5′-NUCLEOTIDASE ACTIVITY OF MOUSE PERITONEAL MACROPHAGES

II. Cellular Distribution and Effects of Endocytosis*

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In the preceding paper (7), we reported on the activity and metabolism of 5′-nucleotidase in resident and inflammatory mouse peritoneal macrophages. In this paper, we deal with the issue of the cellular distribution of the enzyme in these cells and with the distribution and metabolism of this enzyme in cells following two types of endocytic stimulus. In many cells, 5′-nucleotidase appears to be primarily, or even entirely, associated with the plasma membrane (for review, see 3). In mouse macrophages, the enzyme has also been identified in association with the membrane enclosing latex phagolysosomes, presumably as a result of its interiorization on portions of plasma membrane (14). Thus, quantitative information on the cellular distribution of the enzyme could shed light on the processes involved in the metabolism of plasma membrane. Endocytic stimulation, by altering the distribution and fate of plasma membrane, is another approach to an analysis of the cellular controls involved in the metabolism of the plasma membrane. We have used two different endocytic stimuli. Latex is a nondegradable particle which is ultimately included in secondary phagolysosomes. Concanavalin A, however, is a soluble protein which stimulates the formation of pinocytic vesicles which do not readily fuse with lysosomes (5). Thus, although each agent ultimately leads to the formation of an intracellular pool of sequestered membrane, the mechanisms of stimulation and the fate of the interiorized membrane is quite different in the two cases.

The diazonium salt of sulfanilic acid (DASA)[1] is a relatively membrane impermeable reagent, which was originally used by Berg to specifically label externally disposed components of the human red blood cell membrane (1). De Pierre and Karnovsky subsequently exploited it in their study of guinea pig neutrophil plasma membrane enzymes, including the neutrophil 5′-nucleotidase (4). In this study, DASA is used to assess the fraction of total 5′-nucleotidase activity which is externally accessible in the resident and inflammatory macrophage, and to examine the changes in this fraction related to endocytic stimulation.

Materials and Methods

Macrophage Cultures. Cells were harvested and maintained in culture as described in the preceding paper (7).

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[1] *Abbreviations used in this paper:* DASA, diazonium salt of sulfanilic acid; DMEM, Dulbecco's modified Eagle's medium; FCS, heat-inactivated, filtered fetal calf serum.

Preparation and Assay of the DASA. The DASA was prepared by the indirect method exactly as described by De Pierre and Karnovsky (4). The reagent was washed once in iced distilled water and then dissolved in Hanks' balanced salt solution without phenol red. Solutions of 10^{-3} M DASA, or less, had a pH of 7.2. The reagent concentration was assayed by allowing the DASA to react with known concentrations of 1-histidine in distilled water. The absorbance of an alkalinized solution at 530 nm was measured. The molar concentration of histidine for which the reaction was maximal, as indicated by the achievement of a plateau of color development, was taken as equal to the molar concentration of the DASA. This technique was generously suggested to us, in a private communication, by Dr. Ira Goldstein of New York University School of Medicine.[2] In addition, the yield of DASA was directly assessed with [^{35}S]sulfanilic acid and found to be 70–80% of the theoretical yield.

Reagents. Sources of all reagents also used in the preceding paper (7) were as stated there. Chemicals were reagent grade or higher. Concanavalin A (3 × recrystalized, lyophilized) was purchased from Miles Chemical Co., Kankakee, Ill. and stored as a powder until used.

Assays. Assay of 5'-nucleotidase, and cell protein, were carried out as described in the preceding paper (7).

Latex. Polystyrene latex beads (diameter, 1.099 μm) were purchased from Dow Chemical U.S.A., Midland, Mich. Beads were washed once in 70% ethanol and twice in sterile distilled water, and tested for sterility and cell toxicity before use.

Results

Inhibition of 5'-Nucleotidase Activity by the DASA. DASA, a compound that reacts readily with several amino acids (13), has been successfully used by De Pierre and Karnovsky (4) to inactivate the 5'-nucleotidase of intact guinea pig polymorphonuclear leukocytes. As cell membranes are relatively impermeable to this reagent (1), the inactivation of about 95% of the cell complement of 5'-nucleotidase in these experiments was interpreted to mean that the active site of the enzyme was uniformly externally disposed on the outer face of the neutrophil plasma membrane.

