The lymphocytic inflammation correlates with metastatic risk in carcinoid tumours

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The lymphocytic inflammation inside the neoplastic tissue is widely considered expression of immunological reaction and a prognostic factor. This aspect has been not yet considered in carcinoid tumours and this has been the aim of our study. Our researches have been performed on 20 surgical specimens of carcinoid tumours, including gastrointestinal and bronchopulmonary cases. By immunological techniques, we have studied the presence of B, T and NK lymphocytes inside and around the neoplastic tissue. In carcinoid tumours of our series, the tissue immunological response is independent from their anatomical location. The stromal component, neo-lymphoangiogenesis and macrophage infiltration are always scant or absent. Different subtypes of lymphocytes (CD4+ T-helper, CD8+ T-cytotoxic, CD20+ B) can be present inside the proper neoplastic tissue with the same percentage and not organized in lymphatic centres, or in tertiary lymphatic organs. The lymphocytic inflammation can be quantified into three grades: brisk, not brisk or absent. It has been found independent from the mitotic count and perineural invasion, but it is inversely correlated with the presence of hepatic or lymphatic metastases. The scant presence of immunological reaction represents a tumour immuno-tolerance, likely secondary to an intrinsic histological compatibility, or to the local signaling of suppressor molecular mechanisms. On the contrary, a brisk lymphocytic infiltrate can be interpreted as a host reaction, secondary to a tissue incompatibility or to the release of pro-inflammatory molecules. This immunological aspect of carcinoid tumours deserves to be considered as a significative parameter for the metastatic risk.

Keywords: carcinoid tumor; inflammation; immunology; signaling; tumor infiltrating lymphocytes (TILs); metastatic risk


Introduction

Different anatomic characteristics of carcinoid tumours have been adopted as predictors for a clinical prognosis and different histological features have been observed in carcinoids, permitting their subsequent subdivision [1, 2]. For example, the carcinoid tumours have been distinguished in typical and atypical, according to symptoms (carcinoid syndrome), prognosis and biochemical attitude. In fact, a typical carcinoid tumor produces high levels of serotonin and chromogranin A, that can be quantified in the blood, while patients affected by an atypical carcinoid tumor show normal levels of serotonin and chromogranin A in their blood, but high levels of 5-hydroxytryptophan (5-HTP) can be found in
the urine \[3\]. The absence of lymphocytic immune response in small-cell lung cancer (SCLC) has been already demonstrated \[4\]. Here, we have analyzed the impact of lymphocytic inflammation on carcinoid tumours, because they are histogenetically correlated to SCLC, for their common origin from the neuroendocrine cells, belonging to the amine precursor uptake and decarboxylation (APUD) system \[5\].

**Materials and Methods**

We have re-viewed 50 surgical specimens of carcinoid tumours with a follow-up period more than 10 years, re-examining the histological slides. The age of the patients ranged from 40 to 70 years, with a median age of 55 years, equally distributed for gender. We have enrolled 9 cases of bronchopulmonary and 12 cases of midgut carcinoids and we have excluded 29 cases of small carcinoid tumours, with a macroscopic diameter less than 0.5 cm. The presence of hepatic or pulmonary hilar lymph node metastases has been considered as secondary metastatic involvement.

The histological diagnosis was achieved by morphological features and it was confirmed by immunohistochemistry for chromogranin A, synaptophysin and neuron specific enolase (NSE). Moreover, we have analyzed with great attention the tumour infiltrating lymphocytes (TILs), considering the following types of lymphocytes: CD4+ and CD8+ T lymphocytes, CD 20+ B lymphocytes and CD56+ NK lymphocytes. More in detail, new sections from paraffin-embedded tumour block were realised and, after deparaffinization, hydration, endogenous peroxidase blocking, heat-induced antigen retrieval, the tissue sections were incubated for 30 minutes at room temperature with anti-Chromogranin A (clone LK2H10, prediluted; Ventana), anti-Synaptophysin (clone SP11, prediluted; Ventana), anti-NSE (clone MRQ-55, prediluted; Ventana, Tucson, AZ, USA), anti-CD3 (clone 2GV6, prediluted; Ventana), anti-CD4 (clone SP35, prediluted; Ventana), anti-CD8 (clone SP57, prediluted; Ventana), anti-CD20 (clone L26, prediluted; Ventana), anti-CD56 (clone 123C3, prediluted; Ventana). Biotinylated secondary antibody was applied and the staining product detected with avidin-biotin complex (ABC) against a hematoxylin counterstain. Detection of the staining reaction was achieved by an enzyme conjugated polymer complex adapted for automatic stainers from Ventana Medical Systems, with 3-3' diaminobenzidine tetrahydrochloride (DAB) as chromogen. Detection of the staining reaction was achieved by an enzyme conjugated polymer complex adapted for automatic stainers from Ventana Medical Systems, with 3-3' diaminobenzidine tetrahydrochloride (DAB) as chromogen. The immunohistochemical evaluation was independently made by two pathologists, blinded for the presence of metastases. The TILs were simply graded in: absent, not brisk and brisk. Absent TILs denoted the absence of lymphocytes inside the tumour; brisk TILs signified a dense population of
lymphocytes diffusely infiltrating the carcinoid, while not brisk TILs indicated a scant lymphocytic inflammation.

