Phagocytosis of Cancer Cells by Mast Cells in Breast Cancer

FILIPPO DELLA ROVERE[1], ANGELO GRANATA[1], MAURIZIO MONACO[1] and GIACOMO BASILE[2]

[1]*Department of Thoracic Surgery,* [2]*Institute of Anatomic Pathology, Polyclinic G. "Martino", Messina, Italy*

Abstract. *Background: Mast cells (MCs) remain enigmatic more than 100 years after their discovery by Paul Ehrlich. Continuous research over the last 20 years has finally characterized the origin of MCs and determined many of the factors involved in MC differentiation and proliferation. MCs are traditionally known for mediating allergic reactions. In addition, these cells have been implicated in the pathogenesis of clinical conditions. Studies on the role of MCs in cancer have given contrasting results. Materials and Methods: This study included 50 cases of invasive ductal breast cancer not otherwise specified (NOS): 25 of them were highly hormone-receptive (HHR) cancers with estrogen and progesterone receptor values not lower than 50%, 25 were minimally hormone-receptive (MHR) cancers (<5%). In both groups, mast cells were quantified in the peritumoral area. Twenty cases of surgical interventions without cancer were included as controls. Results: It was found that in infiltrating ductal breast cancers having a high hormone receptor content (>50% for both estrogen and progesterone), there was a highly significant increase in MCs with respect to hypo-hormonal cancers in the same location and to controls ($p<0.0001$). MCs have thus proven to be very important cells because they have been found in sites playing an active role in opposing the aggression of the cancer cells (CCs). MCs may represent a protective factor of the human body against cancer aggresion. Two biological phenomena with the same goal can be observed: CCs are first phagocytized by MCs and then completely destroyed by karyocytoplasmic chemolysis through the action of toxicophore granulations. It was demonstrated that one or more CCs are surrounded by an MC's pseudopodia and then engulfed in its cytoplasm. The phagocytized cell progressively loses its chromatic and volumetric characteristics until complete achromia and almost complete reduction of its volume and consistency occur. The cell nucleus soon degenerates to pyknosis and becomes no longer detectable.*

Correspondence to: Filippo della Rovere, Chirurgia Toracica Policlinico "G. Martino" Viale Gazzi 98165 Messina, Italy. Tel: +393 280266230, Fax: +390 902213051, e-mail: fdellarovere@unime.it

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Mast cells (MCs) have a 25-30 μm diameter and can modify their morphology depending on their activity and intratissue location. Their cytoplasm can be so granulated that karyoplasm is no longer visible. MCs are recognisable for their content of metachromatic granules when appropriately fixed and stained with metachromatic dyes such as toluidine blue (1). Granules contain preformed substances, such as histamine, which has a vasodilating action and increases vascular permeability, and heparin, a proteoglycan having an anticoagulat action. MC activation also induces neoformation of molecules deriving from the membrane: leukotrienes and cytokines (*e.g.* TNF-α) (2). MCs have many functions exerted through their ability to produce a host of biologically active substances, the most notable being heparin, serotonin, dopamine, tryptase and chymase. MCs are found to be functionally heterogeneous, possibly site-specific and have the ability to adapt to their environment (3). MCs are still enigmatic cells more than 100 years after their discovery by Paul Ehrlich.

Continuous research over the last 20 years has finally characterized the origin of MCs and determined many of the factors involved in MC differentiation and proliferation. In humans, a pluripotent cell is included in the $CD34^+$ bone marrow population (4). The progenitors differentiate from primitive cells under the influence of cytokines (interleukin-3, IL3). The MC, rich in granules and coated with antigen-specific IgE, migrates from blood circulation to subepithelial regions and completes its differentiation into connective tissue. MCs exist in every organ, but mainly in skin, the respiratory tree, connective tissue and blood vessels (1).

