From immune surveillance to tumor-immune escape: the story of an enemy with multiple strategies of resistance and counterattack

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DE LA INMUNOVIGILANCIA AL ESCAPE TUMORAL:
HISTORIA DE UN ENEMIGO CON MÚLTIPLES ESTRATEGIAS DE RESISTENCIA Y CONTRA-ATAQUE

RESUMEN

Los tumores deben evadir la respuesta inmunológica para ser clínicamente detectables en el paciente. A estos fines, las células cancerosas han desarrollado múltiples estrategias para eludir el ataque inmunológico. Estos mecanismos conspiran en estadíos avanzados de un tumor limitando la habilidad del sistema inmune para inhibir el crecimiento tumoral y la efectividad de estrategias de inmunoterapia en cáncer. Desde la biología tumoral a la clínica, en el presente artículo expondremos los más importantes mecanismos utilizados por tumores para evadir la respuesta inmunológica y su potencial impacto en el diseño de estrategias de inmunoterapia.

PALABRAS CLAVE: Escape tumoral/ Presentación antigenica/ Apoptosis/ Contraataque/ Fas ligando/ Galectina-1.

ABSTRACT

Tumors must circumvent the immune response of the host to become clinically detectable. For this purpose, malignant cells have devised multiple strategies to thwart immune attack. These mechanisms are suggested to conspire in advanced stages of cancer to limit the ability of the immune system to restrain the tumor and the effectiveness of immunotherapy strategies to successfully eradicate malignant cells. From tumor biology to cancer immunotherapy and back again, we will summarize here some of the most important mechanisms used by tumors to evade the immune response and their potential impact in the design of cancer immunotherapy strategies.

KEY WORDS: Tumor-immune escape/ Antigen presentation/ Apoptosis/ Counterattack/ Fas ligand/ Galectin-1.

A BRIEF INTRODUCTION: FROM IMMUNE SURVEILLANCE TO TUMOR-IMMUNE ESCAPE

All living organisms are exposed to different external insults such as viruses, bacteria, fungi, parasites and toxins, among others. To overcome these adverse stimuli, vertebrates have successfully developed a sophisticated system of protection against such foreign pathogens. As a consequence, the immune system displays different and complex innate and adaptive mechanisms in which several cell types are involved.

The first suggestion of a pivotal role of the immune system in recognizing tumor cells as foreign was postulated by Paul Erlich early during the 20th century[1]. Erlich postulated the concept that the immune system would protect the organism, namely «the self», detecting and eliminating the «non-self». The idea that tumor cells are

considered as «non-self» and can be recognized and eliminated by T-cell mediated mechanisms was controversial for many years. The genetic and biochemical changes that a normal cell undergoes during its transition to a malignant cell can frequently lead to the production of new antigens that are not present in normal cells. These new antigens are called tumor-specific antigens (TSA).

However, the first experimental evidence supporting the notion that tumors can induce an immune response emerged four decades later. The original observations of Gross[2], Foley[3], and Prehn[4] demonstrating that different inbred strains of mice can develop resistance to tumor transplantation by preimmunization with the same tumor, were confirmed in different experimental models by several researchers[5, 6]. Nevertheless, those and other experimental results, have not received wide acceptance among the community of tumor biologists, as it was believed that MHC and not TSA antigens would be responsible for the observed immune response. In spite of this controversy, Burnet and Thomas provided a re-birth of Erlich's hypothesis[7, 8] and developed the theory of tumor-immune surveillance. The authors proposed that the majority of the tumors, which appeared frequently, were eliminated efficiently by the immune system, even before they could be clinically detectable. This seminal theory not only induced a great deal of experimental work that meant a significant advance in tumor immunology, but also opened new avenues for immunological intervention in order to eradicate primary tumors and metastases.

THE «GREAT CONTRADICTION»: AN IMMUNOCOMPETENT HOST CANNOT BEAT AN IMMUNOGENIC TUMOR

From its very beginning, the immune surveillance theory had strong supporters but also strong detractors. In spite of the evidences obtained from different experimental models, supporting the existence of an immune response against tumors[9], several arguments were posed against this theory[10]. The lack of increase of tumor incidence in nude mice, which are congenitally athymic and, therefore, incapable of mounting a T cell response, was the main experimental fact that seemed to defeat Burnet's theory. Nevertheless, the experimental evidence obtained later finally confirmed Burnet's hypothesis. It was clearly demonstrated that natural immunity in nude mice could control tumor development. Moreover, it was also shown that nude mice indeed develop extrathymic T cells. After several controversial discussions, this theory is at present in good health, due to the emergence of new experimental

data supporting the concept of immunosurveillance[11-14]. However, if we accept that the immune system of an immunocompetent host is able to detect and respond to the new antigens displayed by the tumors, an important contradiction emerges when we confront this situation with the fact that, very frequently, the immune system is not able to cope with the tumor that grows and, eventually, kills the host. Several mechanisms, known as tumor escape mechanisms, appear to be responsible for the lack of an effective immune response against the tumors.

