Biosynthesis and transport of lysosomal enzymes in human monocytes and macrophages

Effects of ammonium chloride, zymosan and tunicamycin

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Human monocytes and macrophages synthesize lysosomal enzymes as larger precursors. The polypeptide patterns of several lysosomal-enzyme precursors and their mature forms are similar to those observed in human fibroblasts. Like fibroblasts, the monocytes and macrophages release small amounts of lysosomal-enzyme precursors. The lysosomotropic NH_4^+ cation enhances this release. In contrast, zymosan, a degranulating agent, causes release of both the mature and the precursor forms of the lysosomal enzymes. Both NH_4Cl and zymosan inhibit maturation of the precursors. The fractional amounts of mature cathepsin D and β -hexosaminidase released in the presence of zymosan are strikingly different. Probably, in the macrophages several lysosomal organelles are packaged with different relative contents of lysosomal enzymes. The transport of the precursors of cathepsin D into lysosomes is inhibited by tunicamycin. Therefore oligosaccharide side chains are likely to function as signals in packaging of lysosomal enzymes in macrophages also.

Investigations in several laboratories indicate that synthesis of lysosomal enzymes as larger precursors, initially observed for carboxypeptidase in yeast (Hasilik & Tanner, 1976), for cathepsin D in kidney cells (Erickson & Blobel, 1979) and for several other lysosomal enzymes in macrophages (Skudlarek & Swank, 1979, 1981) and fibroblasts (Hasilik & Neufeld, 1980a), is a general phenomenon. In cultured human fibroblasts a portion of the precursors is released, and the release can be enhanced by perturbing the transport into the lysosomes with weak bases (Hasilik & Neufeld, 1980a) or the formation of phosphorylated oligosaccharide side chains (Hasilik & Neufeld, 1980b). Lysosomal enzymes are prominent components of material released from macrophages (reviewed by Page *et al.*, 1978).

Thioglycollate-elicited mouse macrophages have been reported to release exclusively the mature forms of lysosomal enzymes (Skudlarek & Swank, 1981). Interestingly, the fractional rate of release of β -glucuronidase is twice that of β -galactosidase. It is possible to stimulate the release in macrophages with non-inactivated serum (Schnyder & Baggiolini, 1978), certain indigestible particles (Bonney *et al.*, 1978) or weak bases (Riches & Stanworth, 1980). In

the present paper we report on the maturation and release of lysosomal enzymes and their precursors in human monocytes and related cells, and on the effect of certain compounds on these processes. Throughout this paper we use the term 'release' in a strictly operational sense, referring to amounts of radioactive materials or enzyme activity recovered in the medium. We believe that 'release' of radioactive precursors results from secretion rather than from cell death, since these precursors are products of metabolically active cells and are short-lived within the cells.

Materials and methods

Cells and culture conditions

Monocytes were isolated by using leukaphoresis and fractionation in a hypo-osmotic Percoll (Pharmacia, Freiburg, Germany) gradient as described by Feige *et al.* (1982). Monocyte-derived macrophages were prepared from fresh venous blood obtained under sterile conditions from healthy volunteers by using a modification of the procedure of Knight & Soutar (1982): the blood was supplemented with 1 mM-EDTA and centrifuged at 1000 g for 20 min.

