Interaction Between the Immune System and Melanoma

Norma E. Herrera-Gonzalez

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54939

1. Introduction

No scientist can escape being fascinated by the complexity of the interplay between innate and adaptive immunity in order to monitor tissue homeostasis, to protect against infectious pathogens and to eliminate damaged cells. One of the most important tasks of the Immune System is to distinguish between “self” and “foreign”. Cancer is formed of cells that suffer several mutations but form still part of the individual body. All types of cancer are caused by the progressive growth of the progeny of a single transformed cell. Curing this disease requires that all the malignant cells have to be removed or destroyed without damaging the patient. To achieve this task the own body has to distinguish between the cells of “the tumor” and their “other cellular” counterparts.

The initial thought that the immune system has indeed a protective role in tumor development has changed enormously in the past years. In the last few years, it has been experimentally shown that the Immune system itself can facilitate tumor development and progression and functions to promote or select tumor variants with reduced immunogenicity.

Decades of intensive investigation have made it increasingly clear that the interplay between immunity and cancer is complex. Next, there is some information about how the immunosurveillance hypothesis has been confronted through all these years since the early 70’s [1].

2. Immunosurveillance hypothesis confrontation

The first approach to the interplay between cancer and the Immune system, was done by Stutman. He used nude mice and methylcolantreno (MCA) to produce tumors in both, nude and their wild type counterparts [2]. The conclusion was that nude mice did not form more chemically induced tumors compared with the controls, nor did they show a shortened tumor latency period after carcinogen injection. The similarity between immune-competent and nude mice was consistent in subsequent experiments that employed mice of different ages, different doses of carcinogen, etc. These findings were also supported by Rygaard who showed no differences in tumor formation in a study of 10 800 nude mice over a period of 5-7 months [3]. It is now clear that nude mice are not completely immunocompromised since they have detectable populations of functional αβ T cell receptor bearing lymphocytes [4]. Furthermore, these studies were done before the discovery of NK cells, which are thymus independent and ∂γ T cells, a subset of lymphocytes which may develop extrathymically. Later on, experiments based also on models of MCA–induced tumor formation showed that mice lacking either the IFN¥ receptor or STAT1, the transcription factor essential for the signalling of IFN¥ receptor, were found to be 10-20 times more sensitive than wild-type mice to the MCA tumor formation [5]. In studies of mice lacking the TCRβ chain or the TCRγ chain, MCA treatment of either mice increased the incidence of fibrosarcomas as compared with controls, showing that both T cells subsets are critical for protecting in this particular model of tumor development.

Shankaran in 2001 used targeted mice that lack RAG-1 or RAG 2 (recombination activating gene). These enzymes are essential for the repair of double stranded DNA breaks and they are solely in the lymphoid compartment. All this means that RAG deficient mice fail to rearrange lymphocyte antigen receptors and lack of NKT, T and B cells. When these mice were injected with MCA, 26 of 26 RAG deficient mice developed sarcomas. In contrast, 5 of 20 wild type mice developed spontaneous neoplásia [6].

The previous experiments show clearly the participation of some components of the Immune System in order to avoid the formation of tumors. However, these results are specifically for the MCA model, where its carcinogenesis mechanism is different from all the other types of cancer. Even more, these findings were obtained in murine models. However, if cancer immunosurvellience exists in mice, does exists in humans?

3. Is this immune protection similar in humans?

Scientist turned back to look if immunodeficient or immunosuppressed patients and individuals with primary immunodeficiencies had greater incidences of cancer. Early studies of transplanted patients who were subjected to immunosuppressive agents actually showed higher relative risk for cancer development. The answer was affirmative but, most of this higher risk was due to the development of tumors that were of viral origin. For example, non-Hodgking's lymphoma, Kaposi's sarcoma and carcinomas of the genitourinary and anogenital areas where all of these are linked to infection with Epstein-Barr virus, human herpesvirus 8 and HPV. A review of data from thirty years of Transplant Tumor Registry found that transplanted patients showed two times more relative risk to develop melanoma over the general population [7].

It has also been reported that there is evidence showing a positive correlation between the presence of lymphocytes in a tumor (TILs) and an increase in the patient's survival. Sorting more than 500 patients with primary melanoma who had more than 7 years of follow up, showed that patients in the brisk tumor infiltrating lymphocytes response survived two times longer than patients absent of TILs in their tumors. Later on, researchers reported the same prognostic correlation when studied the presence of TIL's in melanomas that had metastasized to lymph nodes [8]. The previous studies show that the presence of lymphocytes in the tumor may increase life survival. However, these patients after some time still die due to the progression and migration of melanoma to other vital organs, therefore, the Immune system do not resolve the tumor.

4. Cancer occur in immunocompetent individuals

The protective role of the Immune System is not completely effective to eliminate tumors. In order to explain this failure, it has been proposed that three stages exist in humans during this process: a) Elimination, where the Immune system is capable of destroying neoplastic cells by the innate immunity effectors; b) Equilibrium, specific effectors that eliminate the tumor are induced but, at the same time, selective pressure is generated on tumor cells, as a result mutated neoplastic variants occur; c) Escape, the tumor variants that survive become more resistant to identification and/or elimination by the Immune System and consequently the tumor grows. This process has been called "Cancer immunoediting" to describe more accurately the dual host-protecting and tumor sculpting actions of the Immune System shaping a neoplastic disease.

This hypothesis explains the observation that tumors often become clinically evident years after their molecular origin. At the end of this equilibrium between the immune system and tumor growth, the immune response allows for the outgrowth of a subpopulation of tumor cells. Factors contributing early neoplastic cells to survive, grow and invade are released by the immune system itself.