MCs appear to be highly engineered cells with multiple critical biological functions. Their best known activation mechanism occurs through the IgE antibody, which forms from plasma cells as a consequence of initial exposure to antigens. Following a second exposure to an antigen, the receptor aggregation of a single antigen molecule to two or more IgE molecules activates the MC. The granules of the activated MC are subjected to degranulation and release preformed and stored substances (5). Two types of

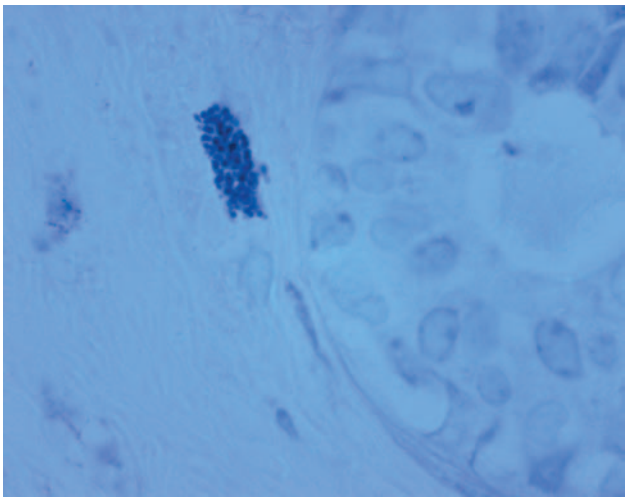

Figure 1. *A mast cell with a cytoplasm full of granules (alert stage).*

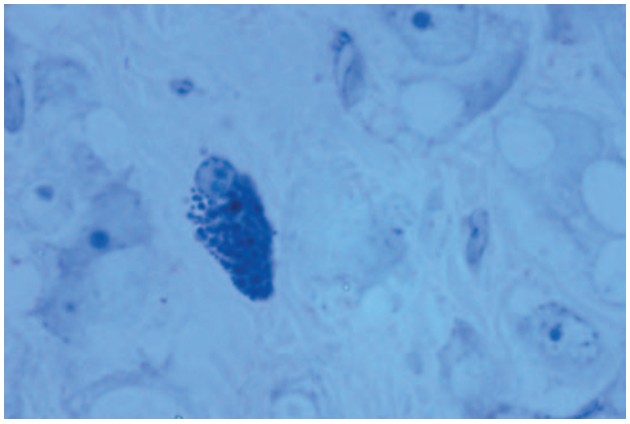

Figure 2. *A mast cell with pseudopodium engulfing a cancer cell (CC), Note the change of the pole that touches the malignant cell. The pole is filled with granules which concentrate in the peripheral area of the neoplastic cell.*

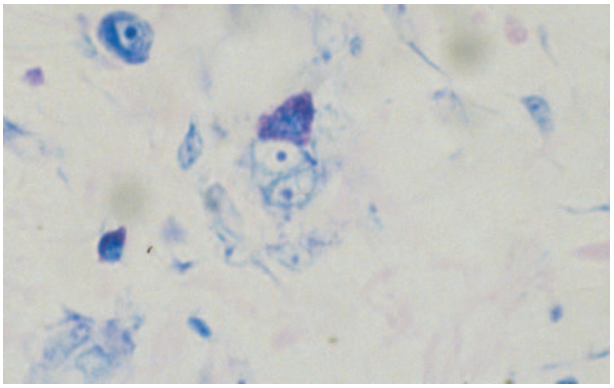

Figure 3. *In the center, an MC engulfing two neoplastic cells at different lysis stages is shown. The one with achromic cytoplasm and pyknotic nucleus is in contact with the MC and lysis occurs. The pole that has touched the CC is less filled with granules than in Figure 2.*