The cells were suspended in 1 vol. of 10 mM-sodium phosphate-buffered saline (0.9% NaCl) containing 1 mM-EDTA, pH 7.4, and fractionated by using Ficoll-Paque (Pharmacia) as recommended by the supplier. The mononuclear cells collected after centrifugation were diluted by adding 3 vol. of the buffered saline/EDTA solution. The cells were pelleted at 800 *g* for 10 min and washed twice with the buffered saline/EDTA solution, and once with Waymouth MAB 87/3 medium (Gorham & Waymouth, 1965) as formulated in the catalogue of Gibco Europe, Karlsruhe, Germany, supplemented with 10% (v/v) foetal-calf serum (Boehringer Mannheim, Mannheim, Germany). After each wash the cells were sedimented by centrifugation at 100 *g* for 10 min. Cells obtained from 20 ml of blood were taken up in 5 ml of the culture medium and transferred into 25 cm² Falcon tissue-culture flasks (Becton-Dickinson, Heidelberg, Germany). After incubation in CO₂/air (1:19) at 37°C for 2 h, the medium and non-adhering cells were removed and adherent monocytes were supplied with 5 ml of Waymouth medium supplemented with 20% (v/v) autologous serum. The synthesis of lysosomal enzymes was studied after 7 days of cultivation, when nearly all cells developed the morphological features of macrophages. Human spleen macrophages were prepared from non-pathological autopsy material. The tissue was cut and homogenized in 10 vol. of RPMI 1640 medium (Seromed, München, Germany) containing 2% human serum. The cells were washed three times with RPMI 1640 medium, suspended in RPMI 1640 medium containing 10% foetal-calf serum (2 × 10⁶ cells/ml of medium), transferred in Falcon flasks (32.5 ml/75 cm² flask) and allowed to adhere at 37°C under CO₂/air (1:19) for 2 h at 10⁶ cells/cm². The adhered cells were cultured for 10 days in McCoy 5A medium (Seromed).

Radioactive labelling and isolation of lysosomal enzymes

Adherent cells were depleted of leucine (Hasilik & Neufeld, 1980a) and incubated in 25 cm² culture dishes with 2.5 ml of Waymouth medium containing 4% (v/v) autologous dialysed serum and 0.2 mCi of L-[4,5-³H]leucine (sp. radioactivity 59.8 Ci/mmol; from NEN, Dreieich, Germany) with or without further additions or manipulations as indicated. The labelling of suspended monocytes was performed in gently shaken 25 ml round-bottom glass flasks containing 25 × 10⁶ cells in 2.5 ml with 0.2 mCi of L-[4,5-³H]leucine as described above, except that dialysed foetal-calf serum inactivated at pH 10.4 (Hasilik & von Figura, 1981) was used and cells were centrifuged at 160 *g* for 5 min for each change of the medium. After the labelling, the medium was concentrated and dialysed as described by Hasilik &

Neufeld (1980a) and stored at -20°C. The labelled macrophages were rinsed with 2 × 3 ml of Hanks solution (Hanks & Wallace, 1949) and lysed with 0.3 ml of 1% Nonidet NP40 and 1% sodium deoxycholate. The lysate was collected in 1.5 ml capped vials in a total volume of 0.5 ml adjusted to 0.15 M-NaCl/10 mM-sodium phosphate, pH 7.4, and frozen at -20°C. The labelled suspended monocytes were collected by centrifugation and homogenized as described for fibroblasts previously (Hasilik & Neufeld, 1980a). Extracts of medium and cells were prepared by thawing the samples, incubating them at room temperature for 0.5 h and then at 4°C for 16 h, followed by centrifugation at 45 000 *g* for 1 h. The cell fraction was subjected once more to freeze-thawing, the incubations and the centrifugation. Lysosomal enzymes were precipitated from the extracts as described previously (Hasilik & Neufeld, 1980a) by using monospecific antisera. Partially purified placental enzymes were added if necessary to adjust the antigen concentration to at least 0.5 µg/ml. The preparation of the antisera against human placental β-hexosaminidase (Hasilik & Neufeld, 1980a), cathepsin D (Hasilik *et al.*, 1983) and arylsulphatase B (Steckel & von Figura, 1982) was described previously. The immune precipitates were collected, washed and solubilized, and the radioactive polypeptides were analysed by electrophoresis in 12.5% polyacrylamide gels in the presence of sodium dodecyl sulphate followed by fluorography as previously described (Hasilik & Neufeld, 1980a). For sequential precipitation of several lysosomal enzymes, the supernatant obtained after each immune-precipitation step was supplemented with another antiserum and processed as described above. The radioactivity in different polypeptides was quantified by densitometry with an Ultro Scan laser densitometer (LKB, Stockholm, Sweden). The activity of β-hexosaminidase was determined in extracts of cells and medium with *p*-nitrophenyl β-N-acetylglucosaminide (von Figura, 1977).

