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PERSPECTIVE

Adequate Antigen Availability: A Key Issue for Novel Approaches to Tumor Vaccination and Tumor Immunotherapy

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Abstract A crucial parameter for activation of the anti-tumor immune response is an adequate antigen availability (AAA) defined here as the optimal tumor antigen dose and related antigen processing and MHC-II-restricted presentation necessary to efficiently trigger tumor-specific TH cells. We will discuss two distinct experimental systems: a) a preventive anti-tumor vaccination system; b) a therapy-induced anti-tumor vaccination approach. In the first case tumor cells are rendered constitutively MHC-II+ by transfecting them with the MHC-II transcriptional activator CIITA. Here AAA is generated by the function of tumor's newly expressed MHC-II molecules to present tumor-associated antigens to tumor-specific TH cells. In the second case, AAA is generated by treating established tumors with neovasculature-targeted TNFα. In conjuction with Melphalan, targeted TNFα delivery produces extensive areas of tumor necrosis that generate AAA capable of optimally activate tumor-specific TH cells which in turn activate CTL immune effectors. In both experimental systems tumor rejection and persistent and long-lived TH cell anti-tumor memory, responsible of defending the animals from subsequent challenges with tumor cells, are achieved. Based on these and other investigators' results we propose that AAA is a key element for triggering adaptive immune functions resulting in subversion from a pro-tumor to an anti-tumor microenvironment, tumor rejection and acquisition of anti-tumor immune memory. Hypotheses of neuro-immune networks involved in these approaches are discussed. These considerations are important also for the comprehension of how chemotherapy and/or radiation therapies may help to block and/or to eradicate the tumor and for the construction of suitable anti-tumor vaccine strategies.

Keywords Tumor vaccination · Tumor immunotherapy · MHC class II transactivator · CD4+ T helper cells · MHC class II-restricted tumor antigen presentation

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Introduction

A complex series of events are responsible for the generation and spreading of cancer cells. These include intrinsic modifications of the tumor cells such as genetic mutations in proto-oncogens and in tumor suppressor genes, which cumulatively impact on the homeostasis of cell cycle and on the mechanisms controlling apoptosis (Hanahan and Weinberg 2000), and the efficiency of host response against tumor cells. Within the latter category, certainly the immune response against the tumor play an important role and components of both innate and adaptive immunity have been shown to participate to this response (Dunn et al. 2004). Nevertheless the fact that the tumor takes off in cancer patients demonstrates that tumor may elude immune defences (Rosenberg et al. 2004; Vesely et al. 2011). Tumors are not simple entities. Tumors are tissues composed of tumor cells, tumor stroma and often of a series of blood-derived infiltrating leukocytes including cells of innate and adaptive immunity (Jochems and Schlom 2011). Extensive studies have demonstrated that tumor-infiltrating leukocytes, including neutrophils, eosinophils, mast cells, macrophages, (de Visser et al.

2006; Sica et al. 2008) as well as T cells with CD4+/CD25+ phenotype and suppressive function on helper and effector T cells, designated regulatory T cells (Tregs) (Nishikawa and Sakaguchi 2010; Sakaguchi et al. 1995), can cooperate in favouring, instead of antagonizing, tumor growth. These findings have created the diffuse belief that a pro-tumor polarization of the innate and adaptive immunity is the cause for tumor cells to survive, replicate and spread (Mantovani and Sica 2010; Ruffell et al. 2010).

However, as seen from the side of the adaptive immune response, the above events can be interpreted not as the cause but simply as the consequence of the tumor strategy to primarily counteract components of the acquired immunity, particularly T lymphocytes as these cells, including CD4+ T helper (TH) and CD8+ cytolytic T lymphocytes (CTL), are mostly involved in the anti-tumor response. In other words, if the specific immune response against the tumor is put on brake, then almost by default all other mechanisms of immunity cannot react satisfactorily and the tumor takes off.

The purpose of this review is to re-establish the role of the acquired immune response, that is specific antigen presentation, stimulation of antigen-specific TH cells and generation of antigen-specific effector T cells, as the leading mechanism of defence against cancer. This principle is of paramount importance if strategies of either preventive or therapeutic vaccination against tumors should be pursued, because adaptive immunity with its capacity to produce specific responses, long lasting protection and memory, is indeed the final goal of vaccination.

