Autocatabolism of Surface Macromolecules Shed by Human Melanoma Cells

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ABSTRACT

The fate of cell surface macromolecules released by human melanoma cells in vitro was studied. Labeled surface macromolecules released by lactoperoxidase-radiiodinated melanoma cells were incubated with unlabeled cells. It was found that some of these macromolecules were autocatabolized to acid-soluble fragments by the cells which had released them. Degradation did not occur in the absence of cells, was almost completely inhibited at 4°C, and was partially suppressed by cytochalasin B (10 μg/ml) and by some inhibitors of energy production, i.e., iodoacetamide (10⁻⁴ M) and a combination of 2-deoxyglucose (18 mg/ml) and 2,4-dinitrophenol (10⁻⁴ M). Radioiodinated surface macromolecules were degraded much more rapidly than radioiodinated serum proteins. Thus, degradation required the presence of cells, was in part an active process, and was selective. These results suggest that one of the pathways for the turnover of surface macromolecules on tumor cells is shedding followed by autocatabolism of the shed material by the cells which they have released.

INTRODUCTION

The turnover of surface macromolecules is believed to have an important impact on the behavior and growth of malignant cells (4, 22). It may lead to the presence of soluble tumor antigens in body fluids (1) which in turn may stimulate or block tumor-specific immune responses and thus influence resistance to cancer. There is considerable evidence to suggest that one of the major pathways for the turnover of the most external macromolecules on the surface of normal and malignant cells is release to the outside of the cells (4, 9, 10). Viable tumor cells release a variety of surface macromolecules including tumor-associated antigens (4, 9). This process occurs very rapidly. In earlier studies, we have found that approximately 60% of the macromolecules and 40% of some tumor antigens expressed on the external surface of human melanoma cells can be released within 3 hr (6). However, little is known about the fate of the shed material following its release.

As an initial step towards understanding this problem, we have studied the catabolism of surface macromolecules released by human melanoma cells in vitro. We have found that some of the shed macromolecules can be degraded by the cells which have released them. The catabolic process requires the presence of cells, is partially energy dependent, and is selective. These findings suggest that one of the pathways for the turnover of membrane macromolecules on tumor cells is autocatabolism of shed material and that, consequently, the ultimate amount of surface material which accumulates in body fluids will depend in part on the balance between the rate of its release and that of its degradation by tumor cells.

MATERIALS AND METHODS

Cells. Human melanoma cells obtained from surgically excised metastatic nodules were established in tissue culture as described previously (8). Stock cells were grown as monolayers in 20-× 100-mm plastic culture dishes containing 10 ml of complete growth medium consisting of Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 10% (v/v) FCS and penicillin (200 units/ml), streptomycin (200 μg/ml), and Fungizone (0.25 μg/ml) (Grand Island Biological Co., Grand Island, N. Y.). To prepare experimental plates, cells from several stock dishes were collected with a rubber policeman and pooled. The cells were resuspended at a concentration of 4 × 10⁶ cells/ml in complete growth medium, and 5 ml of the suspensions were seeded into replicate 60-mm culture dishes. For most experiments, the cells were incubated for 18 hr, at which point they formed confluent monolayers containing approximately 3 to 4 × 10⁶ melanoma cells/dish.

Radiolabeling of Cells. Surface macromolecules on melanoma cells were radioiodinated by the lactoperoxidase technique. This procedure labels only external proteins on viable cells, and we have shown that this is also true for melanoma (8). The cells were suspended with a rubber policeman, collected by centrifugation at 250 × g for 5 min, and resuspended in HBSS at a concentration of 7 × 10⁶ cells/ml. To 2 ml of the cell suspension were added sequentially 1 mCi of ¹²⁵I-sodium (New England Nuclear, Boston, Mass.) in 0.1 ml of PBS, 0.5 mCi of lactoperoxidase (Sigma Chemical Co., St. Louis, Mo.) in 0.5 ml of PBS, and 0.02 ml of 0.3% hydrogen peroxide. The cells were incubated at 27°C and gently agitated at 1-min intervals. After 10 min, 10 ml of HBSS were added to the reaction mixture, and the cells were sedimented and washed 3 times with 10 ml of HBSS.

