

Review

Chronic inflammation, immunosuppression and cancer: New insights and outlook

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A substantial body of evidence supports the notion that chronic inflammation and cancer are connected. This association is apparent under two circumstances: (1) chronic inflammation can predispose an individual to cancer, as demonstrated by the association between inflammatory bowel disease and colon cancer, chronic pancreatitis with pancreatic adenocarcinoma and hepatitis with hepatocellular carcinoma, and (2) developing tumors induce a local and/or systemic chronic inflammatory environment associated with enhanced tumor development and metastasis [1–4]. The common denominator in the two modes by which chronic inflammation associates with cancer is the development of an immunosuppressive environment, enabling escape of the tumor from immune surveillance [5–7]. This review describes the evidence available today explaining the molecular basis for inflammation-associated immunosuppression and its link with cancer development and progression. A thorough understanding of this connection could lead to novel approaches for prevention and treatment of cancer and thus overcome some of the limitations of currently used anti-neoplastic

immunotherapeutic regimens. Moreover, improved knowledge of this link will enable development of preventive therapies for patients with chronic inflammatory diseases, known to be of high risk for developing cancer.

1. Inflammatory responses: the cause and effect

The immune system is composed of a large variety of cells and mediators that interact in a complex and dynamic network to ensure protection against foreign pathogens, which may be encountered during one's life-time, while simultaneously maintaining self-tolerance. The immune system is divided into two arms, the innate and adaptive immune systems, both participating in the generation of acute and chronic inflammatory responses [8,9]. An inflammatory response could be initiated via antigen-dependent or -independent stimulation. Antigen-dependent activation relates to the initiation of an immune response upon the activation of T cells, key players of the adaptive immune system, via their specific and highly rearranged receptors termed the T cell antigen receptors (TCRs). Immune activation via an antigen-independent pathway primarily involves the activation of cells from the innate immune system: granulocytes, dendritic cells (DCs), macrophages, nat-

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ural killer cells (NK cells) and mast cells. These cells express a variety of Toll-like receptors (TLRs), recognizing conserved pathogen associated molecular patterns (PAMPs) or TLR ligands such as lipopolysaccharide (LPS), unmethylated CpG DNA and single stranded RNA, all found on microbes but not expressed on self-tissue [10–12]. Inflammation initiated by antigen-independent activation of the innate system cells is not always a response to infection; it could occur under sterile conditions such as those characterizing autoimmune diseases (rheumatoid arthritis, ankylosing spondylitis, Lupus), cancer (various solid tumors), or as the outcome of sterile trauma and in diseases of unknown cause (psoriasis, atherosclerosis, Alzheimer's, Crohn's) [8,9,12]. These cases of inflammation are characterized by tissue injury and the influx of immunologically active cells. The fact that inflammation can occur under sterile conditions poses a key immunological and medical question of whether the same cells, receptors, proteins and pathways are involved in both infectious and sterile inflammation. There are several cytokines such as TNF α , IFN γ IL-12 and IL-18 common to both conditions, as well as the massive activation of myeloid cells [1–7]. Under pathogenic conditions, it is well established that these cytokines and cells characterize TLR-mediated as well as antigen-dependent inflammatory responses. This leaves unknown the identity of the stimulators inducing chronic inflammation under sterile conditions. In autoimmune diseases and in many tumors, specific antigens stimulate an antigen-dependent inflammatory response. However, other pathologies are characterized by chronic inflammation that does not involve antigen-dependent stimulation. This suggests the possibility that TLRs operate under sterile conditions. If this is the case, what is the identity of their ligands? They must be self-components, which under normal conditions do not activate TLRs. As suggested by Beutler [12], the immune system may be regulated by “innate tolerance” that, if broken, can lead to “innate autoimmunity.” Due to the similarity between the pathogen and sterile-induced inflammatory processes, it is tempting to assume that endogenous TLR ligands do exist. In recent years, it has been suggested that endogenous molecules typically secreted during tissue damage or necrosis such as heat shock proteins, DNA, and compounds of the extracellular matrix also serve as TLR ligands and activate the cells under sterile conditions. However, rigorous measures for the exclusion of ‘exogenous’ (pathogenic) inducers have not always been applied, and the existence of endogenous TLR ligands has not been unequivocally proven [11,12]. There is still a possibility that some of these endogenous compounds activate the TLRs indirectly via other membrane or intracellular adapter molecules as shown for TLR4 and its association with MD-2 and CD14, a ternary complex required for activation with LPS, and CD36 has been identified as a protein that is required for TLR2 activity [13,14]. Today, it is well established that endogenous DNA either of genomic or of mitochondrial origin could serve as a TLR inducer. It was suggested that the adaptive immune response is sustained in autoimmunity (such as in Lupus) by the adjuvant effect of endogenous nucleic acids [15–17].

