

Angiostasis as a way to improve immunotherapy

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Summary

Tumours express tumour-associated antigens that are recognised as self-antigens precluding the induction of effective anti-tumour immune responses. Inflammatory conditions which facilitate appropriate antigen presentation and reduce the immuno-suppressive micro-milieu may break tolerance. However, tumours have evolved mechanisms to escape cytotoxic T-cell attack by expressing inhibitory molecules on their surface, secreting suppressive factors, attracting regulatory T cells to the tumour environment or downregulating MHC molecules. Induction of angiogenesis by tumours may represent another mechanism by which tumours escape from immune attack. It provides an anti-inflammatory milieu that will prevent appropriate activation and maturation of antigen presenting cells, allow tumours

to secrete suppressive factors and inhibit expression of tumour endothelial adhesion receptors, such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin, needed for appropriate interactions with immune cells. Inhibition of angiogenesis may, apart from its direct detrimental effects on the tumour, reverse these processes and contribute to anti-tumour immune reactivity. Without trying to give a complete overview of the field, this paper reviews insights on angiogenesis inhibition in relation to tumour immune responsiveness, mainly based on the Maastricht-Amsterdam experience. This review adds to the hypothesis of improvement of immunodirected therapies for cancer by angiostasis.

Keywords

Angiogenesis and inhibitors, immunity, adhesion molecules

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Introduction

Immunotherapy is, next to surgery, chemotherapy and radiotherapy, one of the treatment modalities that are used to combat cancer. The immune system with its innate (dendritic cell [DC] and natural killer [NK] cell) and adaptive (T and B cell) arms, is equipped to recognise and attack pathogens. However, it is as yet not sufficiently effective in anti-tumour activity, because tumour-associated antigens are also expressed on normal tissues and the immune system may recognise these as self-antigens. Thus, intrinsically, most T cells will be tolerant to tumour cells. However, tolerance may be broken and productive anti-tumour responses elicited when tumour-associated antigens are presented to T cells in the context of major histocompatibility complex (MHC) molecules on the surface of DC in an inflammatory environment (1). This will induce DC to mature and express costimulatory molecules which together with MHC-presented pep-

tides and cytokines are required for T-cell activation, expansion and survival (2) allowing effector T cells to migrate to the tumour sites. In addition, tumours may express inhibitory molecules, secrete suppressive factors or attract regulatory T cells to the tumour environment which inhibit induction of responses or effector functions (3, 4). Because the conditions needed to induce anti-tumour reactivity may also lead to induction of auto-immunity, the treatment window of successful immunotherapy is narrow, and treatments should be conducted in a carefully controlled way.

Recently, clinical benefit is observed with targeted therapies using inhibitors of angiogenesis that limit tumour growth and induce tumour cell death in a dual way: directly through tyrosine kinase inhibition and indirectly through targeting growth factors or their receptors required for blood vessel formation and growth (5). This review will highlight the potential of angiostasis therapy to stimulate immune mediated anti-tumour responses and