In some experiments, melanoma cells were labeled internally with [¹²⁵I]leucine as described previously (7). Briefly, 2 × 10⁶ cells were incubated in culture dishes with 10 ml of complete growth medium containing 0.5 mCi of [¹²⁵I]leucine (30 to 50 Ci/mmol; New England Nuclear) at 37°C. After 48 hr, the medium was removed and the cells were washed 3 times with 5 ml of HBSS and used as described below.

Collection of Shed Macromolecules. Seven × 10⁶ radiolabeled cells were incubated with 10 ml of culture medium, with or without 10% FCS, at 37°C. After 3 hr, the medium, henceforth called labeled medium and containing labeled macromolecules released during this time, was collected. Prior studies (6) have shown that over 60% of labeled surface macromolecules are released by melanoma cells in that time. The radioidination procedure does not appear to have a major effect on release since the amount of protein [measured by the method of Lowry et al. (20)] and of cell surface melanoma-associated antigens (measured by inhibition of specific immunoprecipitation) released by radioiodinated or mock-iodinated melanoma cells does not differ significantly from that released by untreated cells (10). Floating cells and particulate matter were removed by centrifugation at 4000 × g for 10 min.

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1 This work was supported by Grant CA13844-08 from the USPHS.
2 To whom requests for reprints should be addressed, at Department of Dermatology, New York University School of Medicine, 560 First Avenue, New York, N. Y. 10016.
3 The abbreviations used are: FCS, fetal calf serum; HBSS, Hanks’ balanced salt solution; PBS, phosphate-buffered saline; BSA, bovine serum albumin; DNP, 2,4-dinitrophenol; DMSO, dimethyl sulfoxide.
4 Unpublished observations.
Degradation of Macromolecules. The ability of cells to degrade macromolecules which they release was studied by incubating labeled medium with unlabeled cells. Five ml of labeled medium, diluted 1:5 (v/v) with fresh growth medium supplemented with 10% FCS, were added to replicate monolayers of unlabeled cells in 60-mm culture dishes and incubated at 37°. Zero time was set at the addition of labeled medium. At intervals thereafter, 0.4-ml aliquots of medium were collected, and the amount of radioactive associated with acid-insoluble macromolecules was determined as described subsequently. The percentage of degradation was calculated from the following formula:

\[
\text{acid-insoluble cpm at } 0 \text{ time} - \text{acid-insoluble cpm of sample} \times 100.
\]

This and all other experiments were performed on duplicate plates and the average value was used.

The ability of cells to degrade serum proteins was studied in a similar fashion. The stated concentration of radiolabeled protein was incubated in 5 ml of growth medium, without FCS, with replicate confluent monolayers of unlabeled cells in 60-mm culture dishes.

Some experiments were conducted in the presence of metabolic inhibitors or other reagents to study their effect on degradation. The chemicals used included DNP (Grade II), 2-deoxyglucose, crystalline iodoacetamide, colchicine (A grade), cytochalasin B (B grade), amionocaproic acid, soybean trypsin inhibitor, and phenylmethylsulfonylfluoride (all from Sigma). Stock solutions of the chemicals were made up in HBSS to a concentration 100-fold greater than that used experimentally. Stock solutions of cytochalasin B were made up in 100% DMSO and of DNP in 95% ethanol. For use, 0.05 ml of stock solution in 5 ml of labeled medium was added at zero time to dishes or unlabeled melanoma cells. Degradation experiments were otherwise performed as described above. Untreated plates to which 0.05 ml of HBSS was added instead of stock chemicals were used as controls, with the exception of controls for cytochalasin B and DNP experiments to which 0.05 ml of DMSO or 95% ethanol were added, respectively. Inhibition of degradation was calculated from the following formula:

\[
\left( \frac{\% \text{ of degradation in control plates} - \% \text{ of degradation experimental plates}}{\% \text{ of degradation in control plates}} \right) \times 100.
\]

