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Review

New Insights into the Pathogenesis of Warthin's Tumour

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INTRODUCTION

WARTHIN'S TUMOUR (WT) is a relatively common benign epithelial tumour of the salivary gland comprising 5–15% of all parotid neoplasms [1]. After the initial reports by Hildebrand in 1895 [2], much has been written on WT, as this parotid neoplasm continues to fascinate as well as provoke controversy among clinicians, pathologists and other investigators so that even its neoplastic nature is a matter for discussion [3]. This controversy is multifaceted and relates to all aspects of the tumour from its historical beginnings to its histogenesis, pathogenesis and treatment. There are several reasons for such contention, but the most important ones reside in the "unique" histology of WT, characterised by a lymphoepithelial proliferation. In fact, WT consists of two components: a double layer of oxyphilic/oncocytic epithelium and a lymphoid stroma containing lymph follicles. The origin of the epithelial cells and the nature of the lymphoid stroma in WT remain largely unsettled. Most authors agree that the epithelial compartment is derived from the ductal cells [3–5], while it is generally accepted that the lymphoid component is the expression of normal activated lymphoid tissue, characterised by a polytypic B-cell proliferation [3, 6, 7]. The interpretation of the lymphoid stroma as a normal but active lymphoid tissue is also supported by the well-described development of malignant lymphomas in WT [3, 8–11].

HISTOGENESIS OF WT

In the past, clinicians and pathologists elaborated many theories on the histogenesis of WT in an attempt to elucidate the mechanisms responsible for the lymphoepithelial coexistence, characteristic of this parotid neoplasm. The initial theories of Albrecth and Arzt [12], and Thompson and Bryant [13] postulated that WT develops from heterotopic salivary duct epithelium within lymph nodes in the parotid gland, in the periparotid area, or in the neck. This hypothesis was based on the histological and ultrastructural features of the epithelial component of WT, resembling salivary duct cells and was further supported by Dietert who in a histological review of parotid gland specimens from 137 patients with WT was able to identify the WT in an intra-lymphnodal location in 72% of cases [14]. Thus, they postulated that, because of fetal or postfetal disturbance in the ontogenesis of the parotid gland, in association with the enclosure of lymph nodes in the gland, groups of salivary ducts and acini may be included in such lymph nodes [3]. The possible late neoplastic proliferation of this heterotopic parotid ductal epithelium could justify the development of WT.

In 1971, on the basis of morphological analogies between the histological features of WT and those observed in several organo-specific immune disorders, particularly in Hashimoto's thyroiditis, Allegra first postulated that WT might not be a true neoplasm and suggested the involvement of a cell-mediated immune mechanism of the delayed hypersensitivity type in the pathogenesis of such a tumour [15]. More precisely, he suggested that in the early stages of the disease the epithelial cells of the striated ducts undergo oxyphilic metaplasia and produce apocrine secretory activity. The metaplasia triggers proliferation of duct epithelium, characteristic of WT and is followed by an intense stromal reaction with involvement of immune cells. The factor(s) responsible for the initial metaplastic event was unclear. More recently, based on the close association between cigarette smoking and WT, several authors have postulated that tobacco smoke could be involved in the induction of metaplasia in epithelial ductal cells [16] responsible for epithelial and, then, lymphoid cells proliferation. The immune hypothesis has recently been further supported by Ogawa *et al.* [17], who immunohistochemically demonstrated the expression of class II human leucocyte antigens (HLA) on the epithelial component of WT, showing clear analogies with the salivary gland epithelium of the most common organ-specific autoimmune disease involving salivary and lacrimal glands, i.e. Sjögren syndrome (SS) [18]. On the basis of these findings and of the production of interleukin-1 by epithelial cells of WT, the Japanese authors suggested that epithelial cells of WT possess the potential for acting as an antigen-presenting cell (APC) being able to activate lymphoid stroma of WT. In fact, they hypothesised that in WT an immune reaction against unknown antigens presented to T-lymphocytes by HLA-DR positive epithelial cells takes place. Thus, they suggested that the expression of HLA-DR antigens and interleukin-1 production by an epithelial component of WT could specifically activate lymphoid cells within the salivary gland. However, no clear explanations about the mechanisms responsible for the

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aberrant HLA-DR expression by WT epithelial cells and the possible antigens involved in the activation of the lymphoid stroma were done.

