Attack of sea urchin eggs by dogfish phagocytes: Model of phagocyte-mediated cellular cytotoxicity

(Arribia punctulata/Mustelus canis/reverse endocytosis/lysosomal degradation)

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ABSTRACT To test whether lysosomal degranulation of phagocytes is associated with antibody-dependent cytotoxicity, eggs of Arbacia punctulata were used as targets for blood phagocytes of Mustelus canis. Eggs were coated with heat-aggregated dogfish IgM and exposed to phagocytes, and cytolsis of eggs was observed by Nomarski optics. Phagocytes adhered, degranulated, and raised fertilization membranes resembling those induced by sperm or ionophore A23187. Lysis was then observed as damage radiating from the point of phagocyte–egg contact. By 4 hr, coated eggs exposed to phagocytes released 8.9, 12.3, and 7.6% of total catalase (EC 1.11.1.6), β-glucuronidase (EC 3.2.1.31), and superoxide dismutase (EC 1.15.1.1) into the medium. Cytotoxic enzyme release significantly exceeded that from uncoated eggs incubated with phagocytes or eggs alone (uncoated or coated). Because activated eggs release a neutral protease, it was considered possible that this enzyme might be responsible for autolysis of eggs. This possibility was excluded because (i) lysis of eggs was not inhibited by soybean trypsin inhibitor (SBTI) whereas the egg protease was sensitive to SBTI, and (ii) the major trypsin-like activity of phagocytes was not inhibited by SBTI. These experiments demonstrate that IgG-coated cells are first activated, and then killed, when exposed to degranulating phagocytes and suggest that enzymes from attacking phagocytes, and not target cells, are responsible for cell death.

Phagocytes, such as polymorphonuclear leukocytes (PMNs) or macrophages, degranulate and secrete lysosomal enzymes when exposed to immune complexes or aggregated immunoglobulins (1–3). Lysosomal, but not cytoplasmic, enzymes appear in the surrounding medium after the cells' Fc receptors engage immunoglobulins, either in the bulk phase or dispersed on non-phagocytosable surfaces (4). The latter process, called "reverse endocytosis" (5) or "frustrated phagocytosis" (6), is launched when phagocytes encounter aggregated immunoglobulins or immune complexes adsorbed onto large surfaces that the cell cannot ingest. Proteolytic enzymes released from phagocytes by immune stimulation can hydrolyze defined substrates of the extracellular milieu, such as proteoglycans (7, 8), collagen (9), and elastin (10). It is less clear whether phagocytes release materials capable of affecting living target cells, although lysosomal secretion may mediate macrophage-dependent cellular cytotoxicity (11).

To elucidate mechanisms whereby reverse endocytosis brings about injury to living cells coated with antibody, oocytes of sea urchins (Arbacia punctulata) have been coated with aggregated immunoglobulin of the dogfish (Mustelus canis). The target oocytes, too large (76 μm) to be phagocytosed, were exposed to blood phagocytes of the dogfish (10–15 μm). These cells possess surface Fc receptors that mediate lysosomal enzyme secretion when engaged by heat-aggregated, but not native, immunoglobulins (12). Because dogfish cells contain lysosomes (0.7–0.8 μm) easily visible by Nomarski optics, we have combined morphologic observations with biochemical analysis to study whether lysosomal degranulation provokes target cell lysis.

MATERIALS AND METHODS

Reagents. Phenolphthalein glucuronidate, H2O2, dimethyl sulfoxide (MeSO), cytochrome c, hypoxanthine, and Triton X-100 were supplied by Sigma (St. Louis, MO), soybean trypsin inhibitor (SBTI) by Worthington, and xanthine oxidase by Boehringer Mannheim. Antipain and leupeptin were the generous gifts of the U.S.-Japan Cooperative Cancer Program; ionophore A23187, of R. Hersley (Eli Lilly); Cbz-Gly-Gly-Arg-amido-4-methylcoumarin (AMC) and N-acetyl-Ala-Ala-Pro-Ala-AMC, of M. Zimmerman (Merck, Rahway, NJ).

