MAST CELL-MEDIATED TUMOR-CELL CYTOTOXICITY

Role of the Peroxidase System*

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Mast cells have been demonstrated in increased numbers in certain neoplasms in humans (1–3) and in both spontaneous tumors and those induced by carcinogenic agents in animals (4, 5). Eosinophils are often closely associated with mast cells in inflammatory reactions, and these cells also are present in increased numbers in some neoplasms (6). The role of these inflammatory cells in the host defense against tumors is unknown.

A number of distinct peroxidases exist in mammalian tissues which differ in primary structure and in their heme prosthetic group. When combined with H_2O_2 and a halide, peroxidases are toxic to a variety of targets including tumor cells. Peroxidases shown to have tumoricidal activity in vitro include the milk peroxidase (lactoperoxidase) (7), the neutrophil (and monocyte) peroxidase (myeloperoxidase) (8), and the eosinophil peroxidase (EPO)[1] (9). Mast cell granules (MCG) contain a small amount of peroxidase activity (10), and this activity is considerably increased by the firm binding of the positively charged EPO to the surface of the negatively charged MCG (11). H_2O_2 at relatively high concentrations can induce mast cell degranulation (12, 13). When the H_2O_2 concentration is lowered to a level where it is ineffective alone, noncytotoxic mast cell secretion is initiated on the supplementation of the H_2O_2 with EPO and a halide (14). We report here that mast cells are toxic to a line of mouse ascites lymphoma cells when combined with H_2O_2 and a halide. The H_2O_2 initiates mast cell secretion; the released granules with their endogenous peroxidase are toxic to the tumor cells in the presence of H_2O_2 and a halide. In addition, the binding of EPO to the MCG greatly potentiates their tumoricidal activity.

Materials and Methods

Special Reagents. Compound 48/80, guaiacol (anhydrous), and Triton X-100 were obtained from Sigma Chemical Co., St. Louis, Mo.; glutaraldehyde from Polysciences, Inc., Warrington, Pa.; osmium tetroxide from Scientific Chemical Co., Huntington Beach, Calif.; and sodium chloride (Suprapur) was obtained from Matheson Coleman and Bell, Norwood, Ohio. All other reagents were of the highest commercial grade available. EPO was partially purified from

* Supported by U. S. Public Health Service grants AI07763, HD02266, and HL03174 and grants from the Rockefeller Foundation and the Edna McConnell Clark Foundation.

‡ Recipient of U. S. Public Health Service Young Investigator Award AI14805.

§ Supported by U. S. Public Health Service training grant AI07044.

[1] *Abbreviations used in this paper*: EPO, eosinophil peroxidase; LDH, lactate dehydrogenase; MCG, mast cell granule.

 J. EXP. MED. © The Rockefeller University Press · 0022-1007/81/03/0520/14 $1.00
Volume 153 March 1981 520–533

guinea pig peritoneal eosinophils and assayed by guaiacol oxidation as previously described (15).

Target Cells. A Maloney virus-induced ascites lymphoma cell line, designated LSTRA, was maintained by serial intraperitoneal passage in BALB/c mice. After harvesting by peritoneal lavage, the cells were processed and labeled with ^{51}Cr ($Na_2{}^{51}CrO_4$, 100–300 μCi/μg Cr, Amersham/Searle Corp., Arlington Heights, Ill.) as previously described (9). Final cell suspensions contained 2×10^6/ml viable ^{51}Cr-labeled LSTRA in 0.1 M sodium sulfate for experiments with MCG, MCG-EPO complexes, and free EPO, and in 0.154 M sodium chloride for experiments with intact mast cells.

Isolation of Mast Cells. Mast cells were obtained from the peritoneal cavities of male Sprague-Dawley rats and purified by using a 38% bovine serum albumin density gradient as previously described (14). The cell suspensions contained >90% mast cells (<10% mononuclear cells and <1% eosinophils) as determined by toluidine blue staining. The mast cells were always >95% viable as measured by trypan blue exclusion (16).

Preparation of MCG and MCG-EPO Complexes. Membrane-free MCG were obtained by osmotic lysis of purified mast cells followed by differential centrifugation (14). The MCG were washed twice in 0.1 M phosphate buffer, pH 7.0, to remove histamine and other loosely bound mediators. For preparation of MCG-EPO complexes, EPO was incubated with histamine-free MCG as previously described (11). The complexes were washed three times, suspended in water, and assayed for peroxidase activity by guaiacol oxidation.

Cytotoxicity Assay. The components of the reaction mixture (see legends to figures and tables) were incubated in 10- × 75-mm glass tubes for 60 min at 37°C. Cytotoxicity was determined by the ^{51}Cr-release assay as previously described (9). ^{51}Cr release was expressed as a percentage of the maximum releasable activity seen in supernatant fluids of cells treated with 1% Triton X-100.

Measurement of Histamine and Lactate Dehydrogenase (LDH) Release. Mast cells were preincubated for 5 min at 37°C in a water bath oscillating 80 times/min. The components of the reaction mixture (see legend to Table I) were added to a total volume of 0.5 ml with the H_2O_2 added last to start the reaction. After 1 h incubation at 37°C, the suspensions were placed on ice and histamine and LDH release was measured as previously described (14).

Electron Microscopic Studies. Pellets of 2×10^6 mast cells incubated with 2×10^5 LSTRA tumor cells and the various components of the reaction mixture (see legends to figures) were collected by centrifugation at 400 *g* and prepared for transmission electron microscopy as previously described (14). The samples were examined with a JEOL 100B electron microscope (JEOL USA, Electron Optics Div., Medford, Mass.) at 60 kV.

The mast cell (or MCG)-tumor cell preparations (see legends to figures) were prepared for scanning electron microscopy by fixation in 2% glutaraldehyde followed by 1% osmium tetroxide. The samples then underwent ethanol dehydration, critical-point drying with liquid CO_2, and sputter coating with a gold/palladium mixture before examination with a JEOL JSM-35c scanning electron microscope at 19 kV.

Statistical Analysis. The data are reported as the mean ± SE of the combined experiments. Differences were analyzed for significance using Student's two-tailed *t* test for independent means (not significant, $P > 0.05$).

