



# Tumors as elusive targets of T-cell-based active immunotherapy

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**The understanding of tumor–host interactions remains elusive despite significant progress in the identification of tumor antigens (TAs) recognized by autologous T cells. In particular, most human tumors do not regress and continue to grow in spite of spontaneous or immunization-induced immune responses demonstrated in circulating lymphocytes. Indeed, systemic immune responses might insufficiently address the complexity of tumor–host interactions because of factors, such as (1) the lack of productive T-cell receptor (TCR) engagement with epitope owing to qualitative and/or quantitative defects in the generation and maintenance of the immune response, (2) insufficient costimulation provided by the host, (3) the lack of localization of the immune response in target tissues and (4) the complexity of tumor–host interactions within the tumor microenvironment caused by temporal changes in tumor phenotypes and an array of immune mediators expressed in the tumor microenvironment. Here, we will review current knowledge of the different ‘levels’ of immune response that might be necessary for immunotherapy to be effective in the treatment of cancer. Furthermore, we will discuss the information still required in order to understand the mechanism(s) governing tumor rejection by the immune system in response to TA-specific immunization.**

During a surprising metamorphosis in the 1990s, human tumor immunology evolved from the empirical observation of serendipitous tumor regressions in response to immune manipulation into a sophisticated science of molecular accuracy. Crucial in this development has been the molecular characterization of tumor antigens (TAs) that meet the criteria to be used for immunotherapy of malignant diseases [1,2].

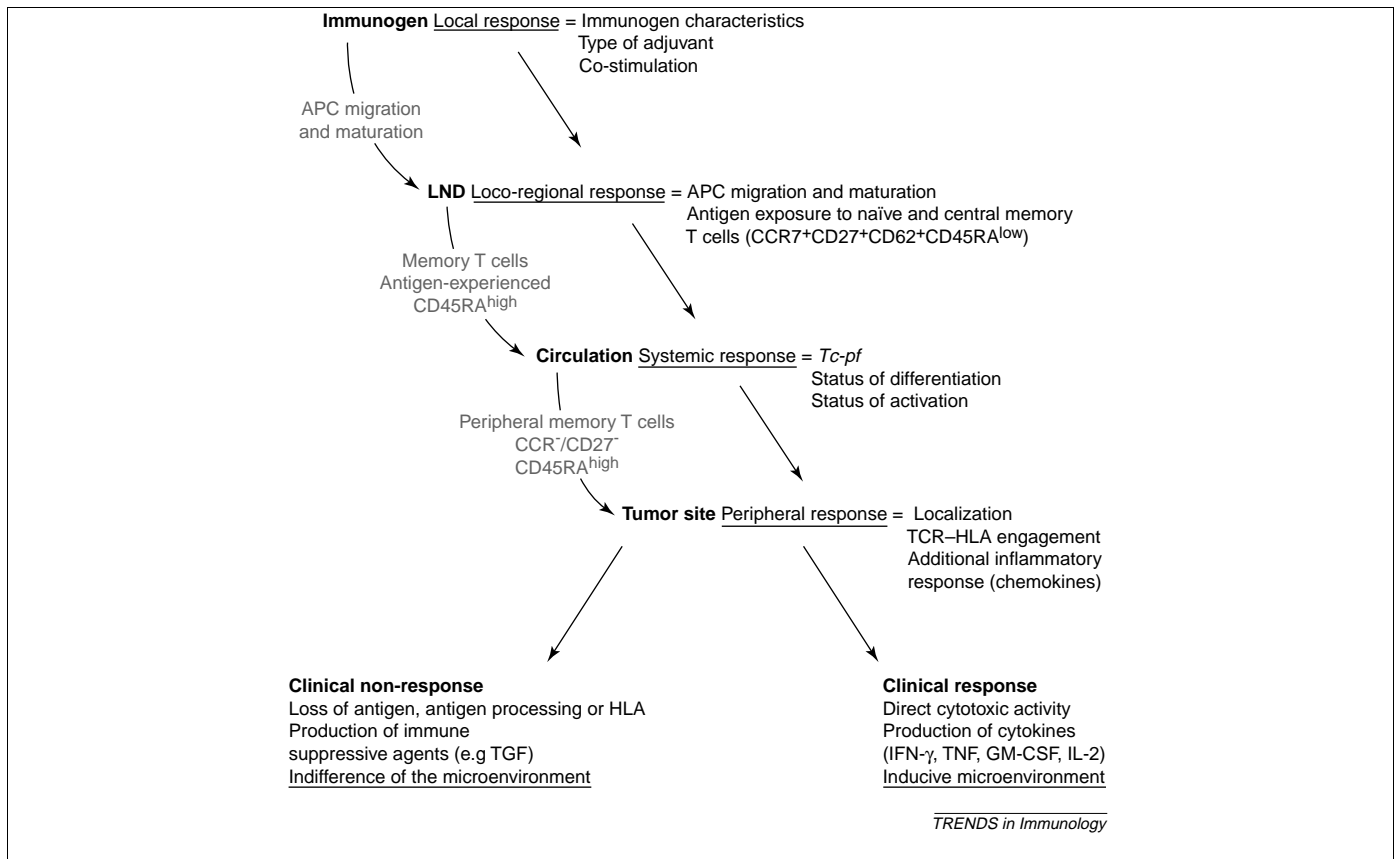
A survey of the TAs identified so far indicates that most are expressed by a relatively broad range of tumor types and by a large percentage of patients within each type of tumor. This is particularly true for (1) the so-called cancer or testis antigens [2], the expression of which in normal tissues is restricted to testes and (2) the tumor differentiation