HLA ANTIGENS, VIRUS INFECTION AND SALIVARY GLAND AUTOIMMUNE DISEASES

Based on their tissue distribution and structure, HLA antigens have been divided into two classes: class I antigens, also termed the classic histocompatibility antigens; class II antigens, including HLA-D, -DR, -DQ, and DP antigens. HLA-A, -B, and -C antigens (class I antigens) are found on virtually every human cells; whereas HLA class II antigens are found chiefly on the surface of the immunocompetent cells, including macrophages/monocytes, activated T lymphocytes and particularly B lymphocytes. The function of HLA class II antigens is to regulate the interactions between immunocompetent cells, controlling all the mechanisms of recognition and co-operation in the immune system, thus restricted at the HLA system [19].

The detection of HLA-DR class II antigens on residing cells that do not normally express these antigens has been reported in various nonlymphoid cells with signs of functional activation, such as vascular endothelial cells, fibroblasts, and various epithelial cells, particularly in organ-specific autoimmune diseases [20–22]. This phenomenon has been explained in different ways. Recently, most authors postulated that the aberrant MHC antigen expression might be linked to the effects of cytokines and/or other substances released after viral infection by epithelial cells [18]. Accordingly, it is well known that virus infection usually leads to the autocrine production of interferons, the major inducers of MHC antigens, by infected cells [17–20].

In several autoimmune disorders, such as Grave's disease, Bottazzo *et al.* [23, 24] postulated that viral infection by eliciting the production of interferons, could activate the ectopic expression of HLA-DR antigens on thyroid cells with the possible recognition of thyroid autoantigens on their surface by T-helper cells, thus triggering an autoimmune reaction. An analogous mechanism has also been postulated in the pathogenesis of other autoimmune organ-specific diseases, including salivary gland diseases.

Among the human viruses, the Epstein–Barr virus (EBV) has been the most prominently considered as a cause of autoimmune diseases because of its ubiquity, persistence and ability to act on the immune system.

A possible role of EBV infection in the activation of autoimmune reactions also in salivary glands has been postulated by the detection of EBV-DNA in epithelial cells from major and minor salivary and lacrimal gland biopsies of SS patients [25–28]. In fact, Fox and associates first reported ductal epithelial staining with anti EBV Early Antigen-D (clone R3) antibodies in eight of 14, and EBV gp350/220-specific antibodies in two of 14 salivary gland biopsies from SS patients, and zero of 10 control glands [25]. Furthermore, Saito *et al.* amplified EBV genomic sequences in 78% of SS gland biopsies as compared to 13% of biopsies from normal controls [26]. Using PCR, Mariette *et al.* detected EBV DNA in 86% of minor salivary gland biopsies from primary SS patients, 60% in secondary SS patients and 29% of normal controls [27]. Also using PCR, Deacon and associates reported the detection of EBV-DNA in 90% of SS minor salivary gland biopsies and in 70% of normal salivary gland biopsies ascontrol [28]. Moreover, more recently Tateishi *et al.* reported the spontaneous massive production of EBV by lymphoblastoid cell lines obtained from peripheral blood mononuclear cells of patients with SS [29]. Taken together, these data strongly suggest a central role of EBV in the pathogenesis of SS. In fact, it is possible that in predisposed subjects ductal epithelial cells, after EBV infection leading to aberrant HLA-DR expression, may be involved in the presentation of EBV-associated antigens to immune T-cells acting as APC, thus triggering an autoimmune reaction [30, 31]. However, because of the detection of EBV-DNA in normal salivary glands from seropositive individuals [32], the exact role of EBV in SS aetiopathogenesis is still controversial.

EPSTEIN–BARR VIRUS AND WT

EBV is the causative agent of infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal undifferentiated carcinoma [33, 34] and is also implicated in B-cell lymphomas and in lymphoproliferative disorders, particularly in immunosuppressed and AIDS patients [33–36].