Results

Mast Cell-mediated Tumoricidal Activity. Incubation of ^{51}Cr-labeled LSTRA tumor cells for 60 min at pH 7.0 with intact mast cells, H_2O_2 (10^{-4} M), and iodide (10^{-4} M), increased ^{51}Cr release from a background of 18.8 to 41.2% ($P < 0.01$) under the conditions used in Table I. The mast (effector) to tumor (target) cell ratio was 10:1. Toxicity to the tumor cells as measured by ^{51}Cr release was abolished by omission of iodide, H_2O_2, or mast cells, or by the addition of the hemeprotein inhibitors azide and aminotriazole. Cyanide at 10^{-3} M did not significantly inhibit the cytotoxic activity of the intact mast cell system. When iodide and H_2O_2 were replaced by

TABLE I

*Cytotoxic Activity of Mast Cells or Mast Cell Granules**

Supplements	^{51}Cr release			
	Mast cells		MCG	
	%	*P*	%	*P*
Background	18.8 ± 1.5 (4)‡		19.9 ± 2.1 (6)	
Mast cells or MCG + iodide + H_2O_2	41.2 ± 5.6 (4)	<0.01	61.9 ± 7.5 (6)	<0.001
Iodide omitted	18.5 ± 3.1 (4)		20.5 ± 2.0 (6)	
H_2O_2 omitted	20.3 ± 2.9 (4)		21.9 ± 1.5 (6)	
Mast cells omitted	19.1 ± 2.5 (4)			
MCG omitted			20.8 ± 2.5 (6)	
MCG heated			22.7 ± 3.5 (3)	
Azide added	20.7 ± 3.4 (3)		21.4 ± 3.4 (4)	
Cyanide added	36.1 ± 2.8 (3)	<0.01	30.2 ± 3.1 (4)	<0.05
Aminotriazole added	17.6 ± 2.7 (3)		22.1 ± 2.8 (4)	
Mast cells + compound 48/80	23.4 ± 2.1 (4)			

* The standard salt solution for the mast cells system consisted of 5×10^{-3} M sodium phosphate buffer, pH 7.0, 1.2×10^{-2} M KCl, 0.11 M NaCl, 2×10^{-3} M $MgCl_2$, and 10^{-3} M $CaCl_2$ and that for the MCG system was 3×10^{-2} M sodium phosphate buffer, pH 7.0, 1.5×10^{-3} M KH_2PO_4, and 1.5×10^{-3} M $MgSO_4$. The reaction mixture contained, in addition, 10^5 LSTRA cells (mean 18,500 cpm ^{51}Cr), 0.005% gelatin, and the supplements where indicated as follows: mast cells, 1×10^6; MCG, 8 μg containing 0.15–0.2 mU peroxidase activity; sodium iodide, 10^{-4} M; H_2O_2, 10^{-4} M; sodium azide 10^{-3} M for mast cell system and 10^{-4} M for MCG systems; sodium cyanide, 10^{-3} M; aminotriazole, 10^{-2} M; compound 48/80, 1 μg/ml. The MCG were heated at 100°C for 15 min where indicated. Probability values for the difference from background are shown where significant ($P < 0.05$).

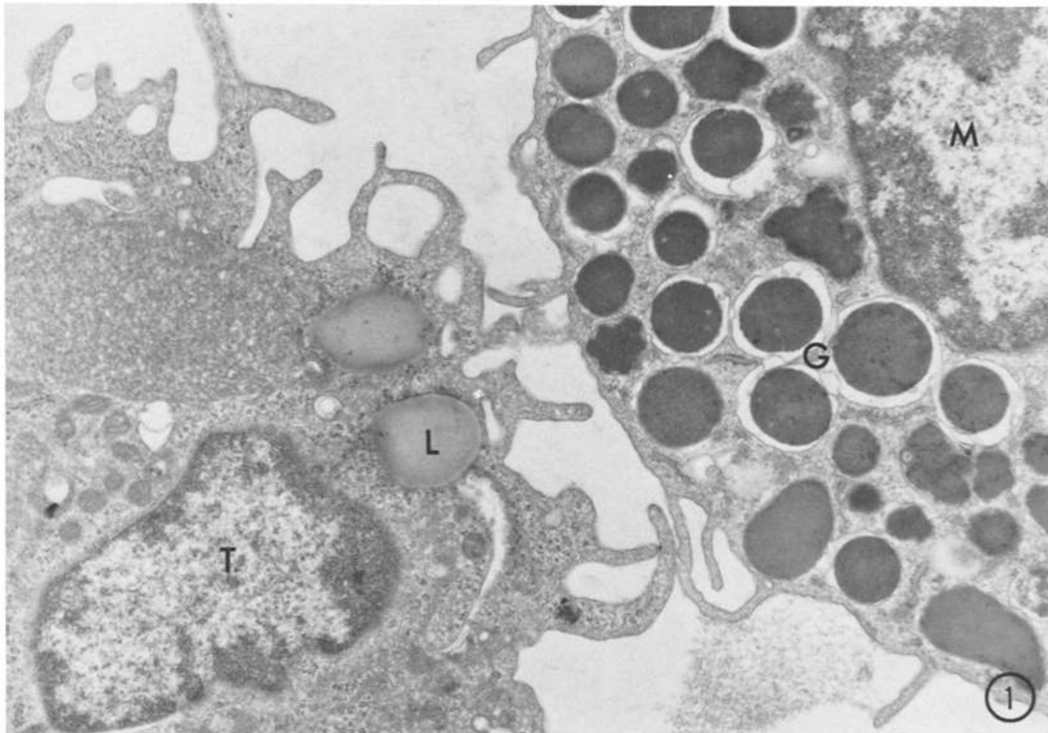
‡ Mean ± SE of experiments (n).

compound 48/80, an agent which stimulates mast cell secretion, no significant toxicity to the tumor cells was observed.

The toxicity to tumor cells of mast cells, H_2O_2, and iodide was confirmed by transmission and scanning electron microscopy. Greater than 90% of mast cells incubated with tumor cells in the standard salt solution alone exhibited the usual morphology of normal cells with numerous electron-dense membrane-bound cytoplasmic granules (Fig. 1) and characteristic surface projections (Fig. 1; Fig. 4). The LSTRA tumor cells also exhibited their usual morphology under these conditions (Fig. 1; Fig. 4). They are smaller than mast cells and contain a single large nucleus, the usual array of cytoplasmic organelles (e.g., mitochondria, endoplasmic reticulum, Golgi vesicles), and lipidlike cytoplasmic inclusions (Fig. 1). Numerous short villous projections are seen on the tumor cell surface (Fig. 1; Fig. 4).