Materials

Radioactive [¹⁴C]methylated standard proteins were purchased from NEN, sodium dodecyl sulphate was from Sigma (München, Germany; cat. no. L-5750), and zymosan A (yeast) was from Sigma. Tunicamycin was given by Dr. R. L. Hamill (Lilly Research Laboratories, Indianapolis, IN, U.S.A.).

Results

Biosynthesis of lysosomal enzymes in monocytes

Monocytes prepared by leukaphoresis and centrifugation in a hypo-osmotic Percoll gradient actively synthesized lysosomal enzymes. As shown in Fig. 1, they synthesized and processed β-hexos-

Fig. 1. Processing and release of β -hexosaminidase and cathepsin D in monocytes isolated by leukaphoresis and centrifugation in a hypo-osmotic Percoll gradient

The cells were incubated for 16 h in the radioactive medium as described in the Materials and methods section. However, 4% dialysed foetal-calf serum inactivated at pH 10.4 (Hasilik & von Figura, 1981) was used in the labelling medium. The enzymes were isolated by immune precipitation and analysed by gel electrophoresis under denaturing conditions, followed by fluorography. The arrows on the left indicate the migration of precursors (Pro α and Pro β) and mature forms (mature α and mature β) of α - and β -chain of β -hexosaminidase. The following ^{14}C -labelled standards were used: phosphorylase b, 92 5000; bovine serum albumin, 69 000; ovalbumin, 46 000; carbonic anhydrase, 30 000; cytochrome c, 12 300.

cathepsin D accumulated in the medium. Little β -hexosaminidase was found in the medium. Here it comprised both the precursor and the mature polypeptides. While our study was in progress, Wakefield *et al.* (1982) reported that monocytes phagocytose Percoll particles. It seemed probable, therefore, that monocytes not exposed to these particles would present a different picture of processing and release of lysosomal enzymes from that shown in Fig. 1. We decided to avoid the use of Percoll in preparation of cells and to study macrophages differentiated from adherent monocytes.

Biosynthesis and transport of lysosomal enzymes in macrophages derived from blood monocytes

Synthesis and distribution of three lysosomal enzymes, β -hexosaminidase, cathepsin D and arylsulphatase B, by macrophages differentiated from adherent monocytes is shown in Fig. 2. After 16 h of labelling and 4 h of chase, the polypeptides of these enzymes were nearly completely retained within the cells. The three enzymes were found predominantly in their mature forms (lane 1). As compared with other human cells (Hasilik & Neufeld, 1980a; Hasilik *et al.*, 1981), in macrophages the α -chain of β -hexosaminidase appears to be processed to a larger product of apparent molecular weight about 58 000. In the medium only small amounts of the precursor polypeptides were present (Fig. 1, Table 1), and no mature enzyme forms were detectable. Whereas released β -hexosaminidase precursor polypeptide accounted for 1.6% (mean of six determinations, with a range of 0.3–3.1%) of total labelled β -hexosaminidase, the relative release of cathepsin D was 5-fold higher (mean 8.1%, range 0.9–16.9%). The relative release of β -hexosaminidase activity (mean 8.9%) is higher than that of radioactive precursors (Table 1), suggesting that some release from metabolically inactive cells occurs. Since no mature enzyme forms are released even during a subsequent chase of 16 h (Fig. 2, lane 5), it is unlikely that the β -hexosaminidase activity released in excess over the precursors originates from secondary lysosomes of metabolically active cells.