Measurement of Macromolecule-associated Radioactivity. The radioactivity associated with macromolecules was measured by precipitation with 10% trichloroacetic acid, as described previously (8). All measurements were made on duplicate 0.02-ml aliquots and the average value was used. In experiments involving [3H]leucine-labeled material, the Millipore filters containing trichloroacetic acid precipitates were dissolved by adding them to glass scintillation vials containing 8 ml of Cocktail D (Beckman Instruments, Inc., Fullerton, Calif.) and 0.2 ml of methanol and counted in a SL-30 liquid scintillation counter (Intertechnique Instruments, Inc., Fairfield, N. J.).

Gel Filtration. The changes in the molecular weight profile of radioiodinated surface macromolecules released by radioiodinated melanoma cells were analyzed by gel chromatography. One-ml samples of labeled medium, obtained prior to or following 24 hr of incubation with unlabeled melanoma cells, were applied to a 1.5 x 35-cm column of Sephadex G-150 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) equilibrated with 0.01 M Tris:0.5 NaCl buffer, pH 8.0, and were eluted with the same buffer. Fractions of approximately 2 ml were collected.

Uptake of Shed Surface Macromolecules by Melanoma Cells. Five-ml aliquots of labeled medium, prepared as described for the degradation experiments, were incubated with replicate confluent monolayers of melanoma cells in 60-mm dishes. At intervals thereafter, the medium was removed from duplicate plates, and the cells were washed 3 times with 2.5 ml of HBSS. At the 24-hr time point, the washed cells were collected with a rubber policeman into 5 ml of HBSS, split into 2 equal aliquots, and sedimented by centrifugation. One aliquot was resuspended in 1 ml of 0.05% trypsin in HBSS, the other was resuspended in 1 ml of HBSS without trypsin, and both were incubated at 37°. Cell viability, measured by trypan blue exclusion, was not affected by trypsinization. After 15 min, 1 ml of undiluted FCS was added to both aliquots, and the cells were collected by centrifugation and washed twice with 2 ml of HBSS containing 20% FCS. All cells were lysed in 1 ml of 0.5% Nonidet P-40, the insoluble material was removed by centrifugation, and the amount of acid-insoluble radioactivity in the lysates was measured. Results were expressed as a percentage of the acid-insoluble radioactivity initially added to the plates.

Radioiodination of Proteins. Purified human IgG and IgM (Immunology, Inc.) and BSA (Grand Island Biological Co.) were radioiodinated by the chloramine T technique (21). After extensive dialysis, acid-insoluble radioactivity was measured by trichloroacetic acid precipitation, and protein concentration was measured by the method of Lowry et al. (20). Concentration of labeled BSA and IgM was adjusted to 42 μg/ml and that of IgG to 21 μg/ml by the addition of unlabeled protein and PBS. The final specific activity of labeled BSA and IgM was 1 x 10^6 cpm/μg and that of IgG 2 x 10^5 cpm/μg.

RESULTS

Degradation of Shed Surface Macromolecules. In earlier studies, we have shown that cell surface macromolecules are rapidly released by viable human melanoma cells in culture (6). To study the fate of the shed material, labeled medium containing radioiodinated surface macromolecules released by melanoma cells in 3 hr was collected and incubated with confluent monolayers of unlabeled cells. Aliquots of medium were collected at the onset of incubation with unlabeled cells and at intervals thereafter. The amount of radioactivity remaining associated with acid-insoluble macromolecules in the medium was measured at each time point. The results of 3 experiments are summarized in Chart 1. There was a steady decline in acid-insoluble radioactivity with time so that approximately 40% of that initially present was lost in 6 hr and approximately 60% in 24 hr. The decrease was associated with a concomitant increase in acid-soluble radioactivity in the medium. Only a small fraction of the disappearing labeled macromolecules was taken up by cells or bound to culture dishes as evidenced by the following experiments. Labeled medium was added to replicate dishes containing confluent monolayers of melanoma cells and incubated at 37°. At intervals thereafter, the cells were col-

