

ORIGINAL ARTICLE

Chronic Allergic-like Inflammation in the Tumor Stroma of Human Gastric Carcinomas: An Ultrastructural Study

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ABSTRACT

Inflammatory cell infiltration around the sites of carcinoma invasion is believed to play important roles in tumor biological behavior. The status of inflammatory cell infiltration at the sites of frank invasion in 92 cases of gastric carcinomas was examined, with special emphasis on tumor-associated tissue eosinophilia (TATE). TATE was found in 7 out of 92 (7.6%) gastric carcinomas (6 of intestinal-type and 1 of diffuse-type). Electron microscopy, selectively performed in the 7 cases of gastric carcinomas with TATE, showed that eosinophils participated in the stromal reaction by interacting with tumor cells, mast cells, and each other. Most of the tumor-infiltrating mast cells exhibited anaphylactic or piecemeal degranulation, indicating that the mast cells had been activated in situ. Some mast cells were noted in close contact to viable tumor cells, suggesting the existence of direct cell-to-cell interactions. There was also extracellular deposition of free eosinophil granules and Charcot-Leyden crystals. These morphologic findings are similar to that described in late/chronic-phase allergic reaction in both human and experimental animals, where angiogenesis and fibrosis/tissue repair are also present. In conclusion, TATE may indicate a chronic allergic-like Th2 host–tumor reaction, and understanding these pathways should create tools to enhance defence and contrast neoplastic disease.

Keywords: Electron microscopy, gastric carcinoma, mast cells, tumor-associated tissue eosinophilia

Eosinophils are innate immune leukocytes implicated in the initiation and maintenance of type 2 immune responses, including helminth infection and exposure to various allergens [1,2]. The ability to store and rapidly secrete preformed cytokines distinguishes eosinophils from most lymphocytes, which must synthesize cytokine proteins prior to secretion [3]. Type 2 inflammatory reactions are also characterized by mast cell/basophil infiltration and by extensive tissue remodeling [3]. The complexity of Th2 inflammation can be appreciated in studies of asthma and other disorders [4–6]. The role of mast cells in allergic reaction has been confined to its acute/early phase, whereas the late/chronic phase of inflammation is usually attributed to the effects of infiltrating eosinophils and leukocytes [7]. However, recent studies in several in vitro and in vivo allergic inflammation conditions have shown a complex network of paracrine

and membrane interaction between mast cells and eosinophils [8,9].

The role of Th2-mediated events in tumor immune surveillance and, in particular, mast cells and eosinophils, has not been thoroughly investigated [10]. Th2-mediated immunity has traditionally been viewed as favoring tumor growth, both by promoting angiogenesis and by inhibiting cell-mediated immunity and subsequent tumor cell killing. However, there also many studies demonstrating the anti-tumor activity of CD4⁺ Th2 cells, by directly recruiting tumoricidal myeloid cells such as eosinophils [10]. It is well established that eosinophils contain cytotoxic granules proteins, such as major basic protein, eosinophil peroxidase, eosinophil cationic protein, and eosinophil-derived neurotoxin, which are capable of inducing tissue damage [1,2], and these mediators could be harnessed to directly kill tumor cells [10]. Mounting evidence indicates that

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mast cells accumulate around tumors and could either promote or inhibit tumor growth, depending on the local stromal conditions [11]. Therefore, it has been suggested that mast cells might act as a new target for the adjuvant treatment of solid tumors through the selective inhibition of tumor-promoting molecules but permitting secretion of cytotoxic cytokines [12–16].

A review of published reports shows that tumor-associated tissue eosinophilia (TATE) and tumor-associated blood eosinophilia may be seen in tumors of different histological types from different anatomical sites, and may occur together or separately [17]. Our previous ultrastructural studies, regarding gastric carcinomas with TATE, described extracellular deposition of membrane-bound eosinophil granules and Charcot-Leyden crystals as well as heterotypic aggregation between activated eosinophils and viable tumor cell [17,18]. Because recent literature data suggest that mast cell–eosinophil interactions form a crucial axis in allergic inflammatory and fibrotic diseases [8,9], we further extended our ultrastructural observations to study the mutual interaction between the frequently occurring eosinophils and mast cells in gastric carcinomas characterized by TATE.