Effects of zymosan and NH_4Cl

Two different types of experiments were designed to study the effect of zymosan or NH_4Cl on transport of lysosomal enzymes (Fig. 2). In one type of experiment the substance was present in the culture in the course of the radioactive labelling. Under these conditions, mainly the influence on the transport of newly synthesized lysosomal enzymes could be traced. Alternatively the cells were labelled under control conditions and the substance was added to the chase medium. The analysis of the cells and the chase medium allowed us to determine the

aminidase and cathepsin D in a way resembling that by other cultured human cells (Hasilik & Neufeld, 1980a; Hasilik *et al.*, 1981). The monocytes, however, appeared also to release processed lysosomal enzymes. Most striking was the massive release of cathepsin D in both the precursor and the mature form; more than half of the labelled

Fig. 2. Effect of zymosan and NH_4Cl on maturation and release of lysosomal enzymes in macrophages

In our experiments cells were cultivated with radioactive medium in the absence (–) or in the presence (+) of 1.2 mg of zymosan/ml or 10 mM- NH_4Cl for 16 h, and then an excess of non-radioactive leucine was added. After 4 h the medium (M) was collected and the cells (C) were harvested. In both experiments in two additional non-treated cultures, after removal of the medium (pulse) the cells were supplemented with medium without or with zymosan or NH_4Cl . After 16 h of incubation, the medium (chase) was collected and the cells (pulse-chase) were harvested. Extracts of medium (M) and cells (C) were prepared and β -hexosaminidase, cathepsin D and arylsulphatase B were sequentially immune-precipitated. Fluorograms of immune precipitates separated by gel electrophoresis are shown. The relative exposure times for the three enzymes were 4:1:10 respectively. We assume that the radioactive band migrating slightly faster than the mature α -chain of β -hexosaminidase, which is well detectable if the labelling is

influence of the added compounds on the release of preformed mature lysosomal enzymes.

If zymosan was present during the labelling, the efficiency of the processing of the precursors was decreased and the radioactivity in the precursor polypeptides was higher than in controls. Concomitantly, the formation of mature polypeptides was diminished, especially of those of arylsulphatase B and cathepsin D. In the latter case the intermediate of M_r 47 000 became a rather prominent species (Fig. 2, lane 6). The apparent inhibition of the formation of mature polypeptides may be secondary to stimulation of the release from cells or inhibition of the transport into the lysosomes. Indeed, in the presence of zymosan the release of the precursors of all three enzymes was enhanced (Fig. 2, lane 7). Besides, the mature polypeptides and processing intermediates of β -hexosaminidase and cathepsin D were also released during the labelling. As mentioned above, few mature polypeptides of arylsulphatase B were found in cells labelled in the presence of zymosan; they were nearly absent also from the medium of these cells. In total, 48–55% of the newly synthesized enzyme polypeptides were recovered in the medium.

In the second type of experiment, cells were prelabelled for 16 h with [^3H]leucine and depleted of

labelled enzyme precursors by incubation for 4 h with an excess of non-radioactive leucine. Then the cells were incubated with fresh medium in the absence or presence of zymosan. In controls, the distribution of the radioactivity in the various polypeptides in the cells and in the medium after the pulse and the chase resembled the situation seen after the pulse-labelling alone, except for the complete absence of the precursors from the cells (Fig. 2, lane 3). Neither the medium collected after the labelling (Fig. 2, lane 4) nor that collected after the chase incubation (Fig. 2, lane 5) contained any mature lysosomal enzyme. Only small amounts of cathepsin D precursor and traces of β -hexosaminidase precursors were found in the media (Table 1). In the cells that were labelled in the absence of zymosan and subjected to chase incubation in its presence, the mature forms again were the only labelled lysosomal enzymes found (Fig. 2, lane 8). Their amounts, however, were diminished, and the corresponding amounts of the mature polypeptides were found in the chase medium (Fig. 2, lane 10). There was relatively little release of mature cathepsin D (<7%), whereas 72% of the mature β -hexosaminidase became released during the incubation with zymosan (Table 1). The differential effect of zymosan on release of the mature forms

Table 1. Release of radioactive β -hexosaminidase and cathepsin D polypeptides and of β -hexosaminidase activity. Radioactive polypeptides (shown in part in Fig. 2), catalytic activity and radioactivity incorporated into material insoluble in cold 5% trichloroacetic acid were determined of the same sample. Some 12.4–13.7% of total radioactivity incorporated was released during the pulse and 2.8–7.8% during the chase. The presence of zymosan or NH_4Cl during the chase was without apparent effect on the release of radioactive material.

