The rate of internalization of different receptor–ligand complexes in alveolar macrophages is receptor-specific

Diane McVEY WARD and Jerry KAPLAN*

Department of Pathology, University of Utah Health Science Center, Salt Lake City, UT 84132, U.S.A.

To probe the mechanisms of endocytosis in alveolar macrophages, we examined the internalization rates of three different receptors. Initial rates of internalization for mannosylated BSA, diferric transferrin and α -macroglobulin–proteinase complexes were all different. Although the absolute rates of internalization varied depending on the cell preparation, transferrin was internalized at 10–20 % and α -macroglobulin–proteinase complex at 40–60 % of the rate of mannosylated-BSA. Incubation of cells with transferrin did not affect the rate of internalization of mannosylated BSA or α -macroglobulin–proteinase complexes, and the rates of internalization were independent of receptor occupancy. These different internalization rates could not be ascribed to different rates of diacytosis. Altering the distribution of unoccupied surface receptors by either trypsin treatment of cells at 0 °C or exposure to hyperosmotic solutions resulted in the absolute internalization rates being affected by the experimental condition, but the hierarchy in receptor internalization rates was maintained. The fact that a variety of conditions affect receptor internalization rates to the same degree implies the existence of co-ordinate regulation at a single rate-limiting step. Based on these results, we suggest that differences in internalization rate reflect the ability of ligand–receptor complexes to be captured by coated pits.

INTRODUCTION

Receptor-mediated endocytosis permits cells to internalize macromolecules needed for cellular processes or provides a route by which potentially injurious molecules can be removed from biological fluids. The receptors which mediate these functions are all capable of recycling. Thus, although the ligand may be catabolized, the receptors are spared from catabolism and may be re-utilized (Goldstein *et al.*, 1979; Anderson & Kaplan, 1983).

The endocytic pathway has been characterized using both biochemical and morphological approaches. Most ligand–receptor complexes are internalized via coated pits. Once internalized, ligand–receptor complexes are localized in a structure defined as the endosome. The endosome is an acidic non-lysosomal compartment where uncoupling or dissociation of ligand–receptor complexes occurs (Van Renswoude *et al.*, 1982; Harford *et al.*, 1983; Geuze *et al.*, 1983). As a consequence of constitutive recycling, the endosome may contain a number of different plasma membrane proteins capable of being recruited to the cell surface (Tietze *et al.*, 1982; Deutsch *et al.*, 1982; Lamb *et al.*, 1983; Buys *et al.*, 1984).

Although the recycling pathway has been described in general outline, a number of questions regarding the movement of molecules through the pathway remain to be answered. We have examined the specific internalization rates of several ligand–receptor complexes in a single cell type. To date, few studies have examined the rate of receptor-mediated internalization for more than one ligand in a single cell type. Those studies which have done so have usually focused on the internalization of hormone–receptor complexes, revealing wide variations in internalization rates depending on the particular hormone studied. Measured half-times for the disappearance of surface receptor–ligand complexes range from 2–3 min for epidermal growth factor (EGF) in human skin fibroblasts (Wiley & Cunningham, 1982) to 2–3 h for somatostatin in rat pituitary cells (Presky & Schonbrunn, 1986). In a number of instances,

internalization of the receptor–hormone complex is clearly associated with termination of a ligand-induced signal (Segaloff & Ascoli, 1981). As such, variations in internalization rate may reveal more about signal transduction than they do about the endocytic machinery.

To elucidate the factors that may regulate internalization rate we have studied those receptors whose major function is either to provide required nutrients to cells or to remove toxic molecules from biological fluids. Since the function of these receptors is to accumulate ligand, one can expect that internalization would occur at maximal rates. Rabbit alveolar macrophages were utilized, as they exhibit a variety of these ‘scavenger’ receptors. Our results indicate that different receptor–ligand complexes can be internalized at different rates. As demonstrated by this study and by Ward *et al.* (1989), internalization is the only receptor-specific rate in the recycling process. That is, once inside the cell, receptor–ligand complexes or ligands move at rates which are determined by the machinery and not by the receptor or ligand.