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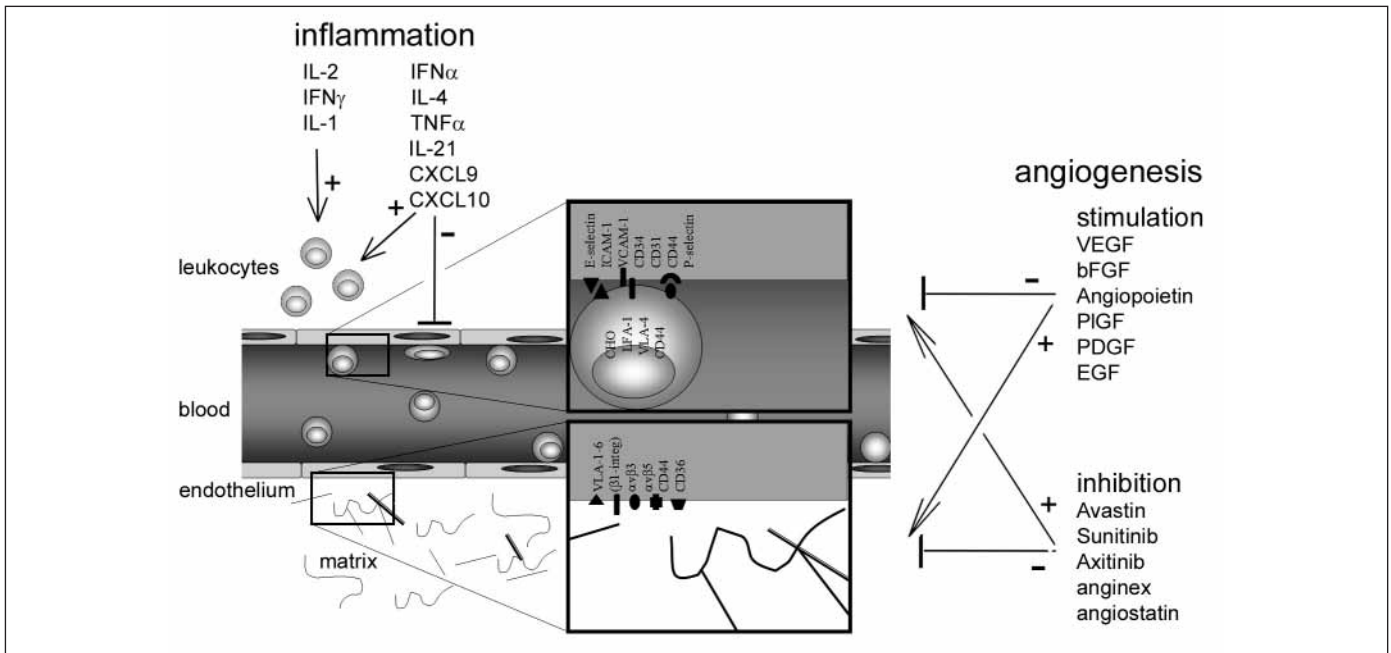


Figure 1: The relationship between angiogenesis and the immune system. A growing tumour protects itself to immunity through angiogenesis. Angiogenesis stimulators, e.g. VEGF and FGF, suppress the expression of endothelial adhesion molecules necessary for leukocyte interactions (e.g. ICAM-1 and VCAM-1). At the same time they induce the expression of endothelial adhesion molecules necessary for cell motility and tube formation (e.g. integrins and CD44). Inhibitors of angiogenesis regulate oppositely: while endothelial growth and sprouting is inhibited, matrix interacting adhesion molecules are suppressed and adhesiveness of the endothelial surface for leukocytes is increased. Angiogenesis inhibition can therefore be applied to stimulate leukocyte infil-

tration into tumours. Anti-tumour immunity is also regulated by inflammatory cytokines. Such cytokines (as listed under 'inflammation') have – through different mechanisms – a positive effect on the anti-tumour immune response. Yet, among these cytokines there are several (e.g. IFN α , IL-4 and IL21) that have a negative effect on endothelial cells, resulting in an angiostatic signal. Interestingly, for several cytokines such as TNF α the angiostatic signal strongly depends on the dose, as very low concentrations rather stimulate angiogenesis. Inflammatory cytokines can have therefore a dual function, both stimulating immune cells and prepare the endothelium for enhanced leukocyte extravasation.

neutralise tumour-mediated immune suppression. In addition, it will speculate on new treatment regimens based on combination of angiostasis and state-of-the-art immunotherapy approaches.

Tumour vasculature

Angiogenesis is required for the outgrowth of tumours and plays a role in metastasis formation and outgrowth as well (6–8). Angiogenesis is intricately regulated by stimulators and inhibitors. In normal tissues angiogenesis is switched off, or there is a balance between angiogenesis stimulators and inhibitors resulting in quiescent vasculature (9). But in the tumour microenvironment the process is stimulated through the production of cytokines such as vascular endothelial cell growth factors (VEGFs) and fibroblast growth factors (FGFs). In this way the tumour ensures sufficient requirements for continuous growth. In tumours changes are observed in the adhesiveness of the tumour vasculature. These changes are associated to activation of endothelial cells and induction of angiogenesis (10). Endothelial cells express a large variety of adhesion molecules. One group of adhesion receptors is involved in the interaction with components of the extracellular matrix. These endothelial molecules, which include for example CD44, α v β 3-, α v β 5- and β 1-integrins, are necessary for endothelial cell migration and are therefore up-