MATERIALS AND METHODS

The Department of Human Pathology (University Hospital, Messina, Italy) had maintained files of 92 cases of surgically resected stomach in which gastric carcinomas were routinely processed for both light and electron microscopic observations. The eosinophils, identified by hematoxylin–eosin (H & E), were counted in the tumor stroma using a light microscope (Zeiss Axioplan). A high-power field (HPF) on this microscope (i.e., using a $\times 40$ objective) measures 0.6 mm in diameter on the slide, giving an area of 1.88 mm². According to Cuschieri *et al.* [19], TATE was recorded as “intense” (an average of 5 or more eosinophils on 10 HPFs), “moderate” (an average of less than 5 eosinophils in 10 HPFs), or “absent” (no eosinophils present).

For electron microscopy, small pieces of the fresh tumor tissue were immediately fixed in 3% phosphate-buffered glutaraldehyde (pH 7.4) and postfixed in 1%

osmium tetroxide. Semithin araldite-embedded sections were stained with Giemsa’s reagent for selection of fields. Two blocks per case were studied ultrastructurally. Thin sections were double-stained with uranyl acetate and lead citrate; they were then examined and photographed in a Zeiss EM 902 electron microscope (Carl Zeiss, Oberkochen, Germany).

No quantization regarding image analysis of eosinophils, mast cells, and tumor cells was undertaken and therefore no statistical data are presented regarding image analysis.

RESULTS

Intense TATE was found in 7 out of 92 (7.6%) gastric carcinomas (6 of intestinal type and 1 of diffuse type). These 7 neoplasms ranged from 3 to 7 cm in diameter, and subsequent H&E-stained sections established the diagnosis in all tumors as advanced gastric carcinomas. Patients ranged in age from 55 to 77 years and comprised 5 males and 2 females. In the 6 cases of intestinal-type adenocarcinomas, eosinophils formed varied-sized aggregates both within the tumor stroma and at the tumor–host interface in the absence of tumor necrosis (Figure 1). Some were seen passing through the neoplastic epithelium, and others were found lying within neoplastic tubules similar to “crypt abscesses” (Figure 1a). Semithin sections revealed mast cells in clusters containing 1 mast cell and 1–3 eosinophils (Figure 1b). In 1 case of diffuse-type carcinoma, eosinophils single or in cluster were found in intimate contact with tumor cells. Semithin sections showed partially degranulated eosinophils and numerous extracellular granules in this case (Figure 2).

Electron microscopy confirmed the presence of homotypic aggregation of eosinophils in the 7 cases of gastric carcinoma with TATE. Eosinophils single or in a group displayed irregular cytoplasmic membrane processes accompanied by extensive piecemeal degranulation, highlighted by the partial and/or complete loss of electron-dense secondary granule crystalline cores and the loss of electron opacity within the granule. Some eosinophils showed ultrastructural features of apoptosis, such as electron-dense condensed

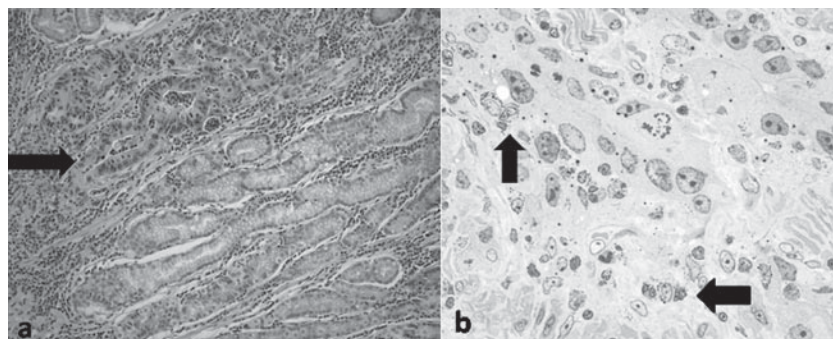


FIGURE 1 (A) Selective infiltration of eosinophils in gastric adenocarcinoma (arrow). H & E $\times 200$. (B) Clusters containing one mast cell and two eosinophils (arrows) are present in the tumor stroma. Giemsa, semithin section, $\times 200$.