One of the most important steps in constructing a protective adaptive immune response is the initial phase of antigen recognition. Many elements, tumor-related and leukocyte-related, may be evoked to justify a scarce initial activation of tumor-specific T lymphocytes (see Fig. 1). Our focus will be mainly on one of this element, the tumor antigen presentation to CD4+ TH cells, because we believe this aspect is crucial in orchestrating all the downstream events leading to an effective anti-tumor adaptive immune response. Here we introduce the concept of "Adequate Antigen Availability" (AAA), intended not only as quantitative tumor antigen production but also as optimal MHC class II (MHC-II)-dependent tumor antigen presentation to efficiently trigger TH cells and, by this, the downstream cascade of adaptive anti-tumor immunity to counteract not only tumor onset but also established tumors. This concept has important consequences not only for conceiving suitable anti-tumor vaccines but also for understanding how chemotherapy and/or radiation therapies may help to block and/or to eradicate the tumor.

Inefficiency of adaptive immunity in tumor-bearing hosts

It has been widely shown that immune cells with specificity for tumor-associated antigens can be found in cancer patients. Based on the groundbreaking studies of Festeinstein and colleagues (Hui et al. 1984), who demonstrated that CTL play a major role in the elimination of tumor cells, most tumor immunologists focussed initially their attention on the presence of CTL in tumor-bearing hosts. The concept of targeting CTL responses has been based on the observation that most cells express MHC-I, the cell surface molecule serving as receptor for peptides derived from endogenously synthesized proteins which are recognized by CTL. Indeed, tumor-specific CTL can be isolated from tumor tissues, they can be amplified in vitro and re-injected into hosts in which, at least in experimental animal models, they can inhibit tumor take or even cure established tumors (De Plaen et al. 1997). On this basis, clinical trials using CTL-defined antigens as vaccines have been performed. In most studies, however, CTL responses were weak and unable to control tumor growth and metastasis (Rosenberg et al. 2008). It was soon apparent that this event was due not only to the frequent loss or reduced expression of MHC-I molecules in tumor cells (Garcia-Lora et al. 2003; Garrido et al. 2010; Kageshita et al. 1999) but also to the poor tumor specific, MHC class II (MHC-II)-restricted T cell help generated in tumor-bearing patients (Huang et al. 1994; Ossendorp et al. 1998), since TH cells are fundamental for optimal induction of both humoral and cellular effector mechanisms (Pardoll and Topalian 1998) and particularly for CTL maturation, clonal expansions and acquisition of cytolytic function (Hung et al. 1998).

TH cell triggering requires recognition of antigenic peptides presented by MHC-II molecule expressed on professional antigen presenting cells (APC) including dendritic cells, macrophages and B cells (Germain and Margulies 1993). Eluding the crucial phase of MHC-II-dependent tumor antigen presentation to and/or activation of TH cells would thus be an effective strategy to block the onset of the adaptive anti-tumor immune response.

Adequate antigen availability: a key element for generating an efficient anti-tumor immunity

In order to obtain a suitable anti-tumor TH cell response, an adequate antigen availability (AAA), must be provided. The nature of the antigen presenting cells is not specified here intentionally, because this role could be played, at least in part, by the tumor cells themselves. We will consider recent results of our group and of other investigators to put forward the concept that AAA is indeed a key element for generating the cascade of immune effector functions and polarizing signals that result in tumor rejection and acquisition of anti-tumor immune memory.

Almost five decades ago Mitchison demonstrated the importance of antigen dose in triggering an adaptive immune response (Makela and Mitchison 1965; Mitchison