Tumors must circumvent the immune response of the host to become clinically detectable. For this purpose, malignant cells have devised multiple strategies to evade or thwart the immune attack. These mechanisms are suggested to conspire in advanced stages of cancer to limit the ability of the immune system to restrain the tumor and the effectiveness of immunotherapy strategies to successfully eradicate malignant cells. In the next sections we will critically discuss some of the most important mechanisms developed by tumors to evade immune recognition and resist immune attack (Figure 1).

TUMOR-IMMUNE ESCAPE STRATEGIES

Alterations in the expression and function of Major Histocompatibility Complex (MHC) molecules

The success in tumor elimination by CD8+ cytotoxic T lymphocytes (CTLs) relies on the efficient presentation of the tumor antigen-derived peptides by MHC class I molecules. Effector T cells specifically recognize those peptides associated with MHC-I and exert their cytotoxic effect by inducing tumor cell apoptosis. Any alteration of the antigen presentation machinery will alter the generation of an effective immune response.

Cancer cells have developed multiple mechanisms aimed at eliminating or, at least, weakening the adaptive immune response. Such a strategy is clearly evidenced by the demonstration of a frequent down-regulation of the expression of MHC class I antigens in several tumor types. The loss of these molecules results in decreased ability to present peptides derived from tumor antigens, thus making these cells resistant to CTLs. Complete loss of class I or loss of single alleles are frequently seen and more than half of all tumors may have one of these alterations. It has been reported by several laboratories that the down-regulation or loss of MHC class I expression prevents the recognition of tumor antigens and the lysis by CTLs. In fact, down-regulation of HLA-A and HLA-B alleles is quite common in different tumor types[15-17]. Moreover, downregulation of MHC I
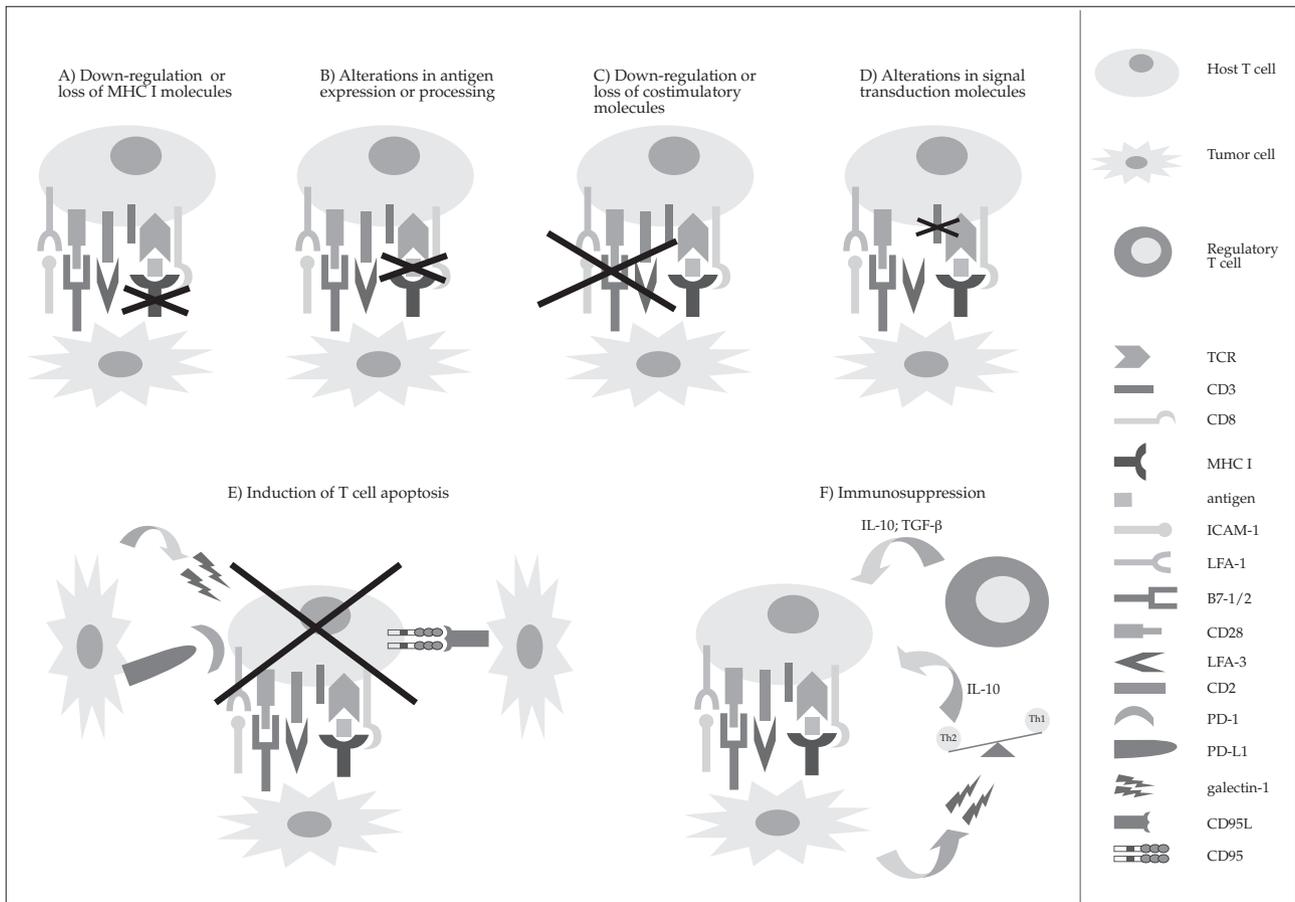
Figure 1. *Mechanisms of tumor-mediated immune evasion: A) alterations (down-regulation or loss) in MHC expression and function in tumor cells; B) alterations in tumor antigen expression and/or processing; C) alterations in the expression of costimulatory molecules; D) alterations in signal transduction molecules (p56lck; p59fyn; CD3-ζ, and others); E) induction of T cell apoptosis by: CD95/CD95L, PD-1/PD-L1, or galectin-1; F) tumor-induced immunosuppression by regulatory T cells, cytokines or galectin-1.*