When compound 48/80 was added to the mast cell-tumor cell suspension, mast cell degranulation was evident morphologically (Fig. 2; Fig. 5). Many swollen and less dense granules were seen in intracellular vacuoles and channels (Fig. 2), and others were seen in the extracellular space and on the surface of adjacent tumor cells (Fig. 5). There was no evidence of cytotoxic damage to either the mast cells or the tumor cells.

When mast cells were incubated with tumor cells in the presence of H_2O_2 and iodide, mast cell morphology was similar to that of cells treated with compound 48/80; that is, noncytotoxic degranulation was observed (Fig. 3a; Fig. 6). However, in contrast to the findings with compound 48/80, when H_2O_2 and iodide were employed the tumor cells showed marked cytotoxic changes (Fig. 3; Fig. 6). Transmission



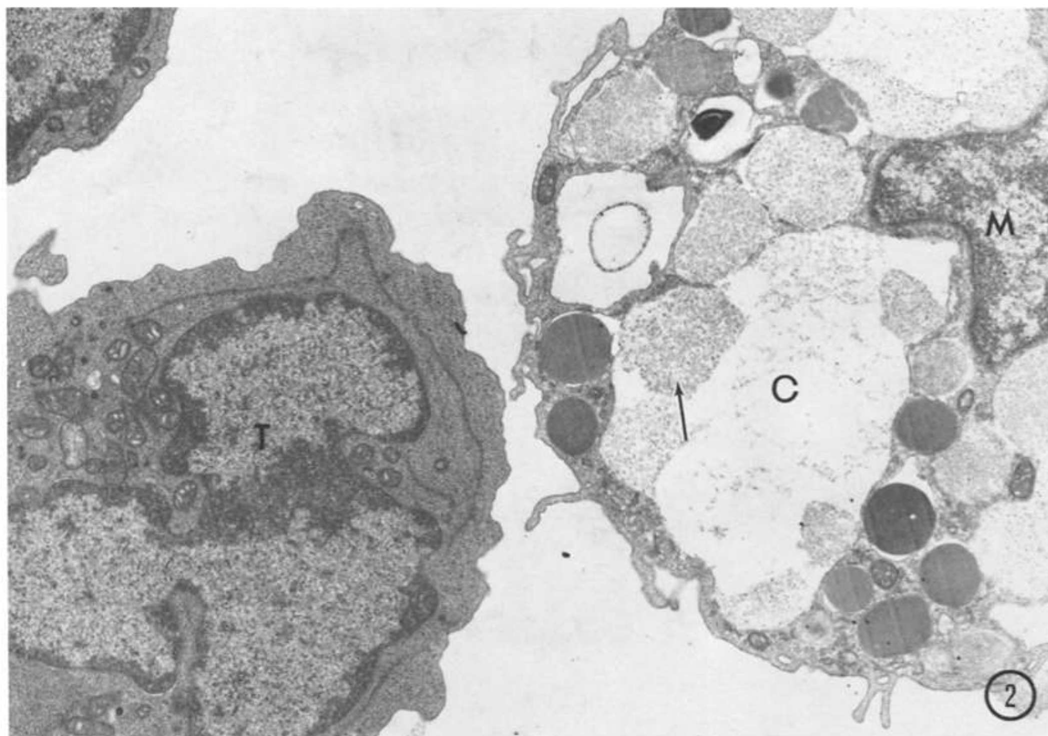


FIG. 1. Mast cell-tumor cell interaction. Transmission electron microscopy. Rat mast cells (M) were incubated with tumor cells (T) in the standard salt solution (see Table I) for 30 min at 37°C. The mast cell shown here contains the typical membrane-bound granules (G) of an unstimulated mast cell. Tumor cells with numerous surface microvilli were of normal appearance, unchanged from cells incubated in standard salt solution without mast cells. Cytoplasmic lipid inclusions (L) were seen in some tumor cells. × 11,750.

FIG. 2. Effect of 48/80 on mast cell-tumor cell interaction. Transmission electron microscopy. Mast cells (M) were incubated with tumor cells in the standard salt solution (Table I) containing 1.0 μg/ml compound 48/80 for 30 min at 37°C. Noncytotoxic degranulation of mast cells was evident with the formation of channels (C) containing swollen granules which had lost their electron density (arrows). The adjacent tumor cell (T) shows no evidence of cytotoxic damage. × 11,750.