regulated during induction of angiogenesis. Another group of adhesion receptors, e.g. intercellular adhesion molecules (ICAMs), vascular adhesion molecules (VCAMs), E-selectin and CD34, is involved in interactions with blood cells and the selection of immune cells that infiltrate into a tissue. Interestingly, these molecules have been found to be suppressed in tumour endothelial cells (8, 11, 12). It has been shown that ongoing angiogenesis, i.e. the production of angiogenic growth factors by tumour cells, is responsible for the disappearance of these adhesion molecules in the tumour vasculature (13, 14). For reference see also Figure 1.

Endothelial cell energy

The observation that adhesion molecules such as ICAM-1, VCAM-1, E-selectin and CD34 are suppressed in tumour endothelium, prompted us to investigate the factors involved and the mechanisms behind this reduced expression. It was found that the disappearance of these adhesion molecules is due to exposure of endothelial cells to tumour-derived angiogenic growth factors (11, 14–16). Both bFGF and VEGF, the strongest mitogenic factors for endothelial cells and also other growth factors, such as epidermal growth factor and placental growth factor, were found to suppress ICAM-1 in endothelial cells, in culture and in in-vivo

conditions. Under angiogenic conditions, the inflammatory response of endothelial cells – in response to cytokines such as interleukin (IL)-1, interferon (IFN)- γ and tumour necrosis factor- α (TNF α) – was heavily impaired (13, 15) and these cytokines did not seem to be able to induce an adhesive phenotype. This phenomenon of endothelium unresponsiveness is called endothelial cell anergy. The mechanisms by which adhesion molecules in endothelial cells are suppressed have also been studied. One of these was discovered in an effort to identify the epigenetic component in tumour angiogenesis (17). By pharmacological inhibition of epigenetic regulatory mechanisms such as promoter hypermethylation and histone deacetylation, it was unexpectedly found that ICAM-1 was among the genes that were most efficiently re-induced (18, 19). Indeed, the ICAM-1 gene contained CpG motive-rich islands. These are CG-rich foci in the sequence that allow methylation of the promoter DNA, which may result in silencing of the gene. Interestingly, it was not the promoter methylation component, but rather the histone deacetylation related mechanism that was responsible for the absence of ICAM-1 in tumour endothelium (18). Another mechanism of angiogenic growth factor mediated induction of endothelial cell anergy was through the sustained activation of p38 MAPK and subsequent inhibition of the transcription factor nuclear factor kappa B (NF κ B), which is under normal conditions heavily involved in the upregulation of ICAM-1 (20). The latter phenomenon was only demonstrated for bFGF but the results strongly suggest that this activity is common to angiogenic growth factors. Whereas these mechanisms give some insight in how endothelial cell anergy is regulated, further studies are needed to fully clarify this process.

Angiostasis enhances leukocyte infiltration in tumours and improves immunotherapy

The role of the presence of leukocytes in tumours and the function of adaptive immune responses that control tumour growth are still controversial, but many reports describe a beneficial effect, indicative of active anti-tumour immune responses (21). In various reports this has been associated to angiogenic growth factor expression and/or ongoing angiogenesis (22, 23). Several studies by us and by others have demonstrated that suppression of blood vessel development by treatment with angiogenesis inhibitors may have immune potentiating capacity, suggesting a role for combining angiostasis and immunotherapy. It has been demonstrated that the angiogenesis inhibitor angiostatin enhances immunotherapy based on gene transfer of the T-cell costimulator B7.1 (24, 25). Another gene therapy-based approach demonstrated improved tumour growth inhibition after implantation of polymer microencapsulated mouse myoblasts genetically modified to deliver angiostatin and IL-2 fusion protein (26). We have demonstrated that inhibition of angiogenesis enhances leukocyte infiltration in tumours, a feature that is suggested to be common to all angiostatic compounds (27). Angiostatin was also used in several other studies in combination with immunotherapy. Combination of adenoviruses expressing angiostatin and IL-12 (28) and the use of immunogenic and non-immunogenic 3LL Lewis lung carcinoma cell lines (29) showed convincing improvement of immunotherapy by angiostasis.