expression and function has been demonstrated to have an important prognostic value[18, 19]. A number of studies have been carried out to assess the different HLA phenotypes and the molecular mechanisms leading to those phenotypes, which are regulated at least in part, through changes in oncogenic factors[20]. Complete loss of expression of the HLA class I alleles is a phenotype found in cervical carcinomas[21], bladder carcinomas[22] and melanomas[23, 24], among others. In the majority of the cases the HLA loss is caused by different mutations involving β_2-microglobulin genes. It has been proposed that tumor suppressor genes and genes implicated in the immune response could undergo inactivation by the same molecular mechanisms[20]. Haplotype loss is the second HLA phenotype that has been described. This alteration is caused by the hemizygous loss of HLA-A, -B and –C alleles, and the underlying mechanism is the presence of large deletions in chromosome 6[25, 26]. Allelic loss of single HLA alleles defines a third HLA phenotype

that is caused by a wide array of genetic defects including point mutations, frame shifts or deletions. These kind of genetic alterations have been detected in melanomas and colorectal carcinomas[27].

It has been well established that cells that express lower levels of HLA-A and HLA-B molecules are more susceptible to lysis by the natural cytotoxicity exerted by NK cells[28]. Such a condition revealed another mechanism of evasion by cancer cells involving the expression of non classical HLA molecules such as HLA-G and HLA-E, which belong to the group MHC class Ib. This group of molecules is involved in immunoregulation, acting as inhibitory signals for NK cell cytotoxicity[29]. It has been shown that expression of HLA-G by melanoma cells contributes to cell evasion of the immune response by inhibiting NK cell recognition[30]. Furthermore, recent evidence indicates that the inhibition of the interactions between NKG2D and its ligands on tumor cells (i.e MICA and MICB proteins) may result in tumor

cell evasion of NK cell responses[11, 12]. The identification of different strategies used by tumors to avoid immune surveillance and the elucidation of the molecular mechanisms involved in these strategies are crucial for the development of novel therapeutic approaches directed to restore an effective immune response.

Alteration in Tumor Antigen Expression and the Antigen Processing Machinery

In recent years a great progress has been made in the identification of tumor-specific antigens (TSA), derived from antigens localized on the surface of tumor cells. Tumor antigens can be classified into two types: 1) those that are unique to transformed cells and do not appear in normal cells (TSA) and 2) those called tumor-associated antigens (TAA), which are frequently found on the surface of tumor cells but are also expressed on the surface of normal cells during the embryonic or fetal life (oncofetal antigens), in selected organs after birth, or in normal cells but at much lower concentration than on tumor cells.

Research over the past decade has led to the identification of different tumor antigens recognized by T cells and the elucidation of their structure and nature at the molecular level[31-35]. The presence of specific antigens is critical for the development of an antitumor immune response. Therefore, if the tumor finds the way of downregulating, modifying or losing its own antigens, it will have found an efficient tactic for avoiding immune recognition. Actually, it has been demonstrated that several of these modifications take place in different tumor models.

Independently of the alterations in the expression of HLA class I antigens, a wide array of modifications in the structure and/or the expression of TSA have been observed in different tumor types. Besides the intrinsic heterogeneity of antigen expression among different tumors, a down-regulation of antigen expression has been demonstrated, which could range from decreased expression levels to a complete loss of one or more tumor antigens[27, 36-38]. Thus, antigen-loss variants that appear or remain within the tumor, particularly after immunotherapy[39, 40] have a selective growth advantage, leading to a more malignant tumor. Melanoma is a prototype tumor in which down-modulation of several differentiation antigens (including Melan-A/MART-1 and tyrosinase)[41-43] has been clearly demonstrated. Down-regulation of Melan-A/MART-1 antigen expression by different mechanisms (including antigen silencing via its promoter)[44] avoids recognition by Melan-A/MART-1-specific T cells and contributes to tumor-immune escape.

