

Pinocytosis in human synovial cells in vitro. Evidence for enhanced activity in rheumatoid arthritis.

K A Krakauer, R B Zurier

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Research Article

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Pinocytosis in Human Synovial Cells In Vitro

EVIDENCE FOR ENHANCED ACTIVITY IN RHEUMATOID ARTHRITIS

KATHRIN A. KRAKAUER and ROBERT B. ZURIER, *Division of Rheumatic Diseases, Department of Medicine, University of Connecticut Health Center, Farmington, Connecticut 06032*

ABSTRACT Human synovial tissue cells in monolayer can be shown to take up and digest a soluble protein, horseradish peroxidase (HRP). Uptake of HRP was linear with increasing concentrations of substrate and cell protein and with time for up to 4 h. Low temperature (4°C), and sodium fluoride, an inhibitor of glycolysis were the most effective metabolic inhibitors of endocytosis. In addition, colchicine, an inhibitor of microtubule assembly, and yeast mannan, an inhibitor of mannose-specific receptors, reduced HRP uptake. Synovial cells from patients with rheumatoid arthritis (RSC) demonstrated a statistically significantly higher rate of endocytosis (247 ± 107 ng HRP/100 μ g cell protein per 2 h.) than cells from control, nonrheumatoid patients (NSC) (100 ± 80 ng HRP/100 μ g cell protein per 2 h.). Thus, it is possible to discriminate RSC from NSC by their quantitatively different rates of endocytosis.

Digestion of HRP by synovial cells is statistically significant by 6 h after uptake. A faster initial rate of digestion was seen in RSC. Over the first 6–8 h of incubation 42% of the endocytosed HRP was still cell-associated in RSC and 67% remained in NSC cultures. However, by 24 h 20–30% of endocytosed HRP was found in both types of cultures. These results indicate that endocytosed molecules may accumulate more rapidly in RSC and persist within their lysosomes for a longer time than in NSC.

The quantitative determination of enhanced endocytosis by RSC compared with NSC suggests that this increased activity may have a role in the pathological function of synovial tissue in rheumatoid arthritis.

INTRODUCTION

Morphologic (1, 2) and biochemical (3) evidence indicate that endocytosis and lysosomal enzyme activity

Dr. Krakauer is a postdoctoral fellow of the Arthritis Foundation. Address reprint requests to Dr. Krakauer, Hospital of the University of Pennsylvania, Philadelphia.

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of synovial tissue from individuals with rheumatoid arthritis are enhanced when compared with control synovium. However, these differences have not been functionally quantitated. Endocytosis and subsequent intra- and extracellular digestion by synovial cells are most likely responsible, at least in part, for removal of microorganisms and erythrocytes, and for metabolism of connective tissue and synovial fluid components. In addition, these processes in synovial cells may mediate the destruction of articular tissue observed in rheumatoid arthritis (4).

Studies of synovial tissue in monolayer cultures have suggested that synovial cells from individuals with rheumatoid arthritis (RSC)¹ may be in a state of "activation" compared with nonrheumatoid synovial cells from individuals with other joint diseases or traumatic injury (NSC). Thus, RSC spontaneously secrete large quantities of collagenase (5), plasminogen activator (6), neutral proteinase (7), and prostaglandin E₂ (5). RSC cultures, therefore, resemble elicited peritoneal macrophages that release similar enzymes spontaneously (8–11) and are considered activated (12) compared with resident peritoneal macrophages. In addition, thioglycolate-elicited macrophages have higher rates of pinocytosis than resident peritoneal macrophages (13). Thus, stimulated endocytosis and subsequent intralysosomal digestive activity may be part of a state of activation of RSC and may contribute to the pathological role of synovial cells in rheumatoid arthritis (4).