In any of these cases, the inflammatory response ongoing under pathogenic or sterile conditions due to the direct or indi-

rect activation of TLRs expressed primarily on myeloid cells triggers a cascade of intracellular signaling events via the adapter molecules MyD88, TRIF, TIRAP and TRAM [10–12]. This results in the activation of NF- κ B-dependent signaling pathways and in increased production of proinflammatory mediators, including cytokines, chemokines, nitric oxide (NO), and activation of araginase and of the complement system, which further induce the recruitment and activation of additional cells of the innate immune system [1–7,10–12,18]. The diverse effector pathways of the innate immune system shape the first line of immune defense against invading pathogens and are critical for the activation and modulation of specific adaptive immune responses.

In contrast to innate immune cells, the adaptive immune cells involved in the initiation of an inflammatory response are T lymphocytes, which express antigen-specific receptors (TCRs) generating an extremely diverse, flexible and broad antigen recognition repertoire, compared to the innate immune system [19]. Following engagement of TCRs by specific antigen—major histocompatibility complex (MHC complexes) displayed on antigen-presenting cells (APCs) such as macrophages, B and dendritic cells, antigen-specific T helper cells are activated [9,20,21]. Under the appropriate conditions, depending on the interacting APC, the nature of the antigen and of the secreted cytokines, an inflammatory Th1-dependent immune response is initiated [8,9]. Although the antigen-specific responding T cells represent a minute population of the total T cells, acute or chronic responses will ensue depending on the duration of the antigenic stimulus. Activated T cells and the secreted cytokines will in turn activate other cells of the innate and adaptive immune systems such as myeloid and natural killer (NK) cells, enhancing an antigen-independent inflammatory response as reflected by the production of pro-inflammatory factors (nitric oxide, hydrogen peroxide and prostaglandins) and cytokines (IFN- γ , TNF α) [8,9]. Thus, this response is initiated by the adaptive arm of the immune system and culminates in the activation of the innate immune system, exhibiting similar features as the response induced upon the direct activation of the innate system via specific TLRs. In both cases, an inflammatory response is generated, enabling pathogen clearance.

2. The inflammatory response: beneficial or harmful?

The inflammatory responses could be divided into two categories: acute and chronic. *Acute inflammation* is a short-lasting (few days) immunological process that in most cases is beneficial to the host due to the clearance of the pathogenic stimulation by the activated innate and adaptive immune cells. Upon clearance, antigenic stimulation disappears, and the immune cells are no longer triggered and return to their quiescent state. An acute response can also occur under sterile conditions during tissue damage, and following clearance of the stimulating agents, the system returns to its normal condition. In contrast, *chronic inflammation* is an inflammatory response of prolonged duration (weeks, months, or even indefinite) whose extended time course is provoked by the persistence of the causative inflammatory stimulus. The inflammatory process inevitably causes

