

Th1 cells in cancer-associated inflammation

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Abstract: The immune system is not only evolved to protect the body from pathogens, but it also recognizes and eliminates cancer cells. CD4+ helper T (Th) lymphocytes are central intercessors differentiated according to the character of physiological or pathological status. Generation of type 1 Th (Th1) cells is primarily associated with a pathological insult that must be removed through immune elimination. Upon interacting with other immune and transformed cells, Th1 cells can hamper cancer progression. Therefore, it is a major obstacle for tumor cells to become insensitive or resistant to Th1-oriented actions. The organism employs various mechanisms to return to a steady state and ensure tissue repair following a destructive inflammatory response. Th1 cells are also tightly regulated during the termination of immune responses. They can reduce the production of inflammatory cytokines, both generate and be prone to inhibitory signals, and undergo activation-induced cell death for inflammation resolution. Additionally, Th1 cells may become hyporesponsive, exhausted, and decorated with many inhibitory receptors and eventually lose functionality. There is growing evidence about tumor cells taking advantage of the strategies used for the resolution of Th1-oriented inflammation. Here, the current insights on Th1 cells during cancer-associated inflammatory responses are reviewed.

Key words: Type 1 helper T lymphocytes, cancer, inflammation

1. Introduction

The immune system comprises cellular and molecular components developed for defending the host against invading microorganisms. Immune cells run a continuous watch through lymphoid organs or act as sentinels in the tissues (Abbas et al., 2010; Sansonetti, 2011). Upon recognition of foreign antigens, these cells deploy defense mechanisms and produce signals for the recruitment of circulating immune cells. In addition, sampling and transport of the foreign material into immune organs are also performed by specialized cells for the expansion of immune responses (Randolph et al., 2008; Peter et al., 2010). Hence, the immune system takes advantage of having diverse components that are specialized, strategically located, and mobile. While it is clearly more successful in coping with foreign (nonself) agents, the immune response is constrained against transformed cells of autochthony (Finn, 2008).

Effective immune responses are triggered against cells infected and transformed by oncogenic viruses (Hislop and Sabbah, 2008; Buonaguro et al., 2011). The viral products and signals generated from infection turn the transformed

cell into a better target than a neoplastic cell generated by spontaneous mutations. A mutation in the coding sequence of a gene can change the structure of a protein (i.e. mutant protein), rendering it into a more discernible subject (as a foreign antigen) of the immune cells (Finn, 2008; Abbas et al., 2010). In addition, this mutant protein may alter the cellular physiology, resulting in a stressed state that can eventually alert the immune system. In a perfect world, all cells bearing mutant products would be eliminated by immune responses. However, cells of the immune system are either incapable of recognizing predominantly self-antigens (cells of innate immunity) or are selected as “nonresponders to self” from an immense pool of many during their ontogenesis (cells of adaptive immunity) (Abbas et al., 2010). Therefore, immune cells are programmed not to give potent responses against the self-antigens, which are also carried by the transformed cells (Abbas et al., 2010). The immunogenicity of a mutant protein and stress signals are interpreted by the immune cells and a decision is made to respond or not.

Immune cells continuously screen the body to determine the distressed areas. This immune surveillance

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generally results in the recognition and eradication of the transformed cells (Dunn et al., 2004; Zitvogel et al., 2006). The major players in this elimination phase (as described extensively in the review by Dunn et al., 2004) are cytotoxic cells, especially natural killer (NK) cells of the innate immune system and cytotoxic T lymphocytes (CTLs) of the adaptive immune system (Dunn et al., 2004). There are many supporting characters playing critical roles in the augmentation and regulation of antitumor immune responses (Zitvogel et al., 2006; Abbas et al., 2010). Indeed, the most destructive antitumor immune responses are exerted under the auspices of the type 1 helper T lymphocytes (Th1) (Ikeda et al., 2002; Wong et al., 2008; Bos and Sherman, 2010; Matsuzaki et al., 2014). In the absence of Th1 functions or the mediators produced by it, it becomes a hard task for immune cells to clear malignant cells out (Kennedy and Celis, 2008).