The major tumor type that occurs with increased frequency in immunodeficient individuals are virus-associated tumors, so immune surveillance is critical for control of this type of tumors, while the immune system does not normally respond to the neoantigens derived from the multiple genetic alterations in spontaneously arising tumors. Studies in mice have also revealed that when these are induced for immunodeficiency, show a high susceptibility to virally induced tumors and a greater tendency to develop spontaneous lymphomas compared with immunocompetent mice [8].

5. Does the immune system control each type of cancer?

All types of cancer share several characteristics, some of the most studied are uncontrolled growth, resistance to apoptosis, motility, proteolytic capacity and adhesion. However, each type of cancer also may have completely different etiologies (physical, chemical and/or biological), cell type of origin, mechanism of transformation, anatomical localization, histolo-pathological features, mortality rate, tumor markers, etc.

6. Melanoma: Its origins and incidence

Melanoma, an aggressive malignancy arising from melanocytes, is one of the most lethal of all skin cancers due to its great capacity to produce metastasis and its high chemoresistance [9]. It causes approximately 80% of skin cancer related deaths worldwide and is considered to be the most common mainly fatal malignancy of young adults mainly in Europe, Australia, New Zealand and United States of America where many people is red-haired with blue or green eyes. This disease is predominantly of populations with lighter skin color (Fitzpatrick I, II III) and the incidence is around tenfold lower in populations with darker skin color (Fitzpatrick IV, V and IV). The list of risk factors in developing melanoma is long but the main risk factors are: blond or red hair, numerous freckles and tendency to burn and tan poorly.

Over the past 55 years, the incidence of melanoma in most developed countries has risen faster than any other cancer type. Incidence rose dramatically between 1950 and 2000 (approximately 10% every year) particularly in some countries where caucasian population are. Melanoma provides one of the best examples of how genetics and environment interact in the pathogenesis of cancer. Incidence is strongly related to race and geographic location [10].

Primary melanoma progresses generally through two phases: a) the radial growth phase, is the horizontal spreading of transformed melanocytic cells inside the epidermis and small groups of invasive cells limited to the upper part of the dermis, and b) the vertical growth phase, is the invasion of melanoma cells into the deeper dermis and subcutaneous tissues.

7. Primary melanoma development

Most melanomas (with the exception of acral melanomas) are caused by exposure to UVB. This radiation can damage melanocyte DNA, causing hundreds of mutations including in genes controlling cell cycle progression and signal transduction pathways. UVB radiation may induce pyrimidine dimers, primarily thymidine di-nucleotides. So, lesions not repaired by nucleotide excision repair can lead to GC---→AT transitions, leaving a mutagenic mark. The epidemiological evidence for a role of solar exposure in melanoma (especially in Caucasian populations) is very strong. Some studies have suggested that total accumulated exposure to sun is a very important factor whereas long-term occupational exposure may be protective. UV irradiation induces also morphological and functional alterations in epidermal Langerhans' cell. The involvement of TNFα in the emigration of Langerhans' cells from UV-exposed skin into the regional lymph nodes has been reported. However, it may exist other non-mutagenic mechanisms involved such as immune suppression, UV induction of melanocyte growth factors by damaged keratinocytes, or UV production of mutagenic oxidative radicals during inflammation. UVB, can also weaken both the innate and adaptive immune systems by promoting the release of IL-10 by Langerhans cells [11] and by favoring the infiltration of IFNγ-producing macrophages. These cytokines possess activities as immunosuppressive and pro-angiogenic respectively [12].

It is important to mention that there are different molecular subtypes of melanomat, which may show a totally different antigenic profile depending on the number and quality of genetic alterations. These subtypes are superficial spreading, lentigo maligna, nodular and acral melanoma. The phenotype of malignancy is a reflexion of genetic events altering the RNA and protein expression patterns of normal cells. It has been observed that in general all metastatic melanoma generally displays resistance to treatment with antineoplastic drugs. Additionally, these therapies are severely toxic to the patients and their side effects include fatigue, malaise and a higher risk for non-melanoma cancers [13]. Superficial spreading melanoma is the most common melanoma in the first world countries and can occur at any site and at any age. About 80% of superficial spreading melanoma occur *de novo.* The classic lesions show variation in pigmentation and pagetoid spread of melanoma cell in epidermis [14]. Superficial spreading melanomas usually occur in younger patients than nodular or lentigo malign melanomas. They typically involve intermittently sun-exposed anatomical sites such as the trunk, back and extremities.

8. From melanocyte to melanoma

The epidermis contains keratinocytes and two types of dendritic cells, a) Langerhans' cells which are professional presenting cells playing an essential role in cellular response mainly to microorganisms but apparently also to tumor antigens; and b) melanocytes. Langerhans' cells are located in the suprabasal layer of the epidermis, whereas melanocytes are located amongst the basal layer of the epidermis, hair bulb, eyes, ears and meninges. Melanin pigment is produced by melanocytes in their specific cytoplasmic organelles called melanosomes. Melanin pigment synthesis by each melanocyte is transferred to an average of 36 keratinocytes. The transferred melanin forms a cap at the top of nucleous of mitotically active basal cells and prevent the UV damaging effects on nucleus.

Melanoma arises through a complex process of cellular mutations and a loss of keratinocyte control over growth and differentiation [15]. As malignant melanoma progresses, it develops through interaction between dysfunctional melanocytes and the tumor microenvironment. This progression is accompanied with changes in both keratinocytes and local adhesion molecules allowing for the formation of nevocyte nests at the dermal-epidermal junction [16].