MATERIALS AND METHODS

Cells

Rabbit alveolar macrophages were obtained by bronchial lavage (Myrvik *et al.*, 1961) and the cells were prepared as described previously (Kaplan, 1980).

Materials

α -Macroglobulin (α M) was isolated from either rabbit or human plasma. α M.¹²⁵I-T was prepared as previously described (Kaplan *et al.*, 1982). Mannosylated BSA (MAN-BSA) was a gift from Dr. Y. C. Lee, Johns Hopkins University, Baltimore, MD, U.S.A. Maleylated BSA (MAL-BSA) was prepared as described (Butler & Hartley, 1972). Human transferrin (Tf) was obtained from Calbiochem–Behring (San Diego, CA, U.S.A.). Human Tf was iron-saturated as described previously (Ward *et al.*, 1982).

Abbreviations used: Tf(Fe)₂, diferric transferrin; MAN-BSA, mannosylated bovine serum albumin; MAL-BSA, maleylated bovine serum albumin; α M.T; α -macroglobulin–trypsin complexes; HBSS, Hanks balanced salt solution; MEM, minimal essential medium; EGF, epidermal growth factor.

* To whom correspondence should be addressed.

MAN-BSA, MAL-BSA and Tf(Fe)₂ were iodinated according to the procedure of Markwell (1982).

Methods

The binding of all ligands was measured by the procedure used to assay binding of aM.¹²⁵I-T (Kaplan, 1980). To distinguish between surface-bound and internalized ligand, the method of Kaplan and Nielson (1979b) was used for aM.T and MAN-BSA. However, in the latter case the EDTA buffer employed was at pH 6.0. A modification of the acid stripping technique (pH of 5.4 instead of 3.8) of Haigler *et al.* (1980) was used to distinguish internalized Tf(Fe)₂ from surface-bound Tf(Fe)₂. Hyperosmotic treatment was performed as described by Heuser & Anderson (1989). Trichloroacetic acid precipitation was measured as described previously (Ward *et al.* 1989).

Additional procedures

Cells were trypsin-treated at 0 °C for 30 min using 0.01 % trypsin (Worthington) in Hanks balanced salt solution (HBSS). The reaction was stopped by adding a 10-fold excess of soybean trypsin inhibitor (Sigma, St. Louis, MO, U.S.A.). Protein determinations were performed as described by Lowry *et al.* (1951), using BSA (fraction V; Sigma) as a standard.

RESULTS

Internalization rates of different receptor-ligand complexes

Two approaches were used to measure internalization rates of different receptor-ligand complexes. First, the specific internalization rate of receptors (K_e) was determined by the method of Opresko & Wiley (1987a). An advantage of this method is that the probability of internalization per occupied receptor can be determined without requiring changes in incubation temperature. We found that internalization of three different ligands, ¹²⁵I-Tf(Fe)₂, ¹²⁵I-MAN-BSA and aM.¹²⁵I-T, occurred at different rates (Fig. 1). However, the absolute value of the specific internalization rate of a given ligand differed with

Fig. 1. Specific internalization rates of aM.¹²⁵I-T, ¹²⁵I-MAN-BSA and ¹²⁵I-Tf(Fe)₂.

Cells were incubated with radiolabelled ligand at 37 °C. At specified times cells were placed at 0 °C, washed three times with cold HBSS, and surface-bound ligand was removed as described in the Materials and methods section. Surface-bound and cell-associated radioactivity were determined and the data were plotted as described by Opresko & Wiley (1987a) as a function of time. ▲, ¹²⁵I-MAN-BSA; △, aM.¹²⁵I-T; □, ¹²⁵I-Tf(Fe)₂.

different cell preparations. For example, the specific internalization rate of MAN-BSA varied by 10-fold in different experiments (see Table 2). Nevertheless, the relative order for internalization of the three ligands remained the same; the K_e of Tf(Fe)₂ was approx. 15 % that of MAN-BSA, while that of aM.T was 50 % of MAN-BSA.