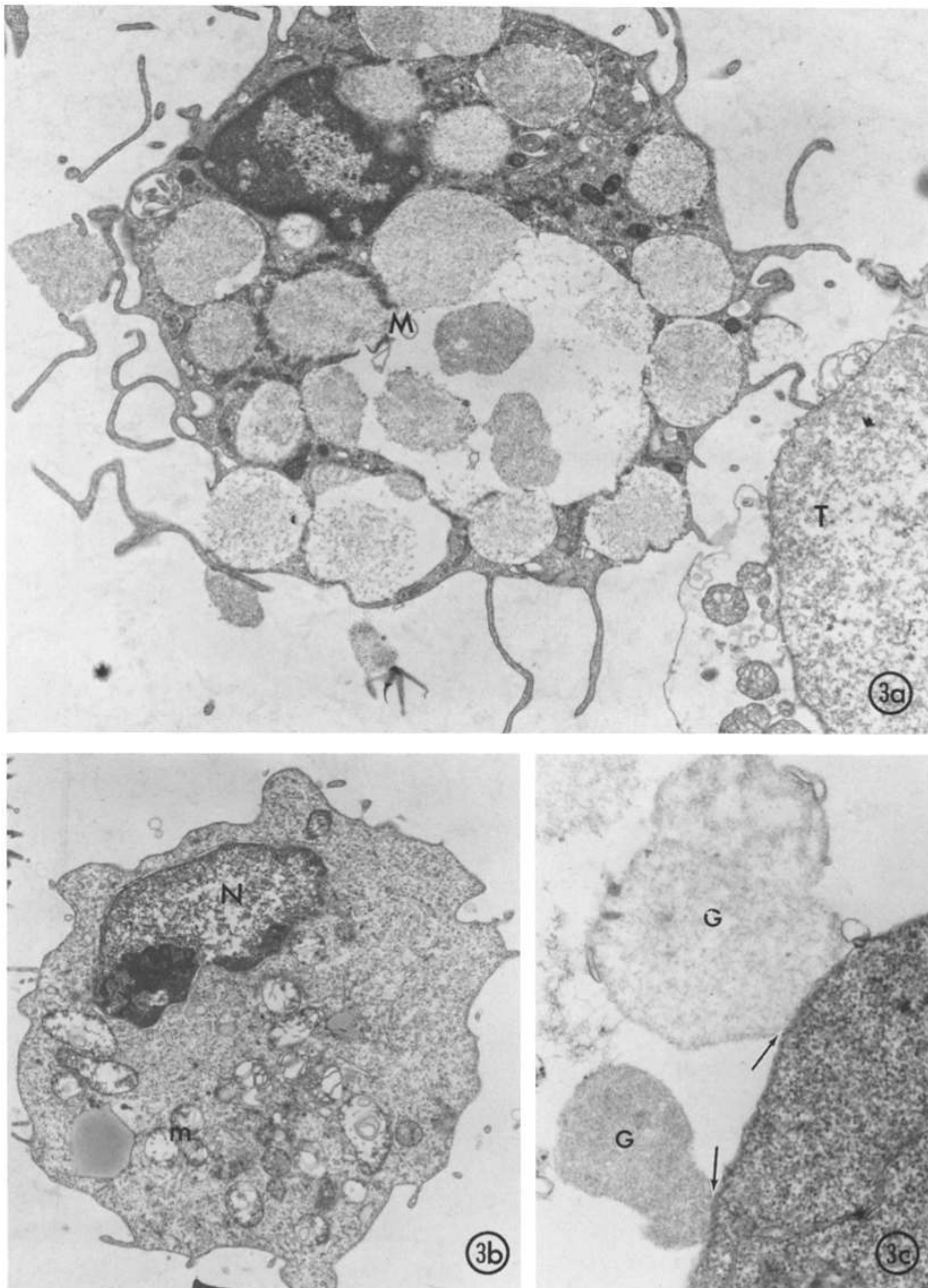


FIG. 3. Effect of H_2O_2 and iodide on mast cell-tumor cell interaction. Transmission electron microscopy. Mast cells were incubated with tumor cells in standard salt solution containing H_2O_2 (10^{-4} M) and iodide (10^{-4} M) for 30 min at 37°C as described in Table I. (a) Mast cells (M) showed extensive degranulation; their cytoplasm, cytoplasmic organelles and surface structure remained otherwise intact. Portion of a tumor cell (T) exhibiting cytolytic changes is shown. × 11,500. (b) The cytotoxic effect of the mast cell-H_2O_2-iodide system is well visualized in this tumor cell. There is nuclear pyknosis (N), aggregation and dilatation of the mitochondria (m), and loss of surface microvilli. × 8,500. (c) Secreted mast cell granules (G) were often noted to adhere to the tumor cell surface (arrows). × 21,000.

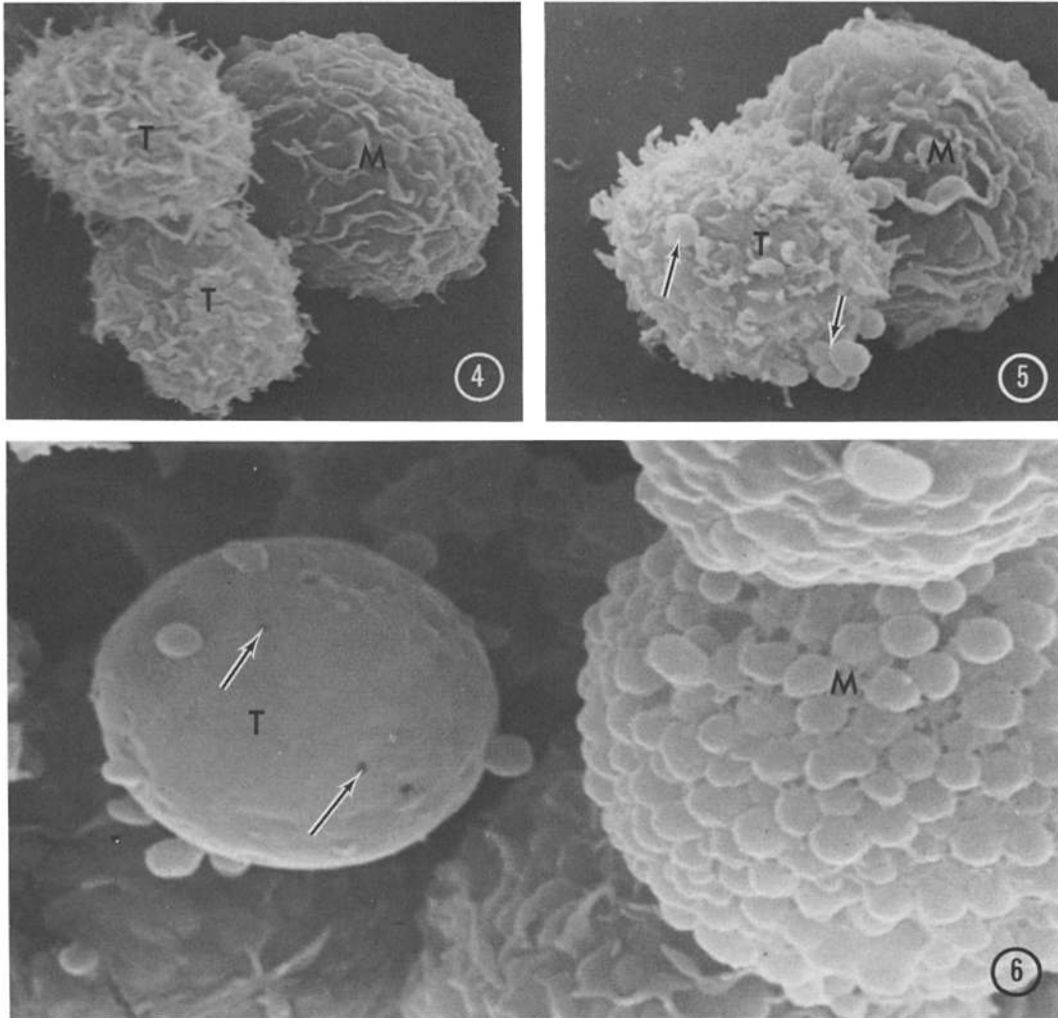


FIG. 4. Mast cell-tumor cell interaction. Scanning electron microscopy. Mast cells (M) and tumor cells (T) were incubated in the standard salt solution as in Fig. 1. The mast cells show the characteristic surface ridges of unstimulated mast cells. The tumor cells which are slightly smaller than the mast cells, have prominent surface microvilli and folds. × 5,100.