tissue damage and is accompanied by attempts at ‘healing and repair’. The exact nature, extent and time course of chronic inflammation depends on a balance between the causative agent and the attempts of the innate and adaptive immune responses to remove it. Chronic inflammation may develop either as a progression from acute inflammation if the original stimulus persists or after repeated episodes of acute inflammation. Etiological agents that can produce chronic inflammation include infectious organisms that can avoid or resist host defense mechanisms and so persist in the tissue for a prolonged period such as *Mycobacterium tuberculosis* that is able to survive within phagocytic cells [22]. In addition, infectious organisms that are not innately resistant but persist in damaged regions where they are protected from host defenses may also initiate inflammation. The common example is of bacteria, which grow in the cavity of an abscess, where they are protected both from host immunity and from blood-borne therapeutic agents, such as antibiotics [23]. Irritant, non-living foreign material such as inhaled compounds and a wide range of materials implanted into wounds that cannot be removed by enzymatic breakdown or phagocytosis could also induce chronic inflammation. Components of damaged tissue such as heat shock proteins, DNA and extracellular matrix components could continuously stimulate the immune system to generate proinflammatory compounds [11,12,15–17]. These are evident in autoimmune diseases and in cases of various cancers. In addition, there are many diseases characterized by a chronic inflammatory pathological process in which the underlying cause remains unknown, as in ulcerative colitis and Crohn’s disease [24].

In conclusion, both the innate and adaptive immune systems are key participants in eliciting the inflammatory responses. Under normal conditions, the process of inflammation is a protective response designed to defeat invasion of the body by pathogens such as bacteria, viruses and/or parasites. The innate and the adaptive immune systems exhibit unique roles in controlling pathogens; but each relies, to a certain extent, upon the effective function of the other in order to efficiently eliminate invading microorganisms. In most cases, the inflammatory response, when acute, is beneficial to the host. However, there are situations in which this complex system is improperly regulated, leading to chronic states of inflammation, which are harmful to the host. This is reflected by the various interconnected abnormalities that are evident under chronic inflammatory conditions, such as local and systemic immunosuppression, persistent tissue damage, opportunistic infections, abnormalities in cell growth, and developing tumors.

3. Chronic inflammation: the cause or consequence of cancer?

The multifactorial process involved in carcinogenesis requires mutations in somatic cells and subsequent alterations of morphology and growth pattern, ultimately resulting in transformation, local invasion, and metastasis. Genes controlling growth (e.g. oncogenes encoding growth factors, their receptors or signal transduction cascades; growth inhibitory genes such as p53, BRCA-1 and -2, or TGF- β), DNA repair (PARP,

DNA-dependent protein kinase), and programmed cell death (apoptosis) are prime targets for the oncogenic process. Chronic inflammation is a serious risk factor associated with cancer: (1) it could alter directly the nature of normally growing cells by affecting the above mentioned genes or by providing the trigger of already modified cells for an uncontrolled growth, and (2) inducing abnormalities in the adaptive immune system affecting T cell function, thus preventing immune surveillance. These two modes are not mutually exclusive; on the contrary, they are closely related and in many cases the cause and the consequence cannot be distinguished. However, in all of these cases, there is a clear link between inflammation and tumor progression [25,26].

3.1. The effect of chronic inflammation on cell growth

The inhibitory environment that is largely orchestrated by inflammatory cells is an indispensable participant in the neoplastic process, promoting proliferation, survival and migration of cells. Inflammatory mediators contribute significantly to neoplasia by inducing transformation of normal cells via activation of oncogenes or loss of anti-oncogenic activity, or by affecting growth of transformed cells (inducing de-differentiation, proliferation, tumor progression from preneoplastic lesions and resistance to apoptosis). For example, various reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced in inflamed tissues react with each other to produce other, much more potent reactive species, primarily to attack invading infectious agents and foreign bodies by nitration, oxidation, and chlorination reactions [25,26]. However, excessive amounts of such reactive species generated in inflamed tissues can cause injury to host cells and induce DNA damage and mutations by the same reactions. It was shown that human phagocytes stimulated by either bacteria or 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced a variety of cytogenetic changes in co-cultured cells [27]. Addition of SOD, catalase, and anti-oxidants inhibited these genotoxic effects. Moreover, alterations of protein structure and function by ROS and RNS that react with proteins to modify amino acid residues by oxidation, nitrosation, nitration, and halogenation may contribute as well to carcinogenesis. Many studies have shown that the levels of modified proteins are elevated in inflamed tissues, such as gastric mucosa of patients with *Helicobacter pylori*-induced gastritis [28,29] and the plasma of lung cancer patients and of cigarette smokers [30]. Moreover, it was demonstrated that mammalian cells incubated with excess NO⁻ accumulate an inactivated form of the p53 tumor-suppressor, which is unable to bind its DNA consensus sequence [31,32]. It was also shown that NO⁻ is capable of activating the p21 protein product of the protooncogene *c-Ha-ras* via *S*-nitrosation [33]. These post-translational modifications of p53 and ras p21 proteins may occur through over-production of NO⁻ in inflamed tissues and may play an important role in carcinogenesis [26]. In the course of inflammation, in addition to the secretion of various cytokines and chemokines, angiogenic factors such as VEGF, bFGF, IL-8 and matrix metalloproteinases [34–37] are released from the surrounding inflamed tissue or by the tumor cells themselves triggering angiogenesis and thus contributing to tumor progression. These factors not only con-