Once the transformed cells evolve to hide from immune surveillance and/or actively suppress the immune attack, it becomes a hard and dysregulated task for immune cells to cope with these highly proliferating, apoptosis-resistant cells. Essentially, reduction in immune-provoking signals derived from the tumor can diminish the effector phase of immune responses (Dunn et al., 2004). Hence, the struggle exerted by the immune system against tumorigenesis does not necessarily end with complete eradication of all transformed cells. It is hypothesized that the tumor cells, which are able to avoid recognition by the immune system, continue to exist in quiescence (the equilibrium phase of cancer immunoediting (Dunn et al., 2004; Zitvogel et al., 2006)). Together with the favorable changes in the host's condition such as loss of immune competence, acquisition of additional mutations can advance the neoplastic transformation. Moreover, the selective pressure applied by the immune system may result in a selection of tumor system that can successfully evade immunity (Pettit et al., 2000). Consequently, heterogeneous populations of tumor cells, which are capable of hiding from immune recognition and/or coping with immune attack, develop and begin to proliferate. During tumorigenesis, transformed cells adapt to grow in a disarranged microenvironment with dysregulated physiology. All in all, these changes divert the immune responses towards a chronic inflammation-like condition (Rakoff-Nahoum, 2006). Thus, immune cells infiltrating tumors, and especially Th1 cells, forfeit certain tumoricidal and destructive features. This review article will focus on the Th1 subset implicated in the destruction of transformed cells and the chain of events that impede their antitumor functions.

2. A rough guide to Th1 cells

Even though they originate from the bone marrow, T lymphocytes need to migrate to the thymus to go through

selection and maturation processes. Within the thymus, thymocytes come across self-antigens, i.e. peptides that are represented on major histocompatibility complex (MHC) molecules by antigen-presenting cells (APCs) such as cortical thymic epithelial cells, medullary thymic epithelial cells, dendritic cells (DCs), and macrophages (Luckheeram et al., 2012). "Helper T lymphocytes-to-be" are selected through a process in terms of their class II MHC binding affinity. In this process, thymocytes expressing T cell receptor (TCR) that is unable to recognize and interact with the MHC-peptide complex undergo apoptosis (death by receptor neglect) (Goldrath and Bevan, 1999). Those that bind with very high affinity to the MHC-peptide complex are destroyed by negative selection (Goldrath and Bevan, 1999). Only thymocytes that interact with the self-peptide-class II MHC complex with sufficient affinity will receive survival signals (positive selection) and differentiate into single positive CD4⁺ helper T lymphocytes (Vrisekoop et al., 2014).

Upon maturation, Th cells travel to the periphery and settle into secondary lymphoid organs such as the spleen, lymph nodes, and mucosal lymphoid tissues where they experience their first encounter with antigens presented by APCs. If the antigen is foreign and presented with appropriate activating stimuli, these naïve lymphocytes get license to become effector cells. Activation and differentiation of helper T lymphocytes requires three types of signals from APCs. The first signal (signal-1) is generated by TCR recognizing the presented antigen, signal-2 is almost simultaneously provided by various costimulatory molecules (especially the activating ligands of the B7 family) (Harris and Ronchese, 1999), and finally signal-3 is mediated by cytokines found in the microenvironment and produced by APCs (Curtsinger et al., 1999). The character and the amplitude of helper responses are critically decided during this cross-talk with APC. In the course of T cell and APC engagement, T cells enhance the binding efficacy by activation of several adhesion molecules. Among these surface proteins, an adhesion molecule from the integrin family called lymphocyte-function-associated protein 1 (LFA-1) binds to intracellular adhesion molecule 1 (ICAM-1) on APCs. It has been indicated that LFA-1 has a crucial role to initiate TCR signaling in the case of lower antigen densities on APCs and 100-fold more antigen was needed to trigger T cell responses in LFA-1-deficient T cells (Katagiri et al., 2002). Furthermore, activation of LFA-1 increases the duration of T cells and APC interaction, which also has an important role for T cells to gain effector function and memory formation. Although CD8⁺ T cells require short-term engagement with antigen for their expansion (van Stipdonk et al., 2001), CD4⁺ T cells need a longer antigen stimulation for proliferation (Iezzi et al., 1998). However,

failure to receive one of the required signals can lead to cell death or anergy.