It has been suggested that chemokines are important regulators of the cross-talk between immunity and angiogenesis (30). Chemokines are multifunctional mediators mainly responsible for leukocyte recruitment to inflamed tissues. While INF-inducible chemokines CXCL9 and CXCL10 were reported to have anti-angiogenesis effects by signalling through CXCR3B (31), these chemokines were discovered to also orchestrate the Th1 cell-mediated immune pathway and to suppress tumour growth by recruitment of mononuclear cells (32, reviewed in [33]). These results suggested that targeting of these chemokines or their receptors might provide a successful therapeutic approach in chronic inflammatory and neoplastic diseases.

Studies have shown that exposure of normal tissue to pellets releasing bFGF and VEGF resulted in impaired upregulation of endothelial adhesion molecules and leukocyte vessel wall interactions within the vasculature in response to TNF α (34). Also, in tumour models intravital microscopy revealed largely impaired interactions of leukocytes with the vessel wall as well as leukocyte extravasation (15). Similar findings were obtained with human tumours. In aggressive ductal breast cancer tissues that are characterised by enhanced angiogenesis potential, a lower amount of infiltrated leukocytes was observed as compared with less angiogenic and less aggressive medullary breast carcinomas. Similarly, a negative correlation was observed between expression of VEGF and the amount of infiltrated T lymphocytes in colorectal carcinoma (23) and in ovarian carcinoma (22). However, results by us and by others showed that angiogenesis inhibitors – among which endostatin, angiostatin, anginex, TNP-470, anti-VEGF antibodies, as well as chemotherapeutic compounds with claimed angiostatic activity – can normalise the expression of adhesion molecules on tumour endothelial cells (see Fig. 1), as well as normalize their responsiveness to inflammatory cytokines both *in vitro* (35–38) and *in vivo* (27).

Interestingly, it has been suggested that not all leukocytes in a tumour have a beneficial impact. It has been demonstrated that the presence of monocytes/macrophages induces growth signals in tumour cells and stimulates angiogenesis (39, 40), leading to a worse prognosis. Moreover, it is likely that the net result of immune cell infiltration into the tumour will depend on the balance between the number of tumour vessels still present, their functional state and the kinetics of the infiltration process. Current research is performed to demonstrate which cell type is involved in an anti-tumour response and which cells can counteract this activity and might contribute to angiogenesis stimulation.

These observations made us hypothesise that inhibition of angiogenesis may revert endothelial cell anergy and may increase leukocyte infiltration in tumours. Whereas increased numbers of lymphocytes infiltrating the tumour may be beneficial (22, 23), ongoing studies will have to show whether in particular tumour infiltrating T lymphocytes are functionally capable of rejecting the tumour.

Different strategies of immunotherapy

In recent years, a variety of immunotherapeutic treatments have been developed aiming at strengthening anti-tumour immune responses. The development of immunotherapy strategies is cur-

rently very rapid. While until the 1980s immunotherapy was mainly performed by treatment with recombinant cytokines (e.g. IL-2, IFN), later strategies involved leukocyte transfer or stem cell transplantations, as well as vaccination strategies using peptides and antigen-loaded DC.

One of the first developed immuno-targeted strategies, is passive immunotherapy with monoclonal antibodies. Some of these strategies are very successful and have made their way into daily clinics, as exemplified by the development and use of rituximab for the management of lymphoma, trastuzumab for breast cancer and cetuximab for colon cancer.