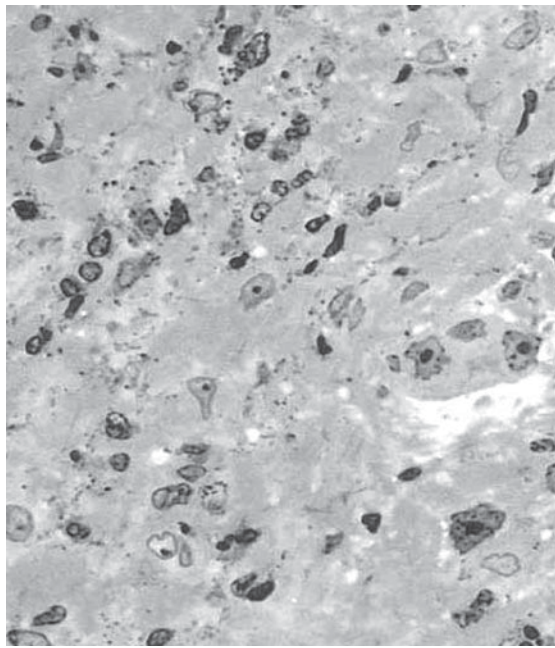


FIGURE 2 Partially degranulated eosinophils and numerous extracellular granules are identifiable in the tumor stroma of a patient with a diffuse-type gastric carcinoma. Giemsa, semithin section, $\times 200$.

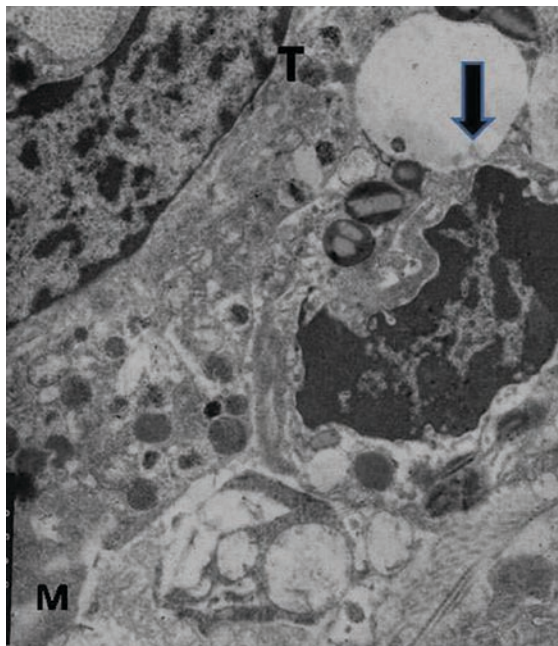


FIGURE 3 Mast cell (M) in close contact with eosinophil showing apoptotic changes (arrow) such as cytoplasmic vacuolization and condensed chromatin. A tumor cell (T) is near the mast cell. $\times 6000$.

nuclear chromatin, focal dilatation of nuclear envelope, preserved plasma membrane, and integrity of the organelles (Figure 3). Eosinophils with signs of exocytosis (extrusion of “whole” membrane-free specific granules) or eosinophils displaying chromatolysis and loss of plasma membrane integrity were not found. Electron microscopy also provided several examples of heterotypic aggregations between mast

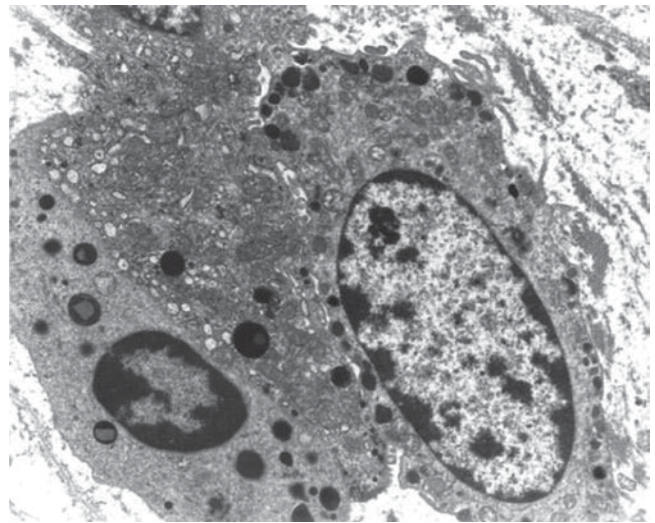


FIGURE 4 Heterotypic aggregation between eosinophil, mast cell, and tumor cell, characterized by extensive areas of plasma membrane contact. $\times 6000$.

