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LETTER TO THE EDITOR

SOLITARY PLASMACYTOMA OF THE TONSILLAR SITE ASSOCIATED WITH ACTINOMYCES INFECTION: THE POSSIBLE ROLE OF IL-6

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ExtraMedullary Plasmacytoma (EMP) is a rare plasma cell tumor. It can occur in the upper aerodigestive tract and presents as a large nodule causing local compressive symptoms. A 79-year old woman presented to Otorhinolaryngology Department with progressive hearing loss and no other symptoms. Following PET/TC examination due to the suspicion of a lymphoproliferative disease, the patient underwent tonsillectomy and the diagnosis of solitary EMP was formulated. In addition to that, the histological examination of the tonsillar tissue revealed large colonies of filamentous bacteria, showing abundant sulphur granules and Splendore-Hoeppli phenomenon; these evidences indicating the presence of a chronic Actinomyces infection. Immunohistochemical analysis demonstrated a marked IL-6 immunoreactivity of the neoplastic plasma cells. Interestingly, a marked IL-6 immunoreactivity was also found in the tissue surrounding the Actinomyces colonies. In the present study we report for the first time a solitary EMP associated with Actinomycosis. It is tempting to speculate that the unsuspected and untreated Actinomyces infection, through chronic IL-6 production, could contribute to the neoplastic transformation of plasma cells.

A 79-year old woman was referred to the Otorhinonolaryngology outpatient department with progressive hearing loss. The patient reported no other symptoms. The neck did not display asymmetry and there were no palpable lymphadenopathy. Oropharyngeal examination showed a large, brownish, irregular mass of approximately 1 cm in the largest dimension. The mass appeared to be arising from the left palatine tonsil and displaced the tonsil medially, but it seem to be contained within the organ. The nodule was mobile during respiration or deglutition. On palpation the mass was soft and not tender. [^{18}F] Fluorodeoxyglucose Positron Emission Tomography scanners with fused Computed Tomographic (^{18}F-FDG PET/TC) examination showed an increased left subcarinal uptake of the radio nucleoside. This picture supported the clinical diagnosis of primary tonsillar neoplasia, likely of a lymphoprolferative nature. The patient, underwent left-side tonsillectomy.

Grossly, the left tonsil consisted of an oval mass of lymphoid tissue measuring 2.6 x 1.6 x 0.9 cm. Cut section revealed a focal dark area measuring 0.8 cm in its maximum diameter. Histopathological analysis of the tonsillar tissue showed sub-epithelial lymphoid aggregates composed of sheets of plasma cells, with varying grades of maturity, in a context of a sparse and fine reticular stroma. In the dark area a high number of atypical plasma cells were observed. The plasma cells that infiltrated the tonsillar tissue, were

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characterized by prominent and eccentric nucleoli with "spoke wheel" chromatin, and by abundant basophilic cytoplasm. Bi-tri-nucleated plasma cells and mitotic figures were also observed (Fig. 1A).

In addition to that, large colonies of filamentous bacteria were noted; they were located within tonsillar crypts (Fig. 1B), very close to the plasmacytoma (Fig. 1C). Colonies showed abundant sulphur granules, and were surrounded by a peripheral darkly staining eosinophilic rim, consisting of polymorphonuclear cells, cell debris and fibrosis (Splendore-Hoeppli phenomenon) (Fig. 1D). These evidences indicate a chronic infection by Actinomyces.

Tumor cells showed positive immunostaining for CD138 (plasma cell marker) (Fig. 1E), CD56 (Fig. 1F) and Lambda light chain (Fig. 1G). They were negative for CD20, CD3, CD15, CD30, CD68, and Kappa light chain immunostaining. The proliferation index, assessed by MIB-1 antibody, was less than 1%. The neoplastic plasma cells were also IL-6 immunoreactive (Fig. 1H). An intense IL-6 immunoreactivity was also present around the Actinomyces colonies (Fig. 1I). On the contrary, normal and non inflamed tonsil didn't show IL-6 positivity (negative control, data not shown). To investigate whether the Actinomyces infection was immunoreactive for IL-6 in the absence of neoplastic plasma cells, IL-6 immunostaining was performed on true vocal cord tissue affected by Actinomycosis. We found a marked IL-6 immunoreactivity in the tissue surrounding the bacterial colonies (Splendore-Hoeppli phenomenon) (Figure, L). Some mononuclear cells within the inflammatory infiltrate in the close proximity of the colonies were also IL-6 positive (data not shown).

The patient was subsequently referred to the Haematological Department for further workup, in order to rule out multiple myeloma (MM) or disseminated disease entirely. Full blood count, urea, calcium, creatine, and β-2-microglobulin were all within normal ranges. No histological evidence of bone marrow involvement on biopsy, or distant bone lesions on radiological skeletal survey were detected. In serum protein electrophoresis, a monoclonal IgG Lambda light chain peak was detected. Urine analysis showed a high level of lambda light chain, but there was no evidence of Bence-Jones protein. After ruling out MM, the patient was diagnosed as having solitary tonsillar EMP.