FIG. 5. Effect of 48/80 on mast cell-tumor cell interaction. Scanning electron microscopy. Mast cells (M) and tumor cells (T) were incubated in the standard salt solution containing compound 48/80 as in Fig. 2. The mast cell contained fewer surface ridges, and bulges were observed over submembranous granules which is typical of the degranulation process. MCG were adherent to the tumor cell surface (arrows) which was otherwise unchanged. × 5,500.

FIG. 6. Effect of H_2O_2 and iodide on mast cell-tumor cell interaction. Scanning electron microscopy. Mast cells (M) and tumor cells (T) were incubated with H_2O_2 and iodide under the same conditions outlined in Fig. 3. Many extracellular granules are noted on the surface of mast cells and tumor cells, and the tumor cell surface showed striking cytotoxic changes. There was a loss of microvilli and membrane folds, and holes (arrows) in the surface membrane were noted. × 12,000.

electron microscopy revealed a striking disruption of the tumor cell nuclear and cytoplasmic structure with aggregation and dilatation of mitochondria and loss of cell-surface microvilli (Fig. 3). Scanning electron microscopy showed a loss of the

normal villous projections, alteration of cell shape, and formation of holes in the cell surface (Fig. 6). MCG were evident on the smooth tumor cell surface (Fig. 3c; Fig. 6).

Tumor cells were unaffected by incubation with H_2O_2 and iodide in the absence of mast cells under our experimental conditions. Their appearance by either transmission or scanning electron microscopy was identical to tumor cells incubated in the standard salt solution alone or with mast cells in standard salt solution (Fig. 1; Fig. 4). Similarly, the tumor cells were morphologically intact when H_2O_2 or iodide was omitted from the mast cell-H_2O_2-iodide system.

H_2O_2-induced Mast Cell Secretion. The mast cell secretion observed when mast cells are incubated with tumor cells, H_2O_2, and iodide (Table I; Fig. 3a; Fig. 6) is due to the added H_2O_2. Under our experimental conditions, mast cells incubated in the standard salt solution with 10^{-4} M H_2O_2 alone for 1 h released 45.3 ± 4.6% (mean ± SE; $n = 6$) of their histamine content (background 8.5 ± 2.7%; $n = 6$; $P < 0.001$) without a corresponding release above background of the cytoplasmic marker, LDH (4.1 ± 2.1% compared with 3.6 ± 1.8% background release; n = 6). Iodide (10^{-4} M) plus tumor cells did not significantly increase histamine release from mast cells above background in the absence of H_2O_2 (9.3 ± 3.1%; n = 3), nor did they affect histamine release by 10^{-4} M H_2O_2 (50.1 ± 6.1%; n = 3). As previously reported (14), lower concentrations of H_2O_2 (2×10^{-5} M) did not induce mast cell degranulation unless supplemented with EPO and a halide.

The induction of noncytotoxic mast cell degranulation by H_2O_2 was confirmed by electron microscopy (Fig. 7). Mast cells incubated in the standard salt solution alone exhibited the usual morphology of unstimulated cells with numerous electron-dense, membrane-bound granules (Fig. 7a). The surface view (Fig. 7b) revealed the typical ridges and villous projections of normal mast cells. H_2O_2 (10^{-4} M) induced the formation of vacuoles containing swollen and less dense granules and extracellular extrusion of the altered granules (Fig. 7c). The reduction in surface ridges, the formation of bulges on the cell surface, and the extrusion of granules revealed by scanning electron microscopy were characteristic of mast cell secretion (Fig. 7d). No concomitant cell damage was evident.

MCG-mediated Tumoricidal Activity. The degranulation of mast cells by H_2O_2 and the associated damage to tumor cells on the addition of iodide raised the possibility that the MCG was the mast cell component required for toxicity. This is supported by the data shown in Table I. Incubation of ^{51}Cr-labeled tumor cells with MCG, iodide (10^{-4} M), and H_2O_2 (10^{-4} M) increased ^{51}Cr release from a background 19.9 to 61.9% ($P < 0.001$). This cytotoxic effect was abolished by omission of any MCG-H_2O_2-iodide system components, by heat inactivation of MCG, and by the hemeprotein inhibitors azide and aminotriazole. Cyanide (10^{-3} M) decreased ($P < 0.05$ vs. complete system) but did not abolish ($P < 0.01$ vs. background) activity.

When MCG were incubated with the tumor cells without H_2O_2 and iodide, the granules adhered to the surface of the cells but no cytotoxic effect was noted by scanning electron microscopy (Fig. 8). The further addition of H_2O_2 and iodide, however, resulted in extensive damage (Fig. 9) comparable to that observed when intact mast cells were employed (Fig. 6).

Effect of EPO on MCG-induced Toxicity. EPO is cytotoxic to the LSTRA tumor cell line when combined with H_2O_2 and iodide (9), and this enzyme forms a firm complex with MCG with retention of peroxidatic activity (11). Table II compares the cytotoxic