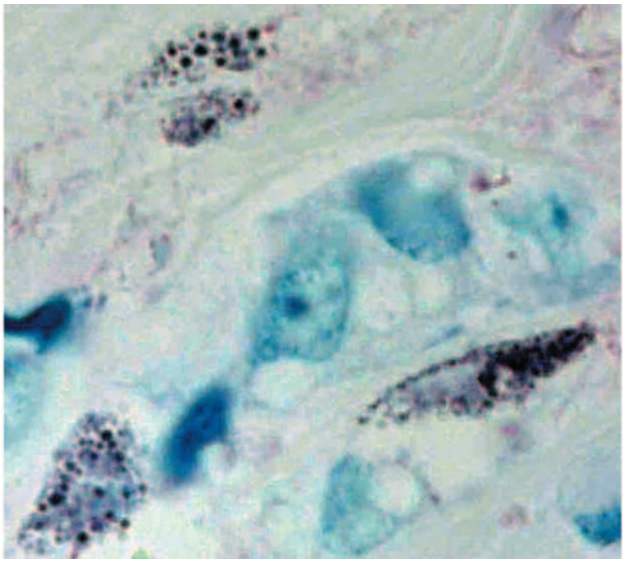

Figure 4. *A group of mast cells that have surrounded a nest of neoplastic cells, some of which are still located on pseudopodial extensions. Some of the neoplastic cells are achromic and without a nucleus due to advanced lysis. The pole that has engulfed the CCs has almost no granules left.*

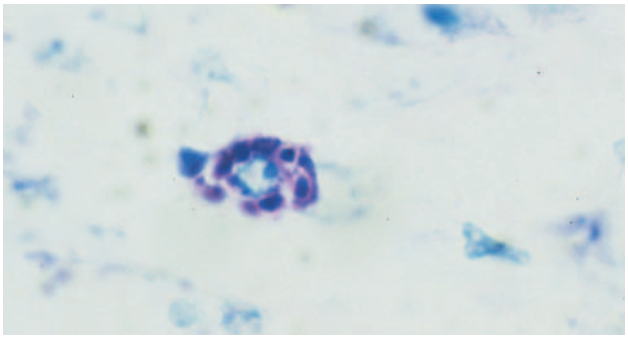

Figure 5. *MC with a pseudopodium containing a neoplastic cell that appears to be achromic due to advanced lysis.*

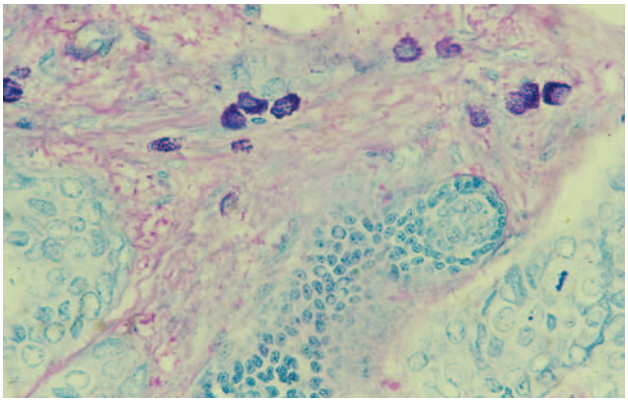

Figure 6. *MCs with their cytoplasm full of granules. MCs act as a barrier against a host of neoplastic cells. Many MCs can be seen here to have engulfed CCs.*

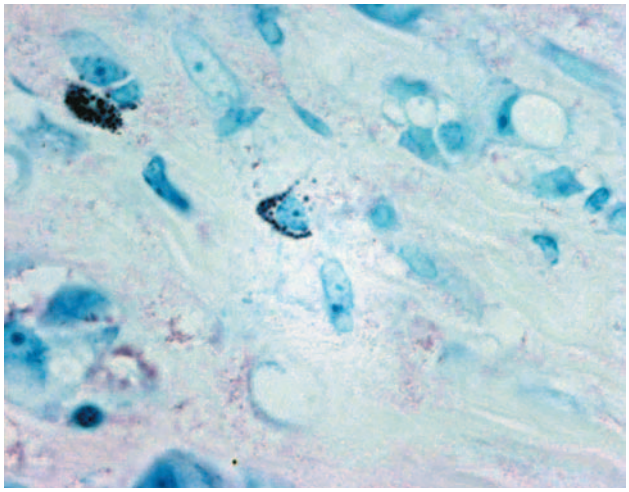

Figure 7. *Top left, MC granules attacking the membrane of two CCs surrounded by pseudopodium. Bottom, right: An MC modifies its shape to capture more CCs.*