DASA will also inactivate 5'-nucleotidase activity in intact mouse peritoneal macrophages (Fig. 1), with maximal inhibition reached at about 5×10^{-4} M DASA when about 80% of the total cell enzyme activity is inhibited. The reaction proceeds rapidly, with maximal inhibition of the enzyme achieved after 10–15 min of incubation at 37°C.

In contrast to the guinea pig neutrophil, residual activity persists in mouse peritoneal macrophages after treatment with DASA (Table I). This fraction is similar in unstimulated cells, endotoxin-stimulated cells, or unstimulated cells cultivated under serum-free conditions, although the absolute size of these residual pools is quite different under these different conditions. This residual activity is not, however, intrinsically insensitive to the DASA reagent. When macrophages are disrupted with detergent, and the lysate is then exposed to DASA, no residual 5'-nucleotidase activity can be detected (Table I).

In an effort to learn more about the metabolic relationship of the DASA-insensitive enzyme activity to the majority of the cell 5'-nucleotidase, we examined the relative and absolute changes in pool size in cells treated with cycloheximide (10 μg/ml). Although there was a considerable decrease in total 5'-nucleotidase activity in cells exposed to the drug for up to 8 h, the fraction of activity which was DASA insensitive remained relatively constant (10.9–21.5%) with perhaps a slight rise at early time points.

Effect of Phagocytosis on 5'-Nucleotidase Activity. As noted earlier by Werb

[2] Goldstein, I., M. Cequeira, S. Lind, and H. B. Kaplan. Submitted for publication.

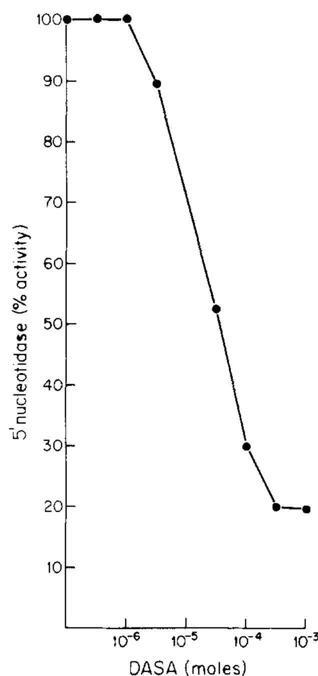

FIG. 1. Inactivation of macrophage 5′-nucleotidase activity by DASA. Cells were exposed to DASA for 30 min at 37°C, rinsed with serum and again with DMEM, lysed in detergent, and assayed for 5′-nucleotidase activity.

TABLE I
*5′-Nucleotidase Activity Remaining after Treating Intact Mouse Peritoneal Macrophages with DASA**

Cell type	5′-nucleotidase activity		5′-nucleotidase activity remaining after DASA
	Total	After DASA	
	nm AMP/min per mg protein		%
Unstimulated (8)‡	52.0 (45.5–58.6)§	10.9 (8.1–11.6)	20.6 ± 5.7
Endotoxin stimulated (2)‡	20.9 (19.0–22.7)	3.6 (1.5–5.1)	17.4
Unstimulated (serum-free medium) (1)	29.0	6.5	22.5
Unstimulated (cell lysate treated) (1)	54.0	<0.5	<1

* Cells were cultivated for 24 h in DMEM-20% FCS or DMEM-0.2% lactalbumin hydrolysate (serum-free medium) as indicated, and then exposed to DASA (10^{-3} M) in HBSS for 15 min at 37°C.

‡ Number of experiments

§ Range of values.

and Cohn (14), 5′-nucleotidase activity decreases for several hours after a brief phagocytic pulse, reaching a minimum level of activity at about 5–6 h after the cells are exposed to the particle load (Fig. 2). The total cell activity then begins to increase, usually returning to normal enzyme levels after an overnight incubation.

