

The role of the inflammatory microenvironment in thyroid carcinogenesis

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Abstract

Immune responses against thyroid carcinomas have long been demonstrated and associations between inflammatory microenvironment and thyroid carcinomas repeatedly reported. This scenario has prompted scientists throughout the world to unveil how the inflammatory microenvironment is established in thyroid tumors and what is its influence on the outcome of patients with thyroid carcinoma. Many studies have reported the role of evasion from the immune system in tumor progression and reinforced the weakness of the innate immune response toward thyroid cancer spread in advanced stages. Translational studies have provided evidence that an increased density of tumor-associated macrophages in poorly differentiated thyroid carcinoma (DTC) is associated with an aggressive phenotype at diagnosis and decreased cancer-related survival, whereas well-DTC microenvironment enriched with macrophages is correlated with improved disease-free survival. It is possible that these different results are related to different microenvironments. Several studies have provided evidence that patients whose tumors are not infiltrated by lymphocytes present a high recurrence rate, suggesting that the presence of lymphocytes in the tumor microenvironment may favor the prognosis of patients with thyroid carcinoma. However, the effect of lymphocytes and other immune cells on patient outcome seems to result from complex interactions between the tumor and immune system, and the molecular pattern of cytokines and chemokines helps to explain the involvement of the immune system in thyroid tumor progression. The inflammatory microenvironment may help to characterize aggressive tumors and to identify patients who would benefit from a more invasive approach, probably sparing the vast majority of patients with an indolent disease from unnecessary procedures.

Key Words

- ▶ thyroid carcinomas
- ▶ inflammatory microenvironment
- ▶ tumor-infiltrating lymphocytes
- ▶ tumor-associated macrophages

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Introduction

Immune responses against differentiated thyroid carcinomas (DTCs) have long been demonstrated (Matsubayashi *et al.* 1995, Modi *et al.* 2003) and associations between inflammatory microenvironment and DTCs repeatedly reported (Yano *et al.* 2007, Muzza

et al. 2010), evidenced by a mixture of lymphocytes and macrophages frequently found within and inside or surrounding primary thyroid tumors (Modi *et al.* 2003, French *et al.* 2010). These data support the hypothesis that immune responses may influence DTC progression

(Gupta *et al.* 2001, Yano *et al.* 2007, Muzza *et al.* 2010). In fact, pathologists have long recognized that some tumors are densely infiltrated by cells of both innate and adaptive arms of the immune system, thereby reflecting inflammatory conditions arising in non-neoplastic tissues. In addition, clinicians have long realized that local inflammatory responses (Matsubayashi *et al.* 1995, Gupta *et al.* 2001) and concurrent chronic lymphocytic thyroiditis (Cunha & Ward 2011, Huang *et al.* 2011) may be associated with the prognostic profile of patients with DTC. All these observations from the bedside have prompted scientists throughout the world to unveil how the inflammatory microenvironment is established in thyroid tumors and what is its influence on the outcome of patients with DTC.

Principles of thyroid carcinogenesis

Although ~50–67% of the population will eventually present a thyroid nodule during their lives (Mazzaferri 2006, Cronan 2008), the malignant transformation of follicular cells is much less common and thyroid cancers account for no more than 2% of all human cancers (Sipos & Mazzaferri 2010). The vast majority of thyroid neoplasms are derived from follicular epithelial cells and can be classified as follows: DTCs, which can be divided into papillary thyroid cancer (PTC) and follicular thyroid cancer, poorly DTC (PDTTC), and undifferentiated thyroid carcinomas or anaplastic thyroid cancer (ATC).

In 2011, Hanahan & Weinberg (2011) stated that a cell acquires ten biological abilities to become a tumor and considered hallmarks of cancer the ability to sustain proliferative signaling, evade growth suppressors, avoid immune destruction, enable replicative immortality, promote inflammation, induce angiogenesis, activate invasion and metastasis, promote genome instability and mutation, resist cell death, and deregulate cellular energetics. Such abilities may appear for different reasons and can be instigated by diverse agents. In the case of thyroid carcinomas, risk factors may include ionizing radiations (Nikiforov 2010, Wartofsky 2010), chronic inflammatory conditions such as obesity (Zhao *et al.* 2012, Han *et al.* 2013), and ingestion of some nutrients such as iodine and others (Marcello *et al.* 2012). Individual genetically inherited profiles of susceptibility (Granja *et al.* 2004a,b, Bufalo *et al.* 2006, Guilhen *et al.* 2009, Xu *et al.* 2012) may explain different responses to both environmental and endogenous aggressions, as well as gender- and gene-associated alterations in the MAPK pathway and PI3K/AKT pathway or in cell-cycle genes, such as *p53* (*TP53*), reflecting in clinical differences.

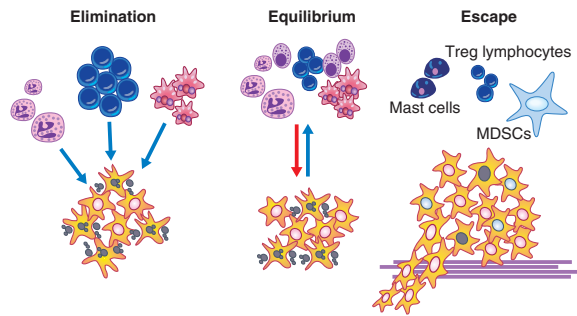
Concepts of tumor immunology

A close relationship between immune response and cancer was first proposed by Virchow in 1863 (Balkwill & Mantovani 2001). A few years later, Coley demonstrated that bacterial products were able to help inoperable cancer patients. The subsequent application of *Bacillus Calmette–Guerin* and other crude immunostimulants showed benefits that led to the regulatory approval of their use in the treatment of some solid tumors such as bladder cancer (Kirkwood *et al.* 2012). Thereafter, the capacity of the immune system to interfere with tumor progression has been proven by a series of clinical and epidemiological evidences. In fact, the success of Herceptin and Ipilimumab in treating breast and metastatic melanomas respectively has prompted scientists to go far beyond the frontiers in tumor immunology.

The field of tumor immunology is based on the fact that, during the tumorigenic process, malignant cells may express tumor-specific antigens and/or tumor-associated antigens. Tumor-associated antigens comprise gene products more frequently found in tumors, whereas tumor-specific antigens are gene products uniquely expressed in tumors (Lewis *et al.* 2003). These groups of antigens are target for immunotherapy, and new targets have actively been searched for all over the world. However, the absolute success of immunotherapy is impaired by tumor immune escape mechanisms.

Tumor cells are initially eliminated by the immune system before they become clinically detectable. This is then followed by an equilibrium phase, where a selection process for less immunogenic tumor variants takes place until tumor cells finally ‘escape’ from immune surveillance, as shown in Fig. 1 (Dunn *et al.* 2002).

One immune escape mechanism is active at the level of the tumor and can be ascribed to abnormalities in the expression of major histocompatibility complex (MHC) class I-restricted antigens, enabling tumors to take on a ‘stealth’ phenotype, hiding from immune cell detection (Poschke *et al.* 2011). The other mechanism results from the ability of the tumor to progress and ‘sabotage’ the host immune system. Certain tumors have the ability to use parts of the immune system to protect themselves against the host immune response. By doing so, tumors induce or recruit immune cells such as myeloid-derived suppressor cells (MDSCs) or regulatory T cells (Tregs). These cells physiologically serve as safeguards against overwhelming inflammation and lead to immune resolution (Poschke *et al.* 2011). The recruitment of these cells makes the microenvironment permissive for cell growth.

