Chapter 6

Discussion:

Local targets for Immune Therapy to Cancer: Tumor Draining Lymph Nodes and Tumor Microenvironment.

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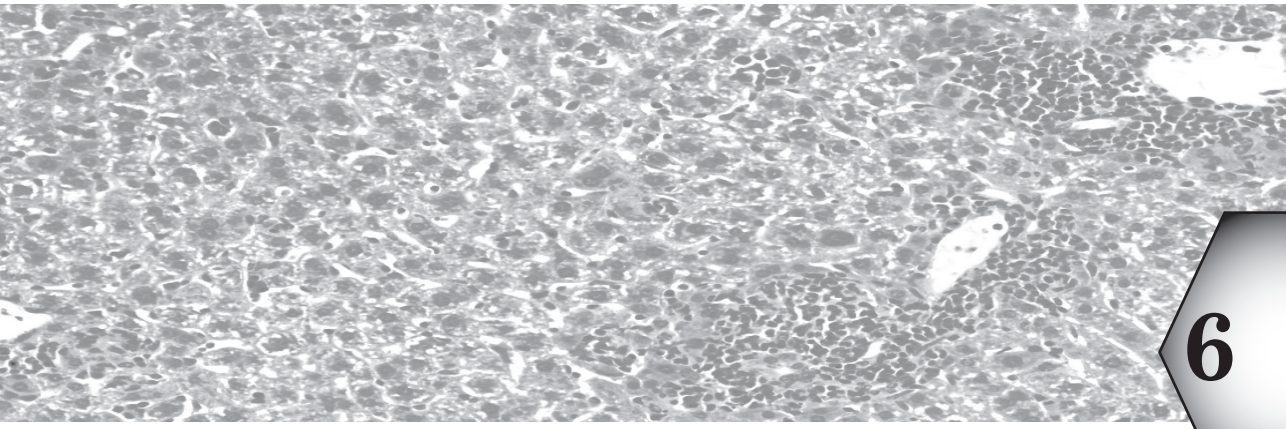

Abstract

In recent years, it has become apparent that in subjects with growing tumors, there is a balance between tumor-eradicating and tumor-promoting immunity. The key players in maintaining this balance are mainly present in the tumor microenvironment and the tumor-draining lymph node. Interventions aimed at shifting the balance towards tumor-eradicating immunity, are therefore most efficient when targeted directly to this area as shown in this thesis. As immune-modulating therapy has been shown to cause many adverse side-effects when administered systemically, we strongly advocate the further development of local treatment for tumor- immune therapy.

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Introduction:

Lymph nodes (LNs) are organs comprised of lymphoid cells that occupy strategic positions throughout the body and play a pivotal role in the immune system. LNs act as sentinels within the system, filtering the afferent lymph and bringing together cells of the innate and adaptive immune system interact, which in case of acute infection, leads generally to robust priming of naïve T cells. Tumor-draining LNs (TDLNs) have a dubious position as they can induce anti-tumor T cell responses but are at the same time under the direct influence of the tumor microenvironment and can act as route for malignant cells towards distant organ metastasis[1-3] Because of this, the controversial role of surgical removal of sentinel LNs has been a matter of debate for decades[4].

The concept of tumor immune surveillance, the potential of the immune system to keep the formation and outgrowth of malignant cells in check, has been described as early as 1891, by William Coley, and has waned and recurred in scientific publications several times over the last 120 years. The experimental evidence supporting the concept of immune surveillance is still growing (see for recent reviews Swann and Smyth[5] and Vesely MD et al[6]) and was recently extended by Schreiber and colleagues who described the association of tumor cells and lymphocytes actively inhibiting the formation and progression of transformed cells and ultimately causing selective evolution of tumor cells that can evade the immune response, a phenomenon called cancer immunoediting[7,8]. Based on this knowledge many tumor intervention treatments have been designed and studied involving immunotherapy in both pre-clinical models and clinical trials with varying successes and pitfalls. The potential for targeted immunotherapy to the tumor area and more specifically to the TDLN has been brought forward in recent years. In this review we would like to discuss the latest insights into tumor immune therapy and the strategies and advantages of local targeting.

Balancing induction and suppression of tumor immunity.

The growth of a tumor often coincides with both the stimulation of anti-tumor T cell responses and the parallel (and often unwanted) induction of immune suppression. Both processes take place mainly in the tumor area and TDLN. This balance between T cell priming and suppression is one of the key aspects in disease prognosis[9].