As mentioned earlier, the progression from healthy melanocyte to melanoma occurs through both mutations within the tumor and through alterations of the cellular environment around the melanoma. In the skin, tissue homeostasis is critical in cellular regulation as well as immune control, and melanoma disrupts this regulation through multiple processes. Differentially expressed genes that are mutated during this multistep process conduct towards the transformation of melanocytes to melanoma. A great number of genes and proteins have been reported to play an essential role in this transformation. Some of these are listed: BRAF, cKIT, PTEN, p16,p53, cyclin1, ARF, K-RAS. Differentially expressed genes between melanocytes and melanoma cells impact in the expression of somehow "different" surface membrane expression of certain proteins (TAAs) that may play an important role for immune recognition and their elimination. In addition to mutation-derived tumor associate antigens (TAAs), melanoma is known to express normal, melanocytic lineage-related antigens (gp100, MART-1) that are not recognized by the immune system owing to some form of tolerance to self antigens [17].

9. The immune system in health

The immune system is highly elaborated, with a diversity of stop and go mechanisms essential to accomplish different tasks. It is composed of many cell types and mediators that interact with non-immune cells in a complex and dynamic way to ensure protection against foreign pathogens but at the same time maintaining tolerance to self-antigens (such as tumor cells in a way). The immune system has two completely different compartments –adaptive and innate, differing these on antigen specificity, timing of activation and cellular composition. These cells have communication networks that allow rapid responses to tissue injury. Innate immune cells, such as dendritic cells (DC) natural killer (NK) cells, macrophages, neutrophils, basophils, eosinophils and mast cells are the first line of defense against foreign antigens and damaged cells.

10. The innate immune system and inflammation

When tissue homeostasis is broken, sentinel macrophages, DC and mast cells release cytokines, chemokines, matrix remodeling proteases (MMP) and reactive oxygen species (ROS), inducing migration and infiltration of more leukocytes into damaged tissue, this process is called inflammation. Although inflammation is important in tissue repair and erradication of harmful pathogens, unresolved, chronic inflammation that happens when the offending agent is not removed, can be detrimental to the host. Immune cells infiltration in the absence of pathogens is also characteristic of cancer, and these cells can definitely influence the growth and progression of this disease. The destructive cycles that are initiated inside the tissues by failure to commit either arm of the immune system, can result in excessive tissue remodeling, loss of tissue architecture due to tissue destruction and finally DNA and protein alterations due to oxidative stress.

So, one might question, why does inflammation potentiate cancer development rather than protect against it. Neoplastic microenvironments enhance chronic pro-tumorigenic inflammatory state [18]. The inflammatory microenvironment of neoplastic tissues is characterized by the presence of host leukocytes both in the supporting stroma and among the tumor cells, with macrophages, dendritic cells, mast cells, and T cells being differentially distributed [19].

Macrophages represent up to 50% of the tumor mass and are key cells in chronic inflammation. These cells constitute an extremely heterogeneous population, which differentiate into distinct macrophages types, identified as M1 (or classically activated) and M2 (or alternatively activated) [20]. These cells respond to microenvironment signals with polarized functional programs [21]. M1 type macrophages produce Th1 cytokines and predominate in earlier stages of the disease. In contrast, M2 type macrophages secrete factors which favor immunosuppression and tumor development, so they prevail in more advanced disease. Initially, these cytokines have regulatory roles in the tumor microenvironment through growth inhibition, but these functions are lost as tumors slowly progress to a state of immunosuppression. This is the case for IL-6 when is released during initial tumor formation by keratinocytes and macrophages inhibits tumor proliferation. However, in late stages of melanoma progression undergoes transition to stimulator [22].

Mutations and genetic polymorphisms in crucial genes that regulate cytokine function, metabolism and leukocyte survival have also been implicated as aetiological factors in chronic inflammation [23]. Population based studies reveal that individuals who are prone to chronic inflammatory diseases have an increased risk of cancer development [24].

11. The adaptive immune system and melanoma

Melanoma usually remains refractory to immunologic control even when these cells are relatively immunogenic compared to other cancer types. Being melanoma a disease generated by autologous cells, should be possible to instruct the organism to fight against it?

The adaptive immune system is composed of the antigen presenting cells (APC) that include dendritic cells (DC), the most effective APCs and CD4+ and CD8+ T cells. CD4+ T cells include both T helper and regulatory T cell (Treg) populations. In order to initiate an adaptive immune response, APC can activate T cells by efficiently processing exogenous as well as endogenous antigens and present them to T cells through the major histocompatibility complex (MHC). T cells recognize their targets by detecting peptide fragments derived from these foreign or damaged proteins. There are two types of MHC, class I and II. One of the most important features of both molecules is an outer extracellular domain that forms a long pocket in which peptide fragments are located. The most important differences between the two classes of MHC molecules are in the source of the peptides they contain and carry to the cell surface. CD8+ and CD4+ T cells interact with melanoma through contact with MHC class I and II on their cell surface, respectively.

12. Defects in antigen processing and presentation

Presentation of tumor-associated antigens (TAA) on MHC class I by APCs is a crucial step for the differentiation and expansion of CD8+ T cells against TAAs and the eventual destruction of tumor cells.

Melanoma cells have been observed to downregulate MHC class I expression, so preventing any T cell activation and tumor elimination [25]. This tumor has strategies to avoid CD8+ detection and activation. There is clinical evidence that support this statement, since patients with metastatic melanoma show detectable CD8+ T cells specific for melanoma antigens, however, the tumor is not eliminated [26]. Other mechanisms that have been described in this context in melanoma include downregulation of MHC class II antigens [27]. One example is the low expression of HLA-DM, a nonclassical Class II MHC responsible for peptide loading into MHC class II and the removal of the invariant chain li peptide (CLIP) [28]. Melanoma cells also differentially express acidic cathepsins which process endogenous and exogenous antigens in endolysosomal compartments. Their limited activity results in poor Ag processing and the generation of useless antigenic determinants, which are unable of stimulating T cells [29]. Melanoma also has been found to lack the IFN∂-inducible lysosomal thiol reductase (GILT), essential enzyme for the functional reduction of cysteinylated or oxidized proteins and peptides. The presence of GILT in endolysosomal compartments enhances the acidic cathepsin processing of TAAs and MHC class II components, and the functional processing of cysteinsylated or oxidized peptides for an excellent CD4+ T cell activation. All these defects result in the presentation of a range of nonfunctional peptides which fail to stimulate interacting CD4+ cells, limiting the effects of CD8+ cytotoxic responses.