Our patients did not require adjuvant radiotherapy, since excision of the entire neoplastic lesion was made. Three months after surgery the patient remained well. Repeated immunoglobulin assay showed renormalization of monoclonal IgG and light chain levels. There was no evidence of multiple myeloma on bone marrow biopsy and no lytic lesions on skeletal survey.

DISCUSSION

Plasma cell tumor (PTC) or Plasmacytoma can occur either in bone (medullary plasmacytoma) or in the soft tissue (extra medullary plasmacytoma (EMP). EMP may present with solitary or multiple lesions. The systemic disease characterized by osseous multiple lesions (multiple myeloma) represents the commonest lesion, and accounts for about 90% of all plasma cell malignancies. In contrast, EMPs are rare; they constitute approximately 4% of all plasma cell neoplasms (1, 2). Solitary EMP typically presents as a well-localized submucosal mass, sometimes with polypoidal configuration (3). Approximately 80% of EMP develop in the upper aerodigestive tract, with the most common sites being in the nasal cavity, paranasal sinus and nasopharinx (4). Tonsillar EMP is rare (about 1% of EMPs), and few cases had been reported in the literature (5, 6). The diagnosis of solitary EMP is based on the detection of a plasma cell tumor in an extramedullary site, on the absence of multiple myeloma on bone marrow examination, radiography, and on the appropriate studies of blood and urine. Solitary EMP is efficiently managed with radiotherapy, due to its radiosensitivity. The role of surgery is usually confined to the excision of small and resectable tumors, or to local-regional recurrences. If a complete surgical resection is obtained, no adjuvant radiotherapy is required. On the other hand, regular monitoring must be warranted, in order to exclude local recurrence and the development of multiple myeloma, which had been observed in about 20% of the cases (4).

PTC development presents special conceptual problems in oncology, since it represents neoplastic derivatives of a terminally differentiated cell in the B-lymphocyte lineage, the plasma cell. It's generally thought that plasma cells have only a limited number