**Figure 1**

The Three Es theory. According to this theory, tumor cells are initially eliminated by the immune system. At this stage, tumors are not clinically detectable. This is then followed by an equilibrium phase, where a selection process for less immunogenic tumor variants takes place. At the third stage, tumor cells are able to 'escape' from immune surveillance.

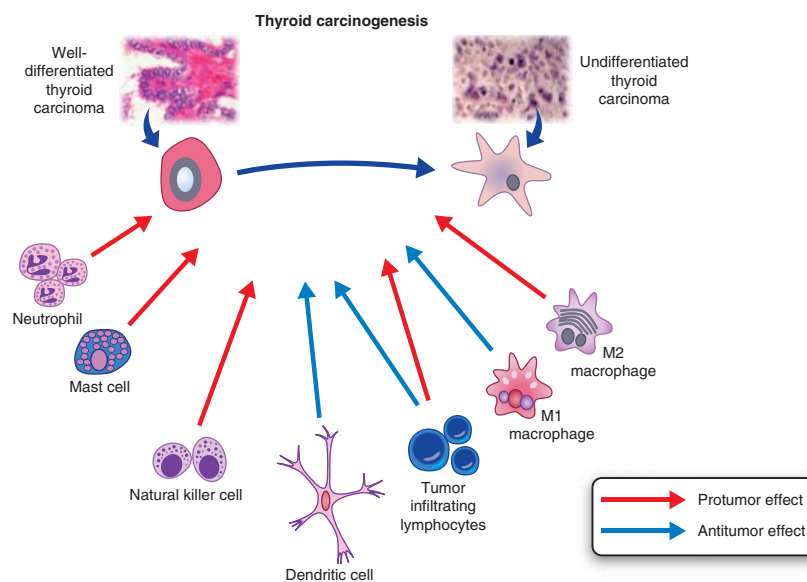
The immune system is a cornerstone determinant of the tumor microenvironment, as shown in Fig. 2. Recent molecular biology studies conducted in cancer patients have revealed that the inflammatory profile of the tumor microenvironment and, in particular, the acute inflammation of host tissues, may define patient prognosis (Hsu *et al.* 2010, Suzuki *et al.* 2011, Chew *et al.* 2012). Unraveling the biology and composition of the inflammatory microenvironment is crucial to understand the nuances of carcinogenesis, as well as to predict the clinical outcome of cancer patients. Herein, we describe the

inflammatory microenvironment in thyroid carcinogenesis by dividing the issue functionally into innate immunity, adaptive immunity, and molecules of the immune system.

Innate immunity

Different from the adaptive immune response, innate immunity comprises mechanisms that protect the host in a nonspecific manner (Murphy 2011). Innate immunity is sustained by cells such as neutrophils, eosinophils, and mast cells, constituting the first line of defensive response. These cells are bone marrow-derived, tissue-dwelling granulocytes found transiently in the blood circulation en route to tissue inflammatory sites (Davoine *et al.* 2013). Special attention has been paid to the neutrophil-to-lymphocyte ratio (NLR).

NLR is derived from white blood cell differentiation counts (Chiang *et al.* 2012). Zahorec (2001) described the prognostic role of NLR in critically ill patients. Highest stress, marked neutrophilia, and lymphopenia were found in intensive care unit patients. Zahorec (2001) concluded that marked neutrophilia and lymphopenia are general innate immune responses to various stressful events. Marked neutrophilia and/or eosinophilia associated with neoplasia are relatively rare findings and are considered paraneoplastic manifestations only after excluding other

**Figure 2**

Immune cells engaged in antitumor or protumor immune response. Most of these cells may play a pleiotropic role in the inflammatory microenvironment. The balance of antitumor/protumor immune responses may be important for immune escape (cancer progression) or cancer elimination.

causes, such as infections, allergy, collagen disease, vascular diseases, and concomitant malignant hematopoietic diseases (Hardy & Balducci 1985). In addition, neutrophilia could represent a nonspecific response to cancer-related inflammation secondary to tissue destruction and cytokine release (Liu *et al.* 2013).

An association between high NLR and increased mortality or recurrence has been observed in various solid organ tumors (Hirashima *et al.* 1998, Walsh *et al.* 2005, Bhatti *et al.* 2010). Liu *et al.* (2013) investigated the total white blood cell and differential counts of 159 patients with DTC and 318 age- and sex-matched controls undergoing thyroidectomy for benign thyroid nodules. They found that cancer patients in the higher NLR tertile had a significantly larger tumor size and a high recurrence risk (Liu *et al.* 2013). There was no difference in NLR between patients having benign thyroid nodules and those having malignant thyroid nodules, suggesting that a high NLR is more important for tumor progression than for tumor initiation (Liu *et al.* 2013). In fact, experimental data indicate that activated neutrophils may directly and indirectly stimulate tumor growth (Fridlender *et al.* 2009). On the contrary, studying the NLRs of 26 patients with benign goiters, 31 patients with incidental papillary thyroid microcarcinoma, 26 patients preoperatively diagnosed with thyroid cancer, and 26 healthy controls, Seretis *et al.* (2013) found NLRs to be significantly elevated in patients with incidental papillary thyroid microcarcinoma and thyroid cancer, suggesting that the NLR should be considered an easily accessible biomarker for detecting incidental papillary thyroid microcarcinoma.

Since their discovery, a large number of studies have demonstrated natural killer (NK) cell-mediated lysis of different types of tumor cells *in vitro*, as well as NK cell-dependent elimination of many tumors *in vivo* (Ljunggren 2008). Phenotypically, NK cells are defined by the expression of CD16 and CD56 (NCAM1) surface markers (Cooper *et al.* 2001a). NK cells produce interferon γ (IFN γ (IFNG)), tumor necrosis factor α (TNF α (TNF)), TNF β (LTA), and granulocyte-macrophage colony-stimulating factor (GM-CSF (CSF2)) (Vivier *et al.* 2011). Gogali *et al.* investigated NK cell infiltration in the thyroid glands of 65 patients with PTC and 25 patients with thyroid nodular goiter (NG). They found a significantly increased number of NK cells in the PTC tissue samples than in the thyroid NG tissue samples. In addition, they demonstrated an inverse correlation between NK cell infiltration and tumor stage, with decreased NK cell infiltration in advanced stages of the disease (Gogali *et al.* 2012). This finding highlights the key role of evasion from the immune

system in tumor progression and reinforces the weakness of the innate immune response toward cancer spread in advanced stages (Gogali *et al.* 2012).

However, does NK cell infiltration correspond to a homogeneous group of cells? The answer is probably no. There are two functionally different subsets of NK cells, CD56dim cells, which are cytotoxic; and CD56bright cells, which play an immunoregulatory role. These NK cell subsets present differences in their cytotoxic potential, cytokine production, and response to cytokine activation (Baume *et al.* 1992, Cooper *et al.* 2001b). The association of NK cells with cells that do not express MHC class I (e.g. tumor cells) makes the targets susceptible to NK cell-mediated lysis (Karre *et al.* 1986). Their ability to kill target cells, without prior sensitization, is regulated by the balance between stimulatory and inhibitory signals (Lanier 2008).