Sea Urchin Eggs. Shedding of eggs from female sea urchins was initiated by injecting 0.5 ml of 0.5 M KCl into the body cavity. Eggs were washed twice in filtered sea water (hand centrifuge), suspended in elasmobranch Ringer's solution (12), and quantified by centrifugation to constant volume and by total protein.

Phagocytes. These were obtained as described (12). Immunoglobulins. These were prepared as described (12). Briefly, euglobulins were precipitated by dialysis at 4°C against 800 volumes of acetic buffer (5 mM, pH 4.5) and redissolved in modified phosphate-buffered saline without Ca or Mg, pH 7.4. Immunoglobulins were further purified chromatographically (Sephadex G-200, 2.6 × 40 cm column) with modified phosphate-buffered saline as eluant. Pooled fresh IgM was heat-aggregated (62°C, 15 min). Eggs were coated with aggregated immunoglobulin (1 mg of protein per ml) by incubation at 23°C in elasmobranch Ringer's solution for 30 min; packed cell volumes ranged from 10 to 15%. After 30 min, eggs were washed free of unbound immunoglobulin by gentle centrifugation.

Incubation. Eggs, phagocytes, and mixtures of the two were

Abbreviations: PMNs, polymorphonuclear leukocytes; MeSO, di- methyl sulfoxide; SBTI, soybean trypsin inhibitor; TAME, tosylarginine methyl ester; AMC, amido-4-methylcoumarin; DFP, diisopropylfluorophosphate.

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incubated in Ringer's solution (500 units of heparin per ml) at 23° for up to 5 hr. Approximate amounts of protein contributed by the two types of cells (when present) to the incubation mixtures (2.5 ml total volume) were: eggs, 1.7 mg; phagocytes, 0.6 mg. At desired times, incubation mixtures were centrifuged at full speed (1 min) in a clinical centrifuge to remove the eggs. Supernatants were then centrifuged at full speed for 10 min to remove phagocytes and debris. Final supernatants were assayed for released enzymes after 1:1 dilution with 0.4% (vol/vol) Triton X-100. To determine total enzyme activities, homogenates were prepared of samples of phagocytes alone or eggs alone; such cells were homogenized (ground-glass hand homogenizer) in buffer containing Triton X-100 (0.2%) and clarified by centrifugation.

**Morphological Studies.** Microscopy was carried out by using a Zeiss Axiomat microscope equipped with Nomarski optics.

**Activation of Eggs by Calcium Ionophore (A23187).** To suspensions of eggs in filtered sea water (10 ml, 5-7% vol/vol) was added ionophore A23187 (20 μM) in Me2SO or Me2SO alone (1% vol/vol), and fertilization was scored visually. After >95% of eggs were activated, the suspending media were centrifuged and the released enzymes were assayed. Collection of “fertilization product” for analysis of enzymes released during the fertilization reaction (using sperm) was performed as described (13).

**Assays.** Protein concentrations were determined by the Lowry method (bovine serum albumin as standard). Superoxide dismutase activity was analyzed as described (14), in filtered sea water. β-Glucuronidase was measured as described (1, 12). Catalase was measured as by Bears and Siger (15); the initial rate of decrease of H₂O₂ concentration was measured at 240 nm. For proteases, synthetic fluorogenic peptides were used as substrates (16); their general formula is F-B-AMC. The hydrolysis product, F-methylaminocoumarin was measured fluorometrically (excitation, 380 nm; emission, 460 nm) in 50 mM triethylenediamine sulfonate buffer, pH 7.5, containing 10% (vol/vol) Me2SO. For the trypsin-like protease, R of the specific substrate was Cbz-Gly-Gly-Arg, and for the elastase-like protease it was N-acetyl-Ala-Ala-Pro-Ala. Generation of fluorescent product was linear with time and enzyme concentration (both proteases) at substrate concentrations of 0.2 mM. A Farrand ratio fluorometer 2 was used with a 7-60 Corning primary filter and a 3-74 secondary filter.