antigens [3], which represent remnants of the cellular lineage of origin of a cancer of a given histology. As a result, many of the identified TAs could act as immunogens to implement specific immunotherapy in large numbers of patients. Several derivatives of these TAs have been, or are being, used in clinical trials to induce or enhance the immune response of the host against cancer cells [4–8]. Surprisingly, induction or enhancement of the immune reactivity of a host towards cancer cells has not been closely correlated with tumor regression [9,10]. Thus, the present decade is left with the daunting task of solving the paradoxical puzzle of the coexistence in the same host of cancer cells and effector immune cells. In this Review, we discuss the role of cellular immunity, microenvironment, susceptibility of tumor cells to immune recognition and their interactions with host immune cells in the outcome of T-cell-based active immunotherapy. In particular, we will emphasize the limitations of currently used immunoassays to monitor patients treated with immunotherapy and the need to take into account tumor-cell susceptibility to immune recognition and destruction.

## Immunological events leading to rejection of cancer cells

Theoretically, the rejection of cancer cells by an immune system sensitized by TA-specific immunization is the end result of a series of events that go from the initiation of an immune or inflammatory response at the site of immunization to a stepwise expansion of the immune response to loco-regional, systemic and peripheral sites (Fig. 1). Various factors might influence the final outcome of immunization. Initiation of an immune response depends on the characteristics of the immunogen, such as solubility, stability and affinity for associated human leukocyte antigen (HLA) alleles. In addition, the use of adjuvants that can enhance the inflammatory response at the site of immunization might potentiate the immune response by attracting inflammatory and immune cells. The milieu induced by the quality and quantity of immunogen might condition the status of activation and migratory properties of antigen-presenting cells (APCs) recruited at the immunization site. The phenotypic characteristics of these APCs will determine their ability to initiate the cognitive immune response in loco-regional lymph nodes.