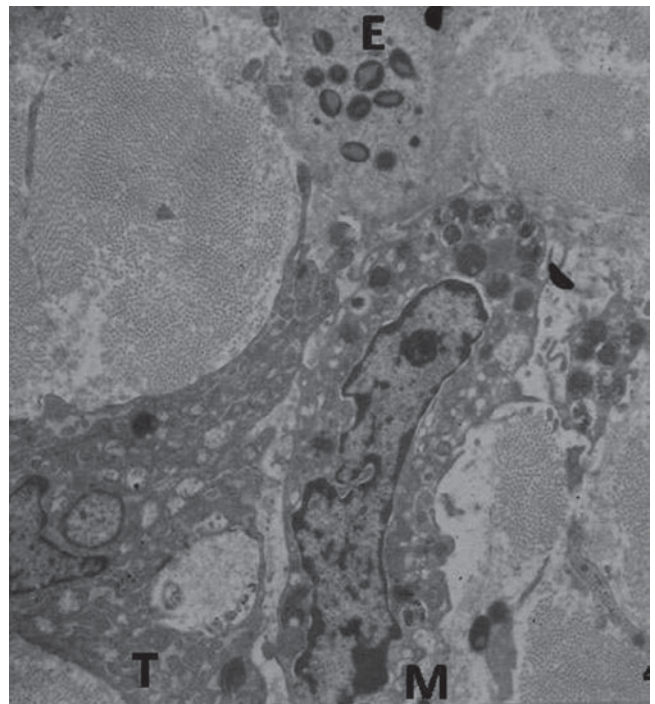


FIGURE 5 Mast cell (M) showing piecemeal degranulation and eosinophil (E) adjacent to a tumor cell (T). Note the extracellular collagen deposition. $\times 6000$.

cells, eosinophils, and viable tumor cells. The type of apposition of eosinophils/mast cells and tumor cells appeared to be “simple,” i.e., plasma membranes running roughly parallel, with no modification of the membrane or cytoplasm (Figure 4). Mast cells included mature cells with full complement of granules displaying homogeneous, scroll, particle, and mixed pattern. The adjacent eosinophils showed no ultrastructural signs of degranulation; i.e., all specific granules had a solid core surrounded by an intact matrix. Other mast cells showed piecemeal degranulation (Figure 5) or

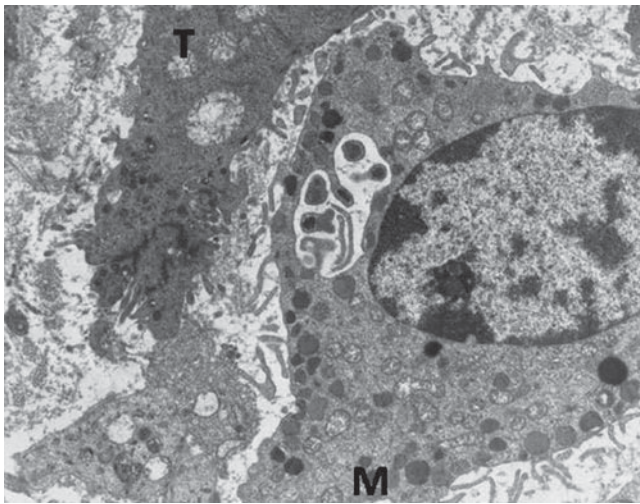


FIGURE 6 Mast cell (M) undergoing recovery from anaphylactic degranulation evidenced by large canaliculi with internalized surface processes. Cytoplasmic portion of a tumor cell is in close proximity to this degranulated mast cell. $\times 6000$.