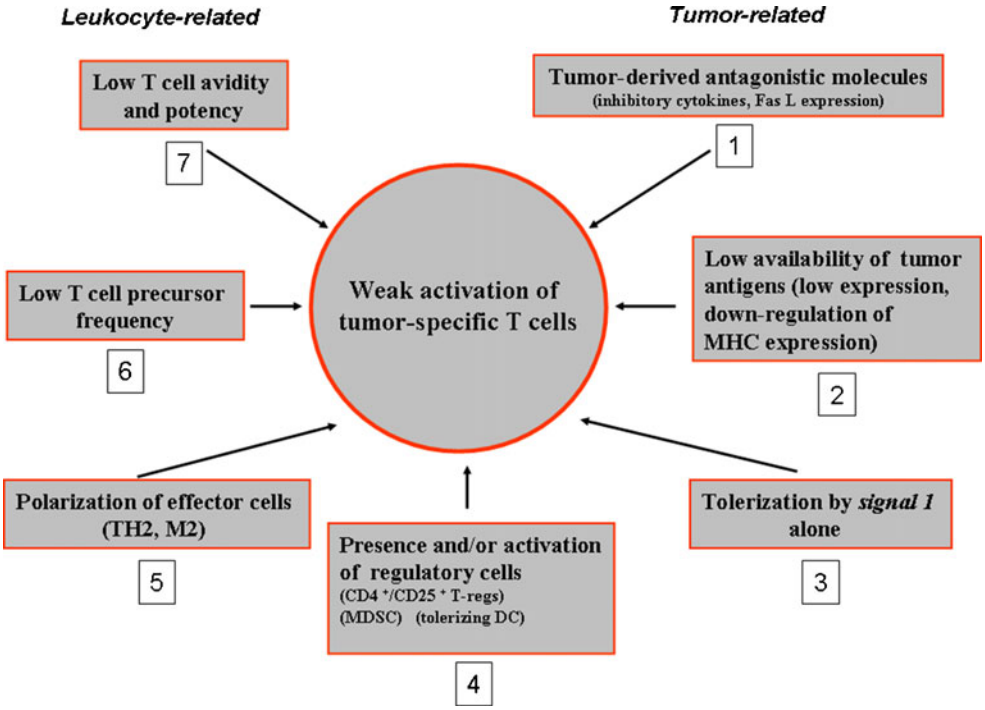

Fig. 1 Elements that can contribute to the inefficient adaptive immune response against cancer. T lymphocytes (including TH and CTL) are considered as the major target subpopulation whose altered/limited function can lead to inefficient anti-tumor adaptive immunity. Weak activation of tumor-specific T cells can result from either tumor-related properties and/or products (1, 2, 3) or leukocytes-related characteristics (4, 5, 6, 7). Abbreviation used: T-regs, regulatory T cells; MDSC, myeloid-derived suppressor cells; DC, dendritic cells; TH2, T helper 2-polarized cells; M2, macrophages polarized toward an M2 phenotype

1964) by showing that low amounts of protein antigen would never trigger an immune response and might even be tolerogenic. The original Mitchison's "low antigen dose-low immune response" paradigm was recently adapted and up-dated by Zinkernagel and coll. who elaborated the concept that for tumor antigens not only the quantity but also the "geographic availability" is crucial to instruct the adaptive immune system (Zinkernagel et al. 1997). Should cells of adaptive immunity not have the chance to encounter the tumor cells because the tumor remains localized at sites out of contact with immune cells, then the tumor will be neglected, ignored by the immune system.

Preventive anti-tumor vaccination and adequate antigen availability: the strategy of CIITA-induced MHC class II expression in tumor cells

In recent years, many attempts have been made to construct preventive anti-tumor vaccines by using as a primary source of antigen whole tumor cells treated by different procedures to make them more immunogenic. Irradiated or genetically modified tumor cells have been used even in clinical trials (Dranoff 2004). These approaches, however, were mostly focussed on providing host's APC with tumor antigens or to generate within the tissue injected with tumor cells a suitable milieu for optimal APC (mostly dendritic cells (DC)) uptake and presentation of tumor antigens via the APC MHC class II molecules.

As a different approach to obtain AAA for triggering the adaptive anti-tumor immune response, a vaccination strategy with tumor cells transduced with the *AIR-1*-encoded MHC-II transactivator CIITA, discovered in our laboratory (Accolla et al. 1986; Accolla et al. 1985b; Accolla et al. 1985c; Steimle et al. 1993) has been explored. The rationale underlying this approach was based on the evidence that, beside controlling MHC-II gene expression, CIITA may positively act on other crucial steps of the antigen processing and presentation mechanism, such as MHC-II transport in endocytic compartments via its function on the upregulation of Invariant chain expression (Accolla et al. 1985a; Meazza et al. 2003) and on antigen loading on MHC-II molecules via its upregulation on the MHC-II accessory molecule DM (Harton and Ting 2000). Thus the idea was that CIITA-transfected tumor cells may act as "surrogate APC" via MHC-II-restricted tumor-associated antigen presentation to tumor-specific TH cells for their optimal triggering. This in turn would facilitate priming and activation of tumor-specific CTL, hopefully resulting in tumor cell killing (see Fig. 2).

Crucial to this approach were the assumptions, verified by previous elegant studies, that endogenous proteins, as most tumor antigens are, could access the MHC class II pathway of antigen presentation (Rudensky et al. 1991; Schimd et al. 2007) and that peptides of these proteins could be recognized and serve as immunogen for TH cell triggering (Chicz et al. 1993; Jaraquemada et al. 1990).