Immunotherapy with (recombinant) cytokines is used to boost T-cell effector functions or other components of the immune response. One of the first cytokines developed for immunotherapeutic strategies is IL-2. Its prime function is to induce and enhance expansion of T- and NK-cell effectors and thereby ameliorate anti-tumour immune responses. It is currently approved for treatment of metastasised melanoma and renal cell carcinoma (41). However, although highly effective in a small number of patients, the total response rates are rather low (approximately 15%). This may be due to a number of factors, including suboptimal effector activation, expansion or traffic to tumour sites and probably also the fact that IL-2 serves as survival factor for regulatory T cells which counteract anti-tumour immune responses. In addition, the use of high-dose IL-2 in the clinic is severely limited by toxic adverse effects like the induction of vascular leakage syndrome. IFNs have widely been used in the clinic as well. IFN α is known for induction of MHC class I molecules on tumour cells and maturation of DC. In addition, it activates cytotoxic T cells, NK cells and macrophages. In addition to its immunologic effects, IFN α can have a cytostatic effect on tumour cells, may promote tumour cell apoptosis and is involved in attraction of immune cells. Treatment of renal cell carcinoma patients with triple therapy consisting of granulocyte/macrophage-colony stimulating factor (GM-CSF), IL-2 and IFN α , was shown to induce active and mature T cells and DC to the tumour site (42). Other cytokines such as IL-15 and IL-21 are currently being developed and have great promise for the treatment of cancer (43, 44). Other immunotherapies make use of the key role of α -GalactosylCeramide-stimulated NKT cells as intermediates for activation of both innate and acquired immune effector cells and immune activation cascades (45). Vaccination is the most common approach to increase the frequency and function of tumour-specific T cells. A number of vaccine formulations, including peptides, antigen-loaded DC (46), DNA vaccines (47), or adenoviruses encoding tumour antigens, have resulted in detectable levels of tumour-specific T cells. In addition, adoptive T cell transfer therapies have been developed (48). Adoptive transfer of tumour infiltrating lymphocytes (49) or T-cell receptor (TCR) gene transfer therapy using genetically engineered cells (50–52) are approaches that have proven therapeutic potential for the biologic therapy of cancer. TCR gene transfer therapy, when combined with host conditioning and vaccination, provides a powerful immunotherapeutic strategy (53) and bypasses the requirement for endogenous T cells that often are anergic in cancer patients. However, this immunotherapeutic approach is only suitable for selected cancers for which tumour-specific antigens are known, e.g. melanoma. Moreover,

improvement of appropriate accumulation and function of adoptively transferred T cells at the tumour site is needed.

Requirements for optimal immunotherapies

Despite the large efforts that are undertaken in the field of immunotherapy, many therapies have only resulted in limited clinical benefit. This may be due either to absence of an endogenous repertoire of T cells capable of recognising tumour antigens, or to the presence of T cells that are anergic and require optimal stimulation conditions to become effective in tumour rejection. In addition, it has become increasingly clear that large tumours often present a suppressive microenvironment that hampers effective antitumour immunity (4).

As has been reviewed recently, a number of requirements and options for successful tumour immunotherapy can be formulated (54–56). First, antigen-presenting cells need to activate tumour-specific T cells to tumour antigens and immune adjuvants should be given to sustain and enhance the ensuing antitumour immune response. Second, tumour-specific T cells need to be activated, expanded and facilitated to traffic to tumour targets. Third, systemic and local negative regulatory elements should be counteracted to allow tumour attack by effector T cells. Fourth, the tumour cells need to be sensitive to the lytic effects of the immune effector cells. Interestingly, as elaborated below, angiogenesis inhibition therapy will provide several of these requirements to improve immunotherapy.

Immunotherapeutic aspects of angiogenesis inhibition

It can be envisioned that angiogenesis inhibition therapy may improve immuno-targeted therapies, because, similarly to immunotherapeutic treatment, it may stimulate anti-tumour immunity and overcome tumour-mediated immune suppression in several aspects considered below.

Enhanced tumour cell lysis stimulates tumour antigen presentation

Therapy-induced tumour lysis can be direct, by tyrosine kinase inhibitors (e.g. Sunitinib and Erlotinib), but also by indirect effects on endothelial cells, through neutralisation of VEGF (e.g. Avastin), resulting in tumour cell death and necrosis (57). Dying tumour cells enhance provision of tumour-associated antigens which will stimulate processing and presentation by DC to T cells. Strong boosting of anti-tumour immunity is expected when angiostasis therapy would be combined with vaccination strategies, including the use of CD70-transduced DC to break tolerance (58). Alternatively, this may be further optimised by CD40-triggering, growth factors or vaccine adjuvants (e.g. IL-12, Flt3L, CpG, PolyI:C, TLR agonists, glycolipids, cytokines, chemokines).