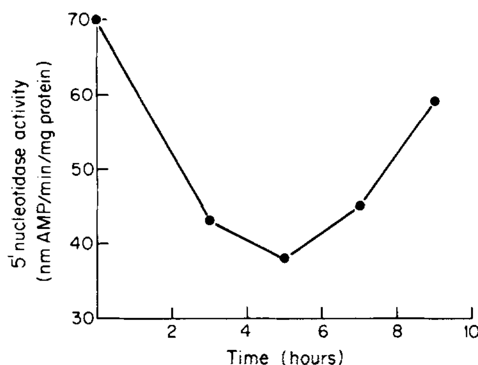

FIG. 2. Effect of latex ingestion on total 5'-nucleotidase activity of mouse peritoneal macrophages. Cells were exposed to latex beads (10^9 particles/ml) in DMEM-20% FCS for 60 min at 37°C, rinsed, and reincubated in DMEM-20% FCS for the periods indicated on the abscissa.

TABLE II
Change in 5'-Nucleotidase Activity of Unstimulated Macrophages Exposed to Polystyrene Beads for 1 h

Latex dilution*	5'-nucleotidase specific activity at times after latex ingestion	
	5 h	20 h
	% of initial activities	
1:20	26.6	70.2
1:50	43.6	84.3
1:100	52.7	97.4
1:200	68.2	113.0
None	100.0	118.0

* Stock suspension contains 1-2 × 10^{11} latex particles/ml.

As summarized in Table II, the decrease in enzyme activity is greater with increasing concentrations of the nondegradable polystyrene particles. The rate of recovery, however, appears to be generally the same, regardless of the initial ingested load. Therefore, cells challenged with higher latex concentrations take more time to return to normal enzyme levels than do cells with lighter particle loads.

Immediately after a 1-h exposure to latex, the DASA-insensitive fraction of the total cell 5'-nucleotidase increases dramatically (Table III), although at this early time the total cell enzyme activity is not changed. The fraction of enzyme which is sequestered from the reagent in these cells generally increases with increases in the latex load offered to the cells. For the latex concentrations used in these experiments, the DASA-insensitive conpartment is expanded some 2–2.5 times, to represent 48.5–64% of the total cell activity.

At 6 h after latex ingestion (Table IV), total 5'-nucleotidase activity is now quite low, having decreased about 35–75% of its original value, depending upon the latex concentration offered to the cells. The DASA-insensitive fraction of 5'-nucleotidase is also considerably reduced, not only from its expanded size immediately after latex ingestion, but also, at higher latex concentrations, from

TABLE III

5′-Nucleotidase Activity After DASA Treatment of Intact Macrophages Immediately after Latex Ingestion

Dilution of latex to which cells were exposed*	Total 5′-nucleotidase specific activity	5′-nucleotidase activity after DASA treatment	DASA-insensitive activity	Increase in DASA-insensitive pool‡
		nm AMP hydrolyzed/ min per mg protein	%	%
1:50	65.5	–	–	–
1:100	63.4	40.6	64.0	38.1
1:150	51.6	29.9	58.0	32.1
1:200	66.8	32.4	48.5	22.6
None	63.0	16.3	25.9	

* Stock suspension contains 1–2 × 10^{11} particles/ml.

‡ Calculated as the difference of the percent DASA-insensitive activity before and after a 1-h exposure to latex.

TABLE IV

Total and DASA-Insensitive 5′-Nucleotidase Activity 6 h after Exposure of Unstimulated Macrophages to Latex Particles

Dilution of latex to which cells were exposed*	Total 5′-nucleotidase activity‡	Initial activity	5′-nucleotidase activity after DASA treatment‡	Total activity at 6 h	5′-nucleotidase activity§	Decrease in 5′-nucleotidase activity remaining after DASA treatment‖
		%		%		
1:50	15.0	(22.9)	1.1	(7.1)	50.5	–
1:100	21.5	(33.9)	3.1	(14.3)	41.9	37.5
1:150	23.6	(45.8)	5.3	(22.4)	28	24.6
1:200	42.2	(63.2)	7.3	(17.4)	24.6	25.1
None	63.0	(100)	16.3	(25.9)	–	–

* Stock suspension contains 1–2 × 10^{11} particles/ml.

‡ nm AMP hydrolyzed/min per mg cell protein.

§ Calculated as the difference in total activity immediately following and 6 h after latex exposure (nm AMP/min per mg).