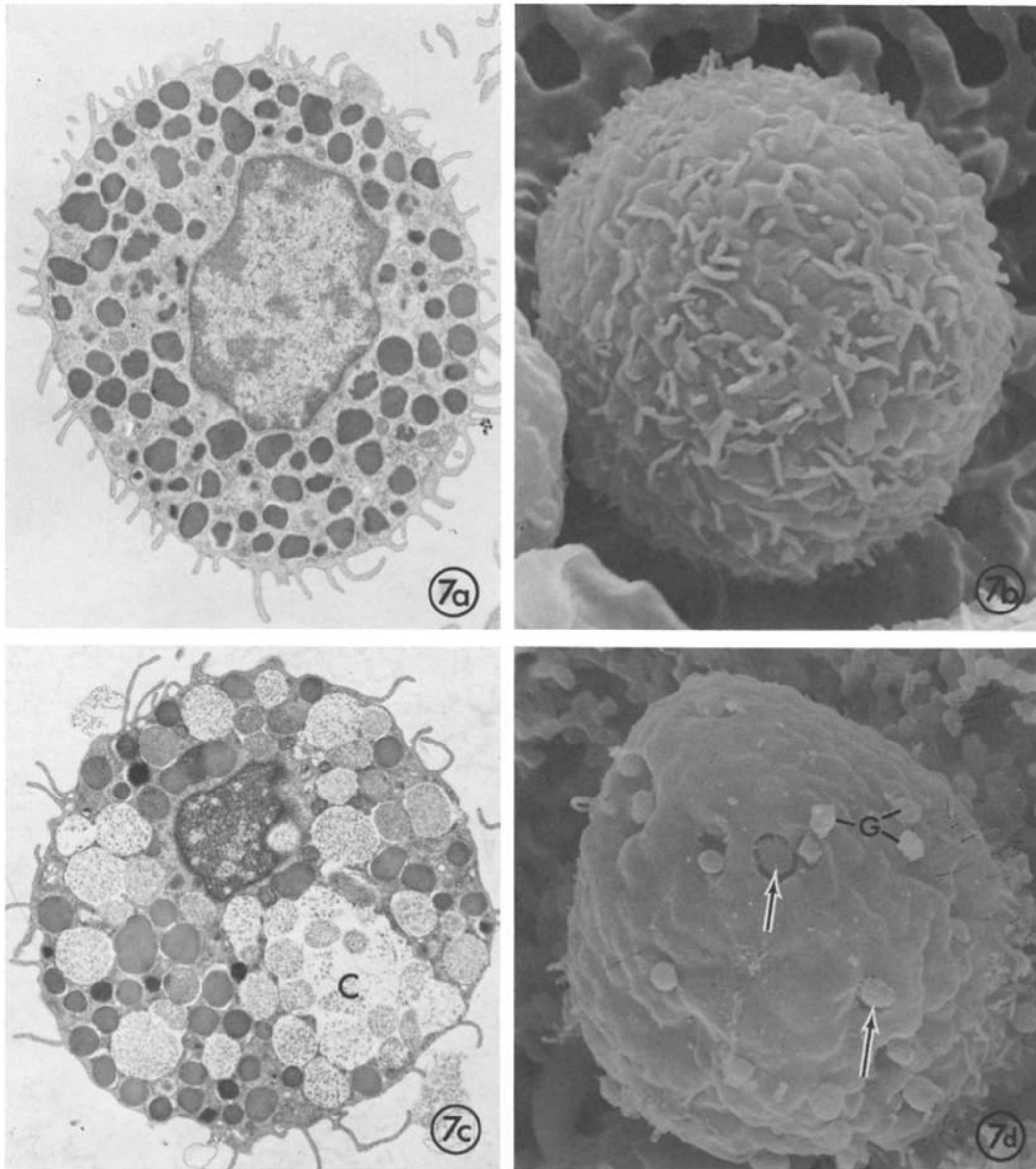


FIG. 7. H_2O_2-induced mast cell secretion. Mast cells were incubated in the standard salt solution without (a,b) or with (c,d) added H_2O_2 (10^{-4} M) for 30 min at 37°C. (a) Transmission electron microscopy shows a typical unstimulated mast cell with its many electron-dense granules and prominent microridges evenly distributed over the surface membrane. × 10,500. (b) The predominant surface microridges were evident by scanning electron microscopy. × 11,500. (c) Noncytotoxic mast cell degranulation was seen by transmission electron microscopy with the typical formation of channels (C) containing swollen granules. × 8,500. (d) Scanning electron microscopy revealed surface structure typical of mast cell degranulation. Pores were open to the outside of the cells and extracellular granules (G) were seen on the cell surface and within the pores (arrows). Surface bulges and the loss of most of the microridges were noted. × 10,500.

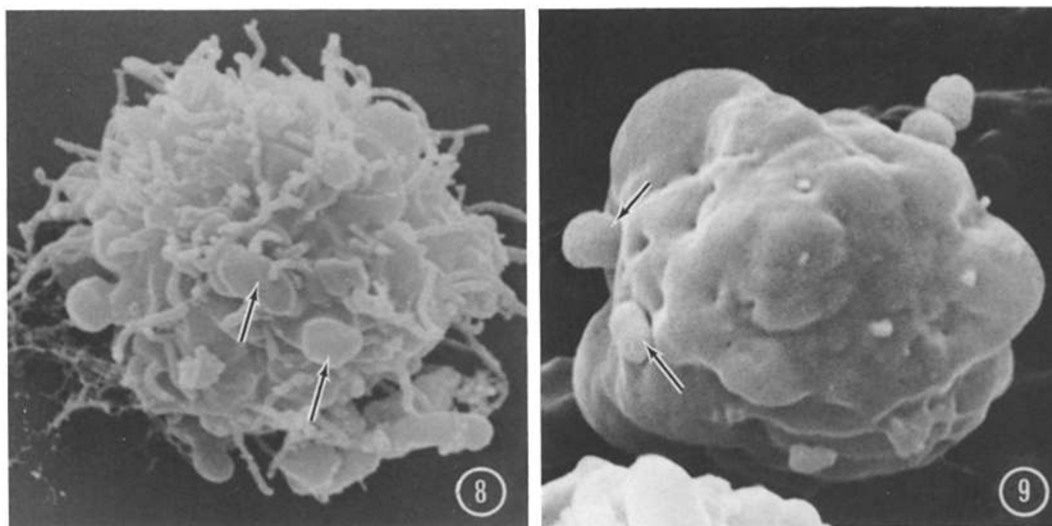


FIG. 8. Effect of MCG on tumor cells. MCG were incubated with tumor cells in buffered salt solution (see Table I) for 30 min at 37°C. The granules attached to the tumor cell surface (arrows) but no cytotoxic damage to the tumor cells was noted by scanning electron microscopy. × 6,500.
FIG. 9. Effect of MCG-H_2O_2-iodide system on tumor cells. MCG were incubated with tumor cells in buffered salt solution containing H_2O_2 (10^{-4} M) and iodide (10^{-4} M) for 30 min at 37°C. Scanning electron microscopy showed that the tumor cell surface structure was greatly altered with loss of microridges, changes in cell shape, and holes in the cell membrane. MCG were adherent to the tumor cell surface (arrows). × 8,500.