13. Failure in costimulatory signals

After the Ag processing and the loading of tumor derived peptides into the MHC class II groove, this complex is translocated to the cell surface for presentation to T cells. CD4+ T cells recognize functional class II complexes with antigenic peptides and tight junction binding occurs between the TCR and the class II/Ag complex. CD4+ molecules on T cells then bind to a different site on the MHC class II molecule and T cells receive their first stimulation signal [30]. A second signal is required for activation of the T cell. If the T cells receive a stimulatory signal from the tumor in the form of CD80/CD86 (B7-1, B7-2) binding to T cell expressed CD28, then T cells become activated and may give an anti-tumor response. The most studied immune checkpoint molecule in activated T cell is CTLA-4. It is a high affinity receptor for the ligand B7 expressed by APCs. Ligation is thought to deliver an inhibitory signal, in contrast to CD28. CTLA-4 blockade is thought to act primarily by increasing effector T-cell function.

Coestimulatory molecules are often modified on melanoma cells inhibiting T cell activation, since it has been shown to express high levels of CTLA-4 [31]. Tumors exploit this process, functionally silencing CD4+ T cell activation and shifting the environment to a T regs setting.

Coinhibitory signaling pathway mediated by PD-1 ligand is expressed by activated T cells. It is considered a marker of T cell exhaustion, as engagements by its ligands PDL-1 (B7-H1] and PDL-2 results in T cell inhibition and apoptosis. Of particular interest is the finding that tumor-infiltrating or peri-tumoral lymphocytes in melanoma patients express PD-1 and have impaired effector function [32]. Study shows that melanoma expresses high levels of the ligand for PD-1, PD-1L which during TCR-MHC interaction sends a death signal to both CD4+ and CD8+ T cells causing them to undergo apoptosis [33]. A number of different subtypes of cancer, as well as lymphocytes and APCs in the tumor environment have also been shown to express ligands for PD-1 which may act to suppress PD-1 expressing T cells [34].

14. Immunosupressive tumor microenvironment and cytokines

An upregulation of immunossupressive cytokines such as IL-6, IL-10, TNFα, TGFβ and VEGF is promoted by melanoma microenvironment. The release of these cytokines attracts immunossupressive cells: myelo-derived suppressive cells, tumor associated macrophages, or tolerogenic DCs in the tumor microenvironment.

Polak reported that melanoma cells, melanoma recruited myeloid suppressor cells and Tregs actively secrete IL-10 to induce tolerized T cells and DC. [35] They showed tolerogenic DCs and Tregs present in all stages of disease progression. However, the expression of IL-10 and IDO increased with melanoma progression with the highest production in positive lymph nodes. Their work also suggests that TGFβ2 renders DCs tolerogenic, although for the case of lymph nodes, IDO and TGFβ1 have a higher impact. This mechanism of tumor-associated immunosuppression probably inhibits the immune response to the tumor and may explain the discrepancy between the induction of systemic immunity by anti-melanoma vaccines and their poor impact in the clinic.

It has also been reported that PGE2 is produced by melanoma associated fibroblasts and immature myeloid cells. Luft found that immature monocytes-derived DC that encountered pro-inflammatory cytokines in the presence of PGE2 acquired migratory capacity, but secreted low levels of cytokines. This suggest that not all mature stages of DCs are destined to migrate to lymphoid organs and the sequence in which stimuli are encountered significantly affects which functions are expressed [36]. Additionally, PGE2 inhibits NK T cells activity, once more resulting in changes in the tumor microenvironment towards immunossupression [37]. COX-2 is a multifunctional enzyme that is involved in prostaglandin biosynthesis, and it is upregulated in neoplastic tissues [38]. In several human epithelial cancers, expression of COX-2 correlates with poor prognosis. The crucial molecules that mediate these effects are not yet known, though they might include the PGE2 receptor EP2 subtype (PTGER2) [39].

15. Upregulation of regulatory T cells

Regulatory Tregs comprise 5% to 10% of the total peripheral CD4+T cell population. The main role of Tregs is to inhibit cytotoxic T cell response against self-antigens and maintain systemic tolerance to self-antigens. Tregs constitutively express CD25 (IL-2 receptor α chain) on their cell surface and suppresses CD4+ and CD8+ effector T cells through the release of immunosuppressive molecules, consumption of IL-2 and direct cell to cell contact [40]. The shift to tumor progression results in part, from the alteration in the type and characteristics of TILs within the tumor. These changes include the enhancement of CD4+CD25+FoxP3+Tregs, since in melanoma, particularly in advanced disease states, Tregs are the primary infiltrating lymphocyte where they inhibit all antitumor activity through direct contact inhibition, and the release of high levels of IL-10 [41]. Once activated these cells, are anergic and are able to block the proliferation of effector cells. A study suggests that high serum concentrations of Tregs are associated with poor prognosis, poor treatment responses and an increased risk of recurrence [42]. Human studies depleting Tregs prior to adoptive cell transfer (ACT) improved the effectiveness of treatment. Patients challenged with melanoma antigen peptides, MelanA/MART-1 and gp-100, developed significant induction of peptide specific CD8+ T cells in 90% of them. This study shows that depletion of Tregs in vivo, results in enhanced immune functions and substantial development of antigen-specific CD8+ T cells in vaccinated individuals [43]. Tregs may be harmful for individuals fighting tumors, since under these circumstances the immune system needs maximal activation, but as common tumor antigens are largely self-antigens, suppression of potentially self-reactive T cells by Tregs may be counterproductive in this specific process.