Dying tumour cells reduce the amount of inhibitory molecules thereby permitting improved immune reactivity

By angiostatic therapy dying tumours and tumour vessels will express lower levels of immune inhibitory molecules B7-H1,

B7-H3, B7-H4 or Bx known to inhibit effector T-cell activation and function, or to induce T-cell apoptosis (3, 59). B7-H1 (also known as PD-L1) expression is abundant on many murine and human cancers and by interaction with its ligand, PD-1 on T cells, found to play an important role in tumour immune evasion (60). B7-H1-expressing cells use several distinct mechanisms to evade T-cell immunity. These are induction of apoptosis, anergy or exhaustion of T cells, forming a molecular shield to protect tumour cells from lysis, inducing production of the immunosuppressive cytokines IL-10 or transforming growth factor (TGF) β and promoting suppression mediated by regulatory T cells (61). In addition, B7-H4 (62) and recently also B7-H3 were shown to relate to clinical behavior and to predict prognosis and survival in renal cell carcinoma patients (63). Tumour cells or tumour accessory cells can deplete tryptophan from its environment by the expression of indoleamine 2,3-dioxygenase (IDO), causing effector T-cell anergy. Blockade of B7-T cell interactions and neutralisation of IDO with enzyme inhibitor MT-1, would be attractive possibilities for anti-tumour immunotherapy in combination with an adjuvant angiostatic therapy.

Such immune suppressive mechanisms are not restricted to B7 expressing tumour cells. It has been suggested that other molecules, such as galectin-1, serve a similar function. Galectin-1 expressed by tumour endothelial cells was found to be a key regulator in tumour angiogenesis, and can induce apoptosis in T lymphocytes (64). The expression of this molecule not only on tumour endothelial cells (65) but also on tumour cells (66), may therefore effectively protect the tumour against effector T-cell activity. So, targeting galectin-1 on tumours may neutralise its angiogenic potential and also block its suppressive effect on T cells. The designer peptide anginex (67), which has been shown to target galectin-1, may combine direct anti-tumour activity, amelioration of anti-tumour immunity and angiostasis in a single compound.

Dying tumour cells release limited immunosuppressive cytokines in the tumour microenvironment thereby permitting improved immune reactivity

Tumours may escape immune attack by secreting immune inhibitory substances like IL-10, IL-6, VEGF, prostaglandin E2 or TGF β . The success of treatment with VEGF-neutralising approaches (e.g. bevacizumab/Avastin) can be ascribed to its direct effect on the tumour, but also to an immune potentiating effect, since VEGF produced by tumours is known to inhibit DC maturation and inhibit T-cell function. Suppressive cytokines IL-10 and IL-6 may lead to upregulation of B7-H4 on antigen presenting cells in an autocrine or paracrine way which in turn down-regulates T-cell activation, as shown in ovarian cancer (68). Further improvement by neutralisation of these cytokines may be an attractive approach to reverse immune escape in combination with angiostatic therapy.

Increased expression of endothelial adhesion molecules leads to enhanced immune cell infiltration into the tumour

To improve expansion and survival of T cells that ultimately will infiltrate the tumour, additional cytokines can be given, e.g. IL-2 for stimulated proliferation and differentiation of T or NK cells,

IL-7 or IL-15 also for enhancing survival of expanding T cells. Combination with IL-21 (44) may be an attractive treatment since this cytokine might dampen the regulatory T-cell response while keeping peripheral T cells in a pre-effector stage. This will enable them to circulate and enter lymphoid organs for interactions with DC potentiating survival and differentiation to memory and effector-cell stages, before migrating to the tumour site (2).