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**Autocatalysis of Surface Macromolecules**

![Chart 1](chart1.png)
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confirmed that melanoma cells can degrade surface macromolecules with macromolecules with molecular weight greater than the total radioactivity in the labeled medium was associated with degradation. In 2 experiments, approximately 1.3% of the added acid-insoluble radioactivity was associated with cells after 3 hr, 3.6% after 6 hr, and 6.8% after 24 hr. Approximately two-thirds of the acid-insoluble radioactivity associated with cells at 24 hr could be removed by trypsin treatment, indicating it was adhering to the surface of the cells. The remainder, approximately 3% of that initially added to the medium, was resistant to trypsin treatment and presumably represented labeled macromolecules taken up inside the cells. Approximately 4 to 6% of added acid-insoluble radioactivity adhered to plates lacking cells. The marked decrease in acid-insoluble radioactivity, the concomitant increase in acid-soluble radioactivity, and the small fraction of radioactivity taken up by cells or binding to culture dishes all suggest that large insoluble macromolecules shed by melanoma cells can be degraded to acid-soluble fragments following incubation with these cells.

To confirm that the loss in acid-insoluble radioactivity was due to degradation, the molecular weight profile of radioiodinated macromolecules in labeled medium was examined by Sephadex G-150 chromatography before and after 24 hr of incubation with unlabeled cells. The results are illustrated in Chart 3. Three peaks of radioactivity were eluted. The major peak had a molecular weight of less than 1000 and represented free $^{125}\text{I}$ in the labeled medium. Following incubation with cells, there was a marked shift in elution pattern consisting of a decrease in labeled macromolecules of higher molecular weight in the first 2 peaks and an accumulation of low-molecular-weight species. Prior to incubation, approximately 33% of the total radioactivity in the labeled medium was associated with macromolecules with molecular weight greater than 50,000 whereas only 18% was present in this fraction following incubation with cells, a decrease of 45%. This experiment confirms that melanoma cells can degrade surface macromolecules which they release.

Rate of Degradation. The rate at which melanoma cells degraded surface macromolecules which they released appeared to be biphasic (Chart 1), suggesting that different macromolecules were degraded at different rates. This was confirmed by several experiments. (a) Approximately 26% of acid-insoluble radioactivity was lost in the 3-hr period immediately following the addition of labeled medium to unlabeled cells, whereas 24 hr later, only 4 to 5% of the acid-insoluble radioactivity still present was lost in a similar 3-hr period. (b) Labeled medium was successively incubated for 6 hr with 2 different plates of melanoma cells. The decrease in acid-insoluble radioactivity during the first incubation was over twice as great as that occurring during the second incubation, even though both plates had similar numbers of cells. And (c) the possibility that spontaneous autocatabolism of partially degraded molecules was responsible for the slow terminal phase of degradation was excluded by the following experiment. Labeled medium was incubated with melanoma cells for 6 hr, and aliquots were subsequently reincubated at 37°C with or without cells. Forty-five % of labeled macromolecules were degraded during the initial 6-hr incubation with cells. An additional 10% of labeled macromolecules were degraded when they were reincubated for an additional 18 hr in the presence of cells, whereas no additional degradation occurred when they were reincubated in the absence of cells for a similar period. These results suggest that different populations of shed macromolecules are degraded at different rates.

Cellular Requirements for Degradation. Several possibilities were entertained to account for the degradation of shed surface macromolecules. These included: (a) dissociation of $^{125}\text{I}$ from labeled molecules; (b) degradation by proteases in FCS; (c) degradation by proteases released by the melanoma cells; and (d) degradation by the cells themselves.