16. Melanoma, an abberrant HLA-G expression

It was previously mentioned that loss or down-regulated of classical MHC class I on melanoma cells is one of the most important mechanisms enabling tumor cells to escape from immune anti-tumor responses. Very similar to tumor cells, fetal cells do not express classical MHC class I molecules, instead these cells express the non-classical HLA-G molecules. The multiple immune suppressive properties of this molecule strongly imply that HLA-G is part of a tolerogenic system. HLA-G exerts exclusively immunossupressive functions, which impair both the innate as well as the acquired immunity by multiple mechanisms. Firstly, the immune effector functions of cytotoxic T lymphocytes and NK cells are inhibited in the case of target cells expressing HLA-G [44]. Secondly, APC expressing HLA-G, inhibit the proliferation of CD4+ T cells, induce CD4+ T cell anergy and cause the differentiation of CD4+ T cells into regulatory cells (Tregs), which as we mentioned previously these cells possess the competence to inhibit the effector function of other T cells. Thirdly, the binding of HLA-G to DC results in disruption of DC maturation, in inhibition of antigen presentation, and in induction of immunosuppressive T cells. Soluble HLA-G molecules fulfill the same tasks as membrane-bound ones. Beyond it sHLA-G molecules are able to inhibit cell cycle progression in T lymphocytes and to mediate the induction of apoptosis on activated T and NK cells [45].

HLA-G is unique in its heterogeneous and unusual molecular structure. Contrary to classical MHC class I molecules HLA-G displays with 23 different alleles a limited polymorphism, in which all amino acid exchanges are located outside the peptide binding groove. Therefore, the diversity of peptides bound by HLA-G is very restricted relatively to classical class I molecules [46]. Thus, it is very unlikely, that this molecule represents a target molecule for the T cell receptor inducing the anti-tumor response. The lymphocyte differentiation marker CD8+ is a classical receptor for MHC class I and as a co-receptor with the TCR during the recognition of peptides being presented by MHC class I. Both, HLA-G and classical HLA class I molecules bind with the same affinity to CD8+. The engagement of sHLA-G molecules with CD8+ results in the induction of apoptosis via the Fas/FasL pathway.

17. Immunotherapy in melanoma: Disappointing results

Recent progress toward an understanding of the interactions between the host's immune system and melanoma has led to the realization that tumor cells have devised many strategies to evade the immune attack. Evasion mechanisms can either be pre-existing, arise through outgrowth of escape mutants or take place during tumor-sculpting actions by the immune system as was proposed in the "Cancer Immunoediting" hypothesis [47]. One of these strategies of tumor-immune escape is represented by the acquistion of FasL expression that may enable cancer cells to deliver death signals to activate Fas-positive T lymphocytes [48]. However, despite all the data accumulated in the support of the FasL counterattack hypothesis, there are many studies in contradiction showing that FasL can also have proinflammatory effects in some contexts [49]. To explain these conflicting findings, it is proposed that the maintenance of immune privileged in tumors depend not only on FasL itsel but also on the production of massive immunosuppressive factors (previously mentioned). The final outcome of an effective antitumor response is determined by a delicate interplay among activating and inhibitory regulatory pathways and the removal of inhibitory signals may be very useful in addition to other therapeutic approaches.

Depending on the subtype of melanoma, patients with metastatic melanoma have a median survival of 8 months and 1 year survival rates of 10 to 15%. The two FDA approved treatments for melanoma are dacarbazine-based chemotherapy and IL-2 with objective response rates below 18% [50]. Several clinical trials in stages IIB-IV of cutaneous melanoma utilizing vaccination with multiple peptides derived from MART-1, gp-100, tyrosinase and MAGE had very limited success in those patients [51].

Nonspecific therapies including the use of monoclonal antibodies against CTLA-4 have leaded to some considerable responses, but this agent has low response rates (12% to 15%) in patients with advanced melanoma.

Active therapeutic immunization has been pursued in clinical trials using a host of tumor vaccines, but these have shown disappointing response rates [52]. The development of therapeutic cancer vaccines is very complex, and it has been learned that stimulation and suppression are the two sides to the coin of manipulation of the immune system and the latter might be increased with the multiple use of a specific vaccine.

Cancer/testis (CT) antigens represent promising targets for immunotherapy because they are expressed in a wide variety of epithelial cancers but are restricted in their expression in normal adult tissues to cells in the testis which lack expression of MHC class I and are not susceptible to damage by T cells that recognize these products. Other members of the CT family of antigens include NY-ESO-1, LAGE-!, SSX1-5, CTp11, CT7, etc. Melanoma cells produce the CT antigens at different frequencies, so more studies are needed to solve if some of these marker proteins might be useful in further clinical studies. The NY-ESO antigen is expressed in 15 to 40% of highly prevalent tumors such as breast, lung, prostate and melanomas. To test the effectiveness of adoptive immunotherapy with genetically engineered cells that target the NY-ESO-1 antigen, phase I clinical trials of cancer vaccines were tried using peptides [53], recombinant vaccinia and fowlpox viruses encoding full-length NY-ESO-1 [54], or recombinant NY-ESO-1 protein [55], but have failed to demonstrate a clinical benefit in patients with advanced disease.

