Data from several laboratories strongly support the existence of a cancer immunosurveillance process whereby the immune system, in the absence of external manipulation, protects the host against tumor development [1]. However, there has been a growing recognition that immunosurveillance represents only one dimension of the complex relationship between the immune system and cancer [1]. Recent work has shown that the immune system may also promote the emergence of primary tumors with reduced immunogenicity that are capable of escaping immune recognition and destruction [1]. These findings prompted the development of the cancer immunoediting hypothesis to more broadly encompass the potential host-protective and tumor-sculpting functions of the immune system throughout tumor development [1,2]. Cancer immunoediting is a dynamic process composed of three phases: elimination, equilibrium, and escape. Elimination represents the classical concept of cancer immunosurveillance, equilibrium is the period of the immune-mediated latency after incomplete tumor destruction in the elimination phase, and escape refers to the final outgrowth of tumors that have outstripped immunological restraints of the equilibrium phase [1,2]. These three phases result from the cross-talk between tumor and host immune cells [2]. Cross-talk may be mediated through direct heterotypic cell–cell contacts or through paracrine secretion of molecules, comprising growth factors, cytokines, chemokines, extracellular matrix, proteins, and lipid products [3]. Contact-dependent communication has several advantages over paracrine interplay, one of which is that it is highly precise, efficiently relaying the signal selectively to the target cells [4]. Eosinophils are multifunctional leukocytes classically described as being involved in helminthic parasitic infections and allergic diseases. Previously restricted to an exclusive role in the release of cytotoxic mediators,
they are now also considered to be immunoregulatory cells and potential effectors in innate immune responses [5]. Eosinophil numbers have also been documented to be elevated in peripheral blood and/or infiltrate the tissues in some malignant disorders [6]. This eosinophilia is called tumor-associated tissue eosinophilia, or TATE.

We previously studied the host response to gastric carcinomas, especially the relationship between eosinophils and cancer cells [7,8]. In the ultrastructural work presented here we have used gastric carcinoma with TATE as a rich source of eosinophils to demonstrate intimate association between eosinophils and tumor cells in vivo.

**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 tumors were routinely processed for both light and electron microscopic observation from 1990 through 2001. None of the patients had undergone preoperative irradiation or immunochemotherapy. The fragments of fresh tumor tissue were divided into two portions with a sharp razor blade. The first member of the pair was processed for routine paraffin embedding together with additional tissue samples taken from the tumor as well as from surgical borders of the specimen. These sections were stained with hematoxylin and eosin (H&E), and the histologic type was defined according to Lauren classification. The second piece of the paired samples was minced into smaller pieces and destined for electron microscopy. This material was immediately fixed in 3% phosphate-buffered glutaraldehyde, pH 7.4, and postfixed in 1% osmium tetroxide. Semithin araldite-embedded sections were stained with Giemsa reagent for selection of fields. For electron microscopy, ultrathin sections were double-stained with uranyl acetate and lead citrate; they were then examined in a Zeiss EM 902 electron microscope. A total of 140 tumor-associated tissue eosinophils were identified and photographed at 6000× magnification.

The eosinophils, identified by 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 ×40 objective) measures 0.6 mm in diameter on the slide, giving an area of 1.88 mm². According to Cuschieri et al. [9], TATE was recorded as “intense” (an average of 5 or more eosinophils on 10 such HPFs), “moderate” (an average of less than 5 eosinophils in 10 HPFs), or “absent” (no eosinophils present).

No quantization regarding image analysis of tumor cells and eosinophils 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. Some were seen passing through the neoplastic epithelium, and others were found lying within neoplastic tubules similar to “crypt abscesses” (Figure 1). In 1 case of diffuse-type carcinoma, eosinophils, single or in cluster, were found in contact with tumor cells. Aggregation, margination, diapedesis, and exudation of eosinophils were seen within blood vessels immediately adjacent to the tumor (Figure 2).

At the ultrastructural level, eosinophils were recognized by their polylobed nucleus, specific granules, lipid bodies, and smooth tubulovesicular structures.
Eosinophils could be seen adhering to viable tumor cells singly or in groups (Figures 3,4A).

Focal intimate contacts were made between eosinophils and viable tumor cells; these consisted of sites approximately 1–1.5 µm in length, where the plasma membranes of the respective cells smoothly paralleled each other and were separated by an extracellular gap of uniform width (approximately 25 nm) (Figure 5). Viable tumor cells in contact with eosinophils showed plasma membrane caveolar invaginations (Figure 3). Sometimes, these caveolae were seen on unopposed surfaces of these tumor cells (Figure 3). Eosinophils showed intact specific granules (with no signs of degranulation, i.e., intact core and matrix) and/or “activated” (various structural changes due to piecemeal degranulation e.g., ragged loss of core material, intragranular tubular structures, and more or less empty granules) (Figures 3–5).