Liapi *et al.* (2013) investigated the distribution of CD3–CD16+CD56dim and CD3–CD16–CD56bright NK subpopulations in tissue and blood samples collected from patients with PTC and NG. Twenty-eight patients with PTC, 13 patients with NG, and 50 healthy donors were included in the study. The distribution of CD16–CD56bright and CD16+CD56dim NK cell subpopulations in the peripheral blood was similar in patients with PTC, patients with NG, and healthy donors. The number of CD16–CD56bright NK cells was increased in the tumor microenvironment of PTC tissue samples. Analysis of NK cell subpopulations in PTC tissue samples revealed that the number of CD16–CD56bright cells was higher than that of CD16+CD56dim cells. Comparison of NK cell subpopulations in the PTC and NG tissue samples revealed that the number of CD16+CD56dim cell was significantly higher in the NG tissue samples than in the PTC tissue samples. By contrast, CD16–CD56bright cells exhibited a higher infiltration in the PTC tissue samples than in the NG tissue samples, suggesting that an immunoregulatory pattern of NK cells is required for thyroid carcinogenesis (Liapi *et al.* 2013). Liapi *et al.* (2013) also found that the number of CD16+CD56dim NK cells was positively correlated with disease stage. The mean cell number in stage IVA was doubled when compared with that in stages I/II. The number of CD16–CD56bright NK cells was inversely correlated with disease stage and thyroid tissue infiltration, being greater in stages I/II. At first look, this result seems contradictory. However, it is well known that the tumor microenvironment of a stage IV tumor is quite different from that of an early-stage tumor (Cunha *et al.* 2012b). This fact should be considered along with the fact that NK cells migrating to lymphoid organs are CD56bright NK cells and seem to be

immature in comparison with CD56dim NK cells (Lanier *et al.* 1986). Recent studies have confirmed that CD56bright NK cells are precursors of the CD56dim subset (Chan *et al.* 2007, Ouyang *et al.* 2007). The tumor microenvironment can be the site where immature CD56bright cells transform into mature cytotoxic NK cells, but this developmental program is not entirely fixed, and mature NK cells can be re-educated by local and environmental factors (Freud *et al.* 2006). This could probably explain the gradual increase or decrease in the percentages of NK cell subpopulations correlating with the tumor stage.

When tissue homeostasis is perturbed, macrophages and mast cells release soluble factors such as cytokines, chemokines, reactive oxygen species (ROS), and bioactive mediators such as histamine, which induces leukocyte migration and infiltration at the site of lesion, characterizing the inflammation (de Visser *et al.* 2006). When the resolution of this response fails, a disease can be initiated (Aggarwal *et al.* 2006, Medzhitov 2010). Active mast cells generate angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor, histamine, heparin, matrix metalloproteinase 9, and several proteases, in addition to leading to the release of more cytokines and chemokines (Ribatti *et al.* 2001). These data suggest that mast cells are critically important for tumor progression. In fact, Melillo *et al.* (2010) showed that human PTC tissues, but not normal thyroid tissues, present an intense mast cell infiltrate and that its intensity is positively correlated with the invasive behavior of thyroid carcinomas. They also found that thyroid carcinoma cell cultures are mast cell chemoattractants mediated by VEGFA. When injected in the tail vein of immunodeficient mice, mast cells were found to be recruited to thyroid cancer cell xenografts. Moreover, when thyroid cancer cells were treated with conditioned media from mast cells, they exhibited higher proliferation rate, survival capacity, and invasive ability. The proliferation of thyroid cancer cells and their vascularization were reverted by sodium cromoglycate (Cromolyn), a specific MC degranulation inhibitor, suggesting that mast cells are cornerstone elements in the inflammatory microenvironment of thyroid cancer (Melillo *et al.* 2010). Proietti *et al.* (2011) evaluated the presence of mast cells in 91 consecutive cases of follicular variant of PTC (FVPTC) and 44 cases of thyroid adenoma. They found that mast cells were highly expressed in the peritumoral compartment of FVPTCs. There was also a significant correlation between the abundance of mast cells and the infiltrative pattern of the tumor in only the FVPTC group.

Inflammation occurring in the tumor microenvironment is characterized by leukocyte infiltration, where it

varies in subset composition and distribution (Liotti *et al.* 2012). Cancer cells recruit monocytes from circulation. Herein, monocytes are induced to differentiate into macrophages by chemotactic factors (Vendramini-Costa & Carvalho 2012). Phenotypically, macrophages can be recognized by the expression of CD68. Functionally, there are two different subsets of macrophages: M1 and M2. The main function of M1 is phagocytosis in response to bacterial stimuli and/or T helper 1 (Th1) cytokines, while the main function of M2 is immunosuppression and trophic activity in response to Th2 cytokines (Mantovani *et al.* 2002, Caillou *et al.* 2011).

Herrmann *et al.* (1994) observed that 75 thyroid carcinomas of follicular cell origin presented rising levels of CD68-positive cell infiltration associated with dedifferentiation. Ryder *et al.* (2008) investigated tumor-associated macrophages (TAMs) in 90 patients with different histological presentations of thyroid carcinoma. An increased number of TAMs in PDTC was found to be associated with capsular invasion, extrathyroidal extension, and decreased cancer-related survival when compared with PDTC with a low density of TAMs, suggesting that TAMs could be associated with disease progression. In fact, the same group experimentally demonstrated that TAMs promote PTC progression (Ryder *et al.* 2013). Using BRAF-induced PTC mouse models, they observed that the conditional activation of BRAF in the thyroids of adult mice induces PTCs, which express macrophage chemoattractants and are densely populated with TAMs. When TAMs were depleted, PTC initiation was impaired. Phenotypic analysis demonstrated an increased expression of M2-related genes such as *Ccr2*, arginase 1, *Ccl22*, and *Il10*, whereas the expression of M1-specific markers (*Il12* (*Il12b*) and *Ros* (*Ros1*)) was not increased. Interestingly, selectively depleted TAMs during advanced stages of PTC induce tumor regression, and CSF1/CSF1R signaling can be pharmacologically targeted to impair PTC initiation (Ryder *et al.* 2013). These results suggest that TAMs may be rational therapeutic targets for patients with refractory advanced PTCs, particularly for those with PDTC and ATC.

Do macrophages play the same role in PDTC and well-DTC? They probably do not. We studied 398 patients with DTC and 132 with nonmalignant tissues (Cunha *et al.* 2012b). We found TAMs more frequently in more aggressive cases, with metastasis at diagnosis; however, paradoxically, macrophage infiltration was correlated with improved disease-free survival (Cunha *et al.* 2012b). As a protumorigenic role was proven previously, how to explain the apparent conflicting results? It is possible that

these different results are related to different microenvironments, as shown in Fig. 3 (Ryder *et al.* 2008). In fact, Fiumara *et al.* (1997) studied 121 well-differentiated PTC samples and found tumors with TAMs and *in situ* evidence of active neoplastic cell phagocytosis. Neoplastic cell phagocytosis by macrophages was positively correlated with the infiltration of both lymphocytes and dendritic cells (DCs), whereas it was negatively correlated with vascular invasion (Fiumara *et al.* 1997). They also found a trend of reduced risk of distant metastases at follow-up in cases with TAMs that, in addition, was associated with lymphocyte infiltration, confirming the complexity of the immunological host reaction to thyroid cancer phenomenon (Fiumara *et al.* 1997, Cunha *et al.* 2012b).