Adoptive cell therapy (ACT) using tumor reactive TILs following host lymphodepletion can lead to objective responsive rates of around 40% and durable responses in patients with refractory melanoma. This ACT used tumor antigen-specific lymphocytes that were initiated in vitro from single-cell enzymatic digests or small fragments of resected tumor specimens and expanded to large numbers before infusion [56]. However, this therapy requires sophisticated cell processing and *in vitro* lymphocyte culturing for long periods. These requirements have technical, regulatory, and logistic challenges that have limited the use of antigen specific TILs as a biological therapy.

Recently, Rosenberg and his group, reported responses of around 45% in patients with melanoma. In their adoptive immunotherapy trial to treat melanoma they used genetic engineering of T cells to express a CT antigen-specific TCR. They reported great variation in levels of T cell persistence between the patients and did not seem to be associated with clinical response to therapy. These findings indicate that treatments using TCRs directed against NY-ESO-1 are effective at mediating tumor regression in some patients (two of eleven patients demonstrated complete regression that persisted after one year) [57]. Given the small number of patients treated in their trial, it is difficult to evaluate the significance of these results.

18. Conclusion

The accumulated data indicate that the outcome of an immune response toward a tumor is largely determined by the type of immune response elicited. A tumor-directed immune response involving CD8+ T cells, CD4+ Th1 cells and NK appears to protect against tumor development and progression.

The future looks promising for melanoma immunotherapy, even with the disadvantages that researchers in the area and clinicians still face nowadays. Most vaccine trials have failed to show an important response rate or an impact on survival. The overall situation, is problematic and it has become clear that large tumors display a setting in which vaccination has a limited role; the most amenable clinical context to assay antitumor vaccination, would be patients in which the existence of micrometastasis, is highly probable. In this case, the immune system has fewer obstacles to surmount. Melanoma patients with satges II and III of the disease could benefit from such therapy.

Of note is the report by Robbins and coworkers in 2011, where they used an adoptive transfer of autologous T cells transduced with a TCR directed against NY-ESO-1, a cancer/testis antigen expressed in 40% of metastatic melanomas, but not in any normal adult tissues except the testis.

They reported two of 11 patients with complete regressions that persisted after one year. Potential strategies that may enhance responses including immunization with recombinant vaccines encoding the NY-ESO-1 antigen, elimination of host Tregs and cotransduction of TCR constructs with genes that encode cytokines such as IL-12 should be taken into account in the future.

Author details

Norma E. Herrera-Gonzalez

Molecular Oncology Laboratory, Posgraduate Section, Superior School of Medicine, IPN, Prol Salvador Diaz Miron y Carpio, Col Sto. Tomas, Mexico

References

[1] Kenneth MurphyPaul Travers, and Mark Walport, Inmunobiología de Janewey, 7th Ed. In: Capítulo 15: Manipulación de la respuesta inmunitaria. Garland Science, New York, NY, pp., 978-0-81534-123-9, 2008-672.

[2] Stutman, O. Tumor development after 3-methylcholanthrene in immunologically deficient athymicnude mice. Science (1974). , 1974, 183-534.

[3] Rygaard, J, & Povlsen, C. O. The mouse mutant nude does not develop spontaneous tumours. An argument against immunological surveillance. Acta Pathol. Microbiol. Scand. (B) Microbiol. Immunol. (1974). , 1974, 82-99.

[4] Maleckar, J. R, & Sherman, L. A. The composition of the T cell receptor repertoire in nude mice. J Immunol. (1987). Jun 1;, 138(11), 3873-6.

[5] Kaplan, D. H, Shankaran, V, Dighe, A. S, Stockert, E, Aguet, M, Old, L. J, & Schreiber, R. D. Demonstration of an interferon γ-dependent tumor surveillance system in immunocompetent mice. Proc. Natl. Acad. Sci. USA (1998). , 1998, 95-7556.

[6] Shankaran, V, Ikeda, H, Bruce, A. T, White, J. M, Swanson, P. E, Old, L. J, & Schreiber, R. D. IFN_ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature (2001). , 2001, 410-1107.

[7] Penn, I. Malignant melanoma in organ allograft recipients. Transplantation (1996). , 1996, 61-274.

[8] Mihm, M. C. Jr. Clemente, C.G. & Cascinalli, N. Tumor infiltrating lymphocytes in lymph node melanoma metastses: a histopathologic prognostic indicator and an expression of local immune response. Lab. Invest. (1996). , 1996, 74-43.

[9] Herrera- González, N. E. Aco Flores A.Y. El melanoma en México. Revista de Especialidades Médico-Quirúrgicas (2010). , 15(3), 161-164.

[10] Balch, C. M, Houghton, A. N, Sober, A. J, Soong, S-J, Atkins, M. B, & Thompson, J. F. Cutaneous Melanoma. 5th ed. St. Louis, MO: Quality Medical Publishing, (2009). 101576262766

[11] Beissert, S, & Schwarz, T. Ultraviolet-Induced Immunosuppression: Implications for Photocarcinogenesis. Cancer Treatment and Research, (2009). Part II, 109-121., 146

[12] Zaidi, M. R, Davis, S, Noonan, F. P, Graff-cherry, C, Hawley, T. S, Walker, R. L, Feigenbaum, L, Fuchs, E, Lyakh, L, Young, H. A, Hornyak, T. J, Arnheiter, H, Trinchieri, G, Meltzer, P. S, De Fabo, E. C, & Merlino, G. Interferon-γ links ultraviolet radiation to melanomagenesis in mice. Nature. (2011). Jan 27;, 469(7331), 548-53.

[13] Kamposioras, K, Pentheroudakis, G, Pectasides, D, & Pavlidis, N. Malignant melanoma of unknown primary site. To make the long story short. A systematic review of the literature. Crit Rev Oncol Hematol. (2010). May;, 78(2), 112-26.