In mice the critical importance of the adaptive immune system, especially of T cells, to prevent tumor development was proven by sophisticated experimental tumor models involving the ablation of the specific regulators of the adaptive immune system[10-12]. In cancer patients, significant numbers of T cells specific to tumor associated antigens have been identified and found to be correlated with improved prognosis[9,13-17]. Tumor antigens are presented by APCs within

the tumor or are first carried from the tumor by tumor cells or APCs traveling through lymphatic channels to become cross-presented in LNs to T cells[18,19,20]. The APCs in the tumor mass and TDLN generally display a certain level of maturation due to the presence of endogenous danger signals from the growing tumor, such as heat shock proteins and uric acid from decaying tumor cells. Compared to pathogenic infections however, the APC maturation signals are much lower, which can lead to inadequate T cell priming[20-23]. Especially, the lack of costimulatory signals (e.g. CD80/86) has been linked to dysfunctional T cells. Likely, also the lower production of the pro-inflammatory cytokines IL-12 and IFN-gamma[20,21,23] contributes to the low state of T cell activation to tumor antigens. This phenomenon of inadequate T cell activation has led to different nomenclatures for these T cells including anergic T cells, division arrest T cells, incomplete differentiated T cells, dysfunctional T cells and tolerised T cells. In tumor settings anergic T cells are characterized by inadequate effector function such as the lack of cytolytic molecules (e.g. perforin or granzyme B), expression of low levels of IFN-γ and a division arrest phenotype, which all contribute to reduced capacity to kill tumor cells[19,24-28]. Besides lower "quality" of T cell priming by APCs, both animal models and human studies show that TDLN also harbor lower numbers of DC. Nevertheless, tumor specific T cells with full cytotoxic capacity have been described with respect to phenotype and function[9,25,29], suggesting that transformed cells can lead to proper T cell activation providing hope for immunotherapeutic strategies.

Immune suppression within the tumor microenvironment and TDLN is characterized by an unfavorable concoction of immunosuppressive cytokines, growth factors, and various suppressing cell populations. Well studied suppressive cytokines, produced by tumors or tumor associated macrophages, are IL-10, TGF-ß, VEGF and IL-6. Chemokines, such as CCL2 and CXCL8, secreted by monocytes and tumor associated macrophages, cause tumor progression, myeloid derived suppressor cell (MDSC) and macrophage infiltration and tumor angiogenesis. Effector T cell suppression is mediated by regulatory T cells, MDSCs and tolerogenic DC[21,22,30]. A special type of factor that inhibits the induction of pro-inflammatory immune responses is IDO (indoleamine 2,3 dioxygenase). IDO, expressed on plasmacytoid DC and some types of tumor cells, causing inhibition of T cell proliferation by enzymatically degrading tryptophan leading to tryptophan starvation. This can also lead to conversion of CD4+ T cells to Tregs in TDLN[31].

The increased presence of regulatory T cells (Tregs) in TDLN compared to non-draining LN has been well established in both animal models and cancer patients, in which accumulation of Tregs in DLN of colorectal cancer patients and not in tumor or peripheral blood is correlated with disease progression[22,30,32,33]. Treg

accumulation in tumor bearing animals can result from either proliferation of natural, thymic differentiated Tregs or conversion of naïve $CD4^+$ T cells into Tregs. The mechanisms of Treg suppression are not fully understood yet, but can include IL-2 deprivation, expression of CTLA-4, and secretion of IL-10 and/or TGF-ß. Recent publications showed that Tregs can also limit DC, NK and $CD8^+$ T cell numbers by direct granzymeB and perforin dependent killing in TDLN[31,34]. Further limiting the immune response is the fact that in elderly patients (the majority of cancer patients), the immune system has undergone aging[35]. This phenomenon, also called immunosenescence, is characterized by loss of immunocompetence which limits immune resistance not only to tumors but also to pathogens such as influenza virus, respiratory synticium virus, pneumococci and tubercle bacilli as well as to, chronic persistent viruses such as CMV. Therapies designed in animal models to boost the immune systems against tumors may be imperfect in elderly patients, because of this phenomenon, and more vigorous therapies or different strategies may be necessary. In this aspect, it is interesting to note that Belloni et al recently reported age-dependent differences in side-effects to systemic anti-IL10 receptor antibodies. IL-10 inhibition caused high mortality in older animals, whereas no mortality was observed in young animals. Since cancer patients are often older individuals, these results imply that systemically blocking the IL-10 receptor should be evaluated carefully[36].