Depending on the signals Th cells receive, they differentiate into diverse effector subsets such as type 1 (Th1), type 2 (Th2), type 9 (Th9), type 17 (Th17), type 22 (Th22), follicular Th cells (Tfh), induced regulatory T cells (iTreg), or type 1 regulatory cells (Tr1). Th subtypes have distinct roles in the regulation of immune responses and are generally distinguished by transcription factors and the cytokines they express. To our knowledge, generation of Th1 cells requires a strong and stable interaction with APC carrying the cognate antigen under the influence of specific cytokines.

APCs determine the character of pathological insults; accordingly, signals provided during the Th-APC cross-talk play a decisive role in the differentiation of specific helper subtypes. Starting from the initial phases of immune recognition, the course of the immune reactions is drawn by cytokines and costimulatory molecules expressed. APC-derived cytokines, and especially interleukin (IL)-12, IL-18, and IL-27, are potent inducers of the Th1 phenotype (Dinarello, 1999; Owaki et al., 2005). The functional differentiation of the Th1 subset is also supported by IL-2, which is an autocrine growth and activation factor for T cells. In addition to the generation of Th1 cells, these cytokines can contribute to functional differentiation of CTLs and NK cells, making them competent for IFN- γ production (Gately et al., 1992; Trinchieri, 1993). These cytokines trigger several intracellular signaling pathways ending up with activation of transcription factors such as T-box expressed in T cells (T-bet), signal transducer and activator of transcription 1 and 4 (STAT1, STAT4), eomesodermin (EOMES), and H2.O-like homeobox 1 (HLX1). These factors have pivotal roles in the regulation of Th1 cell generation (Dong, 2006).

The primary cytokine produced by the Th1 subtype is IFN- γ . IFN- γ favors Th1 responses either directly through mediating a positive-feedback loop of IL-12 production or indirectly through suppression of Th2 generation due to IFN- γ mediated IL-4 inhibition (Sad et al., 1995; Snijders et al., 1998; Schroder et al., 2004). Other than IFN- γ , Th1 cells produce proinflammatory cytokines such as IL-2, tumor necrosis factor (TNF)- α , TNF- β /lymphotoxin (LT)- α , and granulocyte-monocyte colony stimulating factor (GM-CSF) to fuel the innate and adaptive responses involved in destructive immunity and protection against intracellular pathogens (Romagnani, 2000; Knutson and Disis, 2005; Herndler-Brandstetter and Flavell, 2014). LT- α , a member of the TNF superfamily, is involved in lymphoid tissue organogenesis, T cell activation, and cellular migration to the site of infection (De Togni et al., 1994; Roach et al., 2001). Excitingly, many studies have evidenced an alternative role for Th1 cells in the termination of immune

reactions (Jeremias et al., 1998; Van Parijs and Abbas, 1998; Janssen et al., 2005; Nuriev et al., 2009; Saraiva et al., 2009). Along with the expression levels of effector cytokines that are modulated during distinct phases of immune responses, Th1 cells are capable of producing the potent antiinflammatory cytokine IL-10 (Saraiva et al., 2009). Therefore, the presence of IL-10-positive Th1 cells overlaps with the cessation phase of inflammatory responses and contributes to the reestablishment of homeostasis and tissue repair. The immunological parameters associated with Th1 type cells are summarized in the Table.

3. Th1 cells of immune destruction

Once Th1 cells are fully differentiated, they gain capacity for licensing APCs, i.e. macrophages, to destroy ingested foreign material and to more efficiently process and present the antigens together with the secretion of mediators to enhance Th1 functions (Schroder et al., 2004; Janeway, 2005). Production of IFN- γ induces activation of macrophages and upregulation of inducible nitric oxide synthase (iNOS) and the components of the phagocyte oxidation system to produce reactive oxygen species (ROS), leading to intracellular pathogen clearance (Marodi et al., 1993; Schroder et al., 2004). This functional subset of macrophages with antitumor activity is designated as "M1", the producer of inflammatory cytokines such as TNF, IL-1 β , and IL-6, the attractant of Th1 cells through the release of chemokines, i.e. (CXCL)-9 and CXCL-10, and the inducer of antitumor responses by the secretion of cytokines and chemokines (Dunn et al., 2004; Zitvogel et al., 2006; Germano et al., 2008; Mantovani et al., 2008; Sica et al., 2008; Tseng et al., 2013).