These results were verified using an independent approach. Cells were incubated at 0 °C with aM.¹²⁵I-T, ¹²⁵I-MAN-BSA or ¹²⁵I-Tf(Fe)₂ until equilibrium was reached. Cells were washed free of unbound ligand and incubated at 37 °C for various times, after which surface-bound and internalized ligand was measured. In the presence of divalent cations and at neutral pH, the rates of dissociation of aM.T or MAN-BSA and Tf(Fe)₂ are extremely slow (Kaplan & Nielson 1979a; Stahl *et al.*, 1980; Lamb *et al.*, 1983). Consequently, the disappearance of surface-bound ligand is due solely to internalization. These experiments confirm previous studies (Stahl *et al.*, 1980; Lamb *et al.*, 1983) by demonstrating that the rate of loss of surface-bound ligand exhibits first-order kinetics (Fig. 2). Using this experimental approach, we confirm that ¹²⁵I-MAN-BSA and aM.¹²⁵I-T exhibit

Fig. 2. Rates of disappearance of surface-bound ligand

Cells were incubated for 60 min at 0 °C in the presence of aM.¹²⁵I-T, ¹²⁵I-MAN-BSA or ¹²⁵I-Tf(Fe)₂. The cells were washed with cold HBSS to remove unbound ligand and then placed at 37 °C. At specified times aliquots were removed and surface-bound and internalized radioactivity was determined as described in Fig. 1. The data are plotted as the percentage of surface-bound radioactivity remaining as a function of time. ▲, ¹²⁵I-MAN-BSA; △, aM.¹²⁵I-T; □, ¹²⁵I-Tf(Fe)₂.

Table 1. Effect of ligand concentration on the specific internalization rate of ¹²⁵I-MAN-BSA

Cells were incubated in MEM at 37 °C with the designated concentration of ¹²⁵I-MAN-BSA. At specified times aliquots were taken and surface-bound and internalized radioactivity was determined as described in the Materials and methods section. The specific internalization rate was determined as discussed by Opresko & Wiley (1987a) and the correlation coefficient (r) of the slope is included to give an estimation of the accuracy of the data.

Ligand concentration (M)	K_e	r
2×10^{-9}	1.39	0.953
2×10^{-8}	1.43	0.977
2×10^{-7}	1.20	0.998

Table 2. Effects of Tf(Fe)₂ on the specific internalization rates of ¹²⁵I-MAN-BSA and aM.¹²⁵I-T

The experimental protocol was identical with that of Table 1, except that cells were incubated with the radiolabelled ligand in the presence or absence of 1×10^{-5} M-Tf(Fe)₂.

Ligand	Expt.	Tf(Fe) ₂	K _e (internal/integral)	r
¹²⁵ I-MAN-BSA	1	—	0.348	0.987
	1	+	0.362	0.983
	2	—	3.030	0.975
	2	+	2.978	0.967
aM. ¹²⁵ I-T	1	—	0.159	0.961
	1	+	0.151	0.944
	2	—	1.050	0.993
	2	+	1.050	0.974

different internalization rates. The relative differences in rates were similar to the differences in K_e determined above. We did, however, observe the reappearance of ¹²⁵I-Tf on the surface. This result is consistent with the behaviour of Tf, since it is known that apoTf recycles to the cell surface (Karin & Mintz, 1981).

