Case Report

Tumor Associated Macrophages-Friends Turned Foe? A Case Report And Review Of Literature

Jairajpuri Z S[1] *Agrawal U*[2]

Abstract

Most malignant tumors are infiltrated by inflammatory cells and it has long been considered that such infiltrates may be evidence of a host response to the tumor. Although lymphocytes are prominent in the inflammatory infiltrate, macrophages are also present, often in considerable numbers. Breast carcinoma is one such tumor where tumor associated macrophages (TAM) have been reported to be widespread, more so in Western literature. There is paucity of information about the biological and prognostic significance of this phenomenon in India. Ours is a tertiary center which receives approximately100 to120cases of breast carcinoma for pathological evaluation annually. Here we report the first case in our experience which showed large numbers of macrophages infiltrating the stroma around the tumor cells.

Key words: Tumor associated macrophages, breast carcinoma, angaiogenesis

Introduction

Breast cancer is the most common cancer in the women of developed countries and of a major health concern. Moreover, in India, without a significant reduction in carcinoma cervix, women are exposed to the increased risk of breast cancer, infact in some regions it has over taken cancer of the cervix.[1] Metastatic disease is the major cause of cancer mortality and of great clinical significance and concern. This fact indicates that metastases are refractory to traditional modalities of chemotherapy and radiotherapy. The increasing failure of available treatment suggests that biological mechanisms that underlie are poorly understood.

Inflammatory cells infiltrating malignant tumors have long been considered evidence of host response to the tumor, among them, of particular interest is the human breast carcinoma which has the potential for metastasis at a relatively early stage of the disease. Although lymphocytes are prominent, the inflammatory milieu comprises of monocytes and considerable number of macrophages. Tumor associated macrophages have been reported to be wide spread in carcinoma breast[2] Moreover it has been suggested that the presence of macrophages may independently influence the outcome by affecting the metastatic potential of tumors[3]. The identification of this feature is important to pathologists as well as surgeons, considering its prognostic implication. This report highlights the importance of this phenomenon with a review of pertinent literature more so in India where there are very few reports in this context

Case History

A 40 years female presented with a slow growing painless swelling in the left breast for 6 months with history of rapid increase in size. No other significant history was given. On clinical examination, a hard mass measuring 5x4cm was palpable in the left outer quadrant along with multiple smaller masses in the left anterior axillary fold. The mass had irregular borders with restricted mobility and was non tender. A trucut biopsy was done and a diagnosis of infiltrating ductal carcinoma, grade 3 was given. Patient showed a poor response to chemotherapy, infact the disease progressed to a large fungating mass. Even second line chemotherapeutic agents failed to elicit a desirable response. Surgical management was considered and the treatment of choice, Modified Radical Mastectomy was done.

On gross, the specimen measured 15x13x4cm with an elliptical skin flap. Serial sections revealed a hard grey white mass measuring 4x3.5cm with foci of hemorrhage and necrosis. Microscopic examination demonstrated islands of atypical cells in a dense infiltrate of macrophages [Figure I], confirmed by

1. Pr. Jairajpuri ZS, Hamdard Intritute of Medical Sciences and Research Jamia Hamdard, Hamdard Nagar, New Delhi 110062.
2. Dr. Usha Agrawal, Institute of Pathology Indian Council of Medical Research, Safdarjung Hospital Campus, New Delhi 110029

Corresponds to: Dr Usha Agrawal, Deputy Director, Room No. 602, Institute of Pathology, Indian Council of Medical Research, Safdarjung Hospital Campus, New Delhi 110029, ***Email: uburra@yahoo.com***

immunohistochemical staining with CD68 [Figure II], and lymphocytes. Macrophages also expressed vascular endothelial growth factor (VEGF) [Figure III]. Atypical cells were large with eosinophilic cytoplasm. Nuclei were large with prominent nucleoli and irregular margins. Mitosis was brisk. Areas of necrosis were also evident. Metastatic deposits were seen in the sentinel lymph node. Immunohistochemical staining for estrogen and progesterone receptors and Her 2 neu were all negative.

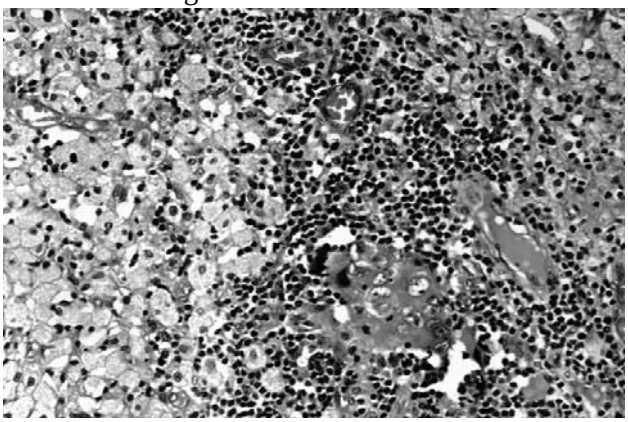

Figure I. *Photomicrograph showing tumour cell nest surrounded by numerous foamy macrophages. (H&E,40X)*