TABLE II
*Comparison of the Cytotoxic Activity of the MCG and MCG-EPO Systems**

Protein	^{51}Cr release		
	MCG	MCG-EPO	*P*‡
μg	%		
0.2	29.5 ± 3.1§	59.3 ± 4.8	<0.002
2.0	39.8 ± 3.4	84.9 ± 5.5	<0.002
8.0	59.6 ± 5.4	89.7 ± 3.5	<0.02
20.0	77.1 ± 5.2	92.3 ± 4.9	

* The reaction mixture was as described for the MCG system in Table I except that the amount of MCG or MCG-EPO complexes was varied as indicated. The peroxidase activity of the MCG was 0.02 mU/μg protein and that of the MCG-EPO complex was 0.20–0.23 mU/μg protein. Background ^{51}Cr release (without MCG, MCG-EPO, H_2O_2, or iodide) was 22.1 ± 3.5%.
‡ Probability values for the difference between the MCG and MCG-EPO systems, where significant ($P < 0.05$).
§ Mean ± SE of five experiments.

activity of the MCG-EPO complex to that of MCG, standardized to equal granule-protein concentrations. In the 0.2–8.0 μg granule-protein range, ^{51}Cr release was significantly increased when EPO was bound to the granules under the conditions employed. This difference was lost when the granule concentration was increased to 20 μg protein. The MCG-EPO complex was also more effective as a component of the peroxidase system than was free EPO over a range of peroxidase activity from 0.1 to

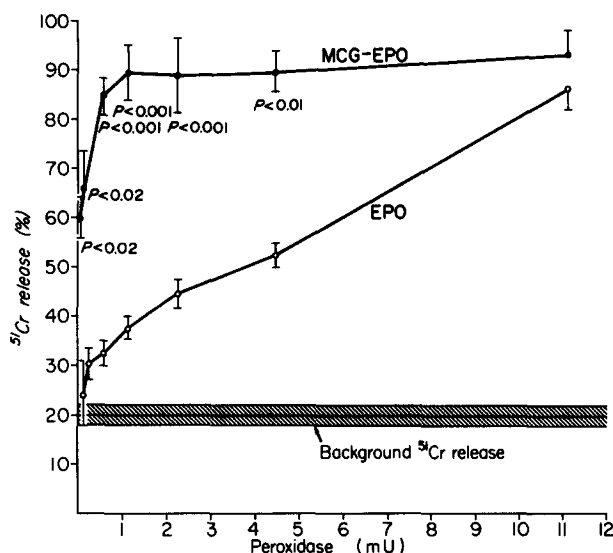


FIG. 10. Comparison of the cytotoxic activity of the MCG-EPO complex and EPO systems. The reaction mixture was as described for the MCG system in Table I except that the MCG were replaced by EPO (○) or MCG-EPO complexes (●) at the indicated peroxidase activity. The data are the mean ± SE of five experiments and the probability values for the difference between the EPO and MCG-EPO systems are shown where significant ($P < 0.05$).

TABLE III

*Effect of Halide Concentration on MCG-EPO-induced Cytotoxicity**

Supplements	^{51}Cr release			
	pH 7.0		pH 6.0	
	%	*P*	%	*P*
Background	19.5 ± 2.8 (5)‡		21.3 ± 1.6 (5)	
MCG-EPO + I^- (10^{-4} M) + H_2O_2	89.8 ± 5.1 (3)	<0.001	95.3 ± 4.0 (3)	<0.001
MCG-EPO + I^- (10^{-5} M) + H_2O_2	60.6 ± 4.5 (4)	<0.001	75.3 ± 6.1 (3)	<0.001
MCG-EPO + I^- (10^{-6} M) + H_2O_2	23.4 ± 3.9 (4)		64.5 ± 5.2 (4)	<0.001
MCG-EPO + I^- (10^{-7} M) + H_2O_2	24.2 ± 3.0 (4)		28.3 ± 2.3 (4)	<0.05
MCG-EPO + I^- (10^{-8} M) + H_2O_2	22.8 ± 2.8 (3)		20.1 ± 3.6 (3)	
MCG-EPO + BR^- (10^{-4} M) + H_2O_2	82.7 ± 3.9 (4)	<0.001	89.8 ± 5.1 (4)	<0.001
MCG-EPO + BR^- (10^{-5} M) + H_2O_2	54.8 ± 4.2 (3)	<0.001	64.7 ± 8.1 (3)	<0.001
MCG-EPO + BR^- (10^{-6} M) + H_2O_2	35.3 ± 5.2 (3)	<0.05	45.7 ± 7.5 (3)	<0.01
MCG-EPO + BR^- (10^{-7} M) + H_2O_2	25.2 ± 3.7 (4)		32.4 ± 4.1 (4)	<0.05
MCG-EPO + BR^- (10^{-8} M) + H_2O_2	21.9 ± 1.9 (4)		21.5 ± 2.3 (4)	
MCG-EPO + Cl^- (10^{-1} M) + H_2O_2	23.0 ± 2.7 (4)		42.0 ± 4.8 (4)	<0.01

* The reaction mixture was as described for MCG in Table I except that the MCG were replaced by MCG-EPO complexes (6 μg protein; 1.2–1.4 mU peroxidase activity) and the concentration of halide was varied as indicated. Probability values for the difference from background are shown where significant ($P < 0.05$).

‡ Mean ± SE of experiments (n).

4 mU guaiacol (Fig. 10). This difference was abolished with a further increase in peroxidase activity.

The MCG-EPO complex-H_2O_2-halide system was cytotoxic to the LSTRA cells at iodide concentrations of 10^{-4} and 10^{-5} M at pH 7.0, and 10^{-4}–10^{-7} M at pH 6.0

(Table III). Bromide was effective from 10^{-4} to 10^{-6} M at pH 7.0, and down to 10^{-7} M at pH 6.0. Chloride at 0.1 M was ineffective at pH 7.0, but could meet the halide requirement at pH 6.0. A 2.5 M solution of the NaCl employed was sent to Bio-Science Laboratories, Van Nuys, Calif., for assay of iodide and bromide. The total iodine was designated as "none detected." Because the assay employed can readily detect 0.1 μg/100 ml of iodide, this would indicate that the iodide contribution by the 0.1 M NaCl solution was $<5 \times 10^{-10}$ M or well below that which was toxic under the conditions employed in Table III. No bromide was detected in the 2.5 M NaCl solution. However the limit of the bromide assay was 0.1 mg/100 ml so that the bromide concentration contributed by the 0.1 M NaCl, although unknown, may be up to 5×10^{-7} M. Bromide is toxic at pH 6.0 at concentrations down to 1×10^{-7} M (Table III); therefore our data does not exclude the possibility that the chloride effect is due partially or totally to bromide contamination.