Very few studies have compared nonrheumatoid with rheumatoid synovial cells. The experiments reported here were performed in an effort to identify quantitative differences between RSC and NSC in monolayer cultures in terms of pinocytic activity and subsequent intralysosomal proteolytic digestion. Our

¹ *Abbreviations used in this paper:* EMEM, Eagle's minimum essential medium; HRP, horseradish peroxidase; LDH, lactate dehydrogenase; NSC, nonrheumatoid synovial cells; RSC, rheumatoid synovial cell.

studies show that, although the kinetics of endocytosis are similar in NSC and RSC, differences in rates of pinocytosis make it possible to discriminate between these cultures. The increased rate of endocytosis evident in RSC is additional evidence that rheumatoid synovial tissue is in a state of activation. Furthermore, endocytosis appears due to a unique synovial cell rather than infiltrating cells or fibroblasts.

METHODS

Synovial cell cultures. For all experiments, synovial tissue specimens were treated identically. Tissue was obtained during surgery, after appropriate informed consent. Monolayers were prepared by the method of Dayer et al. (5). Briefly, tissue was rinsed with Eagle's minimum essential medium (EMEM) (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) containing antibiotics and placed in a petri dish containing 10 ml of 4 mg/ml collagenase (type II, Sigma Chemical Co., St. Louis, Mo.) in EMEM, and then carefully minced into small fragments with a scalpel blade. The tissue was incubated for 2 h at 37°C with frequent pipetting to breakup clumps of cells. An equal volume of trypsin (0.25% in versene, 1:5,000) was added and incubation continued for 30 min. The suspension was then centrifuged (1,000 rpm, 10 min), washed once with trypsin versene solution, twice with Hanks' balanced salt solution and once with 10% fetal calf serum (Associated Biomedical Systems, Inc., Buffalo, N. Y.) in EMEM before being counted in an improved Neubauer hemocytometer. Cells were plated in 35-mm plastic dishes (Lux Scientific Corp., Newbury Park, Calif.) at a concentration of $0.5-1 \times 10^6$ cells/dish. After overnight incubation, the cultures were washed well with 10% fetal calf serum/EMEM and fresh medium added. Cells were routinely grown in 10% heat inactivated fetal calf serum/EMEM containing 100 U penicillin and 100 μ g streptomycin/ml. Primary cultures were used throughout and cells were allowed to grow for 4-40 d before being used.

Measurement of endocytosis. The rate of endocytosis was measured by the method of Steinmann and Cohn (14) using horseradish peroxidase (HRP) as a substrate at a concentration of 1 mg/ml in 10% fetal calf serum/EMEM. After incubation of HRP with cells, culture dishes were carefully washed 10 times over a period of 1 h with 2-4 ml phosphate-buffered saline. Cell lysates were prepared in 0.1% Triton X-100 (wt/vol in water) (Rohm and Haas Co., Philadelphia, Pa.) and assayed for peroxidase activity (14) and for protein (15). For the peroxidase assay, 0.05 ml 1% *O*-dianisidine (Sigma Chemical Co.), 0.06 ml 0.3% hydrogen peroxide, and 6.0 ml phosphate buffer, pH 5.0 were mixed just before use. Change in absorbance over 3 min of 1 ml of this reaction mixture containing cell lysate was measured at 460 nm in a Beckman (Beckman Instruments, Inc., Fullerton, Calif.) 24 spectrophotometer. Endogenous peroxidase activity was similar for both RSC and NSC and never accounted for more than a few nanograms of activity.

Protein and viability determinations. Protein was determined according to the method of Lowry et al. (15). All samples were centrifuged at 3,000 rpm for 5 min before reading to eliminate the flocculant caused by Triton. Bovine serum albumin was the standard protein.

Cell viability was determined by measuring the proportion of cytoplasmic lactate dehydrogenase (LDH) that leaked into the medium. LDH assays were performed by the method of Bergmeyer (16) within 24 h after termination of an experiment.

Metabolic inhibitors, lectins, and charged polymers. Cells

were preincubated for 1 h with each agent and then 1 mg/ml HRP added for an additional 1 h. DEAE dextran (5×10^5 mol wt) and dextran sulphate (5×10^5 mol wt) were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. All metabolic inhibitors and other polymers were purchased from Sigma Chemical Co.