A second immune escape strategy based on modifications of tumor antigens has recently been demonstrated. Tumors can take advantage of the mechanism of antigenic drift, like viruses, and generate point mutations that change the amino acid sequence of the antigen[45, 46]. Then, cells harboring the new antigen variants are selected by their growth advantage, since the specific T-cell response elicited by the original tumor is directed toward an antigen that is no longer represented on the tumor cell surface.

Moreover, even if the expression of HLA I molecules and tumor antigens is conserved, the induction of a specific cytotoxic T cell response may be impaired because of the presence of alterations in the antigen-processing machinery[27]. Down-regulation of the transporters of peptides related to antigen processing TAP-1 and TAP-2, has been associated with the malignant potential of murine[47] and human[48] tumors.

A retrospective analysis of the classical theory of «genetic instability» described by Peter Nowell in 1976[49] provides support to the different tumor cell variants that will harbor, heterogeneity not only in MHC molecules expression, but also in the expression of tumor antigens. The consequence of such variety of phenotypes is rapidly observed: the success of the patrolling immune cells in eradicating susceptible cancer cells is reverted towards rapid tumor growth. These tumor cell variants will have increased malignancy, tumor progression and, finally, metastatic dissemination.

Alterations in the Expression of Costimulatory or Adhesion molecules

Naïve T lymphocytes require at least two different extracellular signals in order to activate and differentiate into effector cells. The binding of the MHC-peptide complex to its specific receptor (TCR-CD3 complex) and to the co-receptors CD4 or CD8, provides the first signal required for the development of a specific immune response. However, this signal is not sufficient for full activation of T cells, requiring the participation of a second signal provided by costimulatory molecules (B7-1/CD80 and B7-2/CD86) expressed by antigen presenting cells (APC). However, other molecules are also involved in nonspecific adhesion mechanisms that are required for the interaction and immunological synapse between effector cells and target cells. Some of these interactions involve ICAM-1 (CD54) binding to LFA-1, CD40L binding to CD40, and members of the TNF receptor family[46]. The majority of the tumors are derived from parenchymal or mesenchymal cells that do not express B7 molecules. Moreover, cells that normally express B7 can down-regulate its expression during the process of tumor progression[50]. Thus, the ability of tumor cells to effectively present tumor antigen derived-peptides might be severely impaired. Consequently, that lack of

costimulation could be an important mechanism of evasion of the immune response, even if tumor cells express normal levels of MHC-I and TSA[51, 52]. In this regard it has been recently demonstrated that low surface expression of B7-1/CD80 represents an immune escape mechanism in colon carcinoma[53]. Tirapu and colleagues showed that silencing of CD80 by interfering RNA led to loss of tumorigenicity of CT26 colon carcinoma in immunocompetent, but not immunodeficient Rag-/- mice[53].

The differentiation of CTLs is stimulated by activated helper T cells and requires the presence of MHC molecules on APC. Thus, for the induction of effective anti-tumor immune responses, a cross-priming by APCs that express costimulatory and MHC molecules is usually required. In this context, a decrease or lack of an adequate cytotoxic anti-tumor response would be provoked by APCs that are not able to efficiently present tumor antigens and activate helper T cells.

Induction of T cell apoptosis: «Tumor Counterattack»

Following the principle that the best defense is a timely attack, cancer cells not only resist immune attack, but have also devised strategies of tumor counterattack.

The CD95/CD95L System

Apoptosis mediated by Fas (CD95)/Fas L (CD95L) interactions is critical for the regulation of the biology and homeostasis of normal tissues. The Fas receptor belongs to the tumor necrosis factor (TNF) family of receptors involved in proliferation and apoptosis and is characterized by an intracellular domain, called «the death domain». Fas ligand (FasL) is a type II transmembrane protein which functions by inducing apoptosis of Fas-bearing cells[54].

In 1995, D.L Vaux observed that FasL-expressing Sertoli cells were not rejected when transplanted into allogeneic mice[55]. This observation was supported by the finding of FasL expression in the eye[56], and allowed the conclusion that FasL expression confers immunological privilege to selected tissues under physiological and pathological conditions. A few years later, the interaction of FasL on tumor cells with its specific receptor Fas on T lymphocytes was implicated in tumor cell evasion of immune surveillance[57].

Binding of FasL to Fas leads to oligomerization of receptors and recruitment of FADD and the pro-caspase 8; these molecules together form a protein complex called the death-inducing signaling complex (DISC). In the DISC, pro-caspase-8 is autocatalytically cleaved and then activates further effector caspases directly or via a mitochondrial pathway, which cleave cellular substrates leading to the typical biochemical and morphological changes of apoptosis. The Fas signaling pathway is regulated by many pro- and anti-apoptotic factors, including members of the Bcl-2 family which regulate apoptosis at the mitochondrial level, FLIPs (FLICE-inhibitory proteins) which interfere with the initiation of apoptosis directly at the level of death receptors, and IAPs/ survivin which bind to and inhibit caspases and apoptosome formation[58].