Adaptive immunity

Granulocytes and macrophages represent the effector mechanisms of innate immunity. Unfortunately, they cannot always eliminate infectious agents or even efficiently combat tumor development. Conversely, cells of the innate immune system play a crucial role in the initiation and subsequent direction of adaptive immune responses that are mediated by lymphocytes (Murphy 2011). The lymphocytes of the adaptive immune system have evolved to provide a more versatile defense system, which, in addition, provides increased protection against subsequent reinfection with the same pathogen (Murphy 2011).

How do tumor-infiltrating lymphocytes (TILs) interfere with tumor progression? CD4+ T cells are central to the

successful orchestration of the immune response. Naive CD4+ T cells differentiate into one of at least four functionally distinct forms: Th1, Th2, Th17, or Tregs. In general, Tregs are identified as FoxP3+ lymphocytes and are thought to contribute to tumor-specific T-cell tolerance (Zhou & Levitsky 2007). The recent discovery of Th17 cells and their important role in host protection against infectious pathogens and in the pathogenesis of various inflammatory and autoimmune diseases have resulted in an explosion of immunological research. However, their role in human cancer is still under investigation (Su *et al.* 2010).

A mixture of lymphocytes are frequently found within and surrounding primary thyroid tumors (Modi *et al.* 2003, French *et al.* 2010). Villagelin *et al.* investigated 157 consecutive patients. They were classified by the degree of lymphocyte infiltration. Lymphocyte infiltration was classified as diffuse, peritumoral (only in or around the tumor), or absent. After a mean follow-up period of 8 years, they observed a significantly high recurrence in the absent lymphocyte infiltration group than in patients in the diffuse and peritumoral lymphocyte infiltration groups, suggesting that the presence of lymphocytes in the tumor microenvironment may favor the prognosis of patients with DTC (Villagelin *et al.* 2011), confirming previous reports (Matsubayashi *et al.* 1995, Gupta *et al.* 2001, Modi *et al.* 2003). Conversely, 100 PTC patients were analyzed for background thyroiditis and TILs by French *et al.* (2010). Patients with TILs exhibited higher disease stage and increased incidence of invasion and lymph node metastasis when compared with patients without lymphocytes or with background thyroiditis. The authors attributed these different results to the fact that concurrent thyroiditis would overshadow the real effect of lymphocytes on tumor progression. It is worth noting that considering only the presence and absence of TILs may obscure the functional and phenotypic diversity adjacent to different lymphocyte subsets.

To define the subset of lymphocytes found in association with PTC, French *et al.* (2010) analyzed primary tumors from ten patients with evident TILs by immunohistofluorescence. CD4+ T-cell frequency was found to be correlated with tumor size, Treg frequency with lymph node metastases, and CD8:Treg ratio inversely with tumor size. Our data demonstrated that the presence of concurrent chronic lymphocytic thyroiditis and infiltration of CD4+, CD8+, CD20+, Th17, and Treg cells are associated with favorable prognostic features in patients with DTC. Immune cells were found to infiltrate malignant tissues more often than benign lesions,

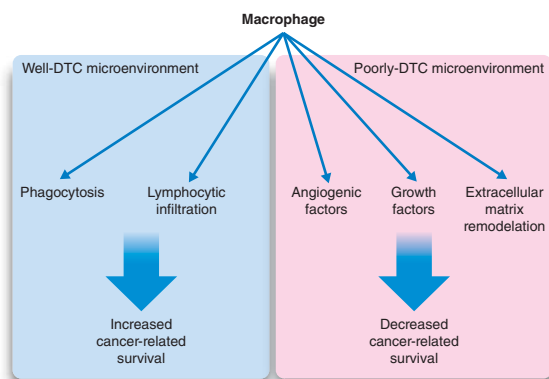


Figure 3 Macrophages and their complex role in thyroid carcinogenesis. The role of macrophages probably depends on the molecular profile of tumor microenvironment. In poorly differentiated tumors, protumor functions are highlighted, whereas well-differentiated cancer microenvironment may support stimuli that are able to elicit antitumor responses.

suggesting an immune reaction of the organism against transformed cells (Cunha *et al.* 2012b). Nevertheless, how the immune system does act is not that simple. In fact, the effect of the immune system on patient outcome seems to result from complex interactions between tumor and immune system cells.

Antitumor immune response is thought to be related to tumor antigenicity. In fact, a correlation between protein expression profile and immune cell infiltration in DTC was observed (Bruland *et al.* 2009). Some researchers have suggested that the expression of MUC1, NIS (SLC5A5), ATM, PTEN, and CD56 might indicate tumor differentiation and tumor progression, demonstrating tumor antigenicity (Larson *et al.* 2007, El Demellawy *et al.* 2008, Morari *et al.* 2010). However, whether these markers are really associated with DTC immune response is still not clear. Aiming to examine the putative association between tumor molecular profile and TIL, TAM, and MDSC patterns, we investigated 398 patients whose tissue samples were maintained in the tissue bank of the institution. Thyroid carcinoma was diagnosed in 266 patients: 253 with PTCs and 13 with follicular carcinomas. We also obtained 132 normal or benign thyroid tissue samples. Immune cell infiltration was closely associated with the immunohistochemical profile of the DTC specimens examined, including CD56, NIS, MUC1, PTEN, ATM, and B7H1 (CD274) (Cunha *et al.* 2012a).

The immune response rarely is enough to eliminate tumors, and the reason for this is the existence of immune escape mechanisms. One of these mechanisms is the upregulation of B7H1 expression. B7H1 is a cell-surface glycoprotein that may be involved in the regulation of local inflammatory responses, and aberrant tumor expression of B7H1 is thought to be associated with the inhibition of the immune system, as shown in Fig. 4 (Hamanishi *et al.* 2007). The expression of B7H1 in tumor cells has been reported to be associated with a poor prognosis in some epithelial cancers (Thompson *et al.* 2006). We demonstrated that the expression of both B7H1 protein and *B7H1* mRNA is upregulated in DTCs, contrasting with the low levels displayed by benign tissues (Cunha *et al.* 2013). This result suggests that tumor cells may acquire B7H1 expression during the tumorigenic process. We also observed an association between high *B7H1* mRNA levels and the presence of some features of tumor aggressiveness, such as higher stages at presentation and increased age at diagnosis, suggesting that high levels of B7H1 expression may help identifying individuals who need a more aggressive approach (Cunha *et al.* 2013). We also studied the expression of B7H1 in 18 patients with