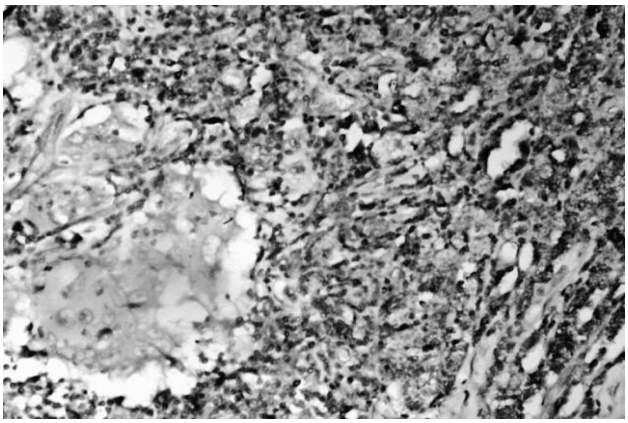

Figure II. *Expression of CD68 in the cytoplasm of the foamy macrophages.(IHC,40X)*

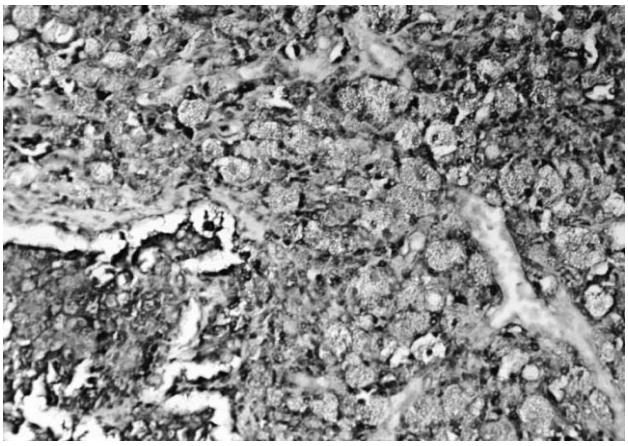

Figure III. *Expression of VEGF in both tumour cells and macrophages.(IHC,40X)*

Discussion

TAM especially in human breast is a well established fact. The biological and prognostic significance of this phenomenon has changed over the years. Some of the earlier reports suggest a positive prognostic effect of the infiltrate, fewer cases with metastases being found among those with high macrophage count. Moreover the available data at the time was more consistent with the view that macrophages in the tumor play an active part in preventing metastatic spread.[3,4] However Morrison et al have interestingly in a multricentric study suggested a negative association.[5] With emergence of conflicting results, existence of TAMs and their role became more and more intriguing. One approach to the investigation of the role of macrophages in human oncology is their quantitation in tumor material. Such a study was conducted by Kelly et al [2] and an analysis was made of macrophage counts according to stage, grade and prognostic index of the malignant tumor. No correlation between macrophage numbers and any of these parameters of breast carcinoma was found.

Over the years, considerable attention was focused on the paracrine activity of TAMs. Production of epidermal growth factor by macrophage is of much clinical significance it promotes the formation of elongated protrusions and cell invasion by carcinoma cells thereby promoting metastatic spread. [6] It has been suggested that growth stimulation and angiogenic activity of TAMs may provide rapid tumor proliferation. Studies have demonstrated the involvement of TAMs in local proteolysis, promoting invasion and metastasis of breast cancer and have the capacity to block local cytotoxic cytokines.[7,8] Evidence of an association between aggressive tumor behavior and intensive macrophage infiltration with worse prognosis (high tumor grade, estrogen receptor negative, high mitotic index) has been suggested,[9] observed in the present case too.

Angiogenesis, is a pre-requisite for tumor growth, cancer cell expansion and spread. There is documentation of specific macrophages expressing VEGF, recruiting to avascular hypoxic areas and in turn promoting tumor progression by releasing VEGF and other pro-angiogenic enzymes and cytokines which

stimulate angiogenesis.[10] In our case also strong positivity for VEGF was observed. Aggressive tumors rapidly outgrow their vascular supply, leading to areas of prolonged hypoxia and necrosis. This in turn may attract macrophages which then contribute to the angiogenic process, giving rise to an association between high levels of angiogenesis and extensive necrosis, a feature of higher grade, larger size and low estrogen receptor status.[11] Strategies that disable these macrophages may be of clinical benefit in the treatment of cancer.