To test these possibilities, labeled macromolecules released into FCS-free medium were incubated at 37°C alone or under various conditions. As can be seen in Table 1, there was no significant decrease in acid-insoluble radioactivity when labeled medium was incubated alone for 24 hr, indicating that $^{125}\text{I}$ does not dissociate from labeled molecules. Nor was there any significant decrease in acid-insoluble radioactivity when labeled medium was incubated in a 10% concentration of FCS...
or with equal volumes of spent medium collected from replicate plates of unlabeled melanoma cells after 6 or 24 hr. These results indicate that degradation was not due to proteases released by the melanoma cells or present in the FCS. By contrast, there was a marked decrease in acid-insoluble radioactivity when labeled medium was incubated with melanoma cells, clearly indicating that cells are required for degradation. The possibility that degradation resulted from interactions between cells and FCS components has not been excluded.

**Effect of Inhibitors of Metabolic, Cell Surface and Protease Activity on Degradation.** To investigate the mechanisms involved in the cell-mediated degradation of surface macromolecules released by melanoma cells, the effect of temperature and of inhibitors of metabolic and protease activity and cell surface mobility on the process was studied.

As can be seen in Table 2, degradation was almost completely suppressed by lowering the temperature to 4°C. Degradation was also partially inhibited by cytochalasin B at a concentration (10 µg/ml) which interferes with pinocytosis. The effect was not due to the DMSO. Colchicine did not significantly alter degradation at concentrations of 10⁻⁵ M. Inhibition was noted at higher concentrations but cannot be ascribed to an effect on microtubules since the drug then has an effect on other cellular functions (2). Degradation was partially reduced by iodoacetamide at concentrations of 10⁻⁴ M and by a combination of 2-deoxyglucose (18 mg/ml) and DNP (10⁻³ M) which individually had little effect on degradation. Several protease inhibitors, i.e., soybean trypsin inhibitor, aminocaproic acid, and phenylmethylsulfonylfluoride, had no discernible effect on degradation. Cell viability was not significantly altered, as measured by trypan blue exclusion, by the above-mentioned drugs.

These results suggest that degradation, at least in part, is an active process requiring cell surface mobility and continued metabolic activity.

**Selectivity of Degradation.** As described earlier, degradation appeared to be biphasic, suggesting that different molecules were degraded at different rates. To study this question further, the ability of melanoma cells to degrade a variety of labeled macromolecules was studied.

Table 3 summarizes the results of several experiments in which various labeled macromolecules were incubated with melanoma cells and the proportion of acid-insoluble radioactivity lost in 24 hr was determined. There were marked differences in the rate of degradation. Surface macromolecules released by melanoma cells were degraded much more rapidly than was a variety of labeled serum proteins or metabolically labeled macromolecules released into medium by melanoma cells.

These observations indicate that surface macromolecules released by melanoma cells are particularly susceptible to degradation by these cells.

**DISCUSSION**

The main finding of this study is that viable human melanoma cells can degrade surface macromolecules which they have released.

Degradation of surface material was evidenced by the conversion of shed acid-insoluble macromolecules to acid-soluble fragments following their incubation with cells. Control experiments excluded the possibility that the loss resulted from binding of molecules to cells or to culture dishes. Sephadex G-150 chromatography of shed surface material revealed a marked shift from high-molecular-weight molecules to lower-molecular-weight species following incubation with melanoma cells, confirming that degradation was occurring. The rate of degradation was biphasic, suggesting that different macromolecules were degraded at different rates. Overall, cells released

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**Table 1**

<table>
<thead>
<tr>
<th>Addition to shed macromolecules</th>
<th>% of degradation at 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.5 ± 2.2</td>
</tr>
<tr>
<td>6-hr unlabeled medium</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>24-hr unlabeled medium</td>
<td>7.0 ± 2.0</td>
</tr>
<tr>
<td>10% FCS</td>
<td>4.5 ± 1.3</td>
</tr>
<tr>
<td>10% FCS + cells</td>
<td>50.8 ± 3.1</td>
</tr>
</tbody>
</table>

*Labeled medium (1 ml), containing surface macromolecules released by radiolabeled confluent monolayers of melanoma cells into FCS-free medium in 3 hr, incubated in 60-mm plastic dishes at 37°C. Volume of all preparations was made up to 5 ml with FCS-free culture medium.