In these experiments, we employed a concentration of ligand equivalent to the K_d, or more precisely, the concentration at which steady-state receptor occupancy is half-maximal. In a previous study we demonstrated that aM.T was internalized at a similar rate at either 10 % or 80 % of surface receptor occupancy (Kaplan & Keogh, 1983). As shown in Table 1, we obtained similar results for the MAN-BSA receptor in that the specific internalization rate was similar at both low and high ligand concentrations. Previously we found that incubation of cells with MAN-BSA did not affect the K_e of aM.T (Kaplan & Keogh, 1983). Similar results were obtained with Tf (Table 2). These results suggest that different receptor–ligand complexes are internalized at different rates, and that the rate of internalization of one ligand is unaltered by the simultaneous internalization of a different receptor–ligand complex.

Differences in specific ligand internalization rates are not due to diacytosis

It is possible that the differences between the specific internalization rates of disparate receptor–ligand complexes might be due to differences in the rate of intracellular ligand accumulation rather than differences in internalization *per se*. If a receptor–ligand complex was internalized and then recycled to the cell surface, our methods of measurement would show a slow 'apparent' rate of internalization. The recycling of receptor–ligand complexes, a process referred to as diacytosis or retroendocytosis, has been observed for a wide variety of receptor–ligand complexes (Auslingkas *et al.*, 1981; Tietze *et al.*, 1982; Greenspan & St. Clair, 1984; Chang & Kullberg, 1984; Marshall, 1985; McKinley & Wiley, 1988). We measured the amount of aM.¹²⁵I-T and ¹²⁵I-MAN-BSA which was retroendocytosed by giving cells a pulse of ligand, removing surface-bound ligand and measuring the amount of intact ligand returning to the medium. Both cell-associated radioactivity (internalized) and that which was stripped from the cell surface after further incubations were quantified. Acid-precipitable radioactivity in the medium was also measured to assess the degradation of internalized ligand. Approx. 5–15 % ligand release was observed following a chase of 2 or 8 min (Fig. 3). It is possible that the stripping procedures affect recycling or diacytosis. In a previous study, however, we compared the rates of recycling of Tf in cells exposed to the stripping procedure versus control cells, and observed no

Fig. 3. Examination of whether internalized ligand is recycled to the cell surface

Cells were incubated at 37 °C with either aM.¹²⁵I-T (a) or ¹²⁵I-MAN-BSA (b) for 3 min. Cells were then placed at 0 °C and surface-bound radioactivity was removed. The cells were resuspended in Hanks MEM and returned to 37 °C for the specified times. At each time point, the cells were placed at 0 °C and radioactivity in the media was determined. Surface-bound (as determined by the stripping technique) and cell-associated radioactivity were also determined. The data are normalized to cell protein. C denotes the amount of radioactivity associated with cells after the initial 3 min incubation at 37 °C; E represents the same sample after stripping surface radioactivity. ▨, internalized radioactivity at the subsequent incubation (1–4 mins) at 37 °C; ■, the sum of radioactivity in the media as well as that removed from the cell surface.

difference (Buys *et al.*, 1987). We also measured the rate of return of receptors to the cell surface under conditions in which cells were stripped or not, and again saw no effect of the stripping procedures on recycling rates (Ward *et al.*, 1989). Therefore the results in Fig. 3 indicate that little internalized ligand was recycled back to the cell surface, and there was no significant difference between the two ligands. The results of these experiments demonstrate that diacytosis cannot explain the observed differences in K_e.

Topological distributions of receptors are not responsible for differences in internalization rates

Differences in internalization rates could be due to differences in the topological distributions of surface receptors. Hopkins (1985) demonstrated that in A431 cells Tf receptors were inserted into the membrane at the cell periphery and then had to migrate to coated pits located at some distance away. This observation leads to the hypothesis that differences in internalization rate may be due to differences in the surface distribution of unoccupied

Fig. 4. Measurement of K_e by surface receptors and those present in the internal pool