fer survival and growth advantages to susceptible tumor cells but also affect local cell invasion characteristics and formation of metastasis [3,38,39]. In many cases, this is enabled by the features of tumor cells that have adopted some of the receptors and signaling molecules of the innate immune system such as selectins, chemokines and their receptors for invasion, migration and metastasis. The persistence of an inflammatory tumor microenvironment depends upon cycles of activation of the innate and adaptive immune cells by either tumor specific antigens or by endogenous ligands characterizing a damaged tissue, respectively. Moreover, tumor cells can express TLRs on their cell surface and thus undergo a direct activation by endogenous or pathogen-derived ligands to secrete inflammatory cytokines and chemokines that will induce tumor cell proliferation by an autocrine pathway, promoting angiogenesis, and favoring invasion and metastasis. Several reports have demonstrated that LPS can elicit such direct effects on tumor cells both in vivo and in vitro [40–42]. Moreover, a recent study demonstrated that melanoma cell lines express TLR4 and could be activated with LPS to secrete IL-8 and to modify their adhesion properties. It was also demonstrated that cells from metastatic melanomas responded by producing higher amounts of IL-8 in cultures stimulated with LPS, compared to cells from primary tumors [43].

The physiological and immunological processes taking place in the surroundings of a developing tumor are similar to those operating during wound healing. However, a critical difference exists between the two events; during tissue injury associated with wounding, cell proliferation is enhanced while the tissue regenerates, and proliferation and inflammation weaken after the assaulting agent is removed, or the repair is completed. In contrast, proliferating cells that maintain DNA damage and/or mutagenic assault continue to proliferate in microenvironments rich in inflammatory cells and growth/survival factors that support their growth. In a sense, tumors act as wounds that never heal [44].

3.2. Immunosuppression links chronic inflammation and cancer

Along with the tumor growth, chronic innate and adaptive immune responses are induced, leading to the generation of an inflammatory tumor microenvironment. The tumor site, which is characterized by a damaged tissue and local sterile chronic inflammatory environment is, in many cases, associated with a burst of proliferating pathogens, most likely enabled due to the local induced immunosuppression that affects the general immune status of the host. In parallel, as mentioned above, chronic inflammatory disease could form the optimal environment for arising tumors, supporting uncontrolled cell growth and inducing local immunosuppression mediated by the chronic inflammatory environment, all resulting in tumor development with no disruption by a potent immune response.

The immunosuppression observed in tumor-bearing hosts is evident in both the adaptive and innate immune systems, as T lymphocytes and NK cells, respectively, lose their ζ chain expression and become dysfunctional. These two cell lineages share the TCR ζ chain, which is associated with key functional