Free radicals such as reactive oxygen intermediates (ROIs), hydroxyl radical (OH \cdot) and superoxide (O $_2^{\cdot-}$), reactive nitrogen intermediates (RNIs), and nitric oxide (NO \cdot) lead to oxidative DNA damage and reduction in DNA repair (Rakoff-Nahoum, 2006). Tumoricidal effects of the reactive mediators have been well documented; however, Th1 help is especially pivotal to their induction (Janeway, 2005). If they are not well equipped with antioxidant systems due to an incompetent DNA repair mechanism, tumor cells become suitable targets for cell death in an oxidizing milieu. In this particular setting, APCs need protection from negative effects of their own radical products. Thus, for their sake, Th1 cells secrete GM-CSF, which exerts a prosurvival function on these myeloid cells (Cousins et al., 2002). Finally, the remnants of tumor cell debris are engulfed by APCs and tumor antigens are presented on class II MHC molecules to Th cells and to CTLs on class I MHC molecules loaded via the cross-presentation pathway.

Not only IFN- γ but also TNF- α and CD40 ligand, a surface molecule belonging to the TNF superfamily,

Table. The factors associated with Th1 cells.

Inducing cytokines	Signal transducers and transcription factors	Produced cytokines and molecules	Functions of produced substances	Surface markers
IL-12 IFN- γ IL-2 IL-18 IL-27	STAT4 STAT1 T bet (Lazarevic et al., 2013) Runx 3 (Kohu et al., 2009) Eomes Hlx Notch (Rutz et al., 2008)	IFN- γ TNF- α TNF- β /LT- α IL-2 Perforin Granzyme A Granzyme B IL-10	Activator of CTL-Th1-NK-NKT cell responses Enhancement of tumoricidal and microbicidal effects Induction of inflammatory responses Inducer of inflammatory response and production of proinflammatory cytokines Mediator of inflammatory, immunostimulatory responses Expansion, differentiation, and survival of T cells Elimination of tumor and infected cells through CTL and NK cell-mediated killing Inhibition of inflammatory responses Suppression of antigen presentation and T effector activation	Costimulatory molecules: CD26 (Seitzer et al., 1997) CD94 (Meyers et al., 2002) CD278 (Wassink et al., 2004) (ICOS) TIM-3 Chemokine receptors: CXCR3 (Groom and Luster, 2011) CCR1 (Weber et al., 2001) CCR5 (Weber et al., 2001) Other receptors and ligands: CD95L (FasL) IL-12R β 2 IL-18 α IL-27Ra NOTCH3 RANKL

contribute to APC-provoking actions of Th1 cells (Janeway, 2005). These mediators promote maturation of myeloid cells, fuel the antigen processing and presentation machinery, and induce pro-survival signals. They are also able to increase the synthesis of free radicals, immunopolarizing cytokines such as IL-12, and consequent cytotoxic responses (Bennett et al., 1998; Ma and Clark, 2009). Moreover, upon engagement with its cognate receptor CD40, CD40 ligand upregulates the expression of potent costimulatory molecules of the B7 family, namely B7-1 (CD80) and B7-2 (CD86) (Ma and Clark, 2009). B7-1 and B7-2 are responsible for delivering signal-2 to naïve T lymphocytes. This reciprocal interaction ensures the activation and differentiation of T lymphocytes recognizing the target antigen presented on APCs. Being located in the same microenvironment, IL-2 produced by Th1 cells also amplifies CD8⁺ T lymphocyte proliferation and acquisition of cytotoxic effector functions (Fearon et al., 1990). If the priming of CTLs happens in the presence of Th1 cells, then they have the capacity to undergo a second round of clonal expansion upon restimulation. Otherwise, CTLs primed without help from Th1 cells can only mediate functions such as cytotoxicity and cytokine secretion upon restimulation. This phenomenon is attributed to the fact that Th1 is able to establish the generation of CD8⁺ T cells' memory (Janssen et al., 2005). Thus, this intercellular communication forms the main axis for the augmentation of antitumor immunity wherein Th1 cells play a central role.