Cells were incubated with saturating amounts of either MAN-BSA or aM.T for 60 min at 37 °C. The cells were placed at 0 °C and washed with HBSS. The cells were then placed at 37 °C in the presence of either aM.¹²⁵I-T or ¹²⁵I-MAN-BSA, and the specific internalization rate was determined as described in the legend to Fig. 1. The specific internalization rate was also determined in cells that were treated identically except that they had not been exposed to non-radioactive ligand. Internalized aM.¹²⁵I-T in control cells (□) and in cells exposed to non-radioactive ligand (■), and internalized ¹²⁵I-MAN-BSA in control cells (△) and in cells exposed to the non-radioactive ligand (▲), was measured.

receptors. Some species of receptors may be located at or near coated pits, whereas others might be randomly distributed and therefore have a greater distance to migrate to coated pits. To test this idea, three different approaches were taken to alter the surface distribution of receptors. Internalization of unoccupied receptors is slower than internalization of occupied receptors, and after exposure of cells to ligand there is a marked reduction in the number of surface receptors, most receptors being present in the internal pool (Ward *et al.*, 1989). The rate of dissociation of MAN-BSA or aM.T is so slow that ligand does not dissociate from surface receptors even at 37 °C. Thus, in cells exposed to non-radioactive ligand, uptake of radioactive ligand would only be mediated by receptors that had previously been within the cell. Exposure of cells to non-radioactive ligand results in a 90% reduction in surface receptor number. This was determined by removing surface-bound non-radioactive ligand followed by measurement of the binding of radioactive ligand. Most remaining receptors were occupied by ligand, as determined by the addition of radioactive ligand without stripping. Thus less than 1.0% of the original surface receptors were unoccupied. Because most are inside the cell at the start of the experiment (addition of radioactive ligand), there should not be any inherent difference in surface receptor distribution. The rate of internalization, mediated by receptors that had been within the cell, was identical with that carried out by receptors present on the surface (Fig. 4).

A similar result was obtained using a different protocol. Cells were treated with 0.01% trypsin at 0 °C. This procedure destroys mannose-terminal glycoprotein receptors but spares those receptors localized within the endocytic apparatus (Stahl *et al.*, 1980). aM.P receptors are not sensitive to trypsin treatment and therefore serve as a control to insure that trypsin treatment does not alter endocytic activity *per se*. Following trypsin treatment, cells were placed at 37 °C and the K_e was measured to demonstrate

Fig. 5. Effect of trypsin on internalization rate

Cells were incubated at 0 °C for 30 min in the presence or absence of 0.01% trypsin and the cells were washed with HBSS containing 0.1% soybean trypsin inhibitor. Cells were shifted to 37 °C in the presence of aM.¹²⁵I-T or ¹²⁵I-MAN-BSA. At specified times, cells were placed at 0 °C and washed with HBSS, and internalized and surface-bound radioactivity was determined. Internalized ligand was calculated as described in the legend to Fig. 1. Internalization plots of ¹²⁵I-MAN-BSA in control (△) and trypsin-treated (▲) cells, and of aM.¹²⁵I-T in control (□) and trypsin-treated cells (■) are shown.

that this manoeuvre did not alter endocytic activity (Fig. 5). Even though trypsin treatment destroyed 90% of surface receptors (results not shown), the K_e of ¹²⁵I-MAN-BSA was similar in trypsin-treated and control cells.