Digestion of HRP. After uptake and thorough washing (eight times) in sterile phosphate-buffered saline, digestion of HRP was followed by the loss of peroxidatic activity from cells over time. Media and cells were monitored for activity. It remains to be investigated whether this loss of activity reflects enzymatic degradation of HRP or denaturation of the active site.

Light and electron microscopy. Following uptake of HRP by synovial cells, cultures were washed in phosphate-buffered saline and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 for 5 min at room temperature (14). These cells and control (unincubated) cells were then stained for peroxidase by incubation for 30 min with 50 mg/100 ml diaminobenzidine in 0.05 ml Tris-HCl buffer, pH 7.6, and 0.01% hydrogen peroxide. Light microscopy was performed using a Nikon inverted phase contrast microscope (Nikon Inc., Instrument Div., Garden City, N. Y.). For electron microscopy, cells were postfixed in 2% osmium tetroxide for 60 min, washed and scraped off the dishes. After dehydration in graded alcohols and embedding in epon-araldite mixture, cells were examined with a Philips 300 electron microscope (Philips Electronic, Mahwah, N. J.) by Mrs. Constance Gillies (Department of Pathology, University of Connecticut Medical School).

RESULTS

Uptake of HRP over increasing HRP concentrations. Uptake of HRP by synoviocytes was linear over a large concentration range. Linearity of uptake by NSC over 1 and 2 h at concentrations ranging from 0.1 to 10 mg/ml is demonstrated in Fig. 1. Similar kinetics were seen for RSC. A load of 1 mg/ml HRP was therefore used routinely in all subsequent experiments.

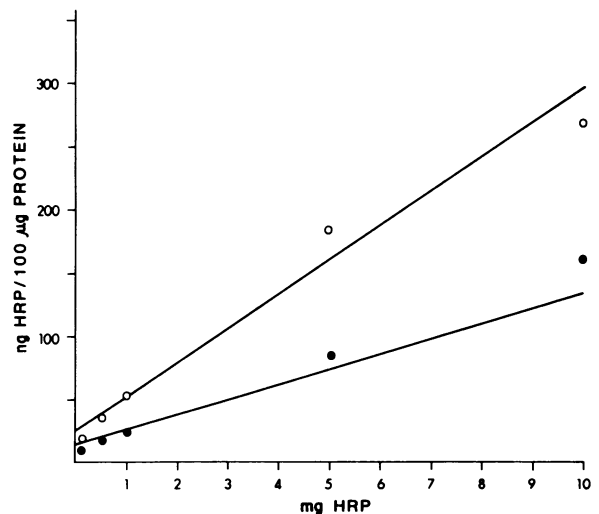


FIGURE 1 Uptake of HRP by NSC with increasing HRP concentrations. Cultures of NSC were incubated for 1 (●) or 2 (○) h at 37°C with concentrations of HRP from 0.1 to 10 mg/ml.

Uptake of HRP over time. Uptake of HRP by RSC and NSC was linear for the first 2–4 h of incubation. Representative experiments for NSC and RSC cultures are shown in Fig. 2. Significant uptake was detectable in both RSC and NSC only after 15 min incubation. In some RSC cultures the rate of uptake appeared to decrease between 2 and 4 h. This may reflect digestion of HRP which could be detected by 2 h after HRP uptake (Fig. 5). The rate of uptake of HRP/100 μg cell protein per min (calculated from Fig. 2) for RSC decreased from 30 min to 4 h (2.66 ng HRP/100 μg cell protein per min at 30 min and 1.35 ng HRP/100 μg cell protein per min at 240 min). For NSC the rate of uptake remained fairly constant (0.93 ng/100 μg cell protein per min for the first 30 min and 0.89 ng/100 μg protein per min by 240 min). These results suggest that an initial rapid rate of removal of HRP by RSC decreased with time and was completely absent in NSC. These results may also indicate that whereas NSC are a homogeneous population with respect to pinocytotic activity, RSC are heterogeneous. RSC cultures appear to consist of a population of cells that has a rapid initial rate of uptake and others that have slower rates and more closely resemble NSC.