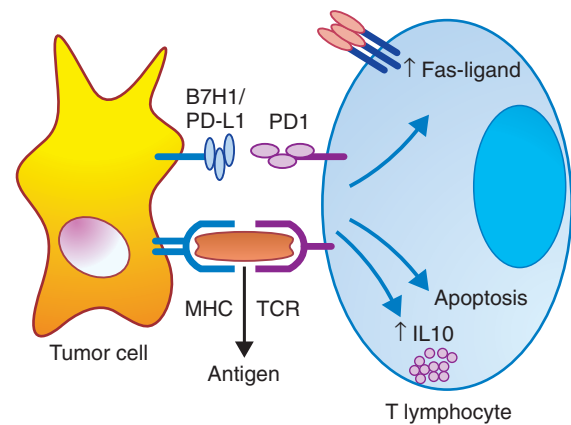


Figure 4

Constitutive expression of B7H1 by exhausted T cells. Tumor cells may acquire B7H1. The interaction between tumor cells and T lymphocytes may induce an inhibition of the immune response mediated by B7H1 expression. B7H1 is associated with IL10 production, induction of Fas ligand expression, and high apoptosis rate.

lymph node metastasis at diagnosis. Paired primary tumor and lymph node metastatic tissue samples were obtained from all the patients. We observed that the lymph node metastatic tissue samples had lower levels of B7H1 than the primary tumor tissue samples, indicating T-cell exhaustion (Cunha *et al.* 2013). French *et al.* (2012) have recently described high levels of IFN γ /CD8 $^{+}$ T cells in 12 metastatic lymph node tissue samples excised during the initial surgery at patient presentation. The authors found that proliferating lymphocytes were evident in tumor-involved lymph node metastases that were enriched with PD1 (ligand of B7H1)+lymphocytes (French *et al.* 2012). They hypothesized that the presence of metastases does not arrest and may, on the contrary, promote an IFN γ response, suggesting the generation of an antitumor response, which may impair tumor evasion. In fact, this could explain the decrease in B7H1 expression that we observed in our matched metastases, suggesting that T-cell exhaustion is a mechanism of tumor progression.

The main players within the context of innate and adaptive immunity are DCs, which induce, coordinate, and regulate the system (Steinman 1991, Banchereau *et al.* 2000). DCs are highly potent antigen-presenting cells with the unique ability of taking up and processing antigens in peripheral blood and tissues. They can migrate into the draining lymph nodes, where they present antigens to naive T lymphocytes and thus induce a cellular immune response involving CD4 $^{+}$ Th1 cells, cytotoxic CD8 $^{+}$ T cells, and B cells (Schott 2006). As DCs can modulate the

whole immune repertoire, these cells are instrumental in the understanding of the inflammatory tumor microenvironment.

The importance of DCs in PTC was documented by an early study showing that patients with a dense infiltrate of S-100+ DCs in the tumor had a more favorable prognosis irrespective of other morphological and clinical features (Schroder *et al.* 1988). A series of 527 consecutive cases of thyroid carcinoma were investigated by Ugolini *et al.* aiming to detect DC infiltration. They found that the intratumoral inflammatory infiltrate (usually composed by mixtures of CD4+, CD8+, and CD20+ cells) was strongly reduced or absent in poorly differentiated and undifferentiated histotypes of thyroid carcinoma. DCs were most frequently detected in PTC cases (classical, follicular, and tall cell variants) and markedly reduced in poorly differentiated and undifferentiated tumors, pointing to the protective role of DCs and infiltrating lymphocytes against thyroid tumors (Ugolini *et al.* 2007). In addition, Hilly *et al.* (2013) have recently demonstrated that DC density in papillary carcinoma is correlated with the concurrent thyroiditis grade and DC density in the surrounding areas of thyroiditis. These data suggest that the infiltration of DCs is part of a more complex series of outcome events that comprises lymphocyte infiltration and thyroiditis establishment.

Proietti *et al.* (2011) studied the infiltration of both immature CD1a+ DCs and mature DC-Lamp+. In the intratumoral or peritumoral area, the expression of immature, but not mature, DCs was more frequently found in FVPTCs than in adenomas. Pusztaszeri *et al.* quantitatively assessed the presence of DCs that were positive for CD1a in the cytological samples of 31 histologically confirmed PTCs and in a control group of 29 benign thyroid nodules. CD1a-positive DCs were identified in 97% of the PTCs in thyroid cytology specimens. By contrast, only 31% of benign thyroid nodules had CD1a-positive DCs (Pusztaszeri *et al.* 2013). The significant presence of CD1a-positive DCs in PTCs suggests that these cells are recruited during malignant transformation.

Aiming to investigate this issue, Scarpino *et al.* established primary cultures of neoplastic thyroid cells and normal cells (from the tumor-free contralateral lobe of the same patients) obtained from eight thyroids removed surgically. Surprisingly, they found that normal thyroid cells and tumor cells were equally effective at releasing chemotactic activity for DCs in culture supernatants (Scarpino *et al.* 1999). This finding is in conflict with the observation that DCs are rare in normal thyroid tissues

and raises the possibility that culture conditions may trigger stimulatory signals for normal thyroid cells. In fact, the same group had previously demonstrated that normal thyroid cells of primary cultures, but not normal thyroid cells of tissue sections, express high levels of MET protein and urokinase-type plasminogen activator receptor (uPAR (PLAUR)) and both factors are engaged in DC recruitment (Ruco *et al.* 1996, Zanetti *et al.* 1998, Scarpino *et al.* 1999). MET protein is present in Hürthle cells of Hashimoto's thyroiditis and hyperfunctioning thyrocytes of Graves' disease (Ruco *et al.* 1996, Zanetti *et al.* 1998), indicating that the upregulation of MET receptor is an early event in thyroid cell alteration. This result corroborates the rare infiltration of DCs in benign thyroid lesions (Pusztaszeri *et al.* 2013).

Immune system molecules in thyroid carcinogenesis

The immune system can produce or stimulate the production of many molecules in response to aggressive or nonproper features. These molecules are called cytokines and may be produced by signals of the innate or adaptive immune response for several types of cells. Cytokines influence the activation, growth, and differentiation of several different target cells, and more than 100 types of cytokines have been identified (Yadav *et al.* 2012). Table 1 summarizes the function of some cytokines. Failure to contain or impede injury can lead to persistent and excessive cytokine production, ultimately leading to harmful tissue destruction. Therefore, the balance of the host reactions to cellular stress, which will cause their production, might affect different stages of cancer (Dranoff 2004).

Interferon γ

IFN γ is a cytokine that mediates pleiotropic effects on the innate and adaptive responses to infection, and its deficiency or deficiency of its receptor has been reported to be related to the development of more tumors in mice exposed to chemical carcinogens (Bach *et al.* 1997, Smyth *et al.* 2000). IFN γ has long been reported to be associated with cytostatic/cytotoxic and antitumor functions (Brown *et al.* 1987). Kaplan *et al.* (1998) reported that approximately one-third of melanoma and lung adenocarcinoma cell lines have inactivating mutations in IFN γ pathway components, suggesting that tumor insensitivity to this cytokine could be an evasion mechanism. Recombinant IFN γ treatment was subsequently tested to

Table 1 Summary of the effects of some cytokines and the cells in which they are produced