A potential caveat to the above experiments is that the rate at which unoccupied receptors regain their original surface distribution might be faster than the rate of ligand binding (Wiley, 1985). Thus, prior to the binding of ligand to receptor, the receptors may have already regained their differential distribution. To test this possibility we employed a condition which randomizes surface receptors. Incubation of cells in hyperosmotic medium results in an inhibition of receptor-mediated internalization (Daukas & Zigmond, 1985; Oka & Weigel, 1988). Correlated with this inhibition is the disappearance of coated pits from the cell surface and a random distribution of previously clustered surface receptors (Heuser & Anderson, 1989). We took advantage of this observation to effect differences in internalization rates. Cells were incubated at 0 °C with ¹²⁵I-MAN-BSA or aM.¹²⁵I-T to occupy surface receptors either in iso-osmotic medium or in medium made hyperosmotic by the addition of sucrose. The cells were then washed and incubated at 37 °C in either hyper- or iso-osmotic medium and the rate of internalization was measured. In hyperosmotic medium there was a marked inhibition of the internalization of both ligands (Fig. 6a versus Fig. 6b). Electron micrographs of control and hyperosmotic-treated cells were analysed for coated pits (results not shown). Few observable coated pits were seen in cells treated with hyperosmotic buffers (approx. 5–8% of control). When cells previously incubated in hyper-osmotic medium were incubated in iso-osmotic medium, internalization recovered during the time course measured, although not to normal levels. The specific internalization rate of ¹²⁵I-MAN-BSA, however, was still faster than that of aM.¹²⁵I-T. These results suggest that the difference in internalization rates is not due to differences in surface receptor distribution.

Fig. 6. Effect of hyperosmotic treatment on the internalization rates of aM.¹²⁵I-T and ¹²⁵I-MAN-BSA

Cells were incubated in either iso-osmotic or hyperosmotic buffers (0.45 M-sucrose in Hanks MEM) at 37 °C for 10 min. The cells were then placed at 0 °C, incubated with ligand, washed extensively and shifted to 37 °C, and at the indicated times surface as well as internalized radioactivity was measured as described in the legend to Fig. 2. (a) Cells maintained in iso-osmotic media; (b) cells either maintained in hyperosmotic buffers throughout the experiment (■) or incubated in hyperosmotic buffers and then placed in iso-osmotic buffers (▲), ¹²⁵I-MAN-BSA, △, aM.¹²⁵I-T.

DISCUSSION

We considered a number of different explanations for receptor-specific internalization rates. Differences in internalization could result from the existence of ligand-specific coated pits, each of which is internalized with a characteristic rate constant. Even though recent studies suggest this possibility (Gorman & Poretz, 1987; Goldberg *et al.*, 1987), two lines of evidence make this unlikely. First, morphological studies have demonstrated that more than one type of receptor-ligand complex can enter the same coated pit (Carpentier *et al.*, 1982). Second, we have demonstrated using the horseradish peroxidase/diaminobenzidine technique that there are no ligand-specific endocytic vesicles in two different cell types using five different ligands (Ajioka & Kaplan, 1987; Ward *et al.*, 1989). These observations do not rule out the possibility that ligand-specific coated pits fuse

rapidly after internalization. There are studies which demonstrate fusion *in vivo* among early endosomes (Salzman & Maxfield, 1988; Ward *et al.*, 1990). We think that the number of receptors is such that it is unlikely that there are a large number of receptor-specific coated pits.

Differences in internalization rates could result from inherent differences in the surface receptor distribution. For example, certain species of receptors may be localized in coated pits, whereas others may be randomly distributed, and differences in internalization rate may reflect the time required for the randomly distributed receptor to migrate to coated pits. A number of experimental approaches argue against this possibility. There were no differences in the specific internalization rates exhibited by receptors present on the surface and those that had been within the endocytic apparatus. Additionally, the difference in relative internalization rates persisted when cells were placed under conditions in which surface receptor distribution was randomized.

A third possibility is that differences in internalization rate may reflect differences in diffusion rates between randomly distributed receptors. Although there are no direct data that would rule out this possibility, several considerations make it unlikely. There is little evidence that diffusion times are rate-limiting for endocytosis (Goldstein & Weigel, 1983; Wiley, 1985). For a given membrane molecule, large structural deletions in either the cytoplasmic or the external domain have little effect on the diffusion rate (Lehrmann *et al.*, 1985; Prywes *et al.*, 1986); suggesting that lateral diffusion rates of receptors are not related to or affected by the size of the membrane protein or that of the ligand.