‖ Calculated as the difference in DASA-insensitive activity immediately following and 6 h after latex exposure (nm AMP/min per mg).

its size in resting cells. The decrease in total cell 5′-nucleotidase can be essentially completely accounted for by the decrease in the DASA-insensitive enzyme fraction. Additional incubation of such depleted cells will permit their full recovery of total enzyme activity, but the DASA-insensitive fraction remains low (2.1% of total activity at 24 h for cells exposed to 1:100 dilution of the stock latex suspension).

Rates of Synthesis and Degradation of 5′-Nucleotidase in Latex-Containing Macrophages. Although the latex-filled cells do eventually recover full 5′-nucleotidase activity, their diminished DASA-insensitive pool raised a question about their metabolism of the enzyme. To examine this issue, cells were exposed

to latex (approximately 10^9 particles/ml) for 1 h, rinsed, and then reincubated for 24 h in Dulbecco's modified Eagle's medium-20% heat-inactivated, filtered fetal calf serum (DMEM-20% FCS). The cells were then transferred to DMEM-20% FCS containing cycloheximide (10 μg/ml), and the rate of decay of 5'-nucleotidase activity was measured over the next 8 h. As shown in Fig. 3, which presents data that are the averages of two separate experiments, the decay is exponential, with a $t_{\frac{1}{2}}$ = 7.6 h. As summarized in the preceding paper (7), it is possible to use this data to calculate directly the rates of synthesis and degradation of the enzyme in these cells. The results of these calculations are presented in Table V, in comparison with similar data obtained earlier for unstimulated macrophages. While the rate of enzyme synthesis is somewhat increased in these cells, as compared to control macrophages, the major difference is in their elevated rate of degradation of the enzyme.

Effect of Concanavalin A on 5'-Nucleotidase Activity. Concanavalin A is a potent stimulator of endocytosis in mouse macrophages (5). However, the pinosomes generated in its presence have an abnormally low rate of fusion with lysosomes and instead tend to persist in the cytoplasm intact for several days (5, 6). It therefore appeared to offer the opportunity to induce the cells to interiorize a considerable amount of plasma membrane in a brief period of time and then sequester the membrane in the cytoplasm. The fate of 5'-nucleotidase in cells containing these sequestered pinosomes could then be compared with that of enzyme in cells containing latex phagolysosomes.

When unstimulated macrophages are exposed for brief periods of time to concanavalin A in serum-free medium, there is a striking decline in total 5'-nucleotidase activity (Fig. 4). The decline is not due simply to the absence of serum, as it does not occur when bovine serum albumin (200 μg/ml) is substituted for concanavalin A, or when serum-free medium is used without any additional supplementation. This decline does not occur if the cells are exposed to concanavalin A at 4°C, but is seen if similar cells are exposed to concanavalin A on ice, rinsed, and then reincubated at 37°C for 60 min. There is no effect, however, on the activity of macrophage lysates when concanavalin A is directly added to them, to final concentrations of up to 2 mg/ml.

The rate of decline and extent of inhibition of activity are both somewhat greater with increasing concentrations of concanavalin A, but even 5 μg/ml of the lectin causes a 35% decrease in the activity of 5'-nucleotidase over a 2-h period. The inhibition of enzyme activity caused by concanavalin A progresses for several hours after the cells are placed in lectin-free medium (Fig. 5), but with further incubation, the total cell enzyme activity then begins to rise. Recovery to normal levels of activity is also quite rapid, occurring in about a 2–4-h period. The recovery process proceeds at about the same rate in medium supplemented with fetal calf serum, or in similar medium to which mannose (50 mM) has been added. Enzyme activity will also recover in serum-free medium, although under these conditions cells do not return to their full resting activities.

Recovery of 5'-nucleotidase activity, after concanavalin A-induced enzyme inhibition, does not require new protein synthesis. As shown in Fig. 6, the rate of enzyme recovery is not affected by cycloheximide (10 μg/ml). This concentra-

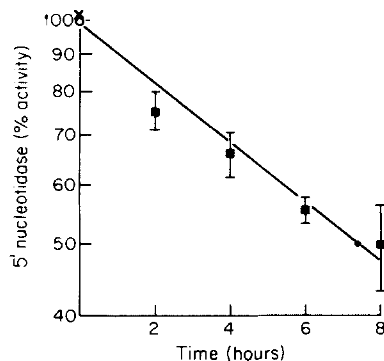

FIG. 3. Decline of 5'-nucleotidase activity in unstimulated macrophages allowed to ingest latex beads, reincubated for 24 h in DMEM-20% FCS, and then exposed at t = 0 to cycloheximide (10 μg/ml).