Experiment	Lane in Fig. 2	Addition	Release into medium (% of total)		
			β -Hexosaminidase		Cathepsin D (radioactivity)
			(catalytic activity)	(radioactivity)	
I Pulse	4	—	2.6	0.9	12.1
Chase	5	—	1.8	0.2	<0.2
Pulse	9	—	2.2	0.4	4.5
Chase	10	Zymosan (1.2 mg/ml)	51.0	71.6	6.9
II Pulse	(not shown)	—	51.1	3.1	9.1
Chase	(not shown)	—	6.9	1.3	0.7
Pulse	14	—	12.8	2.3	16.9
Chase	15	10 mM- NH_4Cl	12.0	1.4	1.3

performed in the presence of zymosan or NH_4Cl , is a processing intermediate of the β -chain of β -hexosaminidase. The β -hexosaminidase polypeptide seen at the bottom of the gel in cell extracts is probably a fragment of the pro- α -chain. In immune precipitates of cathepsin D, the region of the smaller mature fragments may be contaminated and is not considered in our interpretation. The nature of the polypeptide found in the cells labelled in the presence of NH_4Cl between the mature and intermediate forms of cathepsin D is not known. The three mature polypeptides of arylsulphatase B are supposed to be formed from the precursor by alternative cleavages (Steckel & von Figura, 1982). In the Figure only the control from the experiment with zymosan is shown, since the control in the experiment with NH_4Cl was not significantly different.

of the two enzymes has been confirmed in repeated experiments. Under the influence of zymosan, arylsulphatase B (Fig. 2), arylsulphatase A and α -N-acetylglucosaminidase (results not shown) behaved like β -hexosaminidase.

In the presence of 10mM-NH₄Cl there was a partial inhibition of the processing of the lysosomal enzymes (Fig. 2, lane 11). NH₄Cl enhanced release of the precursor polypeptides of all three enzymes studied. It did not induce any release of mature of intermediate polypeptides, whether present during the labelling or during the chase incubation (Fig. 2, lane 12 and 15).

The macrophages appear to be very sensitive to changes of environment. In an experiment we found 15% of total β -hexosaminidase in the medium after 6h of incubation. During the subsequent 16h incubation without additions, or with 1.2mg of zymosan/ml, with 10mM-NH₄Cl, or with both, a further 3%, 72%, 7% or 75% of the activity respectively was released into the medium. Thus the release was little affected by 10mM-NH₄Cl, in agreement with the observation that release of only the newly synthesized lysosomal enzymes is induced. The large amount of β -hexosaminidase released in the presence of zymosan obviously originated from intracellular stores of lysosomal enzymes. This finding parallels the observation on release of prelabelled β -hexosaminidase demonstrated in Fig. 2, lane 10. The distribution of the enzyme activity in the experiments shown in Fig. 2 is presented in Table 1. The release of β -hexosaminidase as a percentage of the total activity was increased from 1.8% in the control to 51% in the presence of zymosan (cf. Fig. 2, lane 10), whereas the release of trichloroacetic acid-precipitable material increased from 2.8% to only 7.8%. The release of the radioactive material was little affected by NH₄Cl. These results and the selective effect of zymosan on the release of β -hexosaminidase and arylsulphatase B as compared with cathepsin D (Fig. 2) indicate that the release in the presence of zymosan is not due to a non-specific disintegration of the cultured macrophages.