Molecule	Producer	Effect	References
IFN γ	Activated macrophages Tumor cells	Cytostatic/cytotoxic Cytoproliferative Stimulation of the secretion of Th1 cytokines	Brown <i>et al.</i> (1987) and Rotondi <i>et al.</i> (2013)
TNF	Activated macrophages CD4+ lymphocytes NK cells Tumor cells	Apoptosis Necrosis Angiogenesis Immune cell activation Differentiation Cell migration Cachexia	Beutler <i>et al.</i> (1985), Wajant (2009) and Lippitz (2013)
TGF β	Myeloid cells T lymphocytes Tumor cells	Epithelial-to-mesenchymal transition Metastasis Immune tolerance Cell survival	Arsura <i>et al.</i> (2003), Bierie & Moses (2006) and Fabregat <i>et al.</i> (2013)
IL1	Leukocytes Tumor cells	Proliferation Differentiation Proangiogenic Prometastatic	Akdis <i>et al.</i> (2011)
IL2	CD4+ lymphocytes CD8+ lymphocytes Activated DCs Activated NK cells	Production of inflammatory molecules Stimulation of antibody synthesis Proliferation of NK cells Differentiation of NK cells	Waldmann (2006)
IL4	Th2 cells Basophils Mast cells Eosinophils Tumor cells	Response against helminths Suppression of Th1 cells Induction of IgE class switching in B cells Expression of class II MHC Expression of B-cell receptors Prolongation of the lifespans of T and B cells Mediation of tissue adhesion and inflammation	Howard <i>et al.</i> (1982) and Akdis <i>et al.</i> (2011)
IL6	Endothelial cells Fibroblasts Monocytes Macrophages	Leukocyte trafficking Leukocyte activation Induction of acute-phase protein production T-cell proliferation B-cell differentiation B-cell survival	Hirano <i>et al.</i> (1985) and Hurst <i>et al.</i> (2001)
IL10	Monocytes T cells (mainly Th2 cells) B cells NK cells Macrophages DCs Mast cells Tumor cells	Immune resolution Immune tolerance Impairment of T-cell activation Survival, proliferation, and differentiation of human B cells	Akdis <i>et al.</i> (2011)
Leptin	Adipocytes	Activation of monocytes and macrophages Angiogenesis Suppression of the production of anti-inflammatory cytokines	Kwon & Pessin (2013)
Resistin	Adipocytes Monocytes Macrophages	Production of IL6 and TNF α	Bokarewa <i>et al.</i> (2005)
Adiponectin	Adipocytes	Insulin sensitivity Inhibition of LPS-induced TNF α production in macrophages Differentiation of M2 macrophages Stimulation of the production of anti-inflammatory IL10 Modulation of T-cell activation and inflammatory function of NK cells	Yokota <i>et al.</i> (2000), Kumada <i>et al.</i> (2004), Takemura <i>et al.</i> (2007) and Li <i>et al.</i> (2009)

treat a variety of tumors: chronic myelogenous leukemia, bladder carcinoma, colorectal cancer, melanoma, ovarian cancer, and adult T-cell leukemia, but the results were considerably heterogeneous (Kurzrock *et al.* 1987, Kloke *et al.* 1992, Miller *et al.* 2009), suggesting that IFN γ has, in fact, two faces: it can have cytostatic/cytotoxic and cytoproliferative effects, depending on the context, similar to transforming growth factor β (TGF β (TGFB1)) and TNF, which also display this kind of dual contrasting behaviors (Roberts & Wakefield 2003, Wajant 2009). IFN γ studies in thyroid tissues have been carried out more frequently in autoimmune diseases, such as Hashimoto's thyroiditis. Some authors have reported that transgenic mice expressing interferon in the thyroid gland develop numerous features of Hashimoto's thyroiditis, including goiter, disrupted thyroid architecture, long-lasting hypothyroidism, and Hürthle cell metaplasia, indicating that IFN γ can transform a thyrocyte into a Hürthle cell by inducing the expression of an immunoproteasome component called LMP2 (PSMB6; Caturegli *et al.* 2000, Kimura *et al.* 2005, 2009). In a recent study, Rotondi *et al.* (2013) have shown that IFN γ stimulates the secretion of CXCL10, a situation associated with Th1 T-cell infiltration typical of autoimmune thyroid disease (AITD), which is closely related to thyroid tumors, leading, in most cases, to a better prognosis of patients (Cunha & Ward 2011, 2012). Conversely, the administration of TNF α has been shown to lead to high levels of CXCL8 (IL8), a chemokine that is not primarily involved in thyroid autoimmunity, but that might induce tumor-related inflammatory infiltrating lymphocytes in thyroid diseases.

Tumor necrosis factor

TNF has a central role in immune homeostasis, inflammation, and host defense. Depending on the cellular context, it can induce diverse processes such as apoptosis, necrosis, angiogenesis, and immune cell activation, differentiation, and migration (Wajant 2009). TNF can also modulate cachexia, mediating cancer-associated fatigue and muscle wasting by its proinflammatory properties (Beutler *et al.* 1985). TNF α and interleukin 1 (IL1) are described as acute-response cytokines. They activate vascular endothelial cells and induce endothelial cells to express adhesion molecules for neutrophils, monocytes, and lymphocytes (Lippitz 2013). Although TNF has a strong role in apoptosis and necrosis, systemic clinical tests with this factor have failed due to the severe inflammatory side effects associated with systemic TNF receptor activation (Wajant 2009). Nevertheless, safe local

administration of TNF in combination with melphalan by isolated limb perfusion was tested as a treatment option for locally advanced soft limb sarcomas, and there are ongoing clinical studies aiming to identify antibodies able to restrict TNF activity to the tumor area or to inhibit the therapy-limiting side effects (van Horsen *et al.* 2006). Increased serum concentrations of TNF α were described in eight tumors: non-small-cell lung carcinoma (NSCLC), breast cancer, colorectal cancer, prostate cancer, chronic lymphocytic leukemia, malignant melanoma, non-Hodgkin lymphoma, and gastric cancer (Lippitz 2013). These studies provide evidences that the production of TNF α in the tumor microenvironment can lead to direct DNA damage; has an apoptotic or antiapoptotic role and, depending on downstream signaling, mitogenic activity; can mediate tumor cell/stroma relationships; and can induce other cytokines and chemokines that promote tumor development (Vendramini-Costa & Carvalho 2012). Zhang *et al.* (2013) have recently demonstrated that the administration of thyroid-stimulating hormone (TSH) can increase TNF α expression, which has already been reported to be related to the chemokine profile of tumor inflammation, possibly indicating that TNF α might play an indirect role in thyroid tumors by the inflammation that it stimulates (Rotondi *et al.* 2013). In Graves' disease, inflammatory mediators, such as ILs and TNF α , stimulate the production of external thyroid-stimulating antibodies that bind to the TSH receptor (Baur *et al.* 2000, Davis 2008).