The fourth possibility, and the one that we favour, is that differences in internalization rates reflect differences in the 'capture' rate of receptors by the coated pit. For example, membrane proteins may be freely mobile, but if they cannot cluster in coated pits they are unable to be internalized (Anderson *et al.*, 1981). These proteins could either be excluded from coated pits, by being immobilized at a distal site, or are simply unable to be captured by coated pits. Mutants in the cytoplasmic domain of the low-density-lipoprotein receptor exist in which the only observable effect is a lack of localization in coated pits (Lehrmann *et al.*, 1985). Similarly, some mutants in the cytoplasmic domain of the EGF receptor also exhibit an inability to both cluster in coated pits and be internalized (Prywes *et al.*, 1986). Studies demonstrate the existence of freely diffusible membrane proteins which are not internalized (Ishihara *et al.*, 1988). These observations suggest that internalization does not reflect a default pathway, but rather is the result of an interaction between membrane receptors and elements present in the coated pit. Several groups have identified proteins associated with coated pits ('adaptins'; clathrin-associated proteins) which are thought to mediate the binding of receptors (Unanue *et al.*, 1981; Pearce & Bretscher, 1981; Pearce, 1985; Prasad & Lippoldt, 1988; Virshup & Bennett, 1988; Ahle & Ungewickell, 1989). However, to date there is little information on the affinity of receptors for these adaptins or on the stoichiometry of adaptins and receptors.

Previously we demonstrated that the internalization of large particles such as zymosan or IgG-coated red blood cells by alveolar macrophages did not affect the internalization rate of a number of different recycling receptors. These observations are similar to those of Ciechanover *et al.* (1983), who demonstrated that in cultured hepatoma cells internalization of asialoglycoproteins and Tf were independent of each other and of receptor occupancy. An inability to saturate internalization might seem to argue against a role for adaptins. Alternatively, the concentration of receptors may be such that under most conditions binding of receptors to adaptins is not rate-limiting. Analysis of the literature

reveals two reports in which the specific internalization rate constant decreases with receptor occupancy. The specific internalization rate of vitellin by *Xenopus* oocytes and of EGF by cultured A431 cells decreases with receptor occupancy (Opresko & Wiley, 1987b; Wiley, 1988). Even at receptor occupancies at which the rate of EGF receptor internalization becomes markedly decreased there is no effect on the internalization of transferrin. It was suggested that saturation of receptors within the endocytic apparatus only occurs at extremely high receptor densities. For example, there is no evidence of saturable internalization of EGF receptors in fibroblasts, a cell type in which the density of EGF receptors is three orders of magnitude lower than in A431 cells. The second conclusion is that different receptors may interact with different components in coated pits, i.e. different adapters, which may vary in concentration.

Our data also indicate that there are elements extrinsic to receptor molecules which are important in specifying internalization rates. We found wide variations in the absolute rate of ligand internalization with different cell preparations. Even though the absolute rate of ligand accumulation varied, the hierarchical distinctions in internalization were maintained. The cells used in this study were obtained from rabbits that had been injected with Freund's complete adjuvant. This agent produces massive pulmonary granulomas and results in a 10–100-fold increase in cell number due to the recruitment of blood monocytes into the lung. While in the lung these cells undergo a number of biochemical changes which distinguish them from blood monocytes or other macrophages. Among such changes are increases in aerobic metabolism, lysosomal enzyme levels and bacteriocidal activity. We suspect that the difference in absolute rate may reflect cells at different developmental stages.

Differences in absolute rates of internalization have been observed for the low-density-lipoprotein receptor introduced by genetic manipulation into human or hamster cells (Beisiegel *et al.*, 1981). Internalization and clustering of the low-density-lipoprotein receptor differs between fibroblasts and epithelial cells (Anderson *et al.*, 1981). The observation that the same receptor molecule (gene) behaves differently in different cell types clearly indicates that there is some feature extrinsic to the receptor which regulates internalization. The fact that one can introduce the same receptor into two different cells or examine two different receptors in the same cell offers an opportunity to define those factors.

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