goeddel, D.V. (1985) J. Immunol. 135, 2492-2497.
5) Berlin, R.D. (1973) J. Biol. Chem. 248, 4724-4730.
6) Varesio, L. (1985) J. Immunol. 134, 1262-1267.
7) Varesio, L., Clayton, M., Radzioch, D. and Bonvini, E.(1987) J. Immunol. 138, 2332-2337.
8) Somers, S.D., Weiel, J.E., Hamilton, T.A. and Adams D.O. (1986) J. Immunol. 136, 4199-4205.
9) Schnyder, J. and Baggiolini, M. (1978) J. Exp. Med. 22, 435-450.
10) Plagemann, P.G.W. and Woffendin, C. (1987) Biochim. Biophys. Acta 969, 1-8.