[14] Bandarchi, B, Ma, L, Navab, R, Seth, A, & Rasty, G. From melanocyte to metastatic malignant melanoma. Dermatol. Res. Pract. (2010).

[15] Shirakata, Y. Regulation of epidermal keratinocytes by growth factors. J Dermatol Sci. (2010). Aug; , 59(2), 73-80.

[16] Hsu, M. Y, & Meier, F. Herlyn M: Melanoma development and progression: a conspiracy between tumor and host. Differentiation (2002). , 70, 522-536.

[17] Mortarini, R, Piris, A, Maurichi, A, Molla, A, Bersani, I, et al. Lack of terminally differentiated tumor-specific CD8+ T cells at tumor site in spite of antitumor immunity to self-antigens in human metastatic melanoma. Cancer Res (2003). , 2003, 63-2535.

[18] Zou, W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nature Rev. Cancer (2005). , 5, 263-274.

[19] Nakayama, T, Yao, L, & Tosato, G. Mast cell-derived angiopoietin- 1 plays a critical role in the growth of plasma cell tumors. J. Clin. Invest. (2004). , 114, 1317-1325.

[20] Martinez, F. O, Helming, L, & Gordon, S. Alternative activation of macrophages: An immunologic functional perspective. Annu. Rev. Immunol. (2009). , 27, 451-483.

[21] Gordon, S. Alternative activation of macrophages. Nat. Rev. Immunol. (2003). , 3, 23-35.

[22] Lu, C. and Kerbel RS: Interleukin-6 undergoes transition from paracrine growth inhibitor to autocrine stimulator during human melanoma progression. J Cell Biol (1993).

[23] Parsonnet, J. Molecular mechanisms for inflammation- promoted pathogenesis of cancer. The Sixteenth International Symposium of the Sapporo Cancer Seminar. Cancer Res.(1997). , 57, 3620-4.

[24] Balkwill, F, Charles, K. A, & Mantovani, A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell (2005). , 7, 211-217.

[25] Cabrera, C. M. The double role of the endoplasmic reticulum chaperone tapasin in peptide optimization of HLA class I molecules. Scand. J. Immunol. (2007). , 65, 487-93.

[26] Harlin, H, Kuna, T. V, Peterson, A. C, Meng, Y, & Gajewski, T. F. Tumor progression despite massive influx of activated CD8 (+) T cells in a patient with malignant melanoma ascites. Cancer Immunol. Immunother. (2006). , 55, 1185-1197.

[27] Goldstein, O. G, Hajiaghamohseni, L. M, Amria, S, Sundaram, K, Reddy, S. V, & Haque, A. Gamma-IFN-inducible-lysosomal thiol reductase modulates acidic proteases and HLA class II antigen processing in melanoma. Cancer Immunol Immunother. (2008). Oct;, 57(10), 1461-70.

[28] Norton, D. L, & Haque, A. Insights into the Role of GILT in HLA Class II Antigen Processing and Presentation by Melanoma. J Oncol (2009).

[29] Goldstein, O. G, Hajiaghamohseni, L. M, Amria, S, Sundaram, K, Reddy, S. V, & Haque, A. Gamma-IFN-inducible-lysosomal thiol reductase modulates acidic proteases and HLA class II antigen processing in melanoma. Cancer Immunol Immunother. (2008). Oct; , 57(10), 1461-70.

[30] Chambers, C. A. The expanding world of co-stimulation: the two-signal model revisited. Trends Immunol. (2001). , 22, 217-223.

[31] Weber J: Overcoming immunologic tolerance to melanoma: targeting CTLA-4 with ipilimumab (MDX-010). (2008). *Oncologist*.

[32] Ahmadzadeh, M, Johnson, L. A, Heemskerk, B, Wunderlich, J. R, Dudley, M. E, White, D. E, & Rosenberg, S. A. Tumour antigen-specific CD8 T cells infiltrating the tumour express high levels of PD-1 and are functionally impaired. Blood (2009). , 114, 1537-1544.

[33] Pilon-thomas, S, Mackay, A, Vohra, N, & Mule, J. J. (2010). Blockade of programmed death ligand 1 enhances the therapeutic efficacy of combination immunotherapy against melanoma. J Immunol 2010; , 184(7), 3442-9.

[34] Thompson, R. H, Gillett, M. D, Cheville, J. C, Lohse, C. M, Dong, H, Webster, W. S, Krejci, K. G, Lobo, J. R, Sengupta, S, Chen, L, Zincke, H, Blute, M. L, Strome, S. E, Leibovich, B. C, Kwon, E. D, & Costimulatory, B. H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci USA (2004). , 101, 17174-17179.

[35] Polak, M. E, Borthwick, N. J, Gabriel, F. G, Johnson, P, Higgins, B, Hurren, J, Mccormick, D, Jager, M. J, & Cree, I. A. Mechanisms of local immunosuppression in cutaneous melanoma. Br J Cancer (2007). , 96(12), 1879-87.

[36] Luft, T, Jefford, M, Luetjens, P, Toy, T, Hochrein, H, Masterman, K. A, Maliszewski, C, Shortman, K, Cebon, J, & Maraskovsky, E. Functionally distinct dendritic cell (DC) populations induced by physiologic stimuli: prostaglandin E2 regulates the migratory capacity of specific DC subsets. Blood (2002). , 100(4), 1362-72.

[37] Balsamo, M, Scordamaglia, F, Pietra, G, Manzini, C, Cantoni, C, Boitano, M, Queirolo, P, Vermi, W, Facchetti, F, Moretta, A, Moretta, L, Mingari, M. C, & Vitale, M. Melanoma-associated fibroblasts modulate NK cell phenotype and antitumor cytotoxicity. Proc Natl Acad Sci U S A (2009). , 106(49), 20847-52.