receptors in both cell populations, the TCR and NK killing receptors (NKP30, NKP46 and CD16). Under normal conditions, in both T and NK cells, the ζ chain plays a key role in the expression and signaling function of the receptors with which it associates. The ζ chain contains in its intracytoplasmic domain three immunoreceptor tyrosine-based activation motifs (ITAMs), which undergo tyrosine phosphorylation during activation enabling this chain to bind and recruit various catalytic and non-catalytic molecules. Thus, the ζ chain is indispensable for coupling antigen recognition by the TCR and NK receptors to diverse signal transduction pathways [20,21,45–47]. In the past decade, many studies have demonstrated aberrations in T cells isolated from hosts (human patients and mouse models) bearing various solid and non-solid tumors [9,48–65]. In all reports, the most pronounced and reproducible observation is the total or partial loss of TCR ζ chain expression, correlated with impaired in vitro T cell function. Although some reports demonstrate aberrations in the expression and function of other molecules such as the CD3 γ chain, tumor necrosis factor (TNF), granzyme B, and the tyrosine kinases Lck and Fyn, the most pronounced and consistent defect was the down-regulation of the ζ chain. During the initial stages of growth of many of these tumors, ζ chain down-regulation and impaired T cell function were detected in tumor infiltrating lymphocytes (TILs) and at a later stage (usually upon tumor progression), this phenomenon was detected in peripheral-blood T cells as well. Several of these reports also demonstrate ζ chain down-regulation in NK cells isolated from tumor-bearing hosts, which correlates with impaired lytic function. Thus, both the adaptive and innate immune systems are affected by the conditions generated in tumor-bearing hosts. It has been suggested that ζ chain down-regulation is one of the many escape strategies developed by various malignancies to evade the immune system [66]. This was based on several studies demonstrating that factors secreted by the tumor are responsible for this immunosuppression, manifested by down-regulation of ζ chain expression and abnormal T cell function [60,67,68]. As research into disease-mediated immune suppression progressed, various studies analyzing T and NK cells in autoimmune diseases noted down-regulation of ζ chain expression and impaired function of T and NK cells, similar to that observed in tumor-bearing hosts. These were evident in patients with systemic lupus erythematosus (SLE) [69,70] and rheumatoid arthritis [71–73]. Moreover, the same phenomenon was observed in patients with infectious diseases such as those infected with HIV [74–76] and *Mycobacterium leprae* [77].

In most pathologies, even though ζ chain levels in the affected T cells are low or absent, normal levels of TCR cell surface expression are observed. This is in contrast to TCR expression in normal T cells, where in the absence of the ζ chain, cryptic TCRs are assembled within cells and targeted for lysosomal degradation [78,79]. It has been shown that in some cases of hosts bearing tumors or with SLE, the Fc ϵ R γ chain can replace ζ chain expression and lead to normal levels of TCR cell surface expression. However, these substituted TCRs still have impaired function upon antigen coupling [48,80]. In most of the studies describing down-regulation of ζ expression, Fc ϵ R γ chain was not detected as part of the TCR. Thus, two possible models

could then explain normal levels of TCR cell surface expression in the absence of ζ : either a small amount of expressed ζ serves as a ‘chaperone’ and transports the assembled TCRs to the cell surface, and/or an as yet unidentified protein, other than the Fc ϵ R γ chain, is expressed under such pathological conditions and replaces the ζ chain. Thus, ζ chain down-regulation can affect both adaptive (T cell) and innate (NK cell) immune responses, leading to partial or total immunosuppression under pathological conditions.