**RESULTS**

**Morphology of Phagocyte-Egg Interaction.** Phagocytes of *A. punctulata* are motile, glass-adherent cells with abundant cytoplasmic granules 0.7-0.8 μm in diameter (Fig. 1A). Within 15-30 min after exposure to IgM-coated *Arbacia* eggs, one to five phagocytes adhered to the cell surface of each egg (Fig. 1B). During the next 15 min, the adherent phagocytes degranulated at the point of cell–cell contact (Fig. 1B and C). Examination of 59 photomicrographs (×580-×1100) showed degranulation at all but six sites of 210 phagocyte–egg contacts; cells not attached remained uniformly granulated. Coincident with degranulation, reddish brown pigment (echinochrome) granules, previously randomly distributed throughout the cytoplasm of the oocyte, collected immediately subjacent to the area in contact with phagocytes; this is characteristic response to fertilization (17). In 40-50% of the cells, this stage was accompanied by elevation of fertilization membranes which enveloped the attached phagocyte (Fig. 1B). For the next 4 hr, various stages of cytology were observed. Whether or not fertilization membranes had been formed, the cells became covered with clusters of phagocytes, localized to discrete sites of the oocyte. The eggs swelled and finally burst; cytoplasmic contents, including yolk and pigment granules, were extruded into the surrounding medium (Fig. 1C and D). Large cytoplasmic blebs appeared in the attacked egg, and these burst, first into the cytoplasm and then into the medium (Fig. 1C and D).

The process was asynchronous; eggs that had only been surrounded by phagocytes were often adjacent to cells that had already undergone cytology (Fig. 1D). At no time were large mononuclear cells or lymphocytes observed adhering to eggs. Once cytosis had proceeded beyond rupture of the egg, with unravelling of the organized fertilization membrane (Fig. 1D), clusters of phagocytes adhered to the injured oocyte. Uncoated cells were also attacked by phagocytes but the phagocytes did not adhere as rapidly, fertilization membranes were not as abundant, and fewer early clusters of phagocytes accumulated.

**FIG. 1.** (A) Phagocyte adherent to IgM-coated egg of *Arbacia punctulata*. Arrow indicates point of contact; degranulation is proceeding at egg–phagocyte interface. (×720.) (Inset) Phagocyte of *Mustelus canis*. (×2300.) (B) At 15 min after A. Degranulation is evident from area of phagocyte in contact with egg (arrow); fertilization membrane has formed, enfolding phagocyte, and dark echinochrome granules have accumulated at point of contact. (×1100.) (C) Another coated egg, undergoing lysis in absence of fertilization reaction; lysis (open arrow) is evident 2 hr after contact with phagocytes (solid arrow). Cytoplasmic blebbing and osmotic swelling are evident. (×720.) (D) At 2 hr after exposure of coated eggs to phagocytes, top egg is lysed (note fertilization membrane) and surrounded by both degranulated and clustered phagocytes. Bottom egg is still intact, and degranulation has just begun. (×580.)
Less than 5% of coated or uncoated cells incubated without phagocytes raised fertilization membranes or underwent cytolysis during observation (3.5 hr). Arbacia eggs exposed to aqueous lysates of phagocytes, at cell concentrations and with β-glucuronidase activity in excess of intact phagocytes (see below), raised fertilization membranes in 25–50% of the zygotes at 3.5 hr; no cytolysis was observed.

Enzyme Activities. Enzymes assayed in the mixed cytotoxicity system are listed in Table 1. The cytoplasmic enzymes (superoxide dismutase and catalase), and the lysosomal enzyme (β-glucuronidase) were present in at least 20-fold greater amounts in the eggs (catalase was not detectable in the phagocytes). Elastase-like proteolytic activity was an order of magnitude greater in the eggs. In contrast, trypsin-like protease activity, abundant in phagocytes, was essentially absent from eggs. The differences in the cellular distribution of these enzymes indicate that cytotoxicity of target cells can be quantitated by means of enzyme analysis.