We could demonstrate that efficient rejection of CIITA-transduced tumor cells of distinct histological origin can be achieved in high percentage of injected immunocompetent syngeneic mice (Frangione et al. 2010; Meazza et al. 2003; Mortara et al. 2006). Furthermore, it was shown that CIITA-tumor vaccinated mice develop an anamnestic response

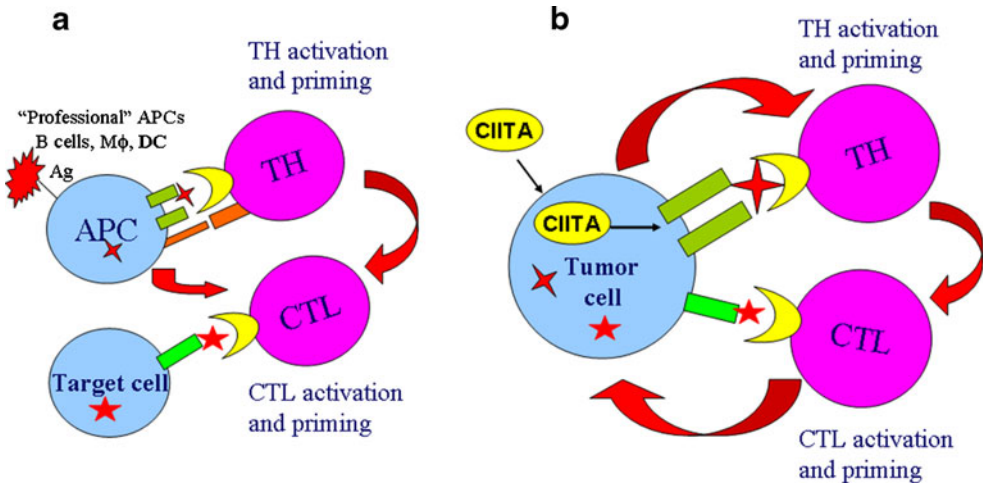

Fig. 2 A model to explain how CIITA-induced MHC class II expression in tumor cells may render these cells "surrogate" APC of their own tumor-associated antigens for MHC-II-restricted TH cells. **a** professional APC, such as dendritic cells (DC), macrophages (Mϕ) and B cells, present in an MHC-II (❚❚)-restricted fashion, processed antigens to CD4+ TH cells. Initial priming of TH cells is mainly due to DC-mediated antigen presentation. TH activation is required to induce optimal activation and effector function of CD8+ naïve CTL that, upon recognition of antigen presented by MHC class I molecules (❚) on target cells may exert their cytolytic activity. **b** transfection of CIITA into tumor cells renders them MHC class II-positive and thus potentially suitable to act as surrogate APC for tumor-specific, MHC-II-restricted TH cells. In turn, activated TH cells induce optimal priming and activation of tumor-specific CTL precursors that will kill the same tumor cells in an MHC-I-restricted fashion

upon challenge with the parental tumor leading to a very efficient rejection of the parental tumor (Frangione et al. 2010; Meazza et al. 2003; Mortara et al. 2006). That CIITA-dependent MHC class II expression in tumor cells was instrumental to trigger the anamnestic protective immune response against the parental tumor, was also demonstrated by vaccination experiments with non replicating CIITA-transfected tumor cells (Mortara et al. 2009). Rejection and/or reduced tumor growth were mediated by tumor specific CD4+ TH and CD8+ CTL (Frangione et al. 2010; Meazza et al. 2003; Mortara et al. 2006; Mortara et al. 2009). Importantly, we could demonstrate that tumor-specific, primed TH cells were long-living memory cells and they could adoptively transfer resistance to tumor take and/or tumor growth even after many months from original stimulation (Frangione et al. 2010), indicating the stabilization of a protective phenotype of effector cells over time.

Critical insight on the mechanisms triggered by CIITA-transfected tumor cells and their possible role as surrogate APCs was provided by the comparative analysis of the tumor microenvironment and of tumor draining lymph nodes in mice injected with parental or with CIITA-transfected tumor cells (Mortara et al. 2006). Tumors derived from parental cells showed little infiltrate, represented mainly by macrophages and neutrophils, very few $CD4^+$ T cells and absence of DC and $CD8^+$ T cells. In contrast the site of CIITA-transfected tumor cell injection was rapidly infiltrated by $CD4^+$ T cells. This was followed 2 to 4 days later by the appearance of DC and CD8+ T cells and by the generation of extensive areas of tumor cell necrosis. Thus $CD4^+$ T cells colonized CIITA-tumor tissue before $CD8^+$ T cells and DC. This finding, along with the capacity of CIITA-expressing tumor cells to process and present antigenic peptides to $CD4^+$ T cells in vitro (Mortara et al. 2006; Sartoris et al. 1998), supports the hypothesis that much of the tumor-specific TH cell triggering and/or restimulation takes place in the tumor tissue itself and is directly mediated by tumor cell-derived MHC class II molecules.