Whereas increased tumour infiltrates have been detected upon angiogenesis inhibition therapy (27, 35), it remains to be established whether infiltrating cells actually are involved in tumour rejection. We have developed assays to detect (tumour)antigen-specific T cells *in situ* (69, 70), and it will be most interesting to investigate whether such cells are detectable within the tumours. However, increased immune cell infiltration may also lead to increased regulatory T cells being attracted to the tumour site. Regulatory T cells, mediating homeostatic peripheral tolerance by suppressing autoreactive T cells, also inhibit host anti-tumour immunity and correlate with reduced survival in ovarian carcinoma patients (71). Sunitinib treatment has been described to induce increased levels of circulating regulatory T cells (72). Neo-adjuvant therapies with angiogenesis inhibitors followed by surgery in our cancer hospital are under way to elucidate effects at the tumour site. If regulatory T cells are outnumbering effector T cells also in the tumour environment, the balance may be restored by combination therapy with anti-CD25, anti-CTLA4 antibody or irradiation. Furthermore, despite necrosis detection by computed tomography scans after Sunitinib therapy (57), our preliminary post-therapy *in situ* examination of the same tumours shows only partial destruction of tumour vasculature, making it unlikely that cell infiltration in the tumour *per se* will be hampered by therapy-induced reduction of blood vessels (Vyth, Griffioen, Bex, unpublished observations).

Induction of a pro-inflammatory microenvironment

Tumour-cell lysis and the stress inflicted to the tumour cells by inhibition of blood and nutrient supplies as result of diminished tumour vessels, will potentiate anti-tumour reactivity by creating a pro-inflammatory microenvironment. Dying cells release RNA, which interacts with Toll-like Receptor 3 on DC, double-stranded DNA, which stimulates macrophages and DC, as well as nucleotides which may stimulate the maturation of DC accompanied by an activation of the NF- κ B pathway (73). Released tumour-associated antigens will be processed and presented by DC leading to release of IL-12 and subsequent secretion of IFN γ by T lymphocytes. Together with IL-1 and TNF α released from the dying tumour, endothelial and stromal cells this will provide a local pro-inflammatory milieu that may potentiate lytic effects of the immune effector cells delivered either through perforin/granzyme or by death receptor ligation (Fas, TRAIL, and TNF). Irradiation may improve tumour cell lysis, because tumour cells exposed to X-rays also express increased numbers of MHC class I molecules and tumour antigens, favoring CTL attack (74).

It is expected that immune potentiation by combination therapies with angiostasis will be most valuable in those cancers for which targeted therapies are the current treatment of choice and for which no tumour-specific antigens are known (e.g. renal cell

carcinoma, lung carcinoma) and therefore adoptive cell therapies are no option. However, even in those cases, particular treatment options potentiating or complementing anti-angiogenesis may be applicable and can be expected to have great impact, e.g. vaccination with CD70-transduced DC or neutralisation of regulatory T cells using host conditioning approach. Selection of the preferred (multi-)combination therapy in individual cases, will depend on the immune characteristics of the relevant tumour type and the patients immune profile. Ongoing improvement in immunomonitoring techniques, including gene profiling (75, 76), will add in this decision making.

Conclusions

It is fundamental to any form of immunotherapy that an induced immune response leads to the generation of tumour-specific memory T cells that are able to reach the tumour cells through the tumour vasculature. We have developed assays to detect specific T cells *in situ* (69, 70). It will be important to determine whether anti-angiogenesis therapies not only will result in enhanced numbers of specific effectors in the peripheral blood, but particu-

larly at the tumour site. As the number of known tumour-specific or tumour-associated antigens for diverse tumour types increases, these investigations will help to determine the effectiveness *in situ* of anti-angiogenic therapy alone, or in combination with other immunotherapeutical approaches.

A careful assessment of the effects of novel targeted therapies on the tumour, tumour microenvironment, and the immune system will be required to define optimal combinations. Several clinical trials are ongoing where angiogenesis inhibition is tested in combination with immunostimulating agents, such as IL-2, IL-21 and anti-CTLA4 antibodies. Such multi-target immunotherapy approaches might be most effective allowing generation and activation of sufficiently strong specific T-cell responses, in a carefully determined kinetic window, with minimal negative regulatory effects. It is expected that anti-angiogenesis therapy may improve future immunotherapy regimens.

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