The fact that the same phenomenon of ζ down-regulation and impaired T and NK functions is observed in a variety of pathologies characterized by different etiology and physiology suggests that there is a common denominator that links the pathologies resulting in immunosuppression. Accordingly, we hypothesized that chronic inflammation and sustained exposure to antigens are the keys for the induced immunosuppression under the pathologies described above, including cancer. By developing an *in vivo* experimental system that mimics chronic inflammatory immune activation we demonstrated that indeed chronic inflammatory environment, whether generated upon exposure to various pathogenic antigens (heat-killed bacteria, viruses) [81] or against purified antigens inducing a sterile response (Vaknin et al. manuscript in preparation), is responsible for ζ down-regulation and T and NK dysfunctions. In these experimental systems the immunological characteristics are: all types of T cells are affected, and not only those responsive to the specific antigen (representing a bystander effect); of all the TCR subunits, only the ζ chain is down-regulated; TCR expression on the cell surface is at normal levels; and *in vitro* and *in vivo* T cell functions (mediated by CD4⁺ and CD8⁺ cells) are impaired. Impaired NK functions are evident as well. Moreover, using interferon γ (IFN γ) knockout mice, we showed that IFN γ plays a key role in the establishment of the immunosuppressive environment by inducing the recruitment/generation of myeloid suppressor cells (MSCs) that induce the immunosuppressive environment and affect ζ down-regulation and impaired T cell function [81] (Ezernitchi et al., manuscript in preparation). A similar population of MSCs with immunosuppressive function, has been reported in various tumor-bearing hosts [5–7] and during acute infection [82]. This population is heterogeneous, and has been detected in lymphoid organs during tumor growth, in graft versus host reactions and in infectious diseases [5–7]. In all cases, impaired T cell responses to TCR-mediated stimuli are observed. MSCs play a key role in the regulation of the inflammatory process and in controlling T cell responses. One of the mechanisms used by MSCs to control T cells is based on arginine metabolism. MSCs use two enzymes to metabolize arginine: inducible nitric oxide synthase (NOS2), which generates nitric oxide, and arginase-1 (ARG1), which converts arginine to urea and L-ornithine. NO inhibits T cell proliferation [83] most likely by preventing activated T cells from entering the cell cycle, and L-ornithine, which is consumed by the MSCs, is not available for the T cells that require it for cell proliferation and differentiation. Moreover, it was recently shown that the availability of L-arginine is necessary for the expression of the TCR ζ chain, through an as yet unknown mechanism [84]. Therefore, the regulation of L-arginine concentration in

the microenvironment could interfere with early signaling pathways and consequently modulate T cell function. Thus, MSC recruitment can serve an important role in controlling excessive immune responses. Expansion of MSCs in lymphoid organs of infected or immunized mice is transient. By contrast, the number of MSCs under conditions of chronic inflammation such as during tumor progression remains high, and immunosuppression is maintained. MSCs suppress the activation of both CD8⁺ and CD4⁺ T cells, in an antigen and MHC-independent manner. Both cell–cell contact between MSCs and T cells, and secreted compounds are required to exert the inhibitory activity of MSCs [85]. Thus, interference with the mechanisms used by MSCs to suppress T cell functions seems to be the most promising therapeutic strategy for restoring T cell function. It is important to note that immunosuppression associated with low ζ chain expression is reversible. Upon antigen withdrawal (as following acute inflammation), T cells recover and ζ chain expression and TCR-mediated functions are restored.

What then is the physiological and immunological rationale, or adaptive advantage for the observed immunosuppression reflected by ζ Chain down-regulation and impaired T and NK cell function under various chronic inflammatory conditions? Based on the cumulative data from different pathologies, which are pathogen-dependent or -independent (sterile), it is evident that chronic inflammation-dependent immunosuppression is the normal outcome of an excessive and potentially hazardous inflammatory immune response. ζ chain down-regulation starts with an “innocent” immune response. Upon antigen recognition, the activated T cells secrete proinflammatory cytokines, proliferate, differentiate to effector and memory cells, and in turn, further activate APCs. In addition or in parallel, APCs could be directly activated by pathogenic or endogenous ligands through their TLRs and other receptors [10–14]. This by itself adds a tremendous burst of proinflammatory compounds. These stimuli will generate an inflammatory response over time. Upon clearance of the pathogen or damaged tissue, the inflammatory environment is reduced, and the system returns to a resting state. To this point, the developing immune response is transient. However, if the antigen is continuously present in the surroundings and the inflammatory environment persists, an antigen-independent process may progress and may be further supported by tissue damage and possible infections due to susceptibility of the host’s immune system. Under these extreme conditions, all T cells will be affected, become hyporesponsive, and down-regulate ζ chain expression. We suggest that in the case of a time-limited exposure to antigen as in acute bacterial or viral infections, the transient ζ chain down-regulation and T cell unresponsiveness may help attenuate the immune response against the pathogen and restore the balance of a “superactivated” immune system. By contrast, in pathological conditions exhibiting ζ down-regulation, such as in tumor-bearing hosts, the continuous presence of antigenic stimulation and the developing chronic inflammation most likely prevent recovery, thus contributing to the pathological nature of the disease.