Effects of tunicamycin

Tunicamycin was used to examine participation of carbohydrate moieties in the transport of lysosomal enzymes in macrophages. As shown in Fig. 3, treating the cells with increasing concentrations of tunicamycin caused a gradual increase in the release of cathepsin D in precursor forms. In cells treated with 1.2 μ M- and 3.6 μ M-tunicamycin, most of the labelled cathepsin D was released into the medium. Precursor forms of the enzyme were released, which contain no or only one oligosaccharide side chain, as judged by size. Normally, the precursor contains two oligosaccharide chains (Hasilik & von Figura, 1981). At the concentrations mentioned, tunica-

Fig. 3. Effect of tunicamycin on the processing and release of cathepsin D in cultured macrophages

The cells were cultured and labelled as described in the Materials and methods section, except for incubation with tunicamycin (0–3.6 μ M as indicated in the Figure) for 3 days before and during the labelling period. Cathepsin D was isolated by immune precipitation and the radioactive immunoprecipitates were analysed by gel electrophoresis under denaturing conditions, followed by fluorography.

mycin severely inhibited the apparent labelling of the enzyme in the cells. The labelling of the material precipitable with 5% (v/v) trichloroacetic acid was inhibited by 20, 40 and 66% in cells treated with 0.12 μ M-, 1.2 μ M- and 3.6 μ M-tunicamycin respectively. The labelling of cathepsin D appeared to be more sensitive to tunicamycin than that of the total protein (cf. Fig. 3). We assume that most of the non-glycosylated precursor became degraded shortly after the synthesis. The remainder was predominantly released and was not segregated into the lysosomes, owing to the lack of carbohydrate. The main mature polypeptide of cathepsin D in the treated cells was probably glycosylated, though its size appeared to be slightly smaller than in the normal enzymes.

Fig. 4. Synthesis, processing and release of β -hexosaminidase and cathepsin D in macrophages from human spleen

The cells were cultivated in the radioactive medium for 16h, and the labelled released and intracellular enzymes were isolated by immune precipitation and analysed by gel electrophoresis under denaturing conditions, followed by fluorography.

Biosynthesis and distribution of lysosomal enzymes in spleen macrophages

Finally, we wished to see whether unstimulated human tissue macrophages also release precursor forms of lysosomal enzymes. Indeed, macrophages isolated from spleen released rather few mature lysosomal enzymes as compared with precursors (Fig. 4). In contrast with macrophages differentiated from peripheral blood monocytes, the tissue macrophages released large amounts of the precursor of cathepsin D (39% of total cathepsin D), and in contrast with monocytes isolated in the presence of Percoll they released little mature cathepsin D (2% of total cathepsin D). The release of the precursor of β -hexosaminidase (6.5% of total) is comparable with that in macrophages differentiated from peripheral blood monocytes.

Discussion

Human macrophages differentiated from peripheral blood monocytes or isolated from spleen release precursor forms of lysosomal enzymes. Ample release of one precursor (cathepsin D) is

observed side by side with an efficient retention of another precursor within the cells (β -hexosaminidase). Depending on the isolation procedure (e.g. use of Percoll), a non-co-ordinate release of mature forms is observed, similar to that of the precursor forms. The selectivity in release or retention of different lysosomal enzymes suggests a heterogeneity among either the vesicles containing lysosomal enzymes of the studied cells. Non-co-ordinate release of β -glucuronidase and β -galactosidase was reported for thioglycollate-elicited mouse peritoneal macrophages (Skudlarek & Swank, 1981). These macrophages released exclusively the mature forms of the enzymes. In contrast, human macrophages release constitutively the precursor forms, whereas they release the mature forms only after certain manipulations.