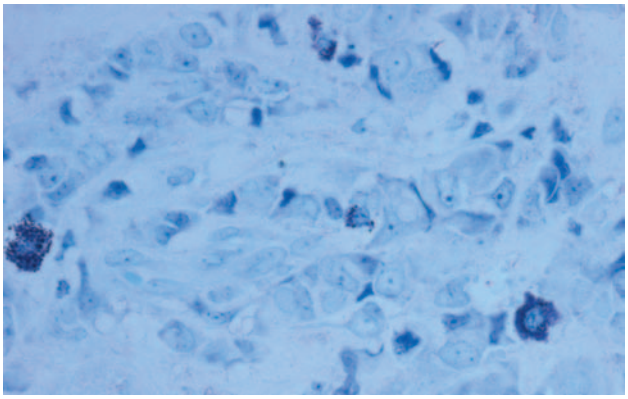

Figure 8. *Various stages of CC engulfment by several MCs. In the center, an MC modifies its shape to capture several CCs by a very long pseudopodium.*

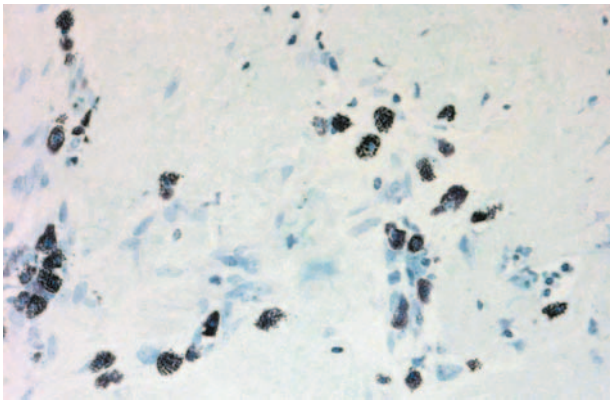

Figure 9. *A very large number of MCs that have surrounded a nest of neoplastic cells, some of which are still on pseudopodial extensions. Some of the neoplastic cells are achromic and have undergone lysis.*

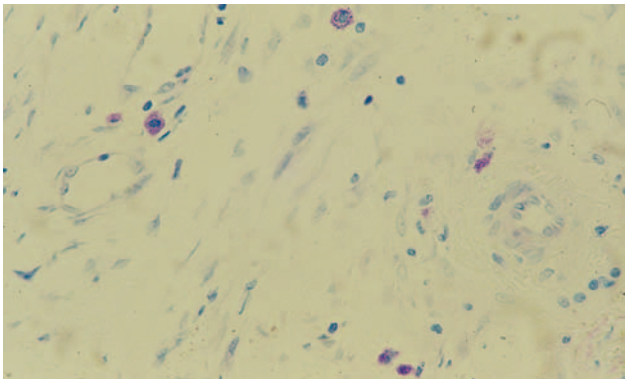

Figure 10. *Many MCs at different stages of CC engulfment.*

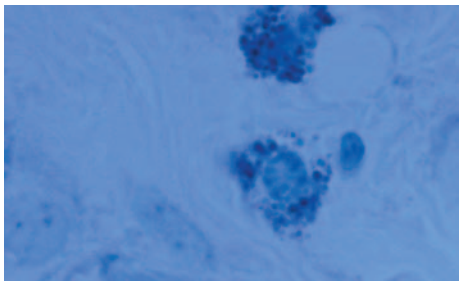

Figure 11. *Top, center: MC with a long transparent pseudopodium is lightly visible containing a neoplastic cell that appears to be achromic due to advanced lysis.*