Expression of FasL has been reported in solid tumors of nonhematopoietic origin, including colon, hepatocellular carcinoma, melanoma, astrocytoma, lung carcinoma, ovarian carcinoma, esophageal carcinoma, glioblastoma, renal cell carcinoma, and head and neck carcinoma. However, considerable heterogeneity in surface expression of FasL has been detected even within a particular lineage of tumor[59]. Although unspecific staining of certain FasL antibodies has been demonstrated[60], it has been shown that FasL-positive tumor cells can kill Fas-positive T cells *in vitro*, demonstrating functional FasL expression[61].

A number of tumors are resistant to apoptosis and express functional FasL constitutively or after chemotherapy. This situation may enable tumor cells to delete anti-tumor lymphocytes and to suppress anti-tumor immune responses, a phenomenon called «tumor counterattack»[62]. Several lines of evidence support the involvement of the Fas system in tumor counterattack. Fas/FasL interactions are proposed to be an important mechanism for the maintenance of immune privilege in selected organs including the eye, placenta, testis, and the central nervous system[63]. Also, various animal models have been used to demonstrate the ability of FasL expressed on tumors to down-regulate anti-tumor immune responses[64, 65]. However, certain experimental evidence seems to contradict the FasL counterattack hypothesis. It has been reported that overexpression of FasL on tumor cells does not confer immune privilege but, instead, induces a strong inflammatory response that accelerates tumor rejection. These reports propose that FasL has a pro-inflammatory function and that gene transfer of FasL can be used in tumor eradication. It has been suggested that locally expressed FasL acts on surrounding cells to induce the production of IL-8 or other leukocyte chemoattractants[66]. Other authors reported that FasL induces the processing and release of IL-1β, which in turn may be responsible for the infiltration by neutrophils[67].

The *in vivo* consequences of FasL expression on tumors are far from clear. So far, many studies of tumor counterattack have been published, but the results are confusing and contradictory because many factors may influence FasL activity *in vivo.* The different levels and the kinetics of FasL expression on tumor cells may be of particular relevance in

tumor-immune system interactions. The sensitivity of T cells to Fas-mediated apoptosis varies considerably depending on the activation status of T cells. Also, T-cell sensitivity to Fas-mediated apoptosis is modulated by many factors, particularly by signaling through various receptors and cytokines[68]. The consequences of FasL expression may also depend on the tumor microenvironment. Various site-specific factors may have a critical impact on the physiology of immune cells, on the interaction of immune and tumor cells and on the tumor itself. In addition the release of different type of cytokines, such as TGF-β and IL-10, the oxygen levels (hypoxia or normoxia), the extent of vascularization and the accessibility to immune cell infiltration, may also influence Fas L-mediated immune privilege.

A further complicating aspect in tumor-immune escape mediated by FasL is the role of soluble FasL[69]. Soluble FasL differs functionally from the membrane-bound form and its biological function is controversial. In some studies soluble FasL was cytotoxic[70], while in other studies Fas L shedding resulted in the absence of killing activity[71]. Finally, other death factors, such as TNF and TRAIL, have also been shown to kill T cells[72] and, thus, might contribute to the suppression of anti-tumor immune responses.

Taken together, the evidence presented suggest that tumor counterattack may be a powerful mechanism of tumor immune escape. To further understand its relevance under physiological and pathological conditions, it will be essential to use defined experimental systems in which all the above mentioned key elements could be controlled or carefully modulated. Thus, inhibition or blocking of tumor counterattack mechanisms might be beneficial for cancer therapy.

Tumor-induced Immunosuppression: Regulatory T cells and Cytokines

Recently, a subset of T cells, co-expressing CD4 and CD25 (IL-2Rα chain), with unique immune regulatory properties has been described[73]. Regulatory CD4+CD25+ T cells (Treg) contribute to the prevention of autoimmune disorders by controlling the activity of autoreactive T lymphocytes and by inhibition of the proliferation of effector T cells. It has been suggested that CD4+CD25+ regulatory T cells may also contribute to immune tolerance at the tumor microenvironment and that depletion of these regulatory cells might be advantageous for boosting the anti-tumor immune response[74]. These cells constitute approximately 5-10% of the total CD4+ T cell population in both humans and rodents, and their removal induces autoimmune disease in target tissues[75]. Besides expressing the CD25 receptor, Tregs express other molecules including the cytotoxic T lymphocyte-associated protein-4 (CTLA-

4), a glucocorticoid-induced TNF receptor family-related protein (GITR) and FoxP3 (a forkhead family transcriptional regulator). Although none of them is a distinct marker of the lineage, FoxP3 has turned out to be a useful marker as well as a critical factor for the differentiation and function of these cells[76]. Association of these markers with the immunoregulatory activity of T regs has provided insights for the identification and molecular characterization of this particular cell subset. It has been recently demonstrated that Gr-1+ CD115+ immature myeloid suppressor cells can mediate the development of tumor-induced T regulatory cells in tumor-bearing hosts[77].