* Calculated as described in "Materials and Methods."

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**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>% of inhibition of degradation (relative to controls after 3 hr of incubation at 37°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Deoxyglucose</td>
<td>18 mg/ml</td>
<td>-</td>
</tr>
<tr>
<td>DNP</td>
<td>10⁻³ M</td>
<td>9.6 ± 7.9</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>10⁻⁴ M</td>
<td>2.9 ± 1.9</td>
</tr>
<tr>
<td>Cytochalasin B</td>
<td>10 µg/ml</td>
<td>32.0 ± 7.7</td>
</tr>
<tr>
<td>Colchicine</td>
<td>10⁻⁵ M</td>
<td>1.6 ± 1.4</td>
</tr>
<tr>
<td>Aminocaproic acid</td>
<td>10⁻³ M</td>
<td>0</td>
</tr>
<tr>
<td>Soybean trypsin inhibitor</td>
<td>10⁻⁴ M</td>
<td>0</td>
</tr>
<tr>
<td>Phenyldimethylsulfonylfluoride</td>
<td>8 X 10⁻⁴ M</td>
<td>9.4 ± 0.3</td>
</tr>
</tbody>
</table>

* Average ± S.E. of 2 to 3 experiments, each performed on duplicate plates.

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**Table 3**

<table>
<thead>
<tr>
<th>Labeled proteins</th>
<th>Concentration (µg/ml)</th>
<th>% of degradation at 24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>5.0</td>
<td>1.08 ± 0.5</td>
</tr>
<tr>
<td>Human IgG</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>Human IgM</td>
<td>5.0</td>
<td>0.45 ± 0.3</td>
</tr>
<tr>
<td>L-Leucine-labeled macromolecules released by autologous melanoma</td>
<td>&lt;15.0</td>
<td>1.6 ± 0.6</td>
</tr>
<tr>
<td>Surface macromolecules released by autologous melanoma</td>
<td>15.0</td>
<td>42.06 ± 5.4</td>
</tr>
</tbody>
</table>

* Inhibited concentrations of labeled proteins were added with 5 ml of FCS-free growth medium to confluent monolayers of melanoma cells and incubated at 37°C.

b Calculated from loss in acid-insoluble radioactivity.

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surface macromolecules more rapidly than they degraded them. Prior experiments have shown that, following radioiodination of melanoma cells, approximately 60% of the radioactivity associated with surface macromolecules is released within 3 hr (6), whereas during the same time, cells degrade only 20 to 30% of the released material. Thus, despite degradation, surface macromolecules can accumulate in culture medium.