Transforming growth factor β

TGF β is a member of the growth factor TGF β superfamily, including bone morphogenetic protein, activins, inhibins, and anti-Müllerian hormone, among others, produced by cancer cells, myeloid cells, and T lymphocytes. TGF β is best known for its antiproliferative effects, and its signaling is an important regulator of the epithelial-to-mesenchymal transition and metastasis (Bierie & Moses 2006). However, tumor cells develop mechanisms to overcome TGF β -induced suppressor effects. Once this occurs, cells may respond to this cytokine inducing other effects that contribute to tumor progression, such as immune tolerance that shields the tumor from immune surveillance (Vendramini-Costa & Carvalho 2012, Fabregat *et al.* 2013). Numerous studies have established that deregulated activation of the NF- κ B and PI3K/AKT pathways by TGF β promotes cancer cell survival (Arsura *et al.* 2003, Lin *et al.* 2007). Interestingly, TGF β routinely suppresses the activation of NF- κ B in normal epithelial

cells by inducing their expression of I κ B α (NFKBIA; Nagumo *et al.* 2005, Sanchez-Capelo 2005). However, this event is inactivated during oncogenic progression, and TGF β stimulation of late-stage cancers of the breast, prostate, and liver has been proven to result in NF- κ B activation, leading to the induction of pro-survival and tumorigenic gene expression profiles (Arsura *et al.* 2003). Both TGF β mRNA and TGF β protein are expressed in thyroid cells. There is evidence that iodine and TSH are regulators of this production. Experimental studies have shown that iodine excess leads to an increased mRNA expression of TGF β , stimulating protein synthesis (Yuasa *et al.* 1992, Cowin & Bidey 1995). In addition, studies in rats treated with TRH showed that during the formation of goiter, there is an increase in Tgf β gene expression (Morosini *et al.* 1996). Kimura *et al.* (1999) proved that goiter and DTC tissues exhibit a higher expression than normal tissues. In a more recent study, Eloy *et al.* (2012) have proposed a new division of malignant tumors, classifying them into well-circumscribed carcinomas (WCPTCs), ones lacking infiltrative features and lymph node metastasis, and poorly circumscribed carcinomas, ones having these characteristics. This allowed the authors to observe that TGF β was less expressed in the center of WCPTCs and that this expression increased as they became invasive forms, also associated with SMAD4 and SMAD7 proteins, probably increasing the ability of these tumors to progress and invade. These findings provided new perspectives for the understanding of TGF β mechanisms for tumor progression, as not only the classically studied pathways, such as PI3K/AKT and MAPK, appear to be involved in malignant TGF β transformation, but also SMAD molecules might play a role in this event.

Interleukins

A large group of secreted proteins that bind to their specific receptors and play a role in the communication among leukocytes are called ILs, and they are divided into families based on sequence homology and receptor chain similarities or functional properties (Akdis 2011). Some of the immune system molecules, such as CD4⁺ Th cells, are classified according to the cytokine profile of the microenvironment where they are present, which is ultimately regulated by the expression of other cytokines, showing a dependence of some ILs on other cytokines to be produced (Fan *et al.* 1998, Akdis 2011). Such a profile might define the specific properties of the cells and their role in immune response to nonproper agents.

IL1 is a proinflammatory cytokine that mediates many inflammatory diseases and interferes in cell proliferation, differentiation, and function in innate and adaptive immunity (Akdis 2011). IL1 can be produced by neoplastic cells or by the microenvironment where it is present, stimulating the production of proangiogenic and prometastatic mediators (Lewis *et al.* 2006). As it has a role in proliferation and can also lead to the production of ROS and RNS, IL1 can favor the accumulation of mutations, increase the invasiveness of tumor cells that are existing already, activate the angiogenic machinery, and induce the production of inflammatory molecules, contributing to the spread of tumor cells (Apte *et al.* 2006). Thus, as expected, high levels of IL1 would be associated with a worse prognosis. In fact, the expression of IL1 has been shown to be upregulated in melanomas and colon, lung, head-and-neck, and gastric cancers (Perrier *et al.* 2009). With regard to thyroid tissues, Rebuffat *et al.* (2013) have recently demonstrated that IL1 β (IL1B) modifies thyroid epithelial tightness by altering the expression and localization of junction proteins in Graves' and Hashimoto's diseases, suggesting that IL1 β could play a role in the pathogenesis of thyroid autoimmunity. Alterations in serum concentrations of IL1 β have been suggested to differentiate PTCs from atrophic thyroiditis (Kammoun-Krichen *et al.* 2012). The inheritance of the polymorphism rs2192752 in IL1 receptor (*IL1R1*) increases the risk of PTC, indicating an association of this IL with DTC (Park *et al.* 2012).

Several studies have also been conducted to test the efficacy of IL2 in stimulating the immune system of cancer patients, as the main functions of IL2 are as follows: regulating the growth of B cells, stimulating the synthesis of antibodies, and increasing the cytolytic capacity of cells, through the increase in NK cell proliferation (Waldmann 2006). As a matter of fact, recombinant human IL2 is used in immunotherapy for advanced renal cancers and melanomas and for treating AIDS associated with HIV (Akdis *et al.* 2011, Liang *et al.* 2012). The administration of high doses of IL2 is associated with a better response rate in the case of melanomas and renal cancers, leading to improvements in disease-free survival, although the side effects of IL2 administration are remarkably hard on most of the patients (Clement & McDermott 2009, Halama *et al.* 2010).

IL4 is produced by Th2 cells, basophils, mast cells, and eosinophils and, similar to most of the cytokines, is able to regulate other immune molecules (Howard *et al.* 1982). IL4 is one of the most important cytokines for the differentiation of CD4⁺ cells into Th1 and Th2 cells, essential

effectors of immune responses (Akdis *et al.* 2011). Besides Th1 and Th2 cell production, IL4 also affects the phenotypes of B and T cells, leading to prolonged cell lifespans, which will affect tissue adhesion and inflammation (Akdis *et al.* 2011). Studies have long shown that IL4 has an inhibitory effect on the growth of human melanomas, renal cell carcinomas, and gastric cells (Hoon *et al.* 1991). On the contrary, IL4 and IL10 have been reported to exert a stimulatory effect on the growth of thyroid cancer cells. The production of IL4 and IL10 has been shown to be related to the promotion of thyroid tumor cell progression through the downregulation of BCL2 and BCL-XL (BCL2L1), leading to the death of a considerable number of cells (Stassi *et al.* 2003). Although through different mechanisms, Vella *et al.* (2004) suggested that concomitant Graves' disease and PTC that exhibited IL4 and IL10 expression were more likely to display apoptosis resistance, probably potentiating anti-apoptotic factors, such as insulin-like growth factors. In the same way, Todaro *et al.* demonstrated that IL4 and IL10 upregulate cFLIP (CFLAR) and PED/PEA15, neutralizing the interaction of CD95L with CD95, which ultimately results in thyroid tumor cell survival and proliferation. The authors observed thyroid tumor cell extinction when they neutralized these cytokines, suggesting that they could be used as therapeutic targets (Todaro *et al.* 2006). In a recent study, Lee (2012) has described the induction of DCs from peripheral blood mononuclear cells purified from patients with thyroid cancer by culturing them in the presence of FL, GM-CSF, IL4, and TNF α . The authors provided morphological evidence that the co-culture of T cells/cancer tissues activated T cells and differentiated cytotoxic T lymphocytes. These cytotoxic T lymphocytes adhered to cancer tissues and lysed cancer tissues, suggesting that DCs could be used as potential vaccines in immunotherapy, resulting in minimum residual disease after the conventional treatments applied to cancer (Lee 2012).