Enzyme Release. From phagocyte–egg interactions. Associated with the attack, and cytosis, of IgM-coated eggs by phagocytes, enzymes were released from the eggs (Fig. 2). In other experiments (not shown), no further release was observed after 5 hr and maximal release was 20–30% of total egg enzyme. In contrast, 70% of β-glucuronidase, catalase, and superoxide dismutase could be recovered in supernatants of distilled water lysates of eggs. Measurable quantities (Fig. 3) of enzyme were released from uncoated eggs alone. Coating of eggs by heat-aggregated IgM did not promote a significant increase in release of enzymes, whereas phagocytes provoked cytolysis of uncoated eggs. Release was maximized from IgM-coated eggs mixed with phagocytes (approximately 10% of total enzyme). In six experiments in which release of β-glucuronidase was measured simultaneously in each of the incubation mixtures (uncoated eggs, coated eggs, and both types of eggs with phagocytes), release was not significantly different from coated and uncoated eggs. Release from uncoated eggs and phagocytes was significantly greater than from eggs without phagocytes, and release from coated eggs and phagocytes was significantly greater still (P < 0.05 for both differences, Student–Newman–Keuls multiple range test). Release of catalase from coated eggs and phagocytes was significantly greater than from uncoated eggs only (P = 0.06). In the comparison of coated eggs to coated eggs and phagocytes, phagocytes caused significant increases in release of all three enzymes measured (β-glucuronidase, P < 0.002, n = 10; catalase, P < 0.02, n = 10; superoxide dismutase, P < 0.05, n = 5; randomization test for matched pairs).

Ionophore-induced fertilization reaction. The fertilization reaction in Arbacia eggs (lifting of the fertilization membrane) can be induced by the calcium ionophore A23187 (13); release of protease activity from cortical granules accompanies this reaction (13). To determine if enzyme release from coated eggs and phagocytes was simply a consequence of a noncytolytic fertilization reaction, release of β-glucuronidase, catalase, and superoxide dismutase was measured. Release of superoxide dismutase and catalase from eggs exposed to A23187 did not exceed that observed from controls. For β-glucuronidase, 2% of total activity was released from control cells and 5% from eggs and A23187 (n = 3), far less than from coated eggs or eggs and phagocytes (Fig. 2).

Contribution from phagocytes. The enzymes utilized to assay cytolysis were present in much higher activity in eggs than in phagocytes (Table 1). Nevertheless, release of lysosomal enzymes from phagocytes (β-glucuronidase) or by cell death (β-glucuronidase and superoxide dismutase) might have contributed to total enzyme activity released from mixtures of eggs and phagocytes. However, total β-glucuronidase activity of

<table>
<thead>
<tr>
<th>Enzyme*</th>
<th>Arbacia eggs</th>
<th>Dogfish phagocytes</th>
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</thead>
<tbody>
<tr>
<td>β-Glucuronidase, µg phenolphthalein released min⁻¹</td>
<td>5.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Superoxide dismutase, units, 50% inhibition of superoxide generating system</td>
<td>13.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Catalase, µmol H₂O₂ reduced sec⁻¹</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Trypsin-like protease, mol-10⁻¹¹ substrate hydrolyzed min⁻¹</td>
<td>&lt;0.09</td>
<td>148</td>
</tr>
<tr>
<td>Elastase-like protease, mol-10⁻¹¹ min⁻¹</td>
<td>8.8</td>
<td>0.09</td>
</tr>
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</table>

*Expressed as total activity (from Triton X-100 extract) per incubation mixture (2.5 ml total volume), which contained 1.7 mg of protein from eggs and 0.6 mg of protein from phagocytes (5 × 10⁷ cells).

Fig. 3. Release of cytoplasmic enzymes from Arbacia eggs in 2–4 hr. Eggs, hatched bars; eggs coated with heat-aggregated IgM, solid bars. (A) Superoxide dismutase, as 50% inhibition of superoxide generating system. (B) Catalase, as mol × 10⁻⁰⁵ of H₂O₂ reduced per min. (C) β-Glucuronidase, as g × 10⁻⁸ of phenolphthalein released per hr. Enzyme activities are corrected for activity released at zero time. Activity released from coated eggs and phagocytes as percentage of activity in Triton X-100 extract: β-glucuronidase, 12.3%; superoxide dismutase, 7.4%; catalase, 8.9%. n is given in parentheses; vertical lines show SEM.
**Table 2.** Inhibition of proteases from *Arbacia* eggs and *M. canis* by various protease inhibitors*