The deadliest part of cancer process, metastasis, appears to rely on help from macrophages, potent immune system cells that usually defend vigorously against disease. The stromal microenvironment, particularly the macrophage component of primary tumors influences their malignant potential. However, at the metastatic site the role of these cells and their mechanism of actions for establishment and growth of metastases remain largely unknown. Using animal models of breast cancer metastasis, a population of host macrophages displaying a distinct phenotype is recruited to extravasating pulmonary metastatic cells regardless of species of origin. Ablation of this macrophage population through three independent means (genetic and chemical) showed that these macrophages are required for efficient metastatic seeding and growth. Importantly, even after metastatic growth is established, ablation of this macrophage population inhibited subsequent growth. This indicates a direct enhancement of metastatic growth by macrophages through their effects on tumor cell extravasations, survival and subsequent growth and identifies these cells as a new therapeutic target for treatment of metastatic disease.[12]

Metastatic disease is the major cause of cancer mortality and if this can somehow be blocked through influencing cells of the metastatic microenvironment the impact on cancer mortality would be enormous. TAMs offer a potentially useful new therapeutic target for anticancer therapy. One attractive possibility is targeting aspects of macrophage behaviour to reduce tumor cell invasion, because macrophages are unlikely to develop multidrug resistance and are genetically stable.[6]

In conclusion, intense macrophage infiltration in breast cancer is associated with poor prognosis and warrants a more aggressive management and follow up of the patient. The present case of infiltrating ductal carcinoma with dense macrophage infiltrate was a poor responder to chemotherapy and was negative for ER, PR and Her2neu. The exact incidence or prevalence of this phenomenon in India is not known, a prospective study of a large number of cases may provide better appreciation of its prevalence in the Indian population.

References

1. Murthy NS, Chaudhary K, Nadavil D, Agarwal UK,Saxena S: Changing trends in incidence of breast cancer: Indian scenario: *Indian J of Cancer* 2009;**46**:73-74.http://dx.doi.org/10.4103/0019-509X.48603 PMid:19282574

2. Kelly PM, Davison RS, Bliss E, McGee JO`D: Macrophages in human breast disease: A quantitative immunohistochemical study. *Br.J.Cancer* 1988;**57**:174-177.http://dx.doi.org/10.1038/bjc.1988.36 PMid:2833921PMCid:2246436

3. Lauder I, Aherne W, Stewart J, and Sainsbury R: Macrophage infiltration of breast tumors: a prospective study. *J clin.Path*, 1977; **30**:563-568.

4. Eccles SA and Alexander P: Macrophage content of tumor in relation to metastatic spread and host immune reaction. Nature (Lond),1974b;250:667-669 http://dx.doi.org/10.1038/250667a0 PMid:4546859

5. Morrison AS, Black MM, Lowe CR, Mac Mahon B, Yuasa S: Some international differences in histology and survival in breast cancer. *Int J Cancer* 1973;**11**:261-267http://dx.doi.org/10.1002/ijc.291011020

6. Goswami S, Sahai E, Wyckoff JB, Cammer M, Cox D, Pixley JF et al : Macrophages promote the invasion of breast carcinoma cells via a colony stimulating factor-1/epidermal growth factor paracrine loop: *Cancer Res*,2005;**65**(12):5278-5283. http://dx.doi.org/10.1158/0008-5472.CAN-04-1853 PMid:15958574

7. Pyke C, Gram N, Ralfkiar E, R?nne E, H?yer-Hansen G, Brunner N et al: Receptor for urokinase is present

in tumor associated macrophages in ductal breast carcinoma: *Cancer Res*1993;**53**:1911-1915. PMid:8385573

8. Roger P, Montcourrier P, Maudelonde T, Brouillet JP, Pages A, Laffargue F et al. Cathepsin D immunostaining in paraffin-embedded breast cancer cells and macrophages: correlation with cytosolic assay. *Hum Pathol* 1994;**25**:863-871. http://dx.doi.org/10.1016/0046-8177(94)90004-3

9. Volodko N, Reiner A, Rudas M and Jakesz R: Tumor-associated macrophages in breast cancer and their prognostic correlations. *The Breast* 1998;**7**: 99-105. http://dx.doi.org/10.1016/S0960-9776(98)90065-0

10. Bingle L, Lewis CE, Corke KP, Reed MWR, Brown NJ: Macrophages promote angiogenesis in human breast tumor spheroids in vivo. *Br J Cancer*, 2006; **94**(1):101-107. http://dx.doi.org/ 10.1038/ sj.bjc.6602901 PMid:16404363 PMCid:2361081

11. Leek RD, Landers RJ, Harris A and Lewis CE : Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. *Br JCancer*1999;**79**:991-995. http://dx.doi.org/10.1038/sj.bjc.6690158 PMid:10070902 PMCid:2362675

12. Qian B, Deng Y, Im JH, Muschel RJ, Zou Y, Li Jet al. A Distinct Macrophage Population Mediates Metastatic Breast Cancer Cell Extravasations, Establishment and Growth. *PLoS ONE* 2009; **4**(8): e6562. doi:10.1371/journal.pone.0006562 http://dx.doi.org/10.1371/journal.pone.0006562 PMid:19668347 PMCid:2721818