In addition to CD4+ CD25+ FoxP3+ natural Treg, recent studies also report the identification of inducible T regulatory cells (Tr1)[78]. The immune regulatory function of Tr1 cells has been attributed to their capacity to secrete immunosuppressive cytokines such as IL-10 and TGF-β[79]. In contrast Treg can suppress the effector immune response by cell-cell contact or by release of immune suppressive factors[80].

However, besides of being responsible for the suppressive effect of regulatory T cells, the unresponsiveness of the immune system in patients with cancer has been attributed to the secretion of immunosuppressive cytokines by the tumor itself. IL-10 is an anti-inflammatory cytokine that participates in the regulation of the immune response at several levels. IL-10 inhibits cytokine production of activated T and NK cells and of monocytes/macrophages and it blocks the antigen presenting activity of macrophages and dendritic cells, along with many other immunological functions. High IL-10 production was found to be associated with the immunosuppressive activity frequently observed in tumor-bearing hosts, and it was speculated that overproduction of IL-10 could be responsible of tumor cell evasion of immune responses[81]. TGF-β is another cytokine that inhibits activation, proliferation, and activity of lymphocytes[82]. High levels of this immunosuppressive cytokine are often found in several malignancies and have associated with poor prognosis and lack of response to immunotherapy[83]. Recent evidence indicates that TGF-β acts on cytotoxic T lymphocytes (CTL) to specifically inhibit the expression of different cytolytic gene products; namely perforin, granzyme A, granzyme B, Fas ligand, and IFN-γ, which are collectively responsible for CTL-mediated tumor cytotoxicity[84]. Consistently, blockade of TGF-β signaling allows the generation of a potent antitumor immune response[85].

Natural regulatory T cells are found at a higher frequency in peripheral blood of cancer patients and may induce tolerance at the tumor microenvironment, facilitating metastatic spread of cancer cells[86]. When Treg were depleted

in mice, transplantable tumors were efficiently rejected by the host immune system[87]. Interestingly, Dannull and colleagues recently demonstrated that vaccine-mediated antitumor immunity can be significantly enhanced following depletion of regulatory T cells[88]. In addition, administration of anti-CD25 monoclonal antibody and/or anti-CTLA-4 for a limited time period also provoked effective tumor-specific immunity against syngeneic tumor cells[89].

It has been postulated that an imbalance in Th1/Th2 cytokine production may also be responsible for the development of cancer, with a shift toward a Th2 response and induction of immunosuppressive cytokines including IL-10, IL-4 and IL-6[90]. Cyclophosphamide (Cy) is an alkylating agent widely used in cancer chemotherapy. It has bimodal effect on the immune system, depending of the dose and schedule of administration[91]. We have demonstrated that a single low-dose of Cy induced a Th2/Th1 shift in the cytokine profile of syngeneic lymphoma-bearing rats, which may be responsible of its antimetastatic effect. Such a treatment reduced the splenic production of IL-10, TGF-β and nitric oxide, restoring the lymphoproliferative capacity[92-94]. Cyclophosphamide has been demonstrated to have selective toxicity to suppressor T cells[95] and, more recently, to CD4+CD25+ regulatory T cells[96], thus promoting tumor growth. Currently, we have preliminary evidence in an experimental lymphoma model supporting the role of Cy-mediated immunopotentiation through a mechanism of selective toxicity against CD4+CD25+ T cells (unpublished results).

T regulatory cells have been proposed as key regulators of antitumor immune responses and their induction is considered a mechanism of tumor-immune escape. The idea that removal and/or inhibition of CD4+CD25+ regulatory T cells or inducible Tr1 cells can abrogate immunological unresponsiveness to syngeneic tumors, has established a novel strategy of evoking tumor immunity that would boost other cancer immunotherapy strategies.

Alterations in Signal Transduction Molecules

Patients in advanced stages of cancer and mice with large transplantable tumors have a compromised systemic immune response with highly decreased delayed-type hypersensitivity (DTH) responses. In this regard, NK and T cells often exhibit alterations in their proliferative and cytotoxic capacities as well as in cytokine secretion[97]. Recent observations showing alterations in signal transduction molecules in T and NK cells from tumor-bearing mice and cancer patients provide a molecular basis to better understand this immune dysfunction. The original observation in mice bearing a mouse colon carcinoma[98] showed an altered

pattern of protein tyrosinase phosphorylation, a reduction of the protein tyrosine kinases p56lck and p59fyn, and a decreased CD3-ζ chain. These findings have subsequently been confirmed and extended to a variety of human tumors, including renal, colorectal, ovarian, liver, gastric, pancreatic, and cervical carcinomas and melanomas[99].