Tumors are seldom infiltrated by B lymphocytes. Following the entry of antigens into the lymph nodes, the activation of T cells is concurrently followed by B lymphocyte responses. As members of adaptive immunity, B cells can specifically recognize soluble antigens concentrated in the secondary lymphoid tissues (Abbas et al., 2010; Delves and Roitt, 2011). Moreover, these cells can internalize the cognate antigen and present it to T lymphocytes. T and B cells, which are capable of recognizing the same antigen, can cross-talk and promote the immune responses. The B cell receptor used for antigen recognition is a membrane-bound immunoglobulin (Ig) molecule (so-called surface antibody) (Vaughan et al., 2011). Upon activation, B cells are also responsible for the production of soluble antibodies. Once the immune response is polarized towards the Th1 type, B cells switch to producing IgG antibodies, which are effective in antitumor immunity (Finkelman et al., 1988; Snapper et al., 1988). IgG production is strictly dependent on the CD40 ligand and IFN- γ provided by Th1 cells (Abbas et al., 2010). Consequently, secreted antibodies can bind antigens with high affinity and trigger cytotoxic mechanisms. Tumor cells coated with antibodies can be easily recognized by NK cells and macrophages and the target is eliminated by a process called antibody-dependent cellular cytotoxicity (Abbas et al., 2010). Additional effector mechanisms can also be triggered by the antibodies such as complement-mediated cell lysis and apoptosis (Manson, 1994; Abbas et al., 2010; Vaughan et al., 2011). Collectively, the interaction

between Th1 cells and APCs forms a vicious cycle that favors elimination of target cells whether they are infected or transformed.

4. Direct actions of Th1 cells on tumor cells

Since the receptors for IFN- γ and TNF- α are widely distributed among all nucleated cells, these mediators produced during a Th1-oriented inflammation exert antiproliferative and proapoptotic effects. This is a defense strategy that hinders the dissemination of intracellular infection and increases the recognition and presentation of antigens through extracting the infectious agents together with the cell debris. If the cell cycle control and apoptotic pathways targeted by IFN and TNF are intact (not mutated to become unresponsive to extracellular signals), the transformed cells are also prone to the direct antitumor actions of Th1 cells. Dead cells collected by APCs give an opportunity to increase the visibility of tumor cells by T lymphocytes and augment the effector immunity (Moretta et al., 2008). TNF- α can enhance ROS and RNI accumulation in premalignant cells, as well (Grivennikov et al., 2010). Tumor stroma and blood vessels are also damaged by IFN- γ , preventing the formation of a favorable microenvironment for disease progression (Briesemeister et al., 2011). Strikingly, like CTLs, Th1 cells can also use a granzyme-perforin-dependent pathway to execute T lymphoma cells (Echchakir et al., 2000). Therefore, accumulating knowledge on the killing of cancer cells directly by Th1 cells underlines the importance of this subset in the fight against cancer.

Accordingly, augmented numbers of CTLs and Th1 cells within the tumor correlate with better disease outcome in the case of various types of cancers such as invasive colon cancer, melanoma, and multiple myeloma (Galon et al., 2006; Swann and Smyth, 2007). The crucial role of Th1 cells in cancer immune surveillance could be verified by Th1 escape mechanisms evolved in the tumors. For instance, epigenetic silencing of CXCL9 and CXCL10 Th1 type chemokines in ovarian cancer cells has been shown to be effective for cancer cells to escape from Th1-mediated tumor rejection due to the prevention of Th1 trafficking to the tumor area. Contrarily, treatment with epigenetic modulators retards the tumor growth through the enhancement of the number of infiltrated Th1 cells (Peng et al., 2015). IFN- γ and TNF- α production by tumor antigen-specific T lymphocytes can inhibit the growth of pancreatic tumors in mice (Muller-Hermelink et al., 2008). In the absence of either TNFR1 or IFN- γ signaling, the same lymphocytes promote angiogenesis and carcinogenesis (Muller-Hermelink et al., 2008). Additionally, while IFN- γ and TNFR1 signaling are strictly required in cancer cell senescence, TNFR1^{-/-} cancer cells resist cytokine-induced senescence and grow aggressively. Therefore, as IFN- γ and

TNF stimulate tumor cell senescence in different cancers, this may be a general mechanism for arresting cancer progression and escape (Braumuller et al., 2013). The mechanisms listed above are summarized in Figure 1.