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**Fig. 1.** Steps leading to immunization-induced tumor regression and potential sources of failure. Antigen(s) or their epitopes might have different immunogenic potential. This can affect their ability to achieve an effective local response that could activate and induce migration of antigen-presenting cells (APCs) to draining lymph nodes (LNDs) and present tumor antigen (TA) to naïve or memory T cells (loco-regional response). On antigen exposure, naïve or central memory T cells (CD45RA<sup>high</sup>, CD27<sup>+</sup> [64,65]) enter the circulation as effector and/or effector or memory (CD45RA<sup>high</sup> or <sup>low</sup>, CCR7<sup>-</sup>CD27<sup>-</sup>) cells (systemic response). Different factors might influence their effectiveness, including the immune-specific T-cell precursor frequency (*Tc - pf*), their status of differentiation and/or activation [29]. Some of these T cells (effector or memory) can localize in peripheral tissues (CCR7<sup>-</sup>) [65] to exert their effector function (peripheral response). On engagement of the T-cell receptor (TCR) with human leukocyte antigen (HLA) or epitope, cytotoxicity occurs. In addition, T cells release cytokines, such as interferon- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-2 (IL-2), which can induce inflammation capable of sustaining the ongoing immune response as well as stimulating a novel local immune reaction. In some circumstances, this secondary inflammatory response might be facilitated by a tumor microenvironment already conditioned towards an inflammatory process by constitutive secretion of chemokines and cytokines by tumor cells or infiltrating normal cells (see Figs 3 and 4). A deficient clinical response could be due to HLA or TA epitope loss. In addition, production of immune-suppressive factors could modulate the extent of the T-cell-induced immune response. Abbreviation: TGF, transforming growth factor.

Successful activation of TA-specific T cells in the lymph nodes by APCs might induce a systemically detectable immune response. Finally, localization of the immune response to the peripheral (tumor) site where its effector function is expected is a required step whose physiology is poorly understood.

#### Interactions between the T-cell receptor (TCR) and HLA or TA epitope and tumor-cell recognition

In recent years, the role of T-cell-mediated immunity in the control of tumor growth has been emphasized. As a result, most immunization strategies adopted in clinical trials have aimed at enhancing TA-specific cellular immunity.

The specificity of cytotoxic T-lymphocyte (CTL)-mediated immune responses is dependent on recognition of the complex of HLA allele with peptide (the T-cell epitope) by T cells through engagement of the TCR. The TCR-epitope interaction, which has been the subject of much debate, is influenced by several variables, including affinity of the peptide for the restricting HLA class I allele and affinity of the TCR for the HLA-epitope complex. Some pre-clinical models suggest that excessive

TCR-epitope interaction might have detrimental effects on T-cell expansion and survival *in vivo* [11–14]. However, the results of clinical trials suggest that peptides with enhanced binding affinity for HLA molecules induce stronger immune responses *in vitro* [15] and *in vivo* [4,16]. In addition, the extent of the immune response elicited by a given immunogen in patients with malignant diseases increases with the number of immunizations through the expansion of T-cell populations with broader- and higher-affinity TCR repertoires [17].

In early studies, an enhancement of TA-specific CTL activity was detected in peripheral-blood lymphocytes isolated from melanoma patients following immunization with a TA-derived peptide and repeatedly stimulated *in vitro* with the immunogen [10]. However, no correlation was found with clinical responses. This finding is not surprising because the immunoassay did not take into account changes in the antigenic profile, which occur frequently in tumor cells because of their genetic instability. This interpretation is supported by the good correlation between intensity of the systemic immune response and the therapeutic outcome observed in mice using a

tumor cell line with a highly stable phenotype [18]. Furthermore, the *in vitro* expansion of epitope-specific CD8<sup>+</sup> T cells might not accurately reflect *in vivo* immune responsiveness because *in vitro* expansion is dependent on exposure to arbitrarily high concentrations of epitope and exogenous cytokines that might exaggerate the extent of the immune responses ongoing *in vivo*. To overcome this limitation, assays have been developed that do not require repeated *in vitro* stimulations.