Results

Our results did not show any difference between the carcinoids originating from the respiratory or enteric tract and their immunohistochemical profile. The stromal network in all our cases was absent or scant, as well as the neo-lymphangiogenesis. We did not observe an evident infiltration by macrophages inside the neoplastic tissue. In case of brisk TILs, the percentage of different types of lymphocytes found in the neoplastic tissue was almost constant: CD4+ T-helper lymphocytes 25%, CD8+ T-killer lymphocytes 50% and CD20+ B lymphocytes 25% (no NK lymphocytes). These immunological competent cells were found not in the peritumoral stroma, nor organized in lymphoid centres or in tertiary lymphoid organs, but inside the neoplastic tissue. The necrotic component of the carcinoid tumours, always limited, was independent from the lymphocytic infiltration. Moreover, the lymphocytic inflammation did not relate to the mean mitotic count or to the perineural invasion, but it was found inversely correlated with the presence of hepatic or lung lymph node hilar metastases. In fact, all the cases with absent TILs (2 bronchopulmonary carcinoids and 2 midgut carcinoids) and a quarter of cases with not brisk TILs (1 bronchopulmonary carcinoid and 2 midgut carcinoids) metastasized during the follow-up period. The remaining cases, including all the cases with brisk TILs (2 bronchopulmonary carcinoids and 2 midgut carcinoids) did not developed metastases at follow-up.

Discussion

Our observations have involved the most frequent carcinoids; they in fact encompass carcinoids of lung and midgut, being rarer those arising from the hindgut. The common origin of all carcinoid tumours from the same enterochromaffin apparatus justifies their strict histogenetic affinity. The subtypes of the investigated lymphocytes are the most common lymphocytic populations researched in the common histological practice, always representing reliable simple immunological indicators. We consider the presence of these immunocompetent cells, directly infiltrating the neoplastic tissue, as a sign of host’s immune response. Interestingly, this reaction does not promote the development of new lymphatic follicles or new lymph nodes, but develops directly inside the carcinoid tissue. The constant presence of both T and B lymphocytes suggest a possible cooperative interaction. The lymphocytic inflammation, as a sign of immunological response, is today largely admitted for many tumours, contemporarily demonstrating that its absence is usually associated with an aggressive clinical behaviour [6-8]. Our findings on carcinoid tumours are aligned with this concept. At first, the absence of immunocompetent reactions can be considered a form of immunotolerance for the neoplastic cells, permitting their escape from the innate surveillance system, their easy invasion into the haematic or lymphatic vessels and subsequent metastatic spread [9, 10]. This immunotolerance can be inherited in particular types of neoplastic cells or can be the result of a primary process of selection and outgrowth of variant tumour cells with an increased immunotolerance. It cannot be excluded the activation of local suppressor signaling by the neoplastic cells, able to depress or abolish the host immunological response. In every case, cellular replication and metastatic spread are favoured [11-13]. On the contrary, an active tissue immune response, as demonstrated by the condition of brisk lymphocytic inflammation and similar to a host reaction, can be explicated by the presence of specific antigens in the neoplastic cells, correlated to a reduced compatibility with the host tissue. In this case, a possible secretion of pro-inflammatory molecules, promoting a subsequent local lymphocytic recruitment, can be considered. This condition opposes to a distant metastatic diffusion, through venous or lymphatic systems [14-18]. We have considered the presence of secondary metastases to the liver or pulmonary hilar nodes, as the result of angio- or lymph-invasion of the tumour, being the lymphatic channels or the venous capillaries the direct ways of diffusion, and the hilar nodes and the hepatic parenchyma the typical distant places of host acceptance.

Figure 2. This midgut carcinoid, which obliterates the muscolaris mucosae at medium magnification (A x10; H&E), overexpresses chromogranin A (B, x5) and denotes a brisk TIL, revealed by immunohistochemistry for CD3 (C, x20). After 11 years of follow-up, there is no evidence of metastasization.
towards the neoplastic tissue. The incidence of locoregional lymph node metastases in intestinal carcinoid was devoid of statistical impact. Moreover, the concomitant presence of metastases was considered more indicative of malignancy than the clinical follow-up, today often deeply influenced by oncological treatment. In conclusion, our study claims attention to the value of lymphocytic inflammation in carcinoid tumours as an immunological criterion inversely correlated to the incidence of distant metastases. This feature could be considered in a possible immunoscore of carcinoid tumours. From a practical point of view, the study of the inflammatory reaction can usefully follow the basic morpho-histological diagnosis in carcinoid tumours.

Conflict of interest

The authors declare to have no conflict of interest.

Author contributions

LR and AM contributed to the design of the study. TP and FP were responsible for data acquisition and analysis. LR, AM and GB interpreted the data. LR and AM prepared the figures and drafted the manuscript. All authors contributed to the critical revision of the drafted manuscript and approved its final version.

References