TABLE V

*Rates of Synthesis and Degradation of 5'-Nucleotidase in Macrophages Exposed to Latex 24 h Earlier**

Cell	5'-nucleotidase activity	DASA-insensitive activity	t½	K_d	K_s
	nm AMP/min per mg protein	%	*h*	h^{-1}	*U/h*
Unstimulated‡	64.4	19.0	13.9	0.052	3.0
Unstimulated + latex (1:100)§	51.5	2.1	7.6	0.091	4.7

* Cells were exposed to latex for 60 min, rinsed, then reincubated in DMEM-20% FCS for 24 h. Rates of synthesis and degradation were estimated using cycloheximide as detailed in reference (7).

‡ Data from reference (7).

§ Stock latex suspension contained 1–2 × 10^{11} particles/ml.

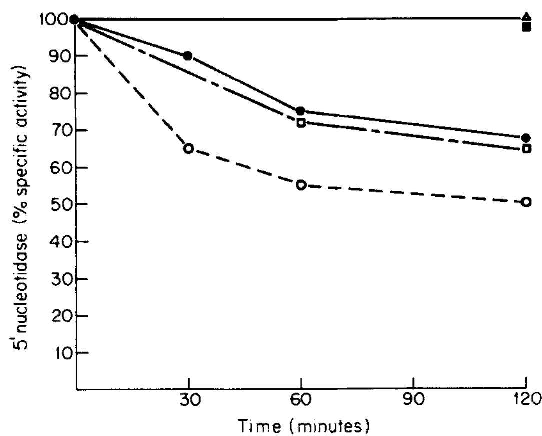

FIG. 4. Effect of concanavalin A on total macrophage 5'-nucleotidase activity. Cells were exposed to one of several concentrations of concanavalin A in DMEM-LH at 37°C, and enzyme activity was assayed at the times indicated on the abscissa. (●) 5 μg/ml; (□) 25 μg/ml; (○) 50 μg/ml. Controls included cells incubated without conanavalin A in DMEM-FCS (■) or DMEM-LH (△).

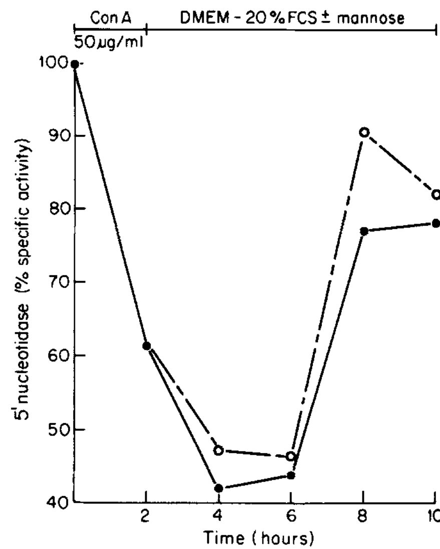

FIG. 5. Time-course of recovery of 5'-nucleotidase activity by macrophages previously exposed to concanavalin A (Con A) (50 μg/ml). Cells were exposed, at t = 0, to concanavalin A (50 μg/ml) for 2 h at 37°C. Cells were then rinsed three times with DMEM containing α-methylmannoside (50 mM) and reincubated either in DMEM-20% FCS supplemented with mannose (50 mM) (×---×) or DMEM-20% FCS alone (●—●).