IL6 is involved in both innate and acquired immunity and its production is affected by stimuli from other cytokines (IL1, IL17, and TNF α ; Akdis 2011). In innate immunity, IL6 is considered a regulator of acute-phase responses, through the activation of leukocytes and also the stimuli for the expression of acute-phase proteins (Hurst *et al.* 2001). IL6 also participates in acquired immunity, promoting B-cell differentiation and survival and plasma-cell production of antibodies (Hirano *et al.* 1985, Akdis 2011). There are strong positive associations between serum IL6 concentrations and tumor size, tumor stage, and disease progression in patients with gastric

cancer, colorectal cancer, bone sarcoma, breast cancer, hepatocellular cancer, nasopharyngeal cancer, renal cell cancer, lung cancer, and melanoma (Niitsu *et al.* 2002, Chow *et al.* 2003, Nikiteas *et al.* 2005, Tas *et al.* 2005, Ahmed *et al.* 2006, Hsia *et al.* 2006, Egler *et al.* 2008, Ikeguchi *et al.* 2009). Although there are several evidences that IL6 is closely related to AITDs, its relationship with thyroid cancers has not been fully investigated (Gillespie *et al.* 2012, Popova *et al.* 2012, Beumer *et al.* 2013).

IL10 is an anti-inflammatory factor and an important regulator of several aspects of immune responses (Akdis 2011). It is very important in the microenvironment where it is located, since it inhibits the expression of many proinflammatory cytokines, chemokines, and chemokine receptors, thus influencing the production and effects of a large number of molecules, indirectly affecting T-cell activation (Akdis 2011). Circulating concentrations of IL10 were investigated in different cancer types and were found to be associated with adverse disease stage or with negative prognosis in bone sarcoma, diffuse large B-cell lymphoma, gastric cancer, colon cancer, Hodgkin's lymphoma, hepatocellular cancer, melanoma, renal cell cancer, NSCLC, and pancreatic cancer (Sato *et al.* 1996, Onishi *et al.* 1999, Suzuki *et al.* 2001, Chen *et al.* 2013). Our group showed that the inheritance of a G allele at *IL10* – 1082A/G polymorphism may favor concurrent thyroid autoimmunity in DTC patients, and this autoimmunity may favor a better prognosis of these patients (Cunha *et al.* 2011). In addition, Yu *et al.* (2013) demonstrated that the concentration of IL10 was significantly higher in patients with PTCs associated with multinodular goiter (MNG) than in patients with MNG alone, suggesting that cancer patients would have a specific type of Treg that affects antitumor responses and may facilitate disease progression and worse prognosis.

Adipokines

Adipokines or adipocytokines are cytokines produced by the adipose tissue. They have different functions, such as regulation of appetite and energy balance, immunity, insulin sensitivity, angiogenesis, inflammation and acute-phase response, blood pressure, and lipid metabolism (Kwon & Pessin 2013). Many current researches involve adipokines such as leptin, adiponectin, and resistin, considering that these adipokines have a direct relationship with the adipose tissue and are directly involved in changes in the immune response and insulin resistance, which can be a risk factor of several diseases associated

with obesity, such as diabetes. Our group has obtained preliminary data suggesting a relationship between obesity and DTC development (Marcello *et al.* 2012), but the molecular mechanisms involved in this relationship are not yet very well understood, although recent literature has been pointing to insulin resistance and potentially adipokines as probable connectors.

The structure of leptin is similar to that of IL2, IL6, and granulocyte colony-stimulating factor, and similar to other cytokines, it can activate monocytes and macrophages, stimulate VEGF and angiogenesis, and suppress the production of anti-inflammatory cytokines (Kwon & Pessin 2013). The induction of inflammatory responses by leptin involves its receptor b (LepR_b), JAK2, and STAT3 signaling pathway (Kwon & Pessin 2013), and all these molecules ultimately have an effect on the PI3K/AKT and MAPK signaling pathways. Thus, leptin interacts with several factors that participate in the main signaling pathways of DTCs and could represent one of the links between obesity and DTC. The expression of leptin and its receptor, OBR (LEPR), has already been reported to be associated with a high risk of lymph node metastases, worsening the prognosis of patients, suggesting that the participation of this adipokine in DTCs might be important for their progression (Cheng *et al.* 2010).

Resistin is primarily related to insulin resistance by the activation of SOCS3 and the suppression of insulin-mediated signaling in rat adipocytes (Steppan *et al.* 2005), but in humans this association is not always true. This might be because resistin is produced not only by adipocytes (as in rodents) but also by monocytes and macrophages in humans (Kwon & Pessin 2013). In macrophages, the expression of resistin is induced by inflammatory cytokines such as IL1 β , IL6, TNF α , and lipopolysaccharide (LPS). In human peripheral mononuclear cells, resistin stimulates the production of IL6 and TNF α through the NF- κ B signaling pathway (Bokarewa *et al.* 2005). Similar to leptin, resistin also binds to TLR4, activating JNK and p38 MAPK to induce insulin resistance (Benomar *et al.* 2013). The expression of resistin has been reported to be associated with the increased proliferation of prostate cancer cells by the stimulation of the AKT pathway, a widely studied pathway in follicular thyroid tumors (Kim *et al.* 2011). There are also reports of the influence of resistin on breast cancer cells (Kang *et al.* 2007, Sun *et al.* 2010). As for other adipokines, there are evidences that the expression of resistin can be modulated by the presence of polymorphisms in its gene and gene receptor (Chen *et al.* 2010). Eke Koyuncu *et al.* (2013) have recently demonstrated significant

correlations between resistin and TSH levels in hyperthyroid individuals, suggesting that the levels of this cytokine are directly related to thyroid dysfunction.

Adiponectin is highly expressed by adipocytes with potent anti-inflammatory properties and its expression can be suppressed by proinflammatory factors such as TNF α , IL6, ROS, and hypoxia (Li *et al.* 2009). The administration of adiponectin or its overexpression in transgenic mice results in improved insulin sensitivity, inhibits LPS-induced TNF α production in macrophages, promotes the differentiation of anti-inflammatory M2 macrophages and phagocytosis to remove apoptotic cells, stimulates the production of anti-inflammatory IL10, and also modulates T-cell activation and inflammatory function of NK cells (Yokota *et al.* 2000, Kumada *et al.* 2004, Takemura *et al.* 2007). Recently, Mitsiades *et al.* (2011) have demonstrated that serum adiponectin concentrations are inversely correlated with DTC, exerting a protective effect against the development of this cancer. Furthermore, the authors demonstrated that thyroid tissues express the receptors ADIPOR1 and ADIPOR2, which facilitate the entrance and functioning of adiponectin, suggesting a relationship between this cytokine and thyroid tissues.

In summary, DTCs are enriched with multiple inflammatory cells and both innate and adaptive immune responses seem to be engaged in thyroid carcinogenesis. In addition, the molecular pattern of cytokines and chemokines helps to explain the involvement of the immune system in tumor progression. The first attempt to use the immune system as a tool for treating patients with thyroid cancer was made in 1975. Active immunotherapy was applied to three patients. Two of them, who were in the terminal stage of the disease, could not develop generalized cell-mediated immunity. One patient developed *in vitro* evidence of cell-mediated immunity against cancer tissue antigens, associated with a decrease in tumor size (Amino *et al.* 1975). Unfortunately, more than 30 years later, no relevant successful result has been obtained for thyroid immunotherapy. On the other hand, the inflammatory microenvironment may help to identify more aggressive tumors and patients who would benefit from a more invasive approach, probably sparing the vast majority of patients with an indolent disease from unnecessary procedures.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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