5. Th1 cells during immune resolution

Taking into account the damage to the organism during autoimmune reactions and in inflammatory diseases, which are generally organized around Th1-associated immunity, understanding how these immune reactions are ceased becomes more critical. T cells are regulated to remove overactivated or autoreactive T cells to maintain peripheral tolerance (Abbas et al., 2010). Notably, the mechanisms employed for dampening the Th1 responses are also hijacked by tumor cells to evade immune recognition and elimination (Khong and Restifo, 2002).

Upon receipt of the stimulatory signals, while T cells undergo activation and massive proliferation they also become sensitive to inhibition. These cells become addicted to prosurvival signals and express receptors that initiate inhibitory signaling cascades. The previously activated T cells trigger a cell-autonomous mechanism, termed activation-induced cell death (AICD), mainly mediated by the death receptor Fas (CD95) and its ligand FasL (CD95L) and by TNF-related apoptosis inducing ligand (TRAIL) and its receptors TRAIL-R1 and -R2 (Sytwu et al., 1996; Jeremias et al., 1998; Janssen et al., 2005). There are many examples of tumor cells expressing the ligands specific for these death receptors forming a trap for infiltrating effector T cells. Critically, Th1-derived factors augment the expression of these ligands or receptors on tumor cells usurping the AICD (Naujokat et al., 1999; Corazza et al., 2004). Especially during the late effector phases of the immune responses where survival factors such as IL-2 and costimulation decrease, T cells expressing both death receptor and ligand pairs prepare for their silent removal (Abbas et al., 2010). This immune resolution mechanism is strengthened especially under the influence of negative regulatory signals derived from antiinflammatory cytokines and coinhibitory ligands. Accordingly, immune checkpoint receptors such as programmed death-1 (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) that are found on activated T lymphocytes can diminish effector responses (Van Parijs and Abbas, 1998). Normally, the interaction of PD-1 and CTLA-4 expressed by activated T cells with their ligands PD-L1 (B7-H1) or PD-L2 (B7-DC) and CD80 (B7-1) or CD86 (B7-2), respectively, on APCs and nonhematopoietic stromal cells maintains peripheral tolerance and facilitates cessation of immune responses (Nurieva and Liu, et al., 2009). However, cancer cells are also able to express these ligands so that they can conceal the efficient induction of antitumor T cell responses (Dong et al., 2002; Yang et al., 2014). In fact, the inhibition of PD-1/