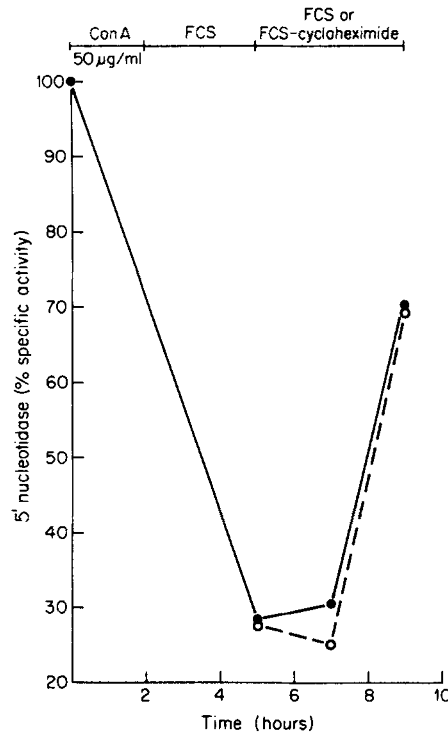

FIG. 6. Effect of cycloheximide on recovery of 5'-nucleotidase activity by macrophages previously exposed to concanavalin A (Con A). Macrophages were exposed to concanavalin A (50 μg/ml) at t = 0 for 2 h, rinsed, and reincubated in DMEM-20% FCS. At t = 4 h, some cells were exposed to DMEM-20% FCS with cycloheximide (10 μg/ml) (O---O), while the other cells remained in DMEM-20% FCS (●—●) and were further incubated as indicated.

tion is sufficient to completely inhibit incorporation of [^{3}H]leucine into trichloroacetic acid-precipitable material in unstimulated macrophages (P. J. Edelson, unpublished observations) within 1 h of incubation.

Discussion

DASA was originally used by Berg (1) to label molecules on the external surface of human erythrocytes. Its major advantages were its reactivity with a wide variety of amino acids (13), including lysine, glutamine, asparagine, histidine, tyrosine, and cysteine, and its relative inability to permeate cell membranes (1). This relative exclusion from the cell interior was exploited by De Pierre and Karnovsky (4) in their study of several phosphatases associated with the guinea pig polymorphonuclear leukocyte. In this cell about 95% of the 5′-nucleotidase activity was inhibited when intact cells were treated briefly with DASA, and no additional inhibition was achieved when broken cell preparations were exposed to the reagent. They therefore concluded that essentially all the specific 5′-nucleotidase activity of the guinea pig neutrophil was associated with the plasma membrane and, in fact, was oriented with its active site facing the exterior.

DASA will also inactivate a fraction of the 5′-nucleotidase activity of cultured mouse peritoneal macrophages. The reaction is rapid, completed in about 10–15 min at 37°C at concentrations of reagent of 5×10^{-4} or more. These conditions are comparable to those required in the guinea pig neutrophil for complete enzyme inactivation.

In the macrophage, though, only 80% of the total 5′-nucleotidase is inactivated when intact cells are reacted with DASA. We believe the remaining 20% represents a pool of intracellular 5′-nucleotidase activity. Macrophages have no detectable nonspecific alkaline phosphatase (7, 14) which might account for this residual activity, and the enzyme assay used in this work appears to estimate the true 5′-nucleotidase activity of these cells (7). In addition, this residual activity is not intrinsically insensitive to DASA, as it is readily inhibited in broken cell preparations.

At least two origins can be imagined for this intracellular enzyme pool. As Farquhar et al. (8) and Widnell (15) have shown in hepatocytes, some of this activity may be associated with the endoplasmic reticulum and Golgi apparatus, presumably as newly synthesized plasma membrane precursor. Additionally, a part of this intracellular pool may be associated with plasma membrane interiorized in pinosomes or phagosomes. Our studies do not allow us to distinguish between these two possibilities. However, some comparisons can be made between the size of the intracellular 5′-nucleotidase pool and other estimates of the pool of internalized plasma membrane in cultural macrophages.

Steinman et al. (12), using stereologic measurements of peroxidase-loaded macrophages, estimated that the surface area of the pool of internalized plasma membrane, both in pinosomes and in secondary lysosomes, is about 18% of the area of the cell surface. Werb and Cohn (14) estimated that the slowly exchangeable cholesterol pool, which also appears to represent the pinosome and secondary lysosome compartment, was about half the size of the rapidly exchangeable pool, which appears to be plasma membrane. If the 20% of the total 5′-nucleotid-

ase activity which is not inactivated by DASA in intact cells is identified with a pinosome and secondary lysosome compartment, it would suggest that this compartment is about 25% of the size of the plasma membrane compartment. These estimates are all roughly compatible with each other, though the specific activities of any given membrane component are not necessarily the same in the two compartments.