Fluorescence-labeled tetrameric HLA-peptide complexes (tHLA) enable direct enumeration and characterization of TA-specific T cells in various tissues [19–21]. tHLA-based enumeration of immunization-elicited T cells has estimated a frequency as high as 5% of total circulating CD8<sup>+</sup> T cells that was not associated with tumor regression [22]. This frequency is significantly lower than that observed during acute viral infections [23,24] or virally induced autoimmune diseases [25,26] and might suggest that the magnitude of the immune response elicited by cancer vaccines is a magnitude insufficient to induce tumor regression. Recent observations suggest that the frequency of TA-specific CTLs might be augmented by increasing the number and frequency of immunizations [17].

An anergic status of T cells might be at the basis of their ineffectiveness [20] although, in most cases, circulating immunization-elicited T cells are TA reactive [27]. Recent work in the context of viral diseases has suggested that interferon- $\gamma$  (IFN- $\gamma$ ) expression by T cells in response to cognate stimulation might not be the functional parameter that best describes their potential as effector cells [28]. Indeed, although immunization-induced T cells display a classical effector phenotype (CD45RA<sup>high</sup>, CD27<sup>-</sup>, CCR7<sup>-</sup> [23]), they do not express perforin and are of small size, which is a phenotype compatible with a resting state [29]. Thus, it remains undefined whether the status of activation of circulating immunogen-induced CD8<sup>+</sup> T cells is adequate to induce tumor regression. In addition, several markers of T-cell differentiation associated with peripheral effector function or ability to migrate to target tissue remain to be characterized [23,30].

Several other variables might influence the effector function of T cells or interfere with their clinical effectiveness, as recently discussed elsewhere [31]. These include the role of natural killer cells and their positive and regulatory receptors, the development of death resistance by tumor cells, the involvement of immune-regulatory T cells and the potential contribution of Fas expression. These variables might have an important role in modulating the immune response at the systemic level as well as the tumor site, and their relevance in the context of human malignancies is currently being explored. The complex requirements for T-cell activation through TCR-HLA-epitope engagement render cellular immune responses heavily susceptible to changes in target molecule expression. This is a significant problem because tumor cells frequently have defects in TAs [32], HLA molecules [33,34] and/or antigen-processing machinery components [35]. HLA-epitope complex loss from tumor cells has an obvious effect on their recognition by TA-specific T cells. In addition, HLA-epitope complex downregulation on tumor

cell membranes correlates with decreased T-cell-triggering capability [36]. Dysfunction of antigen-processing machinery might account for the frequent coexistence of TA-specific T cells with cancer cells expressing the target components necessary for their recognition [37–39].

#### Localization of the immune response at a tumor site

Most studies have demonstrated the presence of TA-specific T cells in the peripheral blood of immunized patients, proving that the primary goal of immunization, which is to induce a systemic TA-specific immune response, has been reached. However, these investigations do not provide sufficient information about the relationship between host and cancer cells in the tumor microenvironment. A possible mechanism underlying the variable behavior of tumors of individual patients might be defects in the localization of TA-specific CTLs at a tumor site. Localization at a tumor site of adoptively transferred tumor-infiltrating lymphocytes (TILs) is necessary, although not sufficient, for a clinical response to occur [40]. By analogy, it could be postulated that immune responses elicited by active specific immunotherapy might not work because they fail to localize at the tumor site. Few studies have addressed this question in humans because of the technical difficulties of studying immune changes *ex vivo* within solid tumors. Comparison of pre- and post-immunization autologous samples obtained from metastatic lesions through fine-needle aspiration (FNA) biopsies has provided indirect evidence that immune responses elicited by active specific immunotherapy can localize at a tumor site. Expansion of tumor-TIL pairs from repeated FNA biopsies of identical lesions in patients undergoing epitope-specific immunotherapy demonstrated that immunotherapy-elicited T cells can be expanded more readily from melanoma metastases after treatment [38]. In addition, comparison by quantitative real-time PCR of cytokine gene expression in pre- and post-immunization FNA samples demonstrated a post-immunization enhancement of IFN- $\gamma$  transcripts in lesions that maintained expression of the targeted TA and of the restricting HLA class I allospecificity [39]. The relationship between IFN- $\gamma$  and TA expression suggests that immunotherapy-induced T cells could engage in a dialogue with tumor cells and/or APCs in the tumor microenvironment. The lack of shrinkage of the lesions in this study excluded lack of tumor localization and TA loss as a single factor hindering the effectiveness of immunotherapy.