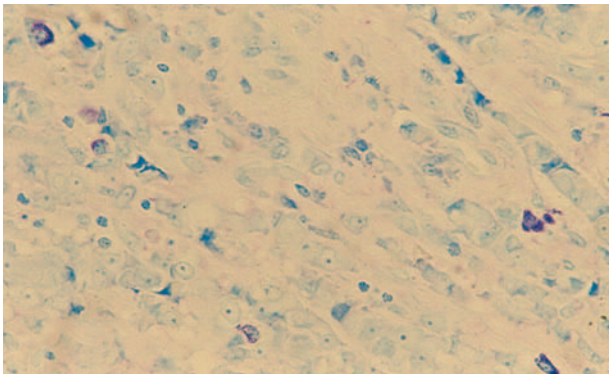

Figure 12. *Several MCs showing antineoplastic activity.*

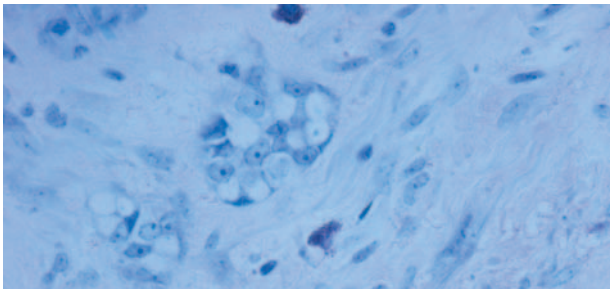

Figure 13. *Three MCs showing different steps of phagocytosis and cytolysis of neoplastic cells. Many CCs can be seen to be achromic and without nucleus. These MCs enter the end stage of their defence role against cancer aggression and can be defined as macromastocytes with pluriphagocytotic activity.*

degranulation may occur. In the case of asynchronous degranulation, rapid granule exocytosis occurs but MC reaction takes place locally. In the case of anaphylactic degranulation, MC reaction can be very widespread and cause anaphylactic shock; in this case extensive degranulation occurs (5). Although MCs are highly differentiated, mature MCs show a remarkable proliferation potential, which might imply a close relationship between MCs and multi-potential progenitors (6). MCs are traditionally known for mediating allergic reactions and more recently have been implicated in a variety of infections (7). In addition, these cells have been implicated in the pathogenesis of autoimmune disorders: atopic and contact dermatitis, bullous pemphigoid, fibrotic lung disease, neurofibromatosis, psoriasis, rheumatoid arthritis, interstitial cystitis, ulcerative colitis and Crohn's disease (7). MCs are involved in non specific inflammatory reaction, fibrosis, angiogenesis and wound healing, and play an important role in the protection against certain bacterial infections (8). MCs have an ability to modulate the host's innate immune response to infectious agents. Like effector cells of the immune innate system, MCs are able to discern a variety of infectious agents and to attach to them. The MC membrane is replete with many receptor molecules including those that promote recognition and binding of bacteria (2).

Though it has been well recognized for over 40 years that in sheep MCs have phagocytic capabilities, the significance and implications of this property have not been adequately examined. Much of the interest in MCs has centred on their exocytic functions (2). MCs have been shown to be a critical source of neutrophil chemoattractants following immune complex injury and allergic inflammation. Of the multiple chemoattractants that can be released by MCs, tumor necrosis factor alpha (TNF-α) was of special interest because MCs have the unique capacity to store presynthesized TNF-α and thus are able to release it spontaneously after activation. Protection against multicellular parasites occurs through vasoactive mediators such as histamine and serotonin, which are released by degranulation. MCs can ingest particles through IgG, IgE and complement receptors (9). Not much is known regarding the precise biological significance of phagocytosis by MCs *in vivo* because attention has been focused on the major contributions of MCs to the host defence against bacterial infection (10). The MCs are key players in the regulation of innate as well as adaptive immunity (11). Studies on the role of MCs in cancer have given contrasting results (12, 13).

Only in the last years has researchers' attention focused on the results of mimetope and MC experiments. Mimetopes are the minimal structural elements which are required to elicit a specific humoral and cellular immune response and consist of 6 to 20 amino acids. They have been experimentally employed in rats as vaccines against many types of solid tumors and the results are promising (14).