[38] Dannenberg AJ & Subbaramaiah KTargeting cyclooxygenase-2 in human neoplasia: rationale and promise. Cancer Cell (2003). , 4-431.

[39] Chang, S. H, Ai, Y, Breyer, R. M, Lane, T. F, & Hla, T. The prostaglandin E2 receptor EP2 is required for cyclooxygenase 2- mediated mammary hyperplasia. Cancer Res. (2005). , 65, 4496-4499.

[40] Camisaschi, C, Casati, C, Rini, F, Perego, M, De Filippo, A, Triebel, F. d. r, Parmiani, G, Belli, F, Rivoltini, L, & Castelli, C. LAG-3 Expression Defines a Subset of CD4+CD25highFoxp3+ Regulatory T Cells That Are Expanded at Tumor Sites. The Journal of Immunology (2010).

[41] Baumgartner, J, Wilson, C, Palmer, B, Richter, D, Banerjee, A, & Mccarter, M. Melanoma induces immunosuppression by up-regulating FOXP3 (+) regulatory T cells. J Surg Res (2007). , 141(1), 72-7.

[42] Vence, L, Palucka, A. K, Fay, J. W, Ito, T, Liu, Y. J, Banchereau, J, & Ueno, H. Circulating tumor antigen-specific regulatory T cells in patients with metastatic melanoma. Proc Natl Acad Sci U S A (2007). , 104(52), 20884-9.

[43] Mahnke, K, Schonfeld, K, Fondel, S, Ring, S, Karakhanova, S, Wiedemeyer, K, Bedke, T, Johnson, T. S, Storn, V, Schallenberg, S, & Enk, A. H. Depletion of CD4+CD25+ human regulatory T cells in vivo: kinetics of Treg depletion and alterations in immune functions in vivo and in vitro. Int J Cancer (2007). , 120(12), 2723-33.

[44] Le Gal FARiteau B, Sedlik C, Khalil-Daher I, Menier C, Dausset J, et al. HLA-G-mediated inhibition of antigen-specific cytotoxic T lymphocytes. Int Immunol. (1999). , 11, 1351-6.

[45] Contini, P, Ghio, M, Poggi, A, Filaci, G, Indiver, F, et al. Soluble HLA-A,-B,-C and-G molecules induce apoptosis in T and NK CD8+ cells and inhibit cytotoxic T cell activity through CD8 ligation. Eur J Immunol (2003). , 33(1), 125-34.

[46] Paul, P, Cabestre, F. A, Ibrahim, E. C, Lefebvre, S, Khalil-daher, I, et al. Identification of HLA-G7 as a new splice variant of the HLA-G mRNA and expression of soluble

HLA-G5,-G6, and-G7 transcripts in human transfec ted cells. Hum Immunol (2000). , 61(11), 1138-49.

[47] Dunn, G. P, Bruce, A. T, Ikeda, H, Old, L. J, & Schreiber, R. D. Cancer immunoediting: from immunosurveillance to tumor escape. Nat. Immunol. (2002). , 3, 991-998.

[48] Whiteside, T. L. Tumor-induced death of immune cells: its mechanisms and consequences. Semin Cancer Biol (2002). , 12, 43-50.

[49] Simon, A. K, Gallimore, A, Jones, E, Sawitzki, B, Cerundolo, V, & Screaton, G. R. Fas ligand breaks tolerance to selfantigens and induces tumor immunity mediated by antibodies. Cancer Cell (2002). , 2, 315-322.

[50] Tsao, H, Atkins, M. B, & Sober, A. J. Management of cutaneous melanoma. N Engl J Med (2004). , 351, 998-1012.

[51] Slingluff CL JrPetroni GR, Olson W, Czarkowski A, Grosh WW, et al. Helper T-cell responses and clinical activity of a melanoma vaccine with multiple peptides from MAGE and melanocytic differentiation antigens. J Clin Oncol (2008). , 26, 4973-4980.

[52] Rosenberg, S. A, & Yang, J. C. Restifo NP: Cancer immunotherapy: Moving beyond current vaccines. Nat Med (2004). , 10, 909-915.

[53] Bender, A, Karbach, J, Neumann, A, Jager, D, Al-batran, S. E, et al. LUD 00-009: phase 1 study of intensive course immunization with NY-ESO-1 peptides in HLA-A2 positive patients with NY-ESO-expressing cancer. Cancer Immun (2007). , 1.

[54] Jager, E, Karbach, J, Gnjatic, S, et al. Recombinant vaccinia/fowlpox NY-ESO-1 vaccines induce both humoral and cellular NY-ESO-specific immune responses in cancer patients. Proc Natl Acad Sci U S A 103:14453-14458, (2006). , 1.

[55] Nicholaou, T, Ebert, L. M, Davis, I. D, Mcarthur, G. A, Jackson, H, Dimopoulos, N, Tan, B, Maraskovsky, E, Miloradovic, L, Hopkins, W, Pan, L, Venhaus, R, Hoffman, E. W, Chen, W, & Cebon, J. Regulatory T-cell-mediated attenuation of T-cell responses to the NY-ESO-1 ISCOMATRIX vaccine in patients with advanced malignant melanoma. Clinical cancer research: an official journal of the American Association for Cancer Research. (2009). , 2166-2173.

[56] Dudley, M. E, Yang, J. C, Sherry, R, Hughes, M. S, Royal, R, Kammula, U, Robbins, P. F, Huang, J, Citrin, D. E, Leitman, S. F, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol (2008). , 26, 5233-5239.

[57] Robbins, P. F, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J. Clin. Oncol. 29, 917-924 ((2011).