Other tumor cells contacted by eosinophils exhibited damage of varying degree (Figure 4B) or were necrotic (Figure 6). Minimal damage (early damage) of the tumor cells display cytoplasmic vacuolization, rows of plasma membrane caveolar invaginations, and mitochondrial swelling (Figure 4B), whereas the remaining part of the tumor cell frequently remains unchanged (Figure 4A).

Necrotic tumor cells were characterized by swelling of mitochondria and endoplasmic reticulum as well as plasma membrane rupture (Figure 6).

**DISCUSSION**

The eosinophil, like the neutrophil, has the capacity to accumulate at sites of inflammation. It has been reported that human eosinophils when activated undergo homotypic aggregation in vitro [10,11]. Our morphologic findings demonstrated stromal and intravascular homotypic aggregation of eosinophils. Therefore, in analogy with the neutrophils, there was morphologic evidence of eosinophil activation both in the stroma and within blood vessels immediately adjacent to gastric carcinomas.
Our ultrastructural study demonstrated that the tumor cells contacted by eosinophils were characterized by necrosis or exhibited damage of varying degree. In particular, relatively early damage to the tumor cell frequently occurred at the contacting regions with the eosinophil, whereas the remaining part of the tumor cell stayed unchanged. Because this was a purely morphologic study, no direct evidence of cytotoxicity could be provided. According to the immunoediting hypothesis [12,13], our morphologic findings suggest an eosinophil-mediated control of gastric carcinoma progression. In their specific granules, eosinophils store several dozen preformed cytokine proteins and four cationic proteins, known to be highly cytotoxic mediators [12]. Despite their apparent fulfilment of the prerequisite criteria as destructive inflammatory cells, we cannot exclude the possibility that eosinophils are not the cause of tumor cell death. Instead, these leukocytes may simply be first responders to signals released by stressed and/or dying tumor cells [13–15]. A recent study by Cormier et al. [15] 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 predominantly localized in the necrotic and capsule region of the tumor [16]. Unfortunately, the lack of kinetic studies in patients or animals models unambiguously defining the timeline between tissue damage and eosinophil recruitment leaves the issue of linkage unresolved [15].

Our ultrastructural study provides several examples of heterotypic aggregations between activated eosinophils and viable tumor cells. The type of apposition of eosinophils and tumor cells appeared to be “simple,” i.e., plasma membranes running roughly parallel, with no modification of the membrane or cytoplasm. These heterotypic eosinophil–tumor cell aggregations are similar to those described in experimental murine tumors in which the local presence of IL-2 and IL-4, whether repeatedly injected at the tumor-growth site or directly released by cytokine-gene-transduced tumor cells, can convert poorly immunogenic tumors into ones that are able to elicit an effective immune response [17]. In these experimental models, contact among eosinophils and tumor cells formed a dominant and recurrent ultrastructural hallmark [18]. At other sites of interaction, eosinophils showed piecemeal degranulation, whereas plasma membrane caveolar invaginations were found in the adjacent tumor cell. Caveolae are small (60–80 nm) invaginations of the plasma membrane, found in almost all cells of the body [19]. They have been shown to play a role in a variety of cellular processes, including endocytosis, cholesterol homeostasis, and signal transduction [19]. The reciprocal change seen in these sites of contact is strong morphological evidence that heterotypic aggregation between eosinophils and viable tumor cells is more than mere topographical association, but may truly represent a immunologic interaction. By interacting, eosinophils and tumor cells build up a synaptic-like chamber that may be considered a very precise, confined, and specific anatomical site of cross-talk between these cells. The fact that caveolae were also present on unopposed surfaces of tumor cells did not detract from the probability that immunologic interchanges were taking place with eosinophils, since interaction between cells is not always associated with close alignment of orientation and may also be by diffusion in intercellular fluids.

In conclusion, our qualitative ultrastructural findings show an intimate association between eosinophils and viable tumor cells characterized by caveolar invaginations in their plasma membrane. Our observations represent an in vivo correlate of in vitro functional studies and provide morphological evidence that in gastric carcinoma with intense TATE at least cross-talk exists between eosinophils and viable tumor cells.

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REFERENCES