Table I. *Descriptive statistics regarding mast cell presence in tumors from high and minimum hormone-receptive breast cancer and controls.*

	HHR (%)		
	MCs	ER	PR
Mean	0.41	70.00	72.00
S.D.	0.16	12.00	9.00
Min	0.22	50.00	50.00
Max	0.64	90.00	85.00
	MHR		
	MCs	ER	PR
Mean	0.17	≤5	≤5
S.D.	0.11	-	-
Min	0.04	<5	<5
Max	0.39	=5	=5
	Controls		
	MCs	ER	PR
Mean	0.13	-	-
S.D.	0.06	-	-
Min	0.06	-	-
Max	0.22	-	-

HHR, High hormone-receptor tumors; MHR, minimum hormone-receptor tumors; S.D., standard deviation; ER, estrogen receptor; PR, progesterone receptor.

In a previous study (15), it was found that in infiltrating ductal breast cancers having a high hormone receptivity (>50 for both estrogen and progesterone), there was a highly significant increase in MCs with respect to hypo-hormonal cancer in the same location and to controls ($p<0.0001$).

In the present study, a closer observation of what happens at the sites where cancer cells (CCs) encounter MCs is desribed.

Materials and Methods

Samples. Fifty samples were selected from the archives of the Pathological Anatomy Institute of Messina. The paraffin-embedded samples included 25 cases of invasive ductal breast cancer not otherwise specified (NOS) with both estrogen and progesterone receptors higher than 50% (HHR) and 25 cases of invasive ductal breast cancer NOS with minimum estrogen and progesterone receptors (<5%) (MHR). These groups were compared with 20 cases of mammary parenchyma from surgery unrelated to cancer which were examined as controls.

MC count. The MC count was obtained through the examination of 4 μm-thick tissue sections that had been dehydrated and stained with Alcian blue (pH 0.3) and Giemsa (pH 0.7). The mean was obtained counting the number of MCs in the mammary parenchyma

per 10 fields under light microscopy at a magnification of ×200. The method of della Rovere *et al*. (15) was employed for percentage quantification. The two staining procedures gave similar MC numbers and the mean of the two readings was considered. MCs present in the peritumoral area were counted, while the overtly abnormal areas were not included. The slides were stained with toluidine blue, except number 6, which was stained with Giemsa.

Statistical analysis. A non parametric combination (NPC test) (16) was performed to assess any difference among the three groups with regard to mast cell percentage. A $p<0.05$ was considered statistically significant.

Results and Discussion

It was found that in infiltrating ductal breast cancers having a high hormone receptor content (>50% for both estrogen and progesterone), there was a highly significant increase in MCs with respect to hypo-hormonal cancers in the same location and to controls ($p<0.0001$) (Table I).

Microscope examination of micronized preparations from tumor exeresis of subjects with very different ages and anamneses have all confirmed CCs phagocytosis by MCs. The occurrence of this phenomenon is well documented by Figures 1-13 that illustrate the five stages that characterize phagocytosis of CCs by MCs in their attempt to counter cancer invasion. The five stages can be thus summarized: a) an MC carried by blood supply approaches CCs; b) the MC protrudes a pseudopodium that completely engulfs the CC; c) the CC is transferred from the pseudopodium to MC cytoplasm and is immediately surrounded by toxic granulations; d) CC chemolysis occurs through nuclear pyknosis and cytoplasmic dyschromia to achromia, then the CC loses its consistency with only a few fragments of cell membrane and karyoplasmatic tissue still visible; e) the MC continues to engulf other CCs that undergo chemolysis until the MC cytoplasm becomes saturated. At the same time, cytotoxic granulations progressively disappear.

Presumably, MCs filled with engulfed CCs remain in circulation in the breast or connective tissue until they are completely destroyed, as commonly happens to other cell elements after apoptosis. At this stage, the MCs assume a characteristic shape, as shown in Figure 13. They could be defined as "pluriphagocytotic macromastocytes" and their presence in the lymphatic circulation in around a tumor and in sentinel lymph nodes may have implications in diagnosis and prognosis.

Acknowledgements

This study is dedicated to the memory of my sister Alba. We thank Dott.sa Irene Olivieri for language review.

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