Of particular interest is the correlation observed between CD3-ζ expression and the disease stage in cancer patients[100]. The TCR-associated CD3-ζ chain is responsible for transduction of signals delivered through the CD3-TCR complex and, therefore, its expression is important for activation of T cells[101]. By quantitative flow cytometry analysis, decreased or absent expression of the CD3-ζ chain in CD4+ or CD8+ T cells as well as in NK cells was demonstrated in patients with malignancies. A similar finding was observed in tumor-infiltrating lymphocytes (TILs). These observations have suggested that the tumor microenvironment has negative effects on immune cells, and that the failure of the host to eliminate tumors might be a consequence of the impaired function of T cells, including the inability of these cells to signal normally upon TCR engagement[102]. Interestingly, evidence has been provided for tumor-induced degradation of the CD3-ζ chain. It has been demonstrated that tumor cells can induce activation of intracellular peptidases in T lymphocytes, and that this tumor-induced enzymatic degradation is responsible for decreased or absent expression of signal transducing molecules, including the CD3-ζ chain in activated T cells [103]. On the other hand, a computer-assisted analysis of the amino acid sequence of the CD3-ζ chain has revealed the presence of sites sensitive to cleavage by caspases, and it has been demonstrated that the CD3-ζ chain is a substrate for proteolysis mediated by intracellular caspases[104]. These results indicate that tumor-induced activation of caspases seems to be responsible for low or absent expression of CD3-ζ and perhaps other signaling molecules in T cells. Finally, other mechanisms, including free oxygen radical generation within the tumor microenvironment, have been proposed to account for decreased CD3-ζ expression in cancer[105].

Although the mechanisms involved in tumor-induced down-regulation of CD3-ζ expression in T cells are under intense scrutiny, there are evidences indicating that direct contact with tumor or tumor-derived factors might participate in degradation of CD3-ζ and perhaps other signaling molecules. In order to prevent signaling defects of T cells in tumor-bearing hosts, it will be necessary to unravel the mechanism(s) involved in tumor-T cell interactions. These studies are of critical importance for the design of future immunotherapy strategies.

Novel Mechanisms of Tumor-immune Escape

Indoleamine 2,3 Deoxygenase (IDO)

An additional mechanism that mediates tumor-induced immunosuppression includes the overexpression of the IFN-γ-inducible enzyme, indoleamine 2,3-dioxygenase (IDO). This enzyme is responsible for the catabolism of tryptophan (the amino acid that is critical for T cell function) and a competitive inhibitor of IDO, 1-methyl-tryptophan (1-MT), can prevent tumor-immune escape[106]. Uyttenhove and colleagues demonstrated that expression of IDO by immunogenic mouse tumor cells prevents their rejection by preimmunized mice[106]. This effect was accompanied by a lack of accumulation of specific T cells at the tumor site and could be partly reverted by systemic administration of 1-methyl-tryptophan, an inhibitor of IDO. These results suggested that the efficacy of immunotherapy strategies might be improved by concomitant administration of an IDO inhibitor.

The STAT3 Pathway

Signal transducer and activator of transcription (STAT)-family proteins are latent cytoplasmic transcription factors that convey signals from cytokine and growth factor receptors to the nucleus. It has been demonstrated that STAT3 signaling in tumor cells suppresses both innate and adaptive anti-tumor immune responses, further enhancing tumor progression[107, 108]. In addition a critical role has been demonstrated for STAT3 in the generation of immune cell tolerance[109]. Proof-of-concept studies in cell culture and animal models have validated STAT3 protein as a promising molecular target for novel cancer therapies, including small-molecule inhibitors of STAT3 signaling.

The PD-1/PD-L1 System and other negative costimulatory signals

PD-1 is an inducible receptor expressed on CD4+ and CD8+ T cells following activation. The expression of one ligand for PD-1, designated PD-L1 or B7-H1, on tumor cells of a variety of histological origins has suggested a potential mechanism for tumor-immune escape[110]. It has been demonstrated that cancer cell-associated PD-L1 increases apoptosis of antigen-specific human T cell clones *in vitro* and *in vivo*[111]. Thus, blockade of PD-L1 (B7-H1) might also be a complementary therapy to augment tumor-specific T cell responses[112].

In addition, Kryczek and colleagues recently demonstrated that B7-H4, a recently identified B7 family molecule, identifies a novel suppressive macrophage population in human ovarian carcinoma and that depletion of B7-H4+ tumor macrophages may represent a novel strategy to enhance T-cell mediated immunity in cancer[113].

Galectin-1: The «Sweet Escape»

Galectins are animal lectins defined by shared consensus amino acid sequences and affinity for β-galactose-containing oligosaccharides[114]. Recent evidence indicates that galectin-1, a proto-type member of this family, has the potential to inhibit T cell effector functions by regulating T cell apoptosis and the Th1/ Th2 cytokine balance[115].

Expression of galectin-1, a prototype member of the galectin family, has been well documented in many different tumor types including astrocytoma, melanoma and prostate, thyroid, colon, bladder and ovary carcinomas[116]. Interestingly, in most cases such expression correlates with the aggressiveness of these tumors and the acquisition of metastatic phenotype.