In summary, chronic inflammation operates on several levels to support tumor development. Upon chronic infection or chronic sterile tissue damage, the ensuing environment could

directly induce cell transformation and support uncontrolled cell growth. In addition, chronic inflammation could induce immunosuppression leading to the unresponsiveness of the immune system to the appearance of abnormal or modified growing cells. In turn, growing tumors could elicit inflammatory conditions by stimulating antigen-independent and antigen-dependent inflammatory responses due to the expression of specific antigens or in response to tissue damage. These in turn will induce immunosuppression enabling persistent tumor growth. Thus, chronic inflammation could serve as a cause for cancer as well as its consequence.

4. Clinical significance of chronic inflammation-dependent immunosuppression and cancer

Over the last decade, there has been a rapid expansion of the field of tumor immunology and convincing evidence has been accumulated that the immune system is capable of interacting with tumor cells. Therefore, the feasibility of stimulating specific anti-tumor immune responses that would be therapeutically effective is currently being pursued. The goal of cancer immunotherapy is to buttress the immune system, rendering it better able to combat cancer cells. The identification of tumor-associated antigens recognized by effectors (cells and antibodies) of the immune system has opened new approaches for cancer immunotherapy. Currently, several forms of immunotherapy are being explored [86], falling into four main categories: monoclonal antibodies directed against specific tumor-associated antigens; immune response modifiers such as cytokines and chemokines; vaccines [87–89] and therapy with engineered or activated T cells [90]. T cell therapy is considered an active therapy as well, since it is expected that the administered T cells will function in vivo and elicit an anti-tumor immune response. In all cases where active immunotherapy is used, the expectations are either that the patient's immune system will be activated (in the case of vaccination) or that the administered T cells (in the case of T cell therapy) will function within the patient. While these modalities have individually shown some promise, the general success rates are limited. Further design details have to be optimized overcoming some critical obstacles such as the induction of graft versus host disease when allogeneic donor T cells are applied and difficulties in the isolation of sufficient numbers of TILs from many tumors. In the case of engineered T cells, difficulties remain in obtaining high and specific responses following immunizations due to an hidden tumor antigenicity, difficulties in overcoming genetic changes during tumor growth and metastasis, and the production of blocking antibodies upon immunization that could enhance tumor growth.

The immunosuppressive tumor microenvironment generated in many cancer patients, depending on the tumor stage, and whether the immunosuppression is local or systemic, plays a critical role in dictating the success rate of the various immunotherapeutic regimens. We expect that the immunosuppressive environment will limit the response to a given vaccination by affecting the endogenous host immune system, as well as suppressing any newly administered T cells, inducing

their ζ chain down-regulation, and impairing their function. Immunotherapy using immune response modifiers such as cytokines and chemokines, also requires a potent immune system and therefore is expected to fail under an immunosuppressive environment. Moreover, it is possible that adjuvants used to enhance a patient's immune response to weakly immunogenic tumor or pathogenic antigens might even result in activation of the regulatory MSCs and thus negatively affect the anti-tumor response. Thus, blockade of negative regulatory pathways such as those mediated by MSCs is a necessary strategy to potentiate the effect of any immunotherapies used.

With this knowledge in mind, the timing of a given therapy must be reassessed. Therefore, it is critical to develop monitoring strategies that may allow the identification of patients who could benefit from cancer immunotherapies [91] and that would help optimize the timing of these interventions.