The mechanism of degradation was not elucidated, although it does require the presence of cells. There was little or no degradation when labeled medium containing radioiodinated macromolecules shed by melanoma cells was incubated alone or with FCS, indicating that degradation was not due to proteases in the FCS, to dissociation of $^{125}$I from labeled molecules, or to binding of labeled molecules to the surface of culture dishes (29). Nor was there significant degradation when shed labeled macromolecules were incubated with medium collected from unlabeled cells, indicating that the process was not due to proteases released by melanoma cells. Degradation was, at least in part, an active process requiring cell surface and metabolic activity. It was partially inhibited by cytochalasin B, which interferes with microfilament function and pinocytosis (33), and by iodoacetamide or a combination of 2-deoxyglucose and DNP which inhibit energy production and macromolecule metabolism. Degradation was almost completely suppressed at $4^\circ$, temperature which depletes metabolic activity and inhibits all endocytic activity (28). Colchicine, at concentrations which interfere with microtubular function (2), had no effect on degradation, indicating these structures were not involved in the process. Several protease inhibitors had no effect on degradation, although this does not exclude the involvement of proteolytic enzymes since there is a great deal of heterogeneity in their susceptibility to inhibitors. These observations suggest that one of the mechanisms of degradation may be similar to that by which most exogenous proteins are degraded by cells, namely endocytosis followed by degradation in lysosomes (3, 11, 13, 14). Melanoma cells were found to take up surface macromolecules which they release, supporting this possibility. Other mechanisms which may be involved in degradation and which were not excluded by these studies include cell surface proteases which may be present on normal (18, 19) and malignant cells (24) including melanoma (17) or active proteases formed as a result of interactions between FCS components and cells, as occur when serum plasminogen is activated to plasmin by cell surface plasminogen activator (12, 26).

An unusual aspect of degradation was its selectivity. Surface macromolecules released by melanoma cells were degraded much more rapidly than was a variety of unrelated serum proteins including human IgG, human IgM, BSA, and FCS proteins. It is unlikely that this was a result of the different methods used to label surface macromolecules and serum proteins. Any impact of radiiodination on degradation should be most pronounced on material labeled by the harsher method, i.e., serum proteins labeled by the chloramine T technique, which was not the case. This observation indicates that the degraded material was not FCS proteins which are known to adhere to melanoma cells in culture and to account for a small proportion of the labeled material released by radioiodinated melanoma cells (10). Surface macromolecules released by melanoma cells were also degraded much more rapidly than were metabolically labeled macromolecules released by the same cells. A possible explanation for this observation is that different populations of melanoma macromolecules (external plasma membrane components labeled by lactoperoxidase versus cytoplasmic as well as internal membrane components labeled by [$^3$H]leucine) differ in their susceptibility to degradation.

The ability of cells to degrade their surface macromolecules after they have been released has a number of biological implications. It adds another general pathway to the several which have been proposed to account for the turnover of surface macromolecules. Besides shedding of external proteins to the outside of cells, these include proteolysis of molecules still attached to the cell membrane (27), dissociation from the membrane and reentry into the cytoplasm of the cell (27), and internalization of membrane macromolecules forming the wall of endocytic vacuoles during pinocytosis followed by their digestion within lysosomes (28, 30, 32). Our findings suggest that, in addition, shed surface macromolecules can be autolysed following their release by the cells which released them. The relative contribution of these different pathways to the total turnover of surface macromolecules is not known. The process we describe in these studies accounts for the degradation of only part of the macromolecules released by cells in vitro. However, since macromolecules are autolysed at different rates, it could account for the degradation of a significant proportion of the more rapidly degraded species. Furthermore, in vivo, the 3-dimensional array of cells may impose restrictions on the diffusion of released molecules that could favor their degradation locally at the site of release.

The ability of melanoma cells to degrade soluble surface macromolecules which they have released may also have an impact on their ability to grow in vivo. It is known that soluble tumor antigens can be released by viable tumor cells (4, 5, 9, 15, 16, 23, 25, 31) and that the release of surface macromolecules including tumor antigens is particularly rapid. In prior studies, we have shown that melanoma cells release in 3 hr over 40% of some of the tumor antigens expressed on their surface (6, 9). This process results in the accumulation of soluble tumor antigens in various body fluids (1) and in the stimulation or blocking of tumor-specific immune defense mechanisms. While the ability of melanoma cells to degrade tumor antigens which they released was not studied, it is evident that the susceptibility or resistance of the antigens to degradation will influence the amount left to accumulate in body fluids. Thus, the final concentration of soluble tumor antigens in body fluids may well depend on the balance between the rate of their release and that of their degradation by tumor cells. This in turn may have an impact on the ability of tumor cells to escape host immune defense mechanisms.

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