#### Requirements for co-stimulation in T-cell activation within the tumor microenvironment

Tumor-host interactions within the target tissue might not adhere to the two-signal model for T-cell activation [41] by not providing the danger signal required for full T-cell activation. This might enable the majority of tumors to thrive in an ignorant immune environment by not sustaining the function and survival of immunization-induced T cells that have localized within the tumor [42,43]. Paracrine secretion of cytokines might promote activation and proliferation of TA-specific CTLs. However, the requirements for interleukin-2 (IL-2) and/or other cytokine levels by CTLs are relatively high [44,45]. CD4<sup>+</sup>

T cells might be necessary to provide this additional stimulation, which could lead to survival and amplification of CTL responses at the tumor site. In this context, CD4<sup>+</sup> T cells might be directly stimulated by tumor cells that might express HLA class II antigens [46]. Furthermore, specialized resident APCs could present TAs shed by tumor cells to CD4<sup>+</sup> T cells or could optimally stimulate CTLs by providing TA-specific stimulation in association with costimulation. However, it is likely that, in most circumstances, such mechanisms are insufficient to sustain an effective adaptive immune response within the tumor microenvironment induced by the vaccine.

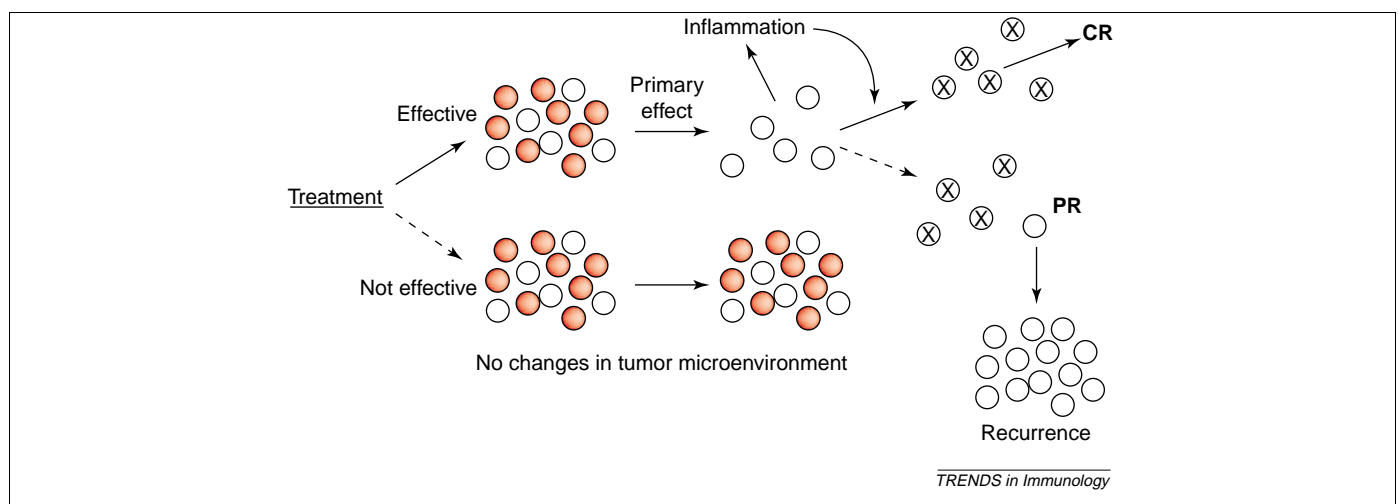
### Temporal changes in the relationship between tumor cells and the host immune system