Interestingly, CIITA-tumor vaccinated mice displayed a polarized CD4+ TH1 cell phenotype in tumor-draining lymph nodes, as compared to a TH2-like cell phenotype found in parental tumor-bearing mice. Moreover, the fact that CIITA-transfected tumor cells could trigger a potent anamnestic and persistent anti-tumor T cell response without an apparent sequel of autoimmunity, suggests that most of the anti-tumor response was directed against tumor-and not self-derived antigens.

The success of this strategy underscores the importance of an adequate MHC-II-restricted antigen presentation, suggests that most tumor cells produce sufficient amounts of tumor antigens and renders unnecessary to know a priori the nature, the identity and the immunogenic hierarchy of the tumor-associated antigen(s). In conclusion, inducing appropriate MHC-II expression in tumor cells may be a way to satisfy the requirement of AAA.

Therapeutic anti-tumor vaccination and adequate antigen availability: the strategy of L19-TNFα conjugate treatment of established tumors

Immune-based tumor therapy approaches are presently an intensive area of investigation for their potential and

promising application to cancer patients. We have performed experiments of tumor therapy in animal models by using mouse TNFα (mTNFα) covalently bound to a Fv antibody (L19) specific for the beta form of Fibronectin, selectively expressed in tumor neovasculature (Borsi et al. 2003). Injection of L19mTNFα conjugate induces a dramatic necrosis of established tumors as it allows concentrating therapeutically active doses of TNFα at the tumor site. When combined with the cytostatic drug melphalan, this treatment dramatically potentiated the effect of melphalan at the tumor site. Moreover L19mTNFα increases intravasal coagulation and trombosis around the tumor further favoring tumor necrosis. The effect of TNFα on vascular permeability and on upregulation of adhesion molecules triggers the inflammatory response at the tumor site by facilitating leukocytes extravasation and dendritic cell migration through lymphatic vessels (see Fig. 3).

Two crucial observations were made in this approach which underline the importance of the adaptive immune response against the tumor and are relevant for the AAA hypothesis. First, a high rate of complete and long-lasting tumor eradication in distinct tumor models, without any apparent adverse side effects and with no recurrence, was observed. Second, tumor-bearing immunodeficient SCID mice were partially resistant to the L19mTNFα treatment even when combined with melphalan (Balza et al. 2006). This prompted us to analyze whether the cured mice developed a tumor-specific immunity. Indeed, all cured mice were resistant to tumor challenge; the tumor rejection was mediated by CTL and, particularly, by long-living, tumor specific TH cells, whereas B cells and NK cells did not play any significant role (Balza et al. 2006). TH cells were crucial for the establishment of what we defined as "therapy induced anti-tumor vaccination" because naive mice depleted of TH cells were unable to reject primary tumors after L19mTNFα/melphalan treatment (Mortara et al. 2007). Moreover, TH cells derived from cured animals treated with L19mTNFα/melphalan, while fully competent in generating tumor rejection when adoptively transferred together with tumor cells in naive mice, were incapable of inducing tumor rejection in CD8-depleted naïve animals, strongly suggesting that a major protective role of primed anti-tumor CD4+ T cells lies in triggering CD8+ naive T cells to become functionally mature antitumor CTL effectors (Mortara et al. 2007). Altogether these findings indicate that the L19mTNFα/melphalan treatment was crucial in generating tumor AAA to efficiently trigger, in an MHC-II-restricted fashion, tumor specific TH cells that subsequently triggered naïve CTL precursors and sustained their cytolytic anti-tumor effector function (see Figs. 3 and 4).

Immunohistochemical characterization of the tumor tissue early after treatment revealed the presence of areas of necrosis with infiltration of granulocytes and macrophages. One week after L19mTNFα/melphalan treatment a remarkable increase in the number of CD4+ and CD8+ T cells infiltrating the tumor was observed as compared to tumors from untreated animals. This was accompanied by a drastic increase in granulocyte infiltration and by the enlargement of areas of tumor necrosis (Mortara et al. 2007). Thus, the tumor necrosis induced by L19mTNFα/melphalan and the earlier infiltration of granulocytes contributing to tumor cell killing could be the important element to generate Adequate Antigen Availability for fuelling professional APC. These APC could then stimulate specific antitumor CD4+ TH cells and CD8+ effector CTL, leading to the complete rejection of the tumor and to the establishment of a critical reservoir of memory effector cells responsible for the accelerated rejection of the tumor upon challenge. Interestingly, more refined phenotypic and functional characterization of the CD4+ T cells involved in the priming of the anti-tumor immune response following therapeutic treatment revealed that, while untreated tumor-bearing mice had in their spleen and tumor-draining lymph nodes IL-4-secreting TH2-type cells, treated mice displayed a mixed TH1- and TH2-type of response with a large percentage of cells secreting IFNγ (Balza et al. 2010; Mortara et al. 2007). Thus, likely the preventive vaccination approach with CIITA-expressing tumor cells, a rapid appearance and conversion toward a TH1 immune phenotype was associated to the protective anti-tumor immune response generated by the treatment with L19mTNFα and melphalan. This preliminary in vivo experimental investigation has paved the way to initiate clinical trials with L19mTNFα and melphalan in patients with selected tumors (Papadia et al. 2012)