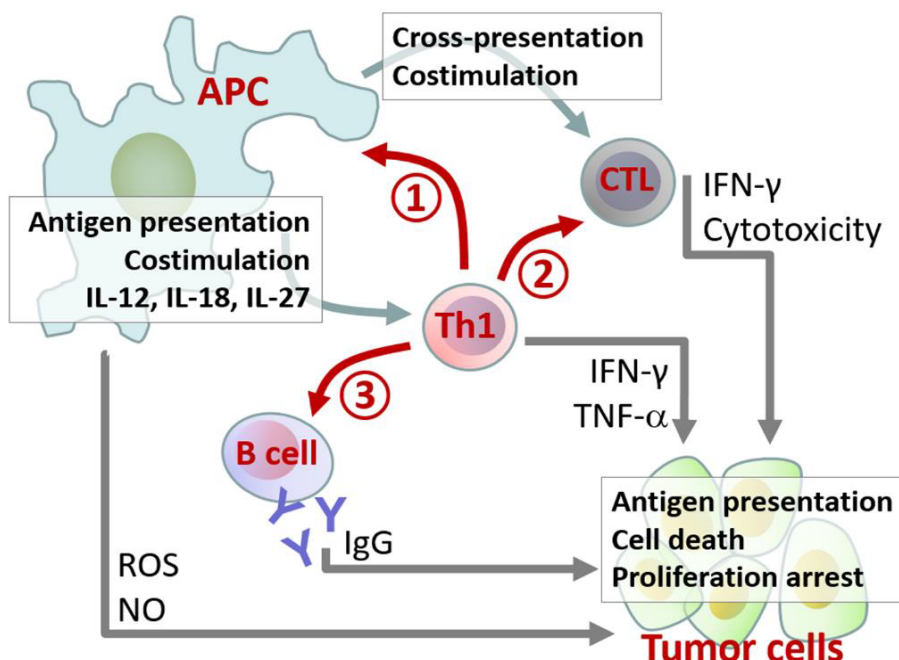


Figure 1. Principal roles of Th1 cells in destructive responses against transformed cells. ① Upon generation of Th1 cells through a strong and stable cross-talk with antigen-presenting cells (APCs) in the presence of Th1-inducing cytokines and potent costimulatory signals, Th1 cells complete the positive-feedback loop by producing cytokines (e.g., IFN- γ , TNF- α) and ligands (e.g., CD40L) that promote APC activities. ② Following the interaction with Th1 cells, APCs can more efficiently activate cytotoxic T cells (CTLs) through cross-presenting antigens and improved costimulation. Additionally, cytokines such as IL-2 directly provided by Th1 cells boost the proliferation and effector capacity of CTLs. ③ Th1 cells, especially via IFN- γ and CD40L expression, induce IgG class-switching and antibody production in B cells. In response to all these Th1-orchestrated inflammatory responses, tumor cells increase antigen presentation, decrease proliferation kinetics, and are eventually eliminated (ROS, reactive oxygen species; NO, nitric oxide).

PD-L1 interaction in several cancers such as melanoma, renal, lung, colon, breast, and sarcoma cancers results in elevated antitumor immunity and reduces tumor growth (Pilon-Thomas et al., 2010; Zhou et al., 2010; Pardoll, 2012; John et al., 2013). It has been well documented that IFN- γ is one of the factors responsible for the expression of PD-1 ligands on tumor cells (Abiko et al., 2015). Collectively, the instruments developed for controlling the collateral damage and immune pathologies are deployed in response to the destructive immunity centrally mediated by Th1 responses. Like untransformed cells of the body, tumor cells take advantage of this negative feedback mechanism and push the antitumor immunity away.

Cancer cells and/or tumor-infiltrating myeloid cells express certain enzymes that deprive the essential amino acids, e.g., indoleamine 2,3-dioxygenase (IDO) and arginase I, catalyzing the degradation of L-tryptophan and L-arginine, respectively, required for T cell expansion (Muller and Prendergast, 2005; Braumuller et al., 2013). Intriguingly, these cells produce certain amounts of NO, a tumoricidal mediator. However, NO appears to have a bimodal effect as it can also restrict the immune activity

(Gabrilovich and Nagaraj, 2009; Mantovani et al., 2009). It has been evidenced that in the presence of Th1 activity, and especially IFN- γ , both IDO and iNOS levels are increased (Munn and Mellor, 2007; Gabrilovich and Nagaraj, 2009). Thus, this metabolic dysregulation hampers both the initiation and the augmentation of T cell-mediated antitumor immune responses (Munn and Mellor, 2007; Vignali et al., 2008; Gabrilovich and Nagaraj, 2009).

During the resolution of inflammation, approximately 90%–95% of the effector T lymphocytes die by apoptosis and the survivors differentiate into long-term memory cells (Kaech et al., 2002; Wherry and Ahmed, 2004). In addition to the inhibitory signals and AICD, for the T lymphocytes under continuous stimulation, an alternative strategy works as an automatic emergency break to control destructive immune responses. Upon chronic exposure to specific antigens, T lymphocytes can undergo functional hyporesponsiveness and fail to wipe cancer cells out (Vignali et al., 2008; Speiser et al., 2014). Here, the stimuli are provided by the tumor antigens, whose levels increase as the disease progresses. This phenomenon is called “T cell exhaustion”. Even though CD8⁺ T lymphocytes have

been demonstrated to act in the direct elimination of virus-infected or malignant cells, recently data started to emerge on the importance of Th1 cell functions under chronic inflammation (Goding et al., 2013; Perreau et al., 2014; Kong et al., 2015). Cytotoxic responses are facilitated and reinforced through the actions of the Th1 subset. Furthermore, CD8⁺ T cell exhaustion is avoided where Th1 cells preserve their fitness (Hunziker et al., 2002; Church et al., 2014). Correspondingly, for the immune system, it becomes even harder to manage infections and cancer where Th1 cells are exhausted.