Werb and Cohn (14) previously showed that 5′-nucleotidase is internalized, in association with phagosome membrane, when cells ingest latex particles. As would be expected, then, for a nonpenetrating reagent, the DASA-insensitive enzyme fraction was found to be increased in cells immediately after their exposure to a pulse of latex. Depending upon the concentration of latex used, from 22–38% of the total cell activity could be transferred from the DASA-sensitive to the DASA-insensitive enzyme fractions. As the plasma membrane activity represents about 80% of the total cell activity, based on its sensitivity to DASA, this represents an apparent internalization of 28–48% of the total plasma membrane 5′-nucleotidase activity. To use these figures to estimate the extent of plasma membrane interiorization as the result of latex ingestion, one must know the specific activity of 5′-nucleotidase in the plasma and phagosomal membrane. If, as earlier work (14) has suggested, these activities are quite similar, then we estimate that about 28–48% of the plasma membrane was interiorized under these conditions.

When latex-filled macrophages are reincubated for several hours, the total cell 5′-nucleotidase activity decreases over a few hours and then slowly recovers to normal levels. The decrease in activity is generally equal to the amount of enzyme initially interiorized, and Werb and Cohn (14) have previously shown a quantitative degradation of enzyme in the phagolysosomal fraction of these cells. However, at higher latex concentrations, total cell activity decreases more than can be accounted for by the quantity of enzyme internalized with the latex particles. In addition to a loss of enzyme associated with the latex phagosomes, the cells also appear to lose a portion of their basal DASA-insensitive enzyme pool. Even after a 24-h reincubation in DMEM-20% FCS, the DASA-insensitive pool may remain so low as to be nearly nonexistent.

This contraction of the DASA-insensitive pool, in the face of a recovery of total cell 5′-nucleotidase activity, is consistent with the concept that the bulk of this pool is not associated with plasma membrane precursor. One possibility is the basal DASA-insensitive pool is continuing to be generated from plasma membrane at the normal rate, but that its intracytoplasmic lifetime is considerably shortened, perhaps as a result of increased fusion with latex phagolysosomes and subsequent enzymatic inactivation of the 5′-nucleotidase activity. To explore this issue we determined the rates of synthesis and degradation of 5′-nucleotidase in latex-filled macrophages which have been allowed to recover their normal enzyme levels. These estimates indicate that, while there may be a slight increase in synthetic rate in these cells, there is a marked increase in rate of degradation of the enzyme, with the half-time for degradation shortened from 13.9 (7) to 7.6 h. These results raise the question not only of whether incoming pinocytic vesicles, with 5′-nucleotidase activity associated with their membrane, may be detoured to latex phagolysosomes, but also whether newly

synthesized plasma membrane precursor, perhaps transported in tiny cytoplasmic vesicles, might not also be diverted to these phagolysosomes for premature disposal.

Several groups of workers have reported that concanavalin A can affect 5′-nucleotidase activity. Riordan and Slavik (10) reported that concanavalin A, in concentrations up to 5 μg/ml, appears to stimulate 5′-nucleotidase activity extracted by detergent from rat liver plasma membrane preparations, while concentrations above 50 μg/ml seem to inhibit the enzyme, both by reducing the V_{max} and increasing the K_m of the enzyme. This suggested that simple site-specific interaction of the lectin with the enzyme was insufficient to explain the inhibition. Little and Widnell (9) have similarly observed an inhibitory effect of concanavalin A on the 5′-nucleotidase of rat liver Golgi vesicles, which were prepared after in vivo ethanol administration. Carraway et al. (2) have reported that concanavalin A can inhibit the 5′-nucleotidase activity of plasma membrane-enriched fractions from rat lactating mammary glands, and that this inhibition is prevented by the simultaneous inclusion of 50 mM α-methyl mannose in the incubation medium. Stefanovic et al. (11), examining the effect of concanavalin A on intact C6 glioma cells, also reported a decrease in enzyme activity, associated with a slight increase in K_m and a marked decrease in V_{max}. It is, however, somewhat difficult to interpret these last experiments, as enzyme activity, before and after concanavalin A treatment, was estimated in intact cells. Presumably, because of the limited ability of intact adenosine-5′-phosphate to penetrate cell membranes, the activity measured would only be that associated with the plasma membrane. Treatment of cells with concanavalin A can lead to extensive internalization of plasma membrane (5), and any enzyme internalized in this way would no longer be expected to be detectable in an assay of intact cells, although it would not necessarily be inactivated by the lectin.