Linearity of HRP uptake with increasing cell protein. HRP accumulation per culture was linear with increasing cell protein (Fig. 3; $r^2 = 0.94$). The specific activity of HRP uptake by RSC (Fig. 3) and NSC (not shown) increased modestly with increased cell density. In Fig. 3 cell concentration is expressed as cell protein per 60-mm culture dishes. The data for RSC indicate that at a protein density $>30 \mu\text{g}/\text{cm}^2$ (or $\sim 1 \times 10^5$ cells/ cm^2) the specific activity was significantly greater ($P < 0.025$) than if below this value. NSC behaved in a similar manner.

The data for rates of HRP uptake by synovial cells

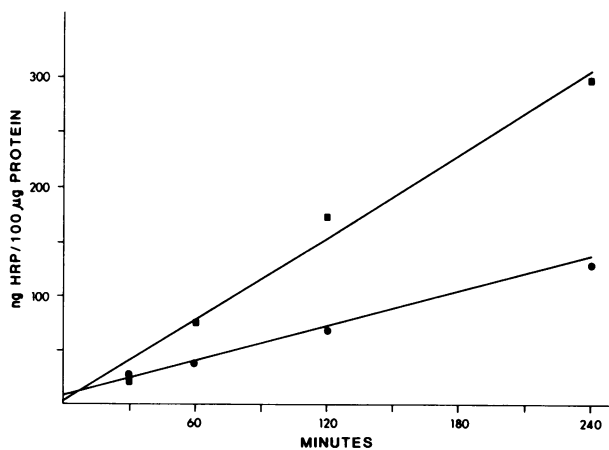


FIGURE 2 Time-course for HRP uptake. RSC (■) and NSC (●) were incubated for 30–240 min at 37°C with 1 mg/ml HRP. The lines were plotted by the method of least squares (RSC, $r^2 = 0.91$; NSC, $r^2 = 0.99$).

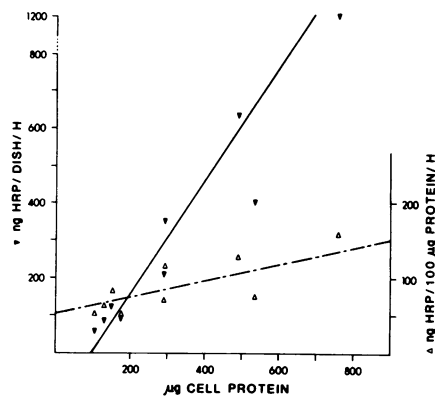


FIGURE 3 Effect of increasing cell concentration on HRP uptake. RSC were incubated for 1 h with 1 mg/ml HRP at 37°C. Cell concentration is expressed as cellular protein per 60-mm dish where 100 μg of protein is equivalent to 3.4×10^5 cells. HRP uptake is expressed as nanograms per dish per hour (▼) and as nanograms per 100 μg cell protein per hour. (△) The lines were plotted by the method of least squares (▼, $r^2 = 0.94$; △, $r^2 = 0.64$).

are summarized in Table I, and indicate that the rates of endocytosis for RSC were statistically significantly greater ($P < 0.005$) than for NSC. RSC exhibited ~ 2.5 times the specific activity of uptake compared with NSC over 2 h incubation when expressed per 100 μg of protein. For every 100 μg of cell protein, however, there were 1.5-fold the number of cells present in RSC as in NSC cultures. Despite this difference, when specific activity is expressed per 10^5 cells, the means of the uptake in these cultures remain statistically significantly different ($P < 0.025$).