One such strategy could be based on measurements of ζ chain expression levels in the host's peripheral T cells. ζ chain expression could serve as a prognostic marker for the appearance of an immunosuppressive environment and for measuring the impact of the disease on the patient's immune system [9,92]. If ζ chain down-regulation is detected in a patient, agents that neutralize the immunosuppressive factors/cells or induce ζ chain re-expression must be applied before or in conjunction with the "traditional" immunotherapy. The neutralizing modalities chosen must be selective, to avoid inhibiting cells that are critical for the induction of a functional immune response. For example, neutralization of the immunosuppressive function of immature MSCs has been achieved by inducing their differentiation (in vitro and in vivo) with GM-CSF [93]. However, cytokines that induce MSC differentiation in vitro might have limited use in vivo due to their pleiotropic activity and the secondary effects that they might exert in patients with chronic disease [94]. An alternative strategy is the use of blocking antibodies specific for cytokines and chemokines involved in the recruitment and/or activation of MSCs [95,96] or secreted by these cells. However, given the large number of cytokines with similar, redundant activity on MSCs, this approach might require the combination of several anti-cytokine, and possibly anti-chemokine, antibodies.

A promising therapeutic strategy would be to target the metabolites used by MSCs to suppress T cell function. For example, compounds affecting arginase-1 and iNOS activity might be a novel class of immune modulators that act by limiting the effects of MSC activity in vivo [97,98]. It has been demonstrated that NO-releasing aspirin, which is a classic aspirin molecule covalently linked to a NO donor group, could interfere with the inhibitory enzymatic activities of myeloid cells. Orally administered NO aspirin normalized the immune status of tumor-bearing mice, increased the number and function of tumor-antigen-specific T lymphocytes and enhanced the preventive and therapeutic effectiveness of the anti-tumor immunity elicited by cancer vaccination [99]. Cancer vaccines and NO aspirin are currently being investigated in independent phase I/II clinical trials; the findings described above offer a rationale for combining these treatments in subjects with advanced neoplastic diseases. Therefore, further identification and char-

acterization of both the subpopulations within MSCs and their related factors that operate in the immunosuppressive environment will enable the development of promising treatments to neutralize this environment, to be applied in conjunction with immunotherapy [100].

Other anti-inflammatory drugs could potentially serve to neutralize the inflammatory environment and reduce cancer initiation by attenuating inflammation, which is associated with the onset of variety of cancers. For example, COX2 inhibitors, NSAIDs, selenium and Vitamin E have all been shown to have efficacy in prostate cancer prevention [100]. Accordingly, anti-inflammatory drugs are expected to neutralize the immunosuppressive environment and thus could serve a dual purpose; they could be used in cancer prevention or as a treatment administered prior to immunotherapy, preparing the host for various immunotherapeutic regimens requiring an optimal environment that will support an anti-tumor immune response.

5. Concluding remarks

Despite compelling data indicating a functional link between inflammation, immunosuppression and cancer, the pathways regulating initiation and maintenance of chronic inflammation during cancer development remain poorly understood. Moreover, the precise identity of MSCs, the specific trigger required for their activation, and the exact immunosuppressive pathway by which they induce TCR ζ down-regulation and impair the function of T and NK cells, await further studies. With our greater understanding of this suppressive mechanism(s), efforts to optimize immunotherapy must be directed toward several clinical and preclinical routes, which are closely related: (1) the ability to evaluate the immunosuppressive environment caused by chronic inflammation in patients suffering from cancer and other pathologies, prior to the initiation of a particular immunotherapy; (2) the investigation of non-steroidal anti-inflammatory drugs, for their use prior to or together with immunotherapy as a supportive treatment of malignant diseases as well as a preventive therapy for arising tumors in cases of chronic inflammatory responses; (3) the precise identification and characterization of the immunosuppressive cells and factors participating in this pathway and (4) identification of molecules/mechanisms that are activated within T and NK cells under chronic inflammatory conditions affecting T cell function and inducing ζ chain down-regulation. Together, these approaches promise to advance our understanding of the mechanisms by which chronic inflammation-induced immunosuppression contributes to cancer development and how these harmful conditions could be artificially regulated and controlled. Exploring these clinical and basic research areas will enable future development of modalities that will neutralize chronic inflammation and resulting immunosuppression, leading to the recovery of the immune response; these approaches may prevent tumor development and/or increase the success rate of immunotherapies applied to cancer patients.

These insights are fostering new anti-inflammatory therapeutic approaches to cancer development. As can be seen these two

conditions that link cancer and inflammation are not mutually exclusive. In many case it is not known which is cause and which is the result.

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