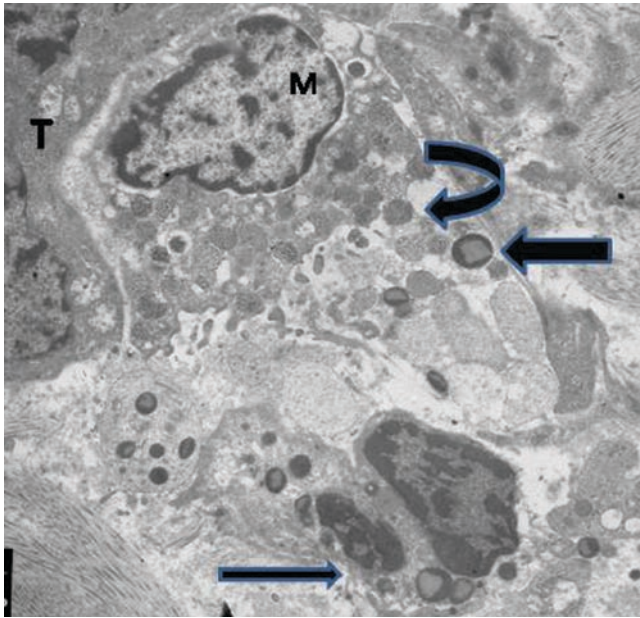


FIGURE 7 Activated mast cell (M) shows anaphylactic degranulation and is in close proximity of tumor cells (T). Note the coexistence of extracellular membrane-bound eosinophil (thick arrow) and mast cell granules (curved arrow). Apoptotic eosinophil is also present (thin arrow). $\times 6000$.

were characterized by many mitochondria, a few cytoplasmic granules, smooth contour, expanded Golgi areas, and intracellular canaliculi that occupied a large portion of cytoplasm (Figure 6). In other areas, mast cells showed anaphylactic degranulation characterized by release of granule content, with formation of channels that were continuous with the extracellular environment (Figure 7). These degranulating mast cells were in close physical proximity to tumor cells and apoptotic eosinophils (Figure 7). Clusters of three or more extracellular membrane-bound secondary

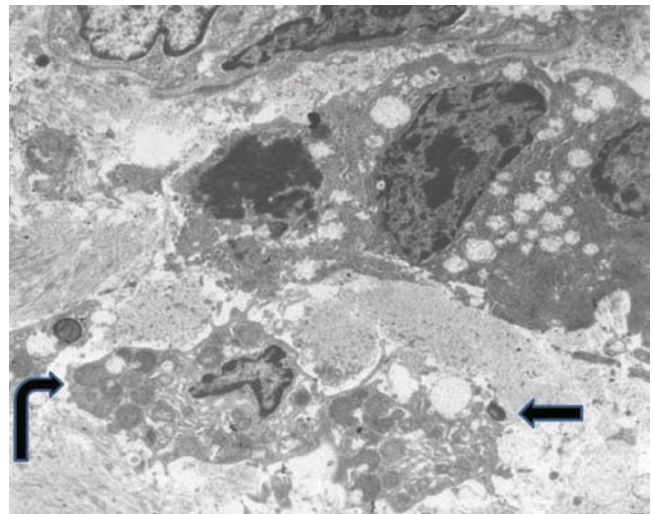


FIGURE 8 Elongate, apparently motile degranulated mast cells (curved arrow) in contact with an extracellular membrane-bound eosinophil granule (arrow). $\times 6000$.

granules were adjacent to apoptotic eosinophils (Figure 7). Bipyramidal, hexagonal, and irregular crystals morphologically similar to Charcot-Leyden crystals were found at the extracellular level or in phagosomes of tissue macrophages (not shown in figure). Some mast cells showed a motile configuration characterized by elongated tail-like structures. Close contacts of mast cells and extracellular membrane-bound eosinophil granules were noted (Figure 8).

DISCUSSION

Our ultrastructural data show that TATE is characterized by complex relations between eosinophils, tumor cells, and mast cells in the tumor microenvironment. Mast cells in proximity of tumor cells showed piecemeal degranulation or were characterized by many mitochondria, a few cytoplasmic granules, smooth contour, expanded Golgi areas, and intracellular canaliculi that occupied a large portion of cytoplasm. These are structures that occur infrequently in *in situ* mast cell populations and increase dramatically in the recovery phase from anaphylactic degranulation [20]. Other mast cells showed anaphylactic degranulation, an explosive and rapid secretory event that is completed within minutes of stimulation [20]. This type of mast cell degranulation is characterized by secretion via degranulation channels or by direct fusion of granule containers with the cell membrane [20].