Light microscopy and cytochemistry. Primary cultures of adherent synovial cells were morphologically heterogeneous. The cells present included (a) fibroblastic cells, derived from B type synovial cells and contaminating subsynovial connective tissue; (b) small, macrophagelike round cells; (c) morphologically intermediate type cells similar to type C synovial cells; (d) stellate or dendritic cells which can be immunochemically identified as cells containing collagenase (17); (e) multinucleated cells; and (f) some T lymphocytes. Fc receptor bearing cells (or the receptors per se) and T lymphocytes were not detectable in cultures older than 7–10 d (5). The proportions of cells present in different cultures varied from specimen to specimen and this variability did not seem to relate to the source of the tissue. Some cultures of NSC and RSC had as much as 80% stellate cells whereas others had a majority of fibroblastic and small, round macrophagelike cells. As the cultures aged, fibroblasts began to predominate. Interestingly, there was no significant difference between the rates of pinocytosis for either RSC or NSC when cells cultured for 1–7 d were compared with cells grown for 8–40 d.

TABLE I
Summary of Specific Activities for HRP Uptake*

RSC	n	NSC	n	RSC vs. NSC
				P
247±107 ng HRP/100 µg protein per 2 h	11	100±80 ng HRP/100 µg protein per 2 h	10	<0.005
3.4±1.3 × 10 ⁵ cells/100 µg protein	20	2.2±0.8 × 10 ⁵ cells/100 µg protein	27	<0.001
66.27±49.2 ng HRP/10 ⁵ cells per 2 h	7	30.62±13.3 ng HRP/10 ⁵ cells per 2 h	11	<0.025

* Results are expressed as mean±SD.

n, numbers of different cultures examined, each run in duplicate.

When RSC were stained for peroxidase activity after uptake of this enzyme, the endocytosed substrate was demonstrated in a variety of cell types. Macrophagelike cells were usually strongly positive as were some fibroblasts and stellate cells. Multinucleated cells and cells present in clumps were frequently positive. In most cases, however, no cell type could be singled out as the endocytic cell in either RSC or NSC cultures. RSC cultures always contained a greater number of cells that stained positively and these cells stained more intensely than those found in NSC cultures.

Electron microscopy. A macrophagelike RSC is illustrated in Fig. 4. The electron dense reaction

product of HRP-H₂O₂-diaminobenzidine is evident in vacuoles throughout the cytoplasm. Staining was not evident on the external surface of the plasma membrane.

Effect on HRP uptake of metabolic inhibitors, charged polymers, lectins, and colchicine. The most effective metabolic inhibitors of HRP uptake were (1) the glycolytic inhibitor fluoride and (2) the nonspecific depressant 4°C (Table II). Differences between NSC and RSC in susceptibility to the metabolic inhibitors studied were not detected; therefore, results for both cell types are combined in Table II. Greater than 85% of the cells remained viable (LDH determination) after incubation with all agents listed in Table II.

Charged polymers, the lectin concanavalin A (Con A), colchicine, and yeast mannan were examined for their abilities to influence synovial cell endocytosis.

TABLE II
Effect of Metabolic Inhibitors on HRP Uptake by Synovial Cells

Inhibitor	Concentration	Percent inhibition of HRP uptake*
mM		
Glycolytic		
Sodium fluoride	10	84±7 (6)
	1	45±5 (4)
	0.1	7 (1)
2-Deoxyglucose	10	10±31 (2)
Respiratory		
Sodium azide	10	8±56 (3)
	1	3±48 (7)
Oxidative phosphorylation		
2,4-Dinitrophenol	1	11±15 (2)
	0.1	0 (1)
Oligomycin	2 µg/ml	41 (1)
	10 µg/ml	46 (1)
General		
4°C		96±4 (4)

* Results are expressed as mean±SD. Numbers in parentheses represent numbers of different cultures examined, each run in duplicate.



FIGURE 4 Ultrastructural localization of HRP. RSC were incubated for 30 min with 1 mg/ml HRP and then stained with diaminobenzidine. A macrophagelike cell is illustrated. Reaction product (H) is visible in recently formed endocytic vacuoles where it forms a lining on the inner aspect of the limiting membrane. A later stage of uptake is visible as a dense reaction product which fills the vacuoles (H'). Some endogenous peroxidase activity (E) (as determined by control cells that were not given HRP) is also visible. Elaborate filopodia (F) are evident on the nonadhered side of this cell. ×12,950.