The immunoregulatory effects of galectin-1 and the correlation between galectin-1 expression in cancer cells and the aggressiveness of these tumors, prompted us to investigate the role of galectin-1 in tumor-immune escape. We hypothesized that tumor cells may impair T cell effector functions through secretion of galectin-1 and that this mechanism may contribute in tilting the balance toward an immunosuppressive environment at the tumor site. By a combination of *in vitro* and *in vivo* experiments using knockdown transfectants, we established a link between galectin-1-mediated immunoregulation and its contribution to tumor-immune escape[117]. Blockade of the inhibitory effects of galectin-1 within tumor tissue resulted in reduced tumor mass (an effect which required intact CD4+ and CD8+ T cell responses) and stimulated the generation of a tumor-specific T-cell response *in vivo.* Our observations support the idea that galectin-1 may contribute to immune privilege of tumors by modulating survival or polarization of effector T cells and suggest a potential molecular target for manipulation of T-cell apoptosis with potential implications in immunotherapy. Recently, Le and collagues confirmed our findings demonstrating a link between tumor hypoxia and galectin-1 expression in head and neck squamous carcinoma cells[118]. Interestingly, the authors found a negative correlation between galectin-1 expression and presence of CD3+ T cells in tissue sections[118].

Challenges for the future will be to employ potent and selective small inhibitors of galectin-1 and, in fact, molecules with such properties have already been developed for galectin-1 or other galectins[119-121]. Furthermore, galectin-1 expression can be modulated by chemotherapeutic and anti-metastatic agents including low doses of Cy[122]. A current challenge is the design of more specific and potent galectin-1 inhibitors for therapeutic purposes with no or minimal adverse effects. Although galectin-1 still remains elusive in terms of our

understanding of its multifunctional modes of action, we are moving closer to unraveling this mystery at a molecular level and to design new therapeutic approaches directed toward modulating its activities. Other galectins, including galectin-2, galectin-3 and galectin-9 promote immune cell apoptosis and modulate cytokine production *in vitro*, suggesting their potential role in tumor-immune escape[114].

Other Mechanisms of Tumor-immune Escape

Other tumor-derived suppressive factors that are able to impair T-cell function systemically include prostaglandin E2 (PGE2), prostate-specific antigen (PSA), sialomucins (MUC1) and RCAS1, a receptor-binding cancer antigen that induces cell cycle arrest and apoptosis of target cells[123].

IMMUNE STIMULATION

This phenomenon was first described by Prehn and Lappé[124] who found that intermediate levels of antitumor immune reactivity might often stimulate rather than inhibit the development of certain tumor types[125,126]. Shortly after, Stewart and colleagues published several data on human breast cancer[127,128] which agreed with Prehn's postulation. They found that certain types of human mammary adenocarcinomas, but not other cancer types, are favored in its growth properties by a mild immune response, at least at the first stages of tumor evolution.

Several molecules secreted by, or expressed on the surface of effector immune cells may be involved in the phenomenon of immunostimulation. Similar to the cross-talk between different immune cell types, cytokines produced by T cells and the stroma can act as paracrine growth factors stimulating tumor growth and progression. Different experimental evidences support this reasoning. IL-10 and its receptor (IL-10R) can stimulate the growth of metastatic cells of a B-cell lymphoma, establishing an autocrine loop[129]. Also, increased production of IL-10, induced by CEA, enhances liver metastasis in human colorectal carcinomas[130]. Moreover, IL-4 stimulates survival of thyroid cancer[131], head and neck squamous cell carcinoma cell lines[132] and B-CLL cells[133]. In addition, other soluble factors released by immune cells and involved in angiogenesis, including vascular endothelial growth factor (VEGF)[134], may also play an important role in immunostimulation. Interestingly, a positive association has been observed between serum levels of VEGF and/or other angiogenic molecules and cancer progression[135]. In contrast, other studies report a critical role for VEGF in the suppression of dendritic cell differentiation and maturation[136].

Taking into account the aforementioned experimental evidence, the implementation of cancer immunotherapy strategies should be preceded by a careful examination of the tumor immunogenicity and the phenomena of tumor escape and immunostimulation. In this regard, recent evidence indicates that inflammatory cells can sculpt the immunogenicity of different tumor types. This phenomenon led to the identification by Schreiber and colleagues of the «*Cancer Immunoediting hypothesis*»[137]. This hypothesis proposes three different mechanisms (*«The Three Es of immunoediting: elimination, equilibrium and escape»),* responsible of eliminating tumors and sculpting the immunogenic phenotypes of tumors that eventually form in immunocompetent hosts[137].

CONCLUDING REMARKS AND PERSPECTIVES

Research over the past few years has contributed to increase enormously the available information about the interactions between tumor cells and the immune system. Currently we accept that tumors have smartly developed a number of evasion strategies which, individually or in combination, may enable these cells to thwart immune attack. The identification of the mechanisms employed by each tumor type will hopefully give the hints required to design novel therapeutic strategies aimed at destroying the tumor «fortress». Such therapeutic approaches should be aimed at overcoming several potential mechanisms that individual tumors may develop at different stages to overthrow or avoid innate and adaptive immunity.

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