In our studies, concanavalin A does seem to have a considerable inhibitory effect on macrophage 5′-nucleotidase, which may be prevented by α-methyl mannose. This effect, however, is not seen at 4°C, can proceed even after the cells have been rinsed and placed in fresh lectin-free medium, and does not occur when detergent lysates are directly treated with up to 2 mg/ml of concanavalin A. These results are not definitive, but suggest that concanavalin A may not be acting only in a direct way, on the enzyme, to inhibit its activity. The temperature dependence may reflect a requirement for endocytosis or a need for some particular physical state of the membrane which is fostered by higher temperatures.

Although the rate of enzyme inhibition is rapid, it is not instantaneous. This would be compatible with a mechanism by which enzyme is interiorized and then degraded by intracellular enzymes, but it seems unlikely that this is the case in this situation. First, concanavalin A vesicles show little fusion with lysosomes (5), and their contents are only very slowly inactivated (6). In addition, enzyme activity can be returned to normal in the face of complete inhibition of protein synthesis and is not dependent on an extrinsic source of enzyme in serum. Thus, as a result of the cell's interaction with concanavalin A, 5′-nucleotidase is apparently reversibly inhibited. This inactivation may occur directly on the plasma membrane or may be associated with the interiorization

of the enzyme in association with pinosome membrane. There is some suggestion, from experiments in which cells are allowed to recover from the concanavalin A-associated enzyme inhibition, that some of this inactivated enzyme may be interiorized. Cells allowed to recover their inactivated activity have an expanded intracellular pool of 5'-nucleotidase, as assessed by DASA insensitivity. However, it has not been possible to demonstrate the quantitative interiorization of the inactivated fraction of enzyme, or to show that the same enzyme which is apparently intracellular after reactivation, does eventually return to the plasma membrane.

A reasonable sequence of events would be to see the concanavalin A as binding to a portion of plasma membrane in which 5'-nucleotidase is sited. This area of membrane, and its associated enzyme, is then interiorized, along with the concanavalin A which has been bound to it (5). Based on the slow tempo of inhibition, in comparison with the rather rapid rate of concanavalin A binding (5), one could expect that the 5'-nucleotidase would be inhibited only subsequent to its interiorization. The inactivated enzyme would remain in association with the intact pinosomes until its subsequent reactivation, perhaps as the result of a displacement of concanavalin A from its binding sites on the inner surface of the pinosome (6). Its subsequent fate is not yet established.

Summary

The diazonium salt of sulfanilic acid (DASA) can inactivate about 80% of the total 5'-nucleotidase of viable macrophages. The remaining 20% can be inactivated if the cells are first lysed in detergent, and presumably represents an intracellular pool of 5'-nucleotidase. The bulk of this pool may represent cytoplasmic vesicles derived from plasma membrane by endocytosis. This internal compartment is expanded up to threefold immediately after the cells have ingested a large latex load. This is consistent with previous observations on the internalization of 5'-nucleotidase in latex phagosomes. In latex-filled cells this intracellular pool of enzyme is inactivated over a few hours, and the cells then slowly increase their enzyme activity to nearly normal levels. However, 24 h after latex ingestion the metabolism of 5'-nucleotidase in these recovered cells is abnormal, as the rate of enzyme degradation is about twice the normal rate, and the DASA-insensitive enzyme pool in these cells is strikingly diminished. This may reflect effects of the accumulated indigestible particles on the fate of incoming pinocytic vesicles or on newly synthesized plasma membrane precursor. Another endocytic stimulus, concanavalin A, also reduces the total cell 5'-nucleotidase activity. This effect, which is time and temperature dependent, can be prevented by the competitive sugar α-methyl mannose. The concanavalin A inhibition can be reversed in the absence of new protein synthesis or in cells cultivated in serum-free conditions. It is not known whether the effect of concanavalin A on 5'-nucleotidase depends upon the interiorization of plasma membrane or is strictly associated with events at the cell surface.

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