Cationic polymers DEAE-dextran (1–10 $\mu\text{g/ml}$), poly-L-ornithine (1–0.01 $\mu\text{g/ml}$), and poly-D-lysine (1–0.1 $\mu\text{g/ml}$) all had no effect on HRP uptake at concentrations that did not reduce viability. The anionic polymer dextran sulfate (100–10 $\mu\text{g/ml}$) had variable effects either not influencing or inhibiting uptake slightly. Hyaluronic acid (1–0.1 mg/ml), poly-D-glutamic acid (1–0.01 $\mu\text{g/ml}$), and heparin (10–1.0 $\mu\text{g/ml}$) all had no influence on HRP uptake. After 30 min simultaneous incubation Con A (100 $\mu\text{g/ml}$) stimulated HRP uptake 15-fold in both NSC and RSC. This stimulation of pinocytosis increased to 30-fold by 1 h.

Table III demonstrates that colchicine, a compound that inhibits microtubule assembly, significantly inhibited pinocytosis in both NSC and RSC at 1 μM . In addition, yeast mannan, an inhibitor of mannose-specific cell surface receptors (18), produced a dose-dependent inhibition of HRP uptake. Neither cell viability nor HRP activity was affected by either of these agents.

Digestion of HRP. In experiments where digestion of HRP was observed, as much as 15% of the cell-associated activity was detected in the medium over 24 h, suggesting that either some exocytosis of undigested HRP did occur or that some membrane- or dish-bound HRP was further eluted into the medium on incubation at 37°. Beyond that, however, further loss of activity appears to be the result of digestion. The rate of loss of cell-associated HRP activity was biphasic with a very rapid initial loss of activity during the first 6 h of incubation, and a much slower loss over the next 20 h (Fig. 5). These results may be further evidence for heterogeneous functioning of the endocytic lysosomal process in synovial cell cultures. The decrease in cell-associated HRP activity was statistically significant by 6 h. RSC had a significantly lower percentage of endocytosed HRP remaining in the cultures by 6–8 h when compared with NSC (42.33 \pm 21.37%, $n = 7$ vs. 61.14 \pm 23.60%, $n = 7$; $P < 0.025$ in RSC and NSC, respectively). The percentage of total HRP remaining at 24 h was similar for

TABLE III
Effect of Inhibitors of Pinocytosis on HRP Uptake by RSC

Agent	Concentration	Percent inhibition of HRP uptake*
Colchicine	10 μM	43 \pm 14% (3)
	1 μM	39 \pm 12% (4)
	0.1 μM	16 \pm 17% (4)
Yeast mannan	500 $\mu\text{g/ml}$	70%
	100 $\mu\text{g/ml}$	50%
	50 $\mu\text{g/ml}$	42%
	10 $\mu\text{g/ml}$	11%

* Results are expressed as mean \pm SD. Numbers in parentheses represent numbers of different cultures examined, each run in duplicate.