Neuro-immune networks and their potential exploitation against the tumors

A relevant observation of our studies was that both in the therapy-induced vaccination generated by treating tumor-bearing hosts with TNFα and melphalan and in the preventive CIITA-tumor vaccination approach a strong reduction of CD4+CD25+ regulatory T cells (Tregs) in tumor-draining lymphonodes was observed (Mortara et al. 2007; Mortara et al. 2009). This suggests that the anti-tumor polarization generated by the two treatments counteracted or prevented the increase and/or the recruitment of Tregs in tumor-draining tissues. Therefore modulating the number and/or the function of Tregs may act as an important "adjuvant" to synergize the protective effect of our vaccination strategies. Within this frame, neuro-immune interactions may play an important role. Indeed, it has been recently shown that Tregs express receptors for neurotransmitters and particularly dopamine (DA) receptors (DARs) (Cosentino et al. 2007). Moreover DA, together with norepinephrine (NE)

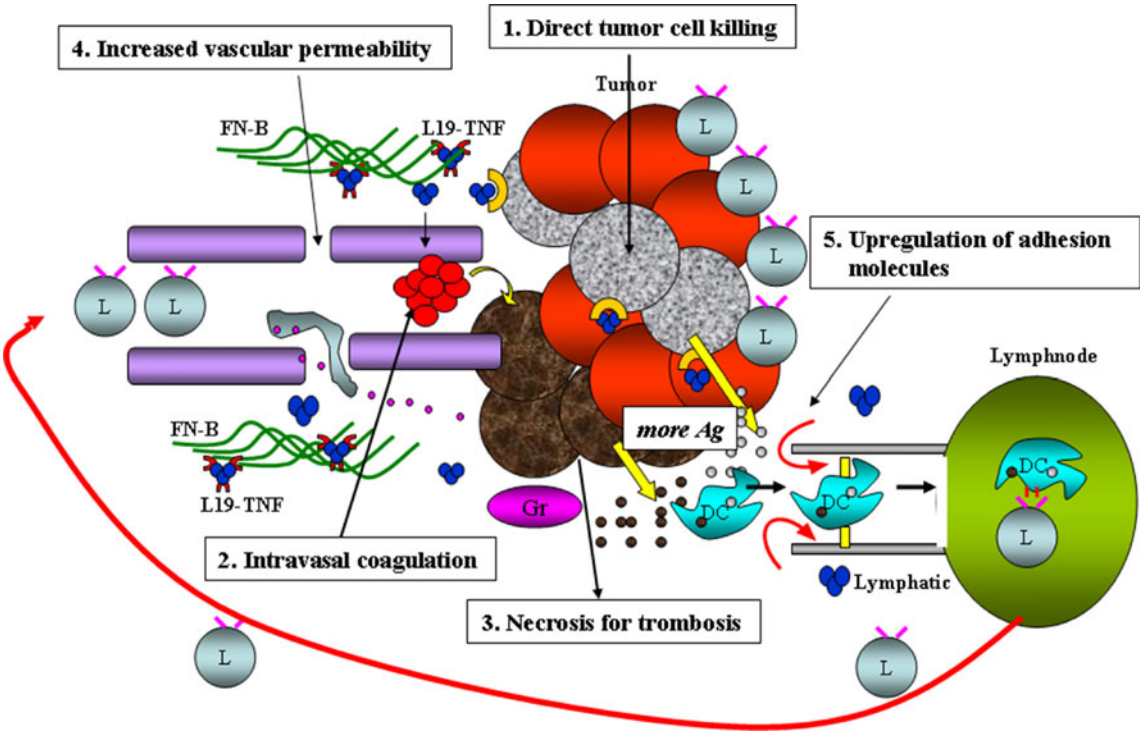

Fig. 3 Major biological effects of TNFα relevant for the triggering of the adaptive immune response against the tumor: a possible scenario. TNFα, targeted to tumor neovessels by covalent binding to a Fv antibody (L19) specific for the beta form of Fibronectin (FN-B), selectively expressed in tumor neovasculature, has direct access to the tumor mass in which may induce direct killing and necrosis (1). Increased concentration of TNFα favors intravasal coagulation and trombosis (2) with additional necrosis of tumor tissue (3). TNFα potently increases vascular permeability (4) with consequent extravasation of blood-derived leukocyte and initiation of an inflammatory reaction which recruits additional lymphocytes (L), dendritic cells (DC) and granulocytes (Gr) in the tumor area. DC capture necrotic cell debris rich in tumor-associated antigens and, via the upregulation of adhesion molecules (5) also contributed by TNFα, migrate to the tumor-draining lymphonodes where tumor-specific lymphocytes can be triggered and further sent via circulation to the tumor site where they can act as immune effectors against the tumor