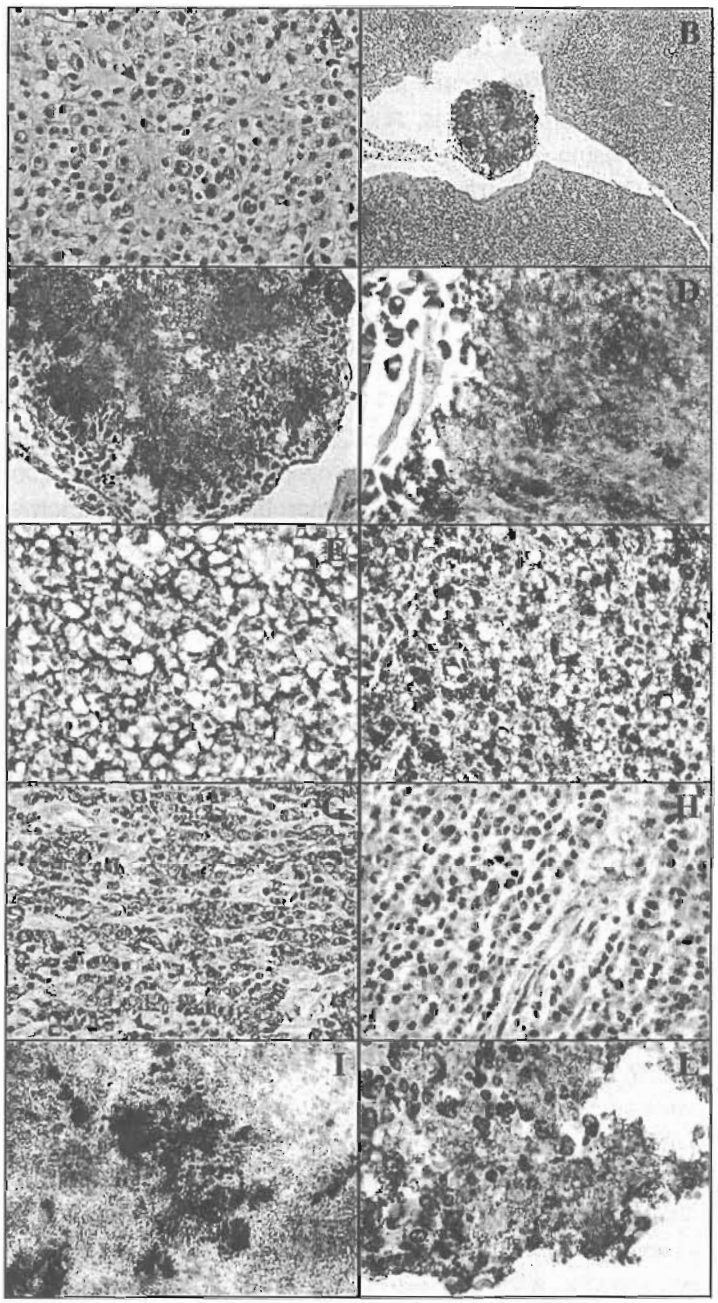

Fig. 1. *Tonsillar extramedullary plasmacytoma. **A)** diffuse atypical plasma cells with eccentric nuclei, prominent nucleoli with "spoke wheel" chromatin, and abundant basophilic cytoplasm. Black arrow: atypical binucleated cells (original magnification x63). Actinomyces colonies within tonsillar crypts (**B**, original magnifications x250) and in proximity of plasmacytoma [(**C**) original magnifications x100] showed typical sulfur granuli, and were surrounded by an eosinophilic peripheral rim, consisting of inflammatory cells, cell debris and fibrosis (**D**, original magnification x630). Immunohistochemical analysis of tonsillar tissue (streptavidine-biotin-peroxidase) showed diffuse CD138 positivity (**E**, original magnification x40), CD56 positivity (**F**, original magnifications x40) and lambda light chain immunoreactivity (**G**, original magnifications x20). Tonsillar EMP cells revealed IL-6 immunoreactivity too (**H**, original magnifications x20). In addition, IL-6 immunoreactivity was seen in the tissue surrounding the Actinomyces colonies (**I**, original magnifications x100). True vocal cord demonstrated Actinomyces colonies and marked IL-6 immunoreactivity in the tissue surrounding the bacterial colonies (Splendore-Hoeppli phenomenon) (L, original magnifications x100).*

of cell divisions before they permanently exit the cell cycle. Thus, plasma cells could be a poor target for neoplastic development. However, PTC occurs in different mammalian species, including humans. A critical point in physiological plasma cell formation is the B lymphocyte-plasma cell (B:PC) transition or differentiation. B:PC transition is associated with multiple changes in gene activity and cell-surface phenotype, such as the down regulation of the antigen binding receptor (BCR) and the major histocompatibility complex class II receptor.

A large number of models for PTC development have been generated. Among these, the most important models are those depending upon naturally arising mutagenic changes (such as pristane-induced PTC), those in which PTC is associated with oncogene activation, those of resistance to apoptosis and those of release growth factors such as interleukin-6 (IL-6) (7). PTC development could start with the extension of the longevity of a B-cell clone through the interaction with antigens (7). Kishimoto et al., for the first time in the '70 isolated and characterized B-cell stimulating factor-2 (BSF-2), also named IL-6 (8). Subsequently, many studies demonstrated a primary role of IL-6 in the development of B-cell neoplasia (9-11). IL-6 is able to regulate the final differentiation of B cells into antibody secreting plasma cells (8). *In vitro* IL-6 stimulates normal B-cells, induces plasma cell differentiation and survival/proliferation of PTCs (8).

The frequent localization of EMP in the upper aerodigestive tract has led to the hypothesis that chronic stimulation (by bacteria, viruses or chemical irritants), may promote tumorigenesis. Lattanzio et al. provided evidence for a role of IL-6 in PTC development, along with a status of chronic inflammation (12). IL-6 deficient mice did not develop B-cell tumors when infected with myc/raf-expressing retrovirus (12).

In early studies it was shown that sonic lysates of *Actinomyces viscosus* displayed mitogenic and adjuvant activities on polyclonal B cells *in vitro* (13).

Actinomyces are filamentous, Gram-positive, anaerobic bacteria, which are part of the normal oral flora. They can be found in calculus, periodontal pockets, carious lesions and oral mucosal surfaces and do not cause any pathology as long as they stay on the surface of the mucosa. If the integrity of the mucosal barrier is compromised, they can initiate a prolonged chronic inflammatory process. It is difficult to obtain Actinomyces cultures, due to the need to rely on a rapid transportation to a specialized laboratory, and to the necessity of immediate incubation in anaerobic environment. Thus, the diagnosis is more often obtained by detecting the typical colonies and "sulphur granules" in histological specimens (14-16). IL-6 mRNA level was increased when human immortalized oral epithelial cells were cultured with exudates from periradicular granuloma of *Actinomyces Viscosus* and *Israelii*, compared with untreated epithelial cells (17, 18). In addition to that, using a multiplexed beadlyte kit, Peyyala and collaborators demonstrated that biofilms of Actinomyces Naeslundii induced high levels of IL-6 in a human oral epithelial cell line (19). In supporting these evidences, we found a clear IL-6 positivity in Actinomyces granuloma arising from other side than tonsil (true vocal cord).

In this context, the adjuvant and mitogenic activity of Actinomyces on B-cells may be partly mediated by their capability to induce IL-6 (20). In other terms, Actinomyces colonies in the tonsillar site may influence B cell proliferation and differentiation through IL-6 production. For this reason the simultaneous presence of Actinomycosis and of a solitary EMP of the tonsillar site is interesting. There is no doubt that specific bacteria and parasites can promote cancer development through different and complex mechanisms. However, clinical evidences regarding the association of each pathogen with malignant diseases vary significantly. For some bacterial species this relationship is well established (21-22), whereas for other pathogens it is still lacking. Unsuspected and untreated Actinomyces infection, through IL-6 production, could promote the transformation process which leads to PTC.

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