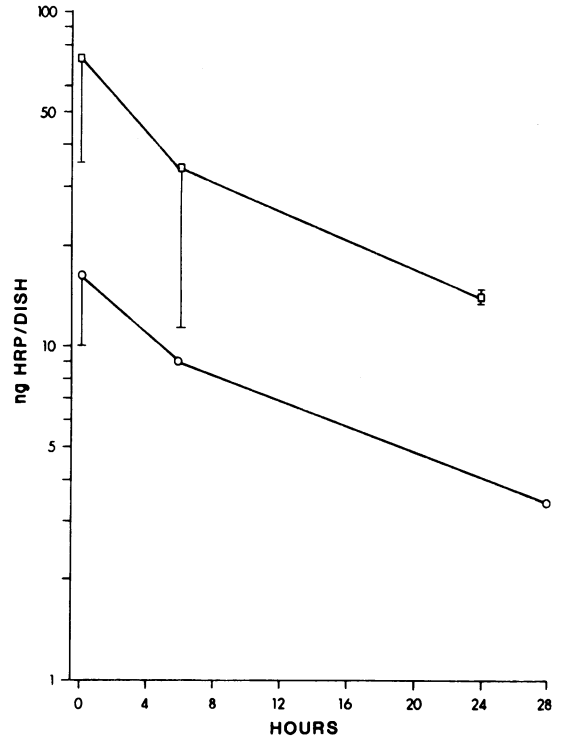


FIGURE 5 Time-course for digestion of HRP. Cells (RSC \square ; NSC \circ) were incubated for 2 h with 1 mg/ml HRP, washed thoroughly and harvested (zero time), or reincubated in HRP-free medium for up to 28 h. Results represent nanograms recovered HRP in both cells and medium. The $t_{1/2}$ for RSC was 5 h and that for NSC was 8 h. For RSC ($n = 4$) the nanograms HRP remaining at 6 h ($P < 0.005$) and 24 h ($P < 0.01$) were significantly less than that present at zero time.

RSC and NSC (20–30%). From Fig. 5 the $t_{1/2}$ for loss of HRP within RSC was \sim 5 h, whereas that for NSC was 8 h.

Digestion did not occur in all cultures. In these experiments as much as 50% of the total cell-associated HRP could be found in the medium after 24 h of incubation.

DISCUSSION

This study demonstrates that primary cultures of synovial cells from individuals with RSC exhibit a significantly higher rate of uptake of a soluble antigen (HRP) than synovial cells from individuals with other joint diseases (NSC). These results also indicate that RSC and NSC have rates of pinocytosis that more closely resemble those of macrophages (13) than of fibroblasts (19). When uptake studies were performed on passaged cultures of synovial cells, a decrease in the primary rate of uptake to that resembling fibroblasts was found in most subcultures. Thus, for the studies presented in this paper primary cultures were used throughout.

Increases in cell density stimulate HRP uptake in synovial cells, L cells (19), and epithelial cells (20).

In addition, we have noted that some RSC cultures, like L cells (21), fail to demonstrate contact inhibition of growth on reaching confluence. Thus, cultures of RSC could achieve higher cell densities than those of NSC during a comparable culture period, and it may be this phenomenon that is at least partially responsible for the enhanced pinocytosis found in RSC on a per cell basis. Enhanced endocytosis may be the result in part, of as yet unknown events induced by high cell density. Some of these may include cell surface phenomena (20), cell-cell interactions, metabolic alterations, and alterations in concentrations of and/or responses to such regulatory molecules as cyclic nucleotides and prostaglandins. As proliferative synovium *in vivo* is the "sine qua non" of rheumatoid arthritis, these events may be important to the pathological process in rheumatoid arthritis.

The mechanism of uptake of HRP by synovial cells probably involves both macro- and micropinocytosis. With the electron microscope many micropinocytotic vesicles are observed in the cytoplasm, and free surface of many cells is covered with an elaborate configuration of microvilli. These microvilli appear to fuse with each other to form endocytic vacuoles. Furthermore, from our studies we can conclude that uptake of HRP appears to be both by fluid phase (21) and adsorptive pinocytosis. Linearity of uptake is demonstrable over a wide range of substrate concentrations (0.10–10 mg/ml) and adsorption of HRP at 4°C never exceeds 10% of the activity found at 37°C. This compares well with the amount of HRP removed by trypsinization after 2 h of uptake.

Con A is thought to stimulate endocytosis by forming bridges between cell surface glycoproteins and "low uptake" or poorly endocytosed forms of soluble glycoproteins such as β -glucuronidase (22). In our experiments Con A may be forming aggregates with a low uptake form of HRP and thus enhancing endocytosis of a portion of this preparation. Although we have not investigated this material, Steinman and Cohn (14) have reported that HRP from the same source (Sigma Chemical Co.) as ours was composed of five separate enzymatic bands on agar and agarose electrophoresis. Each of these forms may be taken up with different avidity. The heterogeneity of this preparation may also help to explain the conflict between linearity of uptake over a large HRP concentration range and the 70% inhibition of uptake we find with mannan (Table III). Mannan is a specific inhibitor of mannose receptor-dependent uptake (18). Addition to cells of mannan, either simultaneously with or before HRP, may inhibit the "high uptake" form(s) of HRP that is rapidly interiorized and is sensitive to inhibition at 4°C. Interiorization of the low uptake form(s) may be by fluid phase and thus would be concentration dependent. These speculations are supported by our observation that maximum inhibition of uptake (67–