The main portal of entry of the virus in humans is the oropharynx, where the virus replicates in many epithelial elements such as acinar and ductal cells of the parotid and other salivary glands, and buccal and pharyngeal epithelial cells [32, 34, 37, 38]. In salivary glands the target of the virus seems to be the ductal epithelium, which may consistently express the C3d/EBV receptor (CR 2/CD21) of B lymphocytes [28]. EBV is ubiquitous and detectable in saliva of apparently healthy subjects [39]. The salivary glands are involved in the EBV primary infection and may constitute a virus reservoir for latent EBV that periodically becomes reactivated [32, 34], as evidenced by increased viral DNA in the saliva and an increased number of circulating B lymphocytes containing EBV genome in patients with some autoimmune disease, including SS patients and rheumatoid arthritis [25, 31].

The detection of clonal episomal EBV genomes and EBV-RNA transcripts in undifferentiated carcinomas of the salivary gland in American Eskimos and Greenlanders [40–42] and, more recently, by our group in Caucasians [43], also suggests a transforming role of EBV in parotid and salivary glands.

More recently, Pflugfelder *et al.* [44] have reported the detection of EBV-DNA in 86%, using ISH, and in 88%, using PCR, of intraductal epithelia and B lymphocytes of lacrimal gland biopsies from SS suggesting a central role of EBV in the pathogenesis of lacrimal gland lymphocytic proliferation associated to SS. The lacrimal gland immunopathology of SS consists of a proliferation mainly of B lymphocytes surrounding epithelial ducts and islands [44], in contrast to the predominant T lymphocyte proliferation of SS salivary glands.

Similar to the immunopathology of lacrimal glands of SS and to other EBV-associated neoplasias [45, 46], a lymphoepithelial pathology is also characteristic of WT. Thus, the lymphoepithelial pathology of WT clearly shows several analogies with SS, including the aberrant expression of HLA-DR on the epithelial cell surface.

The biologic properties of EBV (activation and immortalisation of B lymphocytes, and interactions with epithelial cells, particularly salivary ductal cells) on the one hand, and the above-mentioned analogies between WT and SS on the other,

prompted our group to investigate WT of the parotid gland for the presence of EBV-DNA sequences, using a non-radioactive biotinylated EBV-DNA (BamHI-W fragment) probe and an *in situ* hybridisation (ISH) technique [47, 48]. Our results first indicate that the EBV genome was present in the cytoplasm of neoplastic oxyphilic cells in a significant number of multiple/bilateral WT (86.7%), whereas the EBV genome was documented only occasionally in solitary WT (16.7%). Moreover, the EBV genome was often detected in the cytoplasm of ductal cells (75% of bilateral WT and 33.3% of solitary WT) and occasionally of acinar cells (16.7% of both multiple/bilateral and solitary WT) of the residual normal salivary gland tissue surrounding WT. Although the results of these studies could be influenced by the possible false positive staining of ductal cells due to the endogenous biotin and alkaline phosphatase, the possible role of EBV in WT pathogenesis has been further supported by preliminary results of Taira *et al.* [49]. These authors in fact reported the detection of EBV-DNA in all of the seven cases of WT analysed, using PCR. In spite of higher sensitivity of PCR compared to ISH, our study suggests the possible localisation of the EBV genome in the cytoplasm of epithelial cells and not in the lymphoid stroma of WT. Although the possible co-infection of B-cells in WT cannot be excluded by ISH technique, the detection of EBV-DNA in epithelial cells of WT might justify their proliferation and typical features, and in addition the aberrant expression of HLA-DR antigens on their surface. All together, these data could suggest a pathogenetic role of EBV for WT.

More recently, a retrospective review of clinical records of 140 patients affected by WT in comparison to clinical data from 380 patients with pleomorphic adenoma, the most common parotid gland neoplasm, surgically treated during 1973–1993 at the ENT Clinic of the University of Florence evidenced a higher incidence of autoimmune disorders, particularly organ-specific diseases (i.e. IDDM, Hashimoto's thyroditis) in WT patients than in patients with pleomorphic adenomas (32% versus 4.4%, OR=8.1, $P<0.05$) [50]. In addition, in the sera of 9 further consecutive patients with WT recently treated, we detected significantly higher levels of EBV-VCA-IgG and EBV-EA-IgG than those found in the sera from 20 patients with pleomorphic adenoma or from 20 healthy subjects ($P<0.001$), confirming the original observations of Taira *et al.* [49].