Local Immune therapy: targeting the tumor micro-environment and draining lymph nodes.

Decrease of adverse side-effects by local treatment versus systemic treatment.

Recent reports describe the dangers of toxic side-effects of systemic immune activating treatments, emphasizing the need for more targeted therapies. Together with the growing evidence defining the local suppressive effects of the tumor microenvironment and the unique position of the tumor draining lymph node, this calls for exploring the potential of immune intervention strategies that act mainly locally.

Many different strategies have been proposed to re-activate the TDLN resident anergic T cells, and overcome tumor induced immune suppression, some of which specifically target the tumor, tumor draining area and/or tumor draining LN. Many of these strategies were first described in systemic applications of immunostimulatory strategies in experimental models and later in clinical trials. Numerous pre-clinical studies have described that such systemic therapies can overcome T cell anergy, either by activating DCs (using TLR-ligands or agonistic CD40 antibody), blocking inhibitory signals (blockade of CTLA-4, PD-1 or TGF-ß), or addition of pro-inflammatory cytokines (IL-12, IFN-α or IL-2) [37,38] resulting in tumor eradication. Clinical trials, however, did not show a similar

success rate in clearing tumors as observed in some animal models. Frequently, the relative dose of immune stimulating reagents used in rodents is higher then the maximum tolerated dose used in humans (correlated for body weight). Immunologists using animal models are often less focused on side effects than on efficacy. However, more researchers are starting to become aware that in order for pre-clinical animal models to be more representative to the human situation, lower doses of immune stimulating agents should be used, and toxic side-effects in animal models should be meticulously analyzed,[36,39,40] as described in chapter 2.

Moreover, systemic activation of the immune system can cause serious toxicity as shown in a number of clinical trials and animal studies. An example is the catastrophic clinical trial with CD28 superagonist TGN1412. Indeed, potent systemic activation of the entire immune system is unadvisable, and should be applied with utmost caution[41]. In many other studies, adverse events caused by systemic immune activation were dose-limiting and hampered the efficiency. Agonistic antibodies against CD40 and cytokines IL-12 and IL-2 have all been described to have potent effects in enhancing the anti-tumor T cell response, and all have been causing severe toxicity in patients after systemic administration[42-44]. Even GM-CSF administration, which is not directly immune activating and therefore contains a lower risk of causing toxicity, has been shown to have adverse effects when injected systemically. Serafini et al published a paper in which data was presented showing the increase in MDSC in subjects treated with high dose systemic GM-CSF, causing an impaired immune response[45].

Specific targeting of the tumor microenvironment.

One way of reducing systemic side effects is to target exclusively the tumor lymphoid drainage area. For instance, CpG, a toll-like receptor 9 ligand, injected locally enhances DC maturation and migration to TDLN[40,46-48]. When compared to other administration routes, local injection was superior in DC maturation, T cell priming and tumor eradication, in a preclinical model[48]. In a clinical trial, CpG was administered intradermally directly adjacent to the scar of melanoma resection, before the sentinel lymph node (SLN) resection, and the immune response was analyzed in the SLN and PBMC. Patients displayed higher numbers of DC in the SNL associated with upregulation of costimulatory molecules, increased release of pro-inflammatory cytokines and reduction in immunosuppressive Treg frequencies. Fifty percent of these patients had a measurable pro-inflammatory T cell response against melanoma specific tumor antigens in the SLN and in 40% of the patients, a T cell response was also found in blood. This therapy was well tolerated by patients. In another clinical trial, intratumoral injection of CpG was combined with low dose, local irradiation.

An increase in tumor specific T cells was detected in PBMC of patients, and objective responses were found[49 50].

Induction of inflammation in the tumor lymph node draining area leads to upregulation of several factors, like CCR7 on DC and CCL21 on lymphatic endothelial cells which in turn lead to enhanced migration of DC to the lymph node[51,52]. The influx of mature DC into the LN causes the lymph node to increase in size and cellularity, called reactive lymph node. The inflammatory state of the reactive lymph node influences the activation of T cells, as described recently. Especially important for memory recall responses, T cells that had developed in the presence of a reactive lymph node had a significant quantitative advantage over T cells in mice without a reactive lymph node[53](chapter 4). In animal models and clinical trials, genetically engineered tumor cells secreting GM-CSF, CTLA-4 blocking antibody or CCL20 (a DC attracting chemokine), have been studied as local treatment. By injecting the irradiated tumor cells close to the tumor, they serve as antigen and antibody secreting depot to the TDLN, and cause activation of effective anti-tumor T cell responses and tumor eradication, with lower treatment associated toxicity than upon systemic administration[54,55].