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th><em>M. canis</em> phagocyte lysate, †</th>
<th><em>Arbacia</em> egg lysate, †</th>
<th><em>Arbacia</em> egg fertilization product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td>Trypsin-like, †</td>
</tr>
<tr>
<td>SBTI</td>
<td>No inhib</td>
<td>No inhib</td>
<td>10⁻⁴</td>
</tr>
<tr>
<td>DFP</td>
<td>10⁻³</td>
<td>10⁻³</td>
<td>10⁻³</td>
</tr>
<tr>
<td>Antipain</td>
<td>5 × 10⁻⁴</td>
<td>5 × 10⁻⁴</td>
<td>10⁻⁴</td>
</tr>
<tr>
<td>Leupeptin</td>
<td>10⁻⁴</td>
<td>No inhib</td>
<td>10⁻⁴</td>
</tr>
</tbody>
</table>

* Data expressed as molar concentration of inhibitor that gave 100% inhibition of assay on synthetic substrate. "No inhib" = no inhibition at 0.1 mM.
† Trypsin-like activity assayed on Cbz-Gly-Gly-Arg-AMC; activities of *Arbacia* lysate were too low for inhibitor studies.
‡ Elastase-like activity assayed on *N*-acetyl-Ala-Ala-Pro-Ala-AMC; activities of phagocyte lysate were too low for inhibitor studies.
§ Inhibited 60%; further additions of diisopropylfluorophosphate (DFP) (to 2 mM) yielded no further inhibition.

phagocytes (Triton X-100) was less than the maximal amount released into supernatants of coagulated eggs and phagocytes. Indeed, the total β-glucuronidase activity was (μg of phenolphthalein released in 12 hr) 1.3 in phagocytes and 14.8 in eggs, yielding a total activity in an incubation mixture of 16.1 (Triton X-100 extracts, n = 10). The maximal β-glucuronidase activity contributed by the phagocytes could have been 8.3% of the total whereas, in fact, 12.3% of total was recovered in supernatants of coagulated eggs and phagocytes. When phagocytes were exposed to aggregated IgM in the bulk phase (100 μg/2.5 ml) for 2 hr only 14% of the phagocytes’ β-glucuronidase was released (compared to controls). Moreover, when 90% of phagocytes are killed by crystals of monosodium urate, only 30% of phagocyte β-glucuronidase is released (18). Similar calculations can be performed for superoxide dismutase; catalase is absent from phagocytes.

The effect of SBTI on cytotoxic release of four enzymes from IgM-coated eggs and phagocytes is shown in Table 3. SBTI had no effect on the release of any of the enzymes. Because SBTI did not inhibit the major protease of the phagocytes but did inhibit the trypsin-like protease released from eggs upon fertilization product.

**Table 3.** Effect of SBTI on phagocyte-mediated release of cytoplasmic enzymes from IgM-coated *Arbacia* eggs

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>% release, (SBTI/ control) × 100</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Glucuronidase</td>
<td>108</td>
<td>5</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>109</td>
<td>5</td>
</tr>
<tr>
<td>Catalase</td>
<td>98</td>
<td>5</td>
</tr>
<tr>
<td>Elastase-like protease</td>
<td>88</td>
<td>1</td>
</tr>
</tbody>
</table>

Coated eggs were incubated 3.5 hr (with phagocytes), with and without 0.1 mM SBTI.

**Table 4.** Release of cytoplasmic enzymes from *Arbacia* eggs exposed to lysates of dogfish phagocytes

<table>
<thead>
<tr>
<th>Enzyme activity released into supernatants</th>
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<tr>
<td>Enzyme activity released into supernatants</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
</tr>
<tr>
<td>Catalase</td>
</tr>
<tr>
<td>Elastase-like protease</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>β-Glucuronidase</th>
<th>Catalase</th>
<th>Elastase-like protease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs, t = 0 hr</td>
<td>51</td>
<td>7.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Eggs, t = 3.5 hr</td>
<td>58</td>
<td>24</td>
<td>0.5</td>
</tr>
<tr>
<td>Phagocyte lysate</td>
<td>481</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>Eggs + lysate, t = 3.5 hr</td>
<td>493</td>
<td>45</td>
<td>3.6</td>
</tr>
<tr>
<td>Triton X-100 extract, eggs</td>
<td>2360</td>
<td>3280</td>
<td>30.0</td>
</tr>
</tbody>
</table>

Units of enzyme activity: β-glucuronidase, g × 10⁻⁸ phenolphthalein released × hr⁻¹; catalase, mol × 10⁻⁶ H₂O₂ reduced × min⁻¹. Lysate in incubation mixtures was from 1.4 × 10⁻⁸ cells. Lysate was made in distilled water with a glass homogenizer. Salt concentration was restored by adding NaCl. Values are means from two experiments.