The initial recruitment and activation of eosinophils toward the tumor microenvironment are principally related to Th2 response, although necrotic cells can stimulate both the migration and the activation of eosinophils [21,22]. A recent study by Cormier *et al.* [23] examined the molecular mechanisms of recruitment and accumulation in TATE employing a classical

well-defined B16 melanoma cell tumor model. In this experimental study, the investigators demonstrated that eosinophils were predominantly localized in the necrotic and capsule region of the tumor. By contrast, the common histologic feature of all our cases was a diffuse eosinophilic infiltrate within the tumor stroma unassociated with tissue necrosis. Moreover, a recurrent ultrastructural hallmark was the membrane contacts between eosinophils and viable tumor cells. These data suggest that TATE, observed in our cases of gastric carcinomas, is predominantly related to Th2 response rather than to tumor tissue necrosis.

As suggested by recent studies [8,9], bidirectional interactions between mast cells and eosinophils serve to stimulate each other's functions, thereby perpetuating chronic allergic reactions. Basic proteins and lipid mediators of eosinophils have been found to activate mast cells, and mast cells mediators were shown as eosinophil stimulators. Accordingly, heterotypic mast cell–eosinophil aggregates were detected in the tumor stroma at both the light and the electron microscopic levels. In these complex multicellular aggregates, mast cells showed piecemeal or anaphylactic degranulation, whereas eosinophils displayed morphologic characteristics of activation (e.g., eosinophil homotypic aggregation, irregular cell surface) accompanied by extensive piecemeal degranulation [5,24]. Our ultrastructural findings are similar to that recently described in allergic disorders both in vitro and in vivo [8,9] and, therefore, provide morphological evidence for the existence of an allergic-like reaction in a subset of gastric carcinoma characterized by TATE. In parallel to allergic reaction, it is possible that mast cells recruit eosinophils into tumor stroma and that physical interplay between mast cell–eosinophil can create favorable conditions for sustaining chronic inflammation.

Although extracellular eosinophil granules are visible at light microscopy level, electron microscopy remains a fundamental tool to recognize the distinct core-containing ultrastructure of membrane-bound, extracellular eosinophil granules. The presence of membrane-bound, cell-free granules extruded from eosinophils has long been recognized in tissues involved by allergic diseases and responses to helminthes [25]. However, our previous study provided the first electron microscopic evidence of extracellular deposition of membrane-bound extracellular granules adjacent to late apoptotic eosinophils in the tumor stroma of human gastric carcinomas [18]. Functional capabilities for extracellular granules have recently been demonstrated [25]. Neves et al. [25] reported that extracellular eosinophil granules function as independent organelles after eosinophil lysis. Therefore, extracellular deposition of intact eosinophil granules yields extracellular organelles fully capable of ligand-elicited active secretory responses able to act as functional “minefield” amplifying the differential secretory properties of eosinophils and contributing to

the persistence and exacerbation of the inflammatory response in the tumor stroma. Accordingly, we showed close contact between mast cells and extracellular membrane-bound eosinophil granules. These findings confirm and extend our previous studies showing that mast cells may play an important role in local stromal reaction against cancer cells through an indirect mechanism involving not only adjacent eosinophils but also their extracellular granules [26].

In conclusion, the ultrastructural data presented in this study suggest that events in TATE include homotypic eosinophil aggregation; heterotypic aggregation of eosinophils, mast cells, and tumor cells; piecemeal and anaphylactic degranulation of mast cells; eosinophils undergoing piecemeal degranulation or apoptosis; as well as extracellular deposition of membrane-bound eosinophil granules and Charcot-Leyden crystals. This stromal reaction has several morphologic features in common with Th2-mediated immune reactions recently described in chronic allergic inflammation in both human and experimental animals [7–9]. Therefore, TATE may indicate a chronic allergic-like Th2 host reaction occurring in a subset of gastric carcinomas. These results warrant further investigation of the role of eosinophils and mast cells in the initiation and progression of gastric cancer. Understanding these pathways should create tools to enhance defence and contrast neoplastic disease.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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