70%) is achieved with mannan to HRP ratios of 10:1, 2:1, 1:1, and 1:2. Mannan is not a nonspecific inhibitor of pinocytosis as uptake of [³H]sucrose was unaffected by its presence (unpublished observations).

Studies of metabolic inhibitors presented here indicate that glycolysis is the most important source of energy for endocytosis in synovial cells. NSC and RSC are equally susceptible to these inhibitors. Dingle and Thomas (23) and Castor (24) have demonstrated increased glucose and oxygen metabolism and increased lactate formation in rheumatoid synovial tissue slices and cells in culture. This enhanced metabolism may be partially responsible for the increased rate of endocytosis seen in rheumatoid arthritis cells. All forms of endocytosis in macrophages (macropinocytosis, micropinocytosis, and phagocytosis) are sensitive to inhibitors of glycolysis (25). In fibroblasts, micropinocytosis of HRP is partially inhibited by glycolytic inhibitors and partially by inhibitors of oxidative phosphorylation and respiration (19). Thus, these data suggest that synovial cells resemble macrophages more than fibroblasts in their sensitivity to these inhibitors. Because there are no differences between NSC and RSC in energy requirements for endocytosis, similarly derived cells may be responsible for HRP uptake in both cultures.

Charged polymers stimulate pinocytosis in macrophages and fibroblasts. Cationic polymers induce vacuole formation in fibroblasts (26) whereas anionic polymers are effective in macrophages (25). Neither anionic nor cationic polymers affected HRP uptake under the conditions tested. These studies indicate that synovial cells are different from both fibroblasts and macrophages in their sensitivities to charged polymers; and suggest that endocytosis is occurring primarily in a unique synovial cell rather than contaminating macrophages.

Colchicine, a compound that impairs microtubule integrity (27), partially inhibited uptake of HRP by synovial cells indicating that endocytosis of HRP by synoviocytes depends at least in part, on the presence of intact microtubules. Similar results have been obtained for ¹³¹I-albumin uptake by macrophages (28).

Although variable in amount, digestion of HRP by synovial cells was detected. In some experiments enzyme was simply released into the medium. This was not the result of cell death or loss of cells because peroxidase release was not accompanied by release of LDH. Thus, in contrast to studies in which exocytosis was not observed in macrophages (14), fibroblasts (19), and rat yolk sac (29) during periods when digestion of proteins was being detected, exocytosis appears to occur in synovial cells.

The time it takes for half of the endocytosed HRP to be digested by RSC and NSC ($t_{1/2}$) is approximately the same as it is for fibroblasts (19) and resident peritoneal macrophages (14).

The high rates of endocytosis found in synovial cells resemble those of professional phagocytes. In normal synovial cells this function is probably important to the turnover of joint fluid and synovial tissue constituents such as hyaluronic acid, plasma proteins, collagen, fibrin, and proteoglycans. In rheumatoid arthritis, increased rates of endocytosis may occur in attempts to clear such molecules as rheumatoid factors and their immune complexes, altered collagen and collagen-anti-collagen-immune complexes, immunoglobulins, soluble mediators of inflammation, and/or viral, bacterial, or mycoplasmal antigens.

Because the rate of uptake by RSC is so much greater than NSC, but rates of digestion are similar, endocytosed molecules may accumulate more rapidly and persist longer in RSC. Thus, activated and proliferating synovial cells may become pathogenic to articular tissues by endocytosing large amounts of joint constituents which they only slowly digest. This in turn may induce the release of degradative enzymes and mediators of inflammation that may help perpetuate chronic inflammation and joint tissue injury.

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