**DISCUSSION**

Macrophages (19, 20) and PMNs (21, 22) of mammals provoke injury of normal or tumor cells; injury is enhanced when target cells are coated with antibody. We have now shown that phagocytes of the dogfish can first activate, and then provoke lysis of, oocytes from *A. punctulata*, especially after they are coated with aggregated dogfish IgM. Because aggregated dogfish IgM engages the phagocyte’s Fc receptors and elicits lysosomal enzyme secretion in response to IgM-coated, non-phagocytosable stimuli (12), it is likely that secretion of granule contents from the phagocyte may, at least in part, mediate lysis of the coated target cell.

The blood phagocytes of *M. canis* more closely resemble PMNs rather than macrophages of higher organisms with respect to ultrastructure, pinocytic capacity, and autolytic response to crystals (12, 18, 23, 24). Consequently, the attack of phagocytes from *M. canis* upon IgM-coated *Arbacia* eggs appears to be closely related to previously described mammalian models of PMN-dependent cell injury. Gale and Zhigbelboim (21, 22) have used 51Cr-labeled leukemic cells treated with specific antibody as targets for homologous PMNs. Sinchowitz and Schur (25, 26) have shown that the lectins concanavalin A and phytohemagglutinin render mammalian PMNs lytic to antibody-coated chicken erythrocytes; cytotoxicity was inhibited by agents that prevent fusion of lysosomes with the plasma membrane (27) such as cortisol or chloroquine and was enhanced by the “fusogen” (28) retinol. Concanavalin A stimulates nonphagocytic discharge of the specific granules of the human PMN (29). In both antibody- and lectin-dependent PMN-mediated cytotoxicity, intimate cell-to-cell contact was
required and the process was independent of complement (21, 22, 25, 26). PMNs, by reverse endocytosis, secrete lysosomal hydrolases onto solid surfaces, such as Millipore filters (2, 4), Sepharose beads (30), collagen (31), or cartilage (32), coated with immune complexes or aggregated immunoglobulins (33). Indeed, antibody- or lectin-dependent injury of target cells by mammalian PMNs resembles the simpler model of reverse endocytosis onto inert, immunoglobulin-coated substrates, both with respect to signals (via Fc receptors or surface sugars) and modulation, as by cyclic nucleotides, colchicine, etc. (2, 4, 22, 25, 30).

Cytolysis of Arbacia eggs was accompanied by release of enzymes: cytoplasmic catalase, elastase-like protease, superoxide dismutase, and lysosomal β-glucuronidase. The first two are essentially absent from phagocytes, and amounts of the latter two enzymes recovered in the supernatants could not have been accounted for by extrusion from injured phagocytes. Indeed, when Arbacia eggs were suspended in aqueous lysates of phagocytes, no release of enzymes from the zygotes was observed, despite elevation of fertilization membranes in 25–35% of the eggs. Nor did activation (13) of eggs by ionophore A23187 (>95% fertilization membranes) provoke release of oocyte enzymes. Consequently, the fertilization reaction to phagocytes was not the cause of enzyme release from eggs which, as previously shown (34), do not release lysosomal β-glucuronidase in response to simple fertilization.

Arbacia eggs do release a neutral protease sensitive to SBTI upon fertilization; the enzyme is associated with cortical granules (13, 34) and can be recovered in sedimentable form as part of the “fertilization product” (13). However, whereas SBTI inhibited the neutral protease activity of the fertilization product as well as formation of fertilization membranes in response to sperm or ionophore (13), it inhibited neither the major trypsin-like protease of the phagocytes nor the phagocyte-mediated cellular cytotoxicity. Finally, these experiments have also documented that Mustelus phagocytes lack catalase as well as peroxidase (12); it is unlikely, therefore, that a halide-depending $H_2O_2$-peroxidase system, implicated in PMN-dependent target-cell injury to tumor cells (35), plays a role in this model.

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