Exposure to zymosan affects the maturation as well as the release of lysosomal enzymes. The maturation of some enzymes (arylsulphatase B) is more sensitive than that of others. After stimulation the released material contains precursor, intermediate and mature forms of lysosomal enzymes in approximately the same relative proportions as they are present intracellularly. However, the released cathepsin D is relatively enriched in precursor and intermediate forms. For β -hexosaminidase an extensive secretion of both enzyme activity and radioactively labelled enzyme is induced. Radioactively labelled arylsulphatase B and α -N-acetylglucosaminidase are affected similarly to β -hexosaminidase. It is probable, therefore, that zymosan induces fusion of vesicles containing the newly synthesized enzymes with the plasma membrane. These vesicles may originate from pre-lysosomal and/or lysosomal compartments containing lysosomal enzymes in different stages of maturation. Passage of the newly synthesized lysosomal enzymes through such compartments has been observed (Gieselmann *et al.*, 1983). Since little mature cathepsin D is released in the presence of zymosan, zymosan appears to induce fusion with the plasma membrane of a class of lysosomes that are rich in mature β -hexosaminidase and arylsulphatase B and poor in mature cathepsin D. These observations lend further support to the concept of heterogeneity among vesicles containing lysosomal enzymes and/or the studied macrophages with regard to their contents and responses to certain stimuli.

In macrophages (Fig. 2) 10mM- NH_4Cl causes a moderate inhibition of the maturation of lysosomal enzymes and a moderate increase in the release of the precursors. Its effect on release of the activity of β -hexosaminidase is hardly detectable. NH_4Cl increases pH in macrophage lysosomes (Poole & Ohkuma, 1981). In fibroblasts the elevation of lysosomal pH has been suggested to inhibit the dissociation of lysosomal enzymes from the mannose 6-phosphate-specific receptor and to prevent

the continuous reutilization of the receptor (Gonzales-Noriega *et al.*, 1980). In consequence, newly synthesized lysosomal-enzyme precursors cannot be segregated from the secretory products, and accumulate extracellularly. Elevation of pH and inhibition of receptor reutilization may explain the inhibition of maturation and increased release of precursors induced in macrophages by NH_4Cl .

Evidence is accumulating that in macrophages transport of lysosomal enzymes into lysosomes may occur independently of mannose 6-phosphate residues. Deficiency in phosphorylation of lysosomal enzymes in I-cell disease does not appear to affect lysosomal-enzyme activities in Kupffer cells and leucocytes (McKusick *et al.*, 1978). Receptors other than that for mannose 6-phosphate residues may be involved in the transport of lysosomal enzymes in these cells (Ullrich & von Figura, 1979). In two mouse macrophage cell lines, which lack the mannose 6-phosphate receptor, large amounts of lysosomal enzymes are inserted into lysosomes (Gabel *et al.*, 1983). Nevertheless, macrophages synthesize phosphorylated lysosomal enzymes (Goldberg & Kornfeld, 1981; Jessup & Dean, 1982). In contrast with phosphorylation, glycosylation appears to be indispensable for segregation of lysosomal enzymes in macrophages. Non-glycosylated cathepsin D synthesized in the presence of tunicamycin is released and not transported into lysosomes. The effects of tunicamycin on the macrophage cathepsin D as shown in Fig. 3 resemble very closely those on lysosomal enzymes in fibroblasts (von Figura *et al.*, 1981). In these cells also the release of cathepsin D and β -hexosaminidase is enhanced, whereas the total radioactive labelling appears to be diminished. This latter effect may indicate instability of non-glycosylated lysosomal enzymes. Similar effects of tunicamycin on cathepsin D were observed in cultured rat hepatocytes (Rosenfeld *et al.*, 1982).

In conclusion, our experiments demonstrate that human macrophages are able to release in a controlled manner certain lysosomal enzymes. The release may concern selectively the precursor or the mature forms of the enzymes. Further studies are needed to characterize the effectors of the differentiation status of the cells responsible for the specific patterns of the material released. The differences in the behaviour of the different lysosomal enzymes indicate alternative routes in their transport and probably the presence of separate classes of lysosomal organelles in macrophages, differing in their acid hydrolase complements and in responses to exocytosis stimuli.

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