Discussion

We report here that mast cells are toxic to LSTRA tumor cells when combined with H_2O_2 and iodide. Our studies suggest the following sequence of events: H_2O_2 in the concentration used in the tumoricidal system (10^{-4} M) initiates noncytotoxic mast cell secretion; MCG contain a small amount of endogenous peroxidase (10); the MCG peroxidase reacts with H_2O_2 and iodide to kill the tumor cells. Thus H_2O_2 would serve here both to induce mast cell secretion and as a component of the MCG-H_2O_2-iodide cytotoxic system. Evidence in support of this sequence is as follows:

H_2O_2 at 10^{-4} M induces mast cell secretion (as measured by histamine release) without associated cell lysis (as measured by LDH release). The initiation of noncytotoxic mast cell degranulation by H_2O_2 is supported by ultrastructural studies (Fig. 7). Transmission electron microscopy revealed many swollen granules with decreased density in vacuoles and channels, some open to the outside of the cell; extracellular granules were seen by both transmission and scanning electron microscopy. There was no evidence of mast cell damage. This effect of H_2O_2 confirms the earlier findings of Ohmori et al. (12, 13) who reported that xanthine oxidase when incubated with its substrate, hypoxanthine, induced noncytotoxic histamine release from mast cells. Histamine release by this oxygen radical- and H_2O_2-generating system was inhibited by catalase but was unaffected by either the superoxide anion scavenger, superoxide dismutase, or the hydroxyl radical scavenger, mannitol, suggesting a role for H_2O_2. They also reported that H_2O_2 alone could initiate mast cell degranulation at concentrations of 5×10^{-5} M or greater (12, 13).

Isolated MCG when combined with H_2O_2 and iodide are toxic to the tumor cells, as measured by ^{51}Cr release and confirmed by electron microscopy. The properties of the mast cell- and MCG-mediated systems were comparable: each component of the systems (mast cells or MCG, H_2O_2, iodide) was required, and cytotoxic activity was abolished by the addition of the peroxidase inhibitors azide and aminotriazole. Cyanide was only partially inhibitory of the MCG system and did not affect the intact mast cell system under our experimental conditions. The relative insensitivity of the MCG peroxidase to cyanide was similar to the response of EPO to cyanide under comparable experimental conditions (9, 15). MCG were seen by electron microscopy to adhere to the surface of LSTRA tumor cells but cytotoxic damage (severe nuclear and cytoplasmic disruption, loss of surface folds, formation of holes in the surface

membrane) was induced only when H_2O_2 and iodide were added (Figs. 3, 5, 6, 8, and 9). Although our studies were performed only with mast cells, basophils, the blood-borne leukocytes with many properties similar to those of tissue mast cells, may react similarly. In an ultrastructural study of the immunologic rejection of syngeneic, chemically induced tumors implanted intradermally in previously sensitized guinea pigs (17), basophils were observed to be in close contact with both living and dead tumor cells and to degranulate. In vitro studies (18) indicated that basophils stimulated to degranulate either by antigen or lectin could exert a modest cytotoxic effect when cultured for 1–24 h with the tumor cells ($<10\%$ of tumor cells affected). Isolated basophil granules were observed to adhere to the tumor cell surface (18) with minimal or no damage resulting from this interaction. This is analogous to the attachment of unsupplemented MCG to the tumor cell surface in our studies. In our mast cell-mediated tumoricidal system, H_2O_2 and iodide were necessary for toxic activity, and it is not known whether these substances were required for the basophil-mediated damage.

Eosinophils may influence mast cell tumoricidal activity in a number of ways. Eosinophils in common with other phagocytes form and release H_2O_2 on appropriate stimulation (19–21) and thus could supply this needed component of the tumoricidal system. Degranulation of eosinophils can occur with the release of their granule peroxidase (22, 23). Although as reported here and by Ohmori et al. (12, 13), H_2O_2 alone can cause noncytotoxic mast cell secretion, when the H_2O_2 concentration is reduced to a level where it is no longer effective in this regard, the addition of EPO and iodide leads to a return in secretory activity (14). EPO, a positively charged protein, binds tightly to the secreted histamine-free MCG, forming a stable complex which dissociates only when suspended in hypertonic salt solutions (0.75 M or greater) (11). The MCG-EPO complex-H_2O_2-iodide system has significantly greater bactericidal and iodinating activity than the granule-free EPO system when the two preparations are standardized to equal guaiacol units of peroxidase activity (11). We report here that when combined with H_2O_2 and iodide, the MCG-EPO complex has greater tumoricidal activity than does either an equivalent weight of MCG without bound EPO or an equivalent amount of free EPO (as measured by guaiacol oxidation). At pH 7.0, iodide was effective at 10^{-4} and 10^{-5} M and bromide at 10^{-4} to 10^{-6} M; whereas at pH 6.0, significant cytotoxic activity as measured by ^{51}Cr release was seen at iodide or bromide concentrations down to 10^{-7} M. Chloride at 10^{-1} M could meet the halide requirement at pH 6.0 but not at pH 7.0.

These findings suggest that the following sequence of events could occur at tumor sites characterized by an eosinophil and mast cell response. Eosinophils attracted to the inflammatory site may be activated there with the secretion of EPO. H_2O_2 generated by the stimulated eosinophils or by other phagocytes in the region would be expected and the H_2O_2 either alone or combined with EPO and a halide may initiate mast cell secretion. The release of the chemical mediators of immediate hypersensitivity, together with the formation of stable MCG-EPO complexes with potentiated cytotoxic activity may then contribute to the host defense against the neoplastic cells.

Summary

Mast cells, when supplemented with H_2O_2 and iodide, are cytotoxic to mammalian tumor cells as determined by ^{51}Cr release, and transmission and scanning electron

microscopy. H_2O_2 at the concentration employed (10^{-4} M) initiates mast cell degranulation, and mast cell granules (MCG), which contain a small amount of endogenous peroxidase activity, are toxic to tumor cells when combined with H_2O_2 and iodide. This toxicity is greatly increased by binding eosinophil peroxidase (EPO) to the MCG surface. Each component of the mast cell, MCG, or MCG-EPO system was required and toxicity was inhibited by the addition of the hemeprotein inhibitors azide or aminotriazole, which is compatible with a requirement for peroxidase in the cytotoxic reaction. A sequence of reactions is proposed in which mast cells, stimulated to release their granules by H_2O_2 generated by adjacent phagocytes, react with H_2O_2 and a halide to damage tumor cells. EPO release from eosinophils may contribute to this sequence of reactions, both by stimulation of H_2O_2-induced mast cell secretion and by combination with MCG to form a complex with augmented tumoricidal activity. These reactions may play a role in the host defense against neoplasms.

We thank Coralie Baker, Gertrude Chiang, and Chuan Teh for technical help, and Caroline Wilson for secretarial assistance. We also thank Dr. Beate M. Czarnetzki for helpful discussions.

Received for publication 30 September 1980.

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