Previously we reported that targeting the tumor-draining area with a low dose of agonistic CD40 antibody in a slow-release formulation overcomes tumor-induced immune suppression and induces excellent systemic tumor-specific T cell responses capable of killing metastatic cells located elsewhere in the body. Local therapy therefore can thus lead to systemic responses, with only a fraction of the toxic side-effects[56](chapter 2). Local, slow-release administration of CTLA-4 blocking antibody is also capable of activating tumor-eradicating CD8+ T cells as a monotherapy, as described in chapter 3. This treatment severely reduced the serum-levels of CTLA-4 blocking antibody compared to systemic administration, reducing the risk of auto-immune related side-effects.

It is likely to assume that slow-release formulations are functional in targeting immune stimulating agents to the TDLN, because they keep the tumor-draining area, or regional basin, in a pro-inflammatory status for a prolonged period of time, allowing the T-cell response to fully develop and the immune suppression to remain blocked. In addition, the concentration of immune stimulatory agent remains high only locally and not systemically, thereby preventing undesirable side-effects and unspecific overstimulation. Slow-release formulations such as montanide ISA 51, have been studied for their efficiency in delivering immune modulating antibodies (such as anti-CD40) to the TDLN with strong systemic anti-tumor responses as a result, but no systemic toxicity[56](chapter 2 and 3). The discovery of several new sustained release systems, such as PLGA-based microparticles, opens up possibilities for targeted treatments which can be explored for tumor immunotherapy. [57,58] However, as described in chapter

5, dextran-based microparticles have unexpected local side-effects, causing enhanced tumor-outgrowth, making them inferior as slow-release delivery system to Montanide. Slow-release formulations should therefore be carefully analyzed for their suitability in tumor-area targeted therapy.
Another aspect that strengthens the use of local immunotherapy lies in the fact that many immunosuppressive mechanisms that inhibit tumor-specific effector T cell responses, as described before, are not uniquely operable in the tumor microenvironment, but are mechanisms that have evolved to keep the immune system from attacking self tissue. Interfering with these interactions on a systemic scale, therefore, is risky. Not surprisingly, examples of systemic immunostimulatory tumor immunotherapy causing severe autoimmunity are abundant [38,59,60].

Potential hurdles for local immunotherapy.
Recent studies have shown that elevated levels of MDSCs are present in cancer patients and tumor bearing mice. Since these cells are described to incite systemic suppression, rather then local suppression, targeting of the TDLN is not likely to overcome suppression by these cells[61,62]. Several studies mentioned in this review describing local targeting of the TDLN have been able to overcome local suppression by activating robust anti-tumor T cell responses, which are able to withstand systemic suppression and eradicate distant tumors. However, systemic suppression by MDSC was not analyzed in these studies, and might have been weak, where in other models, it could be stronger, and therefore harder to overcome.
Targeting TDLN might cause the practical problem of inaccessibility of a draining node, since in several types of cancer TDLN's are not within easy reach. In order to solve this problem, new approaches are being studied such as delivery of nanoparticles coupled to tumor-antigen-specific antibodies, which can be injected systemically but, deliver their immuno-modulating content selectively into the tumor from where it will eventually drain to the TDLN[40,46].

Concluding remarks.
The tumor microenvironment and especially the TDLN are the key locations for important anti-tumor immunological processes, and therefore the quintessential targets for immune-modulating therapies in tumor bearing subjects. Since both priming of tumor-specific T cell responses and immune suppression occur in this area, local therapies designed to balance this equilibrium toward more effective anti-tumor T cell response will be most efficient. Whether tumor eradication is most efficiently achieved by promoting the stimulation of DCs presenting tumor

antigens, enhancing tumor antigen presentation, abolishing immune suppressive pathways, or a combination of these, remains to be defined experimentally and clinically. Notably, since most of the tumor-immunotherapy strategies harbor the risk of causing serious toxicity and/or auto-immunity, targeting the TDLN and/or the tumor microenvironment instead of systemic administration should be a focus of future immuno-therapeutic strategies.

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