and epinephrine (E), is also produced and utilized by immune cells themselves (Cosentino et al. 2003). In human lymphocytes the synthesis of DA, NE and E is induced upon activation through tyrosine hydroxylase (the first and rate-limiting enzyme in their synthesis), resulting in intracellular accumulation, release and subsequent action on lymphocytes themselves and/or neighbouring cells. It has been shown that Tregs constitutively express tyrosine hydroxylase and DARs and contain high amounts of DA, NE and E, which subserve an autocrine/paracrine loop involving DAergic D1-like (D5) receptors and resulting in down regulation of Treg inhibitory functions (Cosentino et al. 2007). The existence in lymphocytes of an endogenous DAergic/adrenergic system may thus represent a novel and so far unexploited target for the selective modulation of the immune response, particularly in cancer-bearing hosts undergoing immunotherapeutic approaches as the one described in the present paper. Indeed, while functional suppression of Treg is mediated by D1-like D5 receptors (Cosentino et al. 2007), physiological concentrations of DA can even drive resting human T cells into functional activation through D2, D3, and D1/D5 DA receptors (Besser et al. 2005). The use of selective D1/D5 agonists in vivo might thus result in functional activation of effector T lymphocytes both directly as well as through the functional suppression of Treg function. Interestingly, it ha been recently reported that beta2 adrenergic receptor (beta2-AR) expression is reduced in astrocytes in pathological states as Multiple Sclerosis (MS) (De Keyser et al. 2010). Astrocytes may express CIITA and thus MHC-II molecules and they may act as surrogate APC for autoantigen in autoimmune diseases like MS (Soos et al. 1998). Since stimulation of beta2-AR increases cAMP, leading to activation of protein kinase A (PKA) a suppressor of CIITA expression, beta2-AR deficiency in astrocytes of MS may result, in turn, in the reduction of the suppressive action of PKA on CIITA, potentially contributing to the perpetuation of the disease. Thus it would be important to investigate whether certain tumor cell types, susceptible to express MHC class II molecules, may express functional beta2-AR, because beta2-AR antagonists can be used as a potential additional tool to maintain MHC-II expression and surrogate tumor antigen presenting function in cancer patients.