All together these findings could indicate a role of EBV in WT aetiopathogenesis. In fact, because of its possible pathogenetic role in several autoimmune diseases [31], it is possible to postulate that EBV could be involved in the pathogenesis of both WT and autoimmune disorders frequently associated in WT patients.

Because of the well-known association between HLA antigen(s) and autoimmune diseases, including SS (HLA B8, DR3, DW52a, DQw2 haplotype strongly associated with primary SS) [51], we investigated a possible association between HLA antigen(s) and WT. In our WT population without autoimmune diseases, a statistically significant association between HLA-DR6 antigen and WT ($P<0.05$) was found (unpublished data), suggesting a genetic predisposition to develop WT. The exact significance of such an association is still unclear, but according to other HLA associations [52], HLA-DR6 positive subjects could be associated with a decreased efficiency of the immune system, particularly in its anti-EBV activity.

In conclusion, in predisposed individuals EBV in latent sites of infection, such as the parotid gland [53], could infect ductal

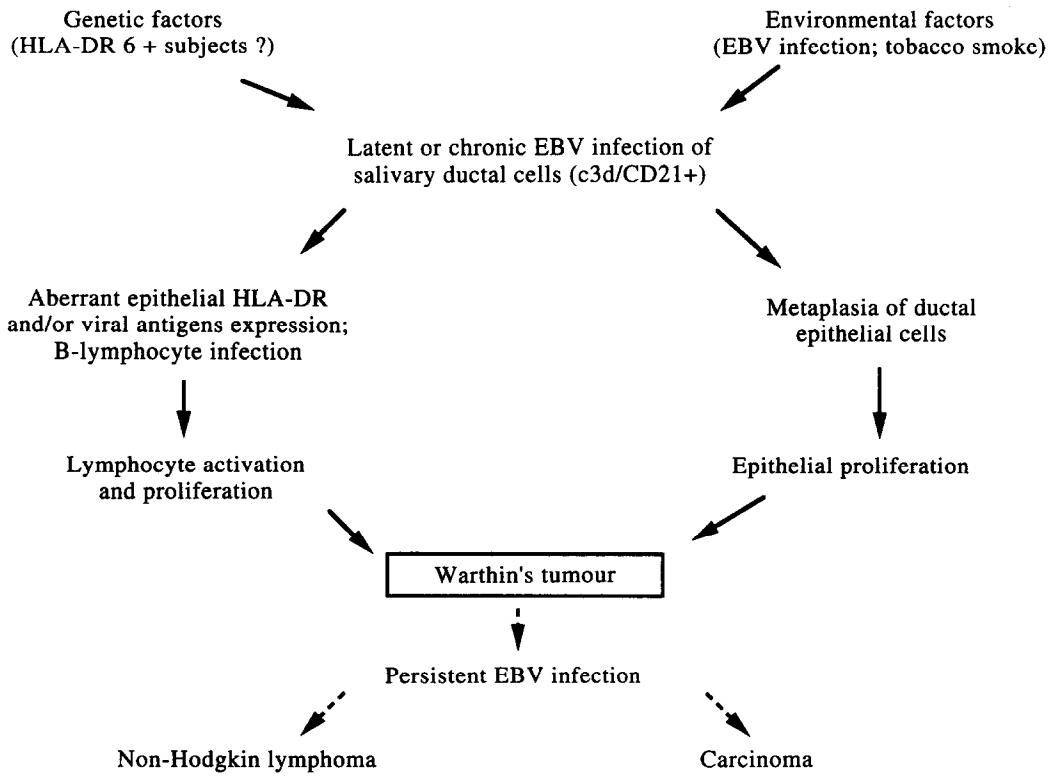

Fig. 1. The hypothesis of Warthin's tumour pathogenesis.

epithelial cells. This would induce the release of cytokines, in particular interferon-gamma, by infected cells, which in turn would induce HLA-DR expression by epithelial cells. EBV gene products and/or cytokine release by infected cells may activate lymphoid tissue or stroma within the parotid gland and result in a polyclonal B-cell response. The possibility of developing malignant lymphomas in both SS [54] and WT [8–11], and the well-known involvement of EBV in the pathogenesis of generalised lymphoreticular malignant diseases [45, 46], further support this hypothesis (Fig. 1).

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