In an inflammatory scenario ending with T cell exhaustion, initially generated effector cells gradually lose their functions in a hierarchical manner as the immunogen persists (Wherry et al., 2003). Certain properties such as IL-2 production, cytotoxicity, and proliferation are forfeited at first, while TNF- α production is diminished later on (Wherry et al., 2003). Finally, at an advanced stage of exhaustion, IFN- γ production is lost (Wherry et al., 2003). In the most severe situation with high levels of antigen and absence of Th1 help, antitumor immunity becomes completely impaired or T lymphocytes are devoid of effector functions, and they can even be deleted (Matloubian et al., 1994; Ou et al., 2001; Fuller and Zajac, 2003; Wherry et al., 2003; Fuller et al., 2004).

Exhaustion is mainly constituted by immune regulation in which soluble factors (e.g., IL-10), immune regulatory cells (e.g., regulatory T cells), and inhibitory receptors such as programmed cell death-1 (PD-1) play fundamental roles (Freeman et al., 2006). Even though there are some shared expressions of certain inhibitory

receptors, they can be transiently found on effector T cells during activation. Both CD8⁺ and CD4⁺ exhausted T cells are marked by the stable expression of multiple inhibitory receptors such as PD-1, CTLA-4, T-cell immunoglobulin and mucin domain-containing protein-3 (TIM-3), CD160, 2B4 (CD244), lymphocyte activation gene (LAG)3, and B and T cell lymphocyte attenuator (BTLA) (Virgin et al., 2009; Legat et al., 2013). Collectively, in exhaustion, effector functions of Th1 cells are repressed by the actions of a great variety of inhibitory receptors assuring their hyporesponsiveness. The tumor escape strategies in Th1-mediated immune responses are depicted in Figure 2.

6. Conclusion

Although it is difficult to overcome cancer due to its ability to evade immune system attack and acquire multiple resistance mechanisms, tumor immune surveillance, whereby immune cells can recognize and eliminate the newly transformed cells, is a critical process to protect the host from tumorigenesis. Nonetheless, these immune responses are inflammatory and should be ceased to protect the host and maintain homeostasis, and Th1 cells play central roles both in elimination and evasion of transformed cells. Having different outcomes of adoptive TIL therapy dominated by CD4⁺ T cells could be due to Th1 antitumor versus protumor functions. As such, a very small percent of the patients who receive adoptive TILs dominated by CD4⁺ T cells have tumor regression while others have bad clinical outcomes (Powell et al., 2005; Prieto et al., 2010; Wu et al., 2012). Therefore, it is pivotal to understand these cells' behavior and discover potential

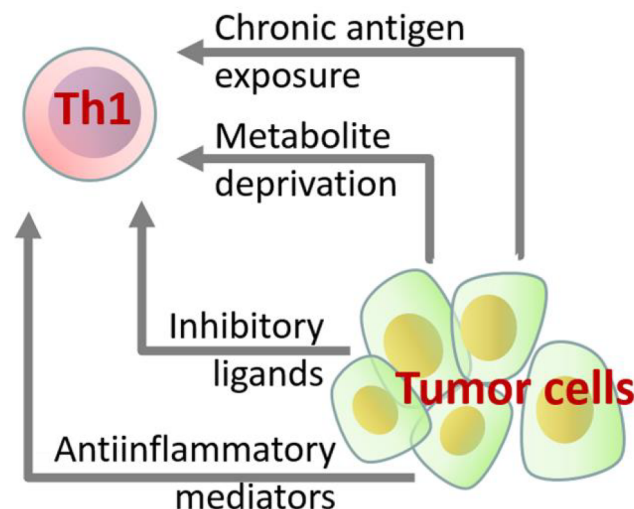


Figure 2. The factors utilized by tumor cells to evade Th1-oriented immune responses. Expression of inhibitory ligands (e.g., PD-L1, PD-L2), secretion of antiinflammatory mediators (e.g., IL-10 and TGF- β), deprivation of essential metabolites for type 1 effector functions (e.g., tryptophan metabolism via IDO), and continuous exposure to tumor antigens leading to a hyporesponsive state are general strategies for cessation of destructive responses in order to avoid immunopathologies.

approaches to intervene in their struggle against cancer, either by employing other approaches as combination therapies, such as radiation therapy, chemotherapy, or

targeting costimulatory pathways, or by selecting patients for personalized therapies.

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