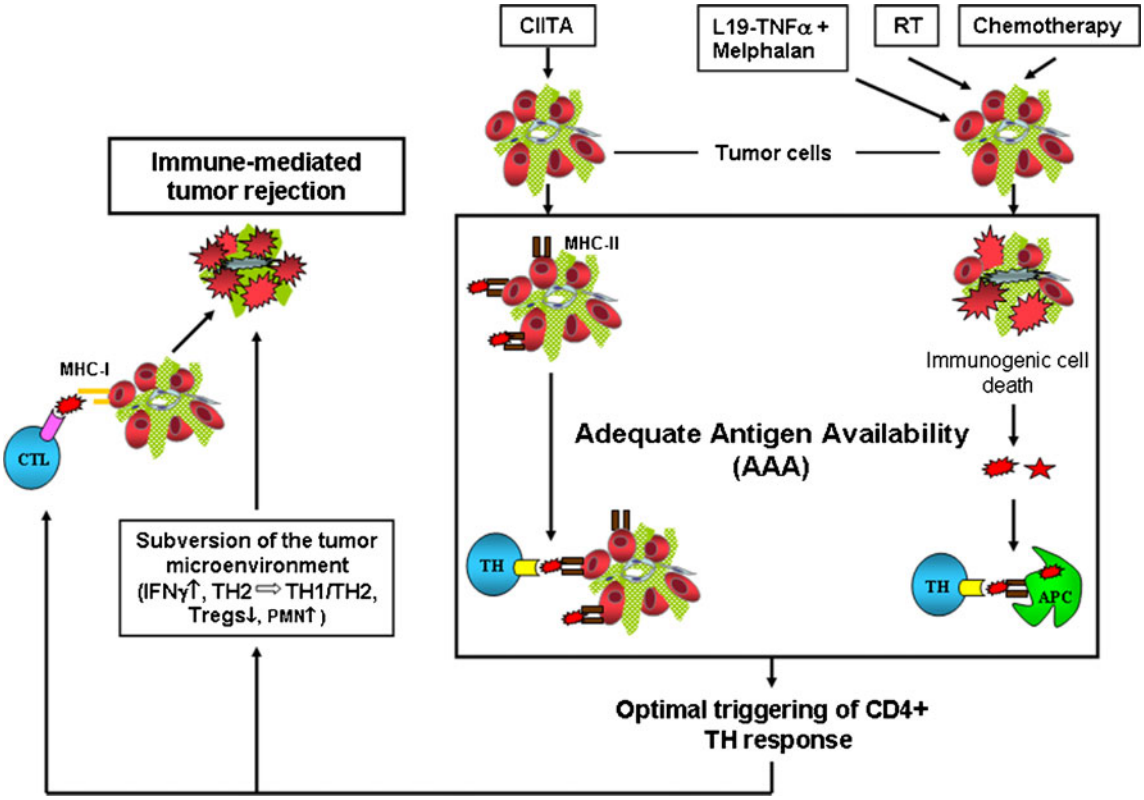

Fig. 4 Adequate Antigen Availability (AAA) as a key parameter for an effective anti-tumor adaptive immune response. AAA as defined in the text may result from either surrogate antigen presenting activity by tumor cells expressing CIITA-dependent MHC class II (MHC-II) molecules or by increasing the amount of tumor antigen availability for classical antigen-presenting cells (APC) via therapeutical approaches resulting in immunogenic cell death, such as radiotherapy (RT), chemotherapy, and biotherapy with, for example, L19-TNFα and melphalan (L19-TNFα + Melphalan). AAA generated by the two approaches is instrumental for the optimal triggering of anti-tumor CD4+ T helper cells (TH). These MHC-II-restricted anti-tumor TH cells have a dual action in the process of immune-mediated tumor rejection: **a** they are required for the activation, proliferation and cytolytic activity of CD8+ anti-tumor cytotoxic T lymphocytes (CTL); **b** they are major players in the subversion of the tumor microenvironment toward an anti-tumor milieu by polarizing, for example, the lymphocyte infiltrate toward a mixed TH1/TH2 or exclusive TH1 population with an increased frequency of IFNγ secreting cells, by favouring the recruitment of inflammatory cells such as polymorphonucleates (PMN), by inhibiting the function and/or the recruitment of leukocytes with suppressive action on TH cells and on tumor-specific CTL such as regulatory T cells (Tregs)

Radiotherapy and chemotherapy as procedures to potentially offer AAA

The experimental evidences gathered from our approaches of either preventive or therapy-induced anti-tumor vaccination clearly demonstrate that a subversion toward an anti-tumor microenvironment can be generated by promoting AAA not only before the tumor onset but also after cancer has developed (see Fig. 4).

In consideration of the above findings and taking into account that an AAA can be obtained in different ways, a large series of previously published studies on the appearance of an anti-tumor immune response following conventional anti-tumor therapy, may be re-interpreted also as a way to offer AAA to the adaptive immune system.

For example, tumor site-specific radiation therapy (RT), given alone (Takeshima et al. 2010) or in combination with immunostimulatory cytokines (IL-2 and TNFα) (Lee et al. 2000; Yamini et al. 2007) enhances its efficacy by generating anti-tumor specific CTL responses. RT can also increase CTL stimulation by up-regulating MHC class I expression in tumor cells (Lugade et al. 2005; Reits et al. 2006) and, more interestingly, by positively influencing antigen processing and presentation by dendritic cells (Liao et al. 2004). Similar findings have been reported also after treatment of localized tumors with thermoablative techniques (Haen et al. 2011)

Chemotherapy can also increase the immunogenicity of tumor cells which are recognized by immune effectors (Zitvogel et al. 2011). Central to this role of RT and Chemotherapy is the concept of immunogenic cell death that, induced by the therapeutical agent, finally ends up in the triggering of an adaptive immune response (Lake and Robinson 2005), a concept that can be easily accommodated within the hypothesis of AAA (see Fig. 4).

This will possibly help to better understand the initial phases of the immune response against tumors and offer potential new strategies to prevent and fight cancer.

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Conflict of interest The authors declare that they have no conflicts of interest.

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