Cancer is characterized by extreme genetic instability. The resulting tumor-cell heterogeneity evolving through time, compounded by the additional effect of immune selection on the drifting phenotypes of progeny cells, is a major obstacle to the success of immunotherapy [31,47]. Comparison of the antigenic profile of autologous primary and/or metastatic melanoma lesions surgically removed at different time points throughout the natural course of the disease or following immunotherapy has demonstrated temporal changes often associated with the specificity of the immunization [36]. In particular, differentiation antigen expression by tumor cells can be lost in association with TA-specific immunization [32,48]. This finding might provide an explanation for the paradoxical co-existence of cancer cells with TA-specific immune cells in the tumor-competent host. However, the antigenic heterogeneity of synchronous metastases raises the possibility that differences in the expression of various markers among distinct lesions might simply reflect the intrinsic heterogeneity of metastases rather than time- or treatment-induced changes [35,49]. This possibility can be tested by the use

of serial FNA biopsies [37,50], which provide the opportunity to study the kinetics of gene expression within the same metastasis at time points relevant to the disease process or its treatment.

Analysis of FNA serially obtained before and soon after immunization with gp100 antigen from 52 melanoma metastases unexpectedly showed a rapid decrease in gp100 expression in metastases that regressed following immunization but detected no change in lesions that did not regress [51]. This surprising finding suggests that a successful immunization primarily induces killing of cells expressing the target TA and simultaneously triggers a broader immune response, possibly through release of cytokines that can, in turn, activate other components of the innate or adaptive immune response (Fig. 2). If the inflammatory process is of sufficient magnitude, tumors disappear completely or partially. However, if the tumor cell destruction is not complete, tumor recurrence or progression will ensue. Frequently, in such cases, the immune selection induced by the originally successful localization of TA-specific T cells might lead to immune escape in recurring lesions by loss from cancer cells of complexes of HLA with TA epitope [32]. Thus, it is likely that tumor escape variants will emerge most frequently in the context of effective immunotherapies [31].

High-fidelity antisense RNA amplification (aRNA) has facilitated the comprehensive characterization of changes in the genetic profile of sequentially sampled metastases [50,52]. Analysis of the molecular phenotypes of serially sampled melanoma metastases identified two subsets that underlie the extent of the temporal instability of cancer [53]. One subset of metastases enriched with earlier samples (cluster I) contained a transcriptional machinery closely aligned with normal human epithelial melanocytes (NHMs), whereas a second cluster (cluster II) portrayed a late-progression expression profile because its members



**Fig. 2.** Postulated mechanism underlying tumor antigen (TA)-specific T-cell-based immunotherapy and effect on target antigen expression. Localization of TA-specific T cells at a tumor site appears to be necessary but not sufficient for tumor elimination [38,39]. If no response occurs, the interaction between T cell and tumor cell is probably not sufficient (or sustained) to start or maintain an inflammatory process. If the treatment induces an adaptive response of a quality and/or intensity that exceeds a certain 'threshold', then direct interaction of T cells with antigen-expressing (red circles) tumor cells leads to their destruction, as well as to the production of proinflammatory and pro-apoptotic cytokines that induce secondary activation of adaptive and innate immune effectors capable of clearing tumor cells that have lost antigen expression (white circles). If the tumor-cell destruction is not complete, the proliferation of remaining cells leads to recurrence. In those cases, the recurring tumors have lost the surface expression of the epitope relevant to the vaccination [66,67]. Abbreviations: CR, complete response; PR, partial response; X, cells killed by the therapy.

include an inordinate proportion of samples obtained later in the progression of the disease. Thus, cancers represent an extremely movable target that could rapidly adapt to the immune pressure induced by immune stimulation.

### Modulation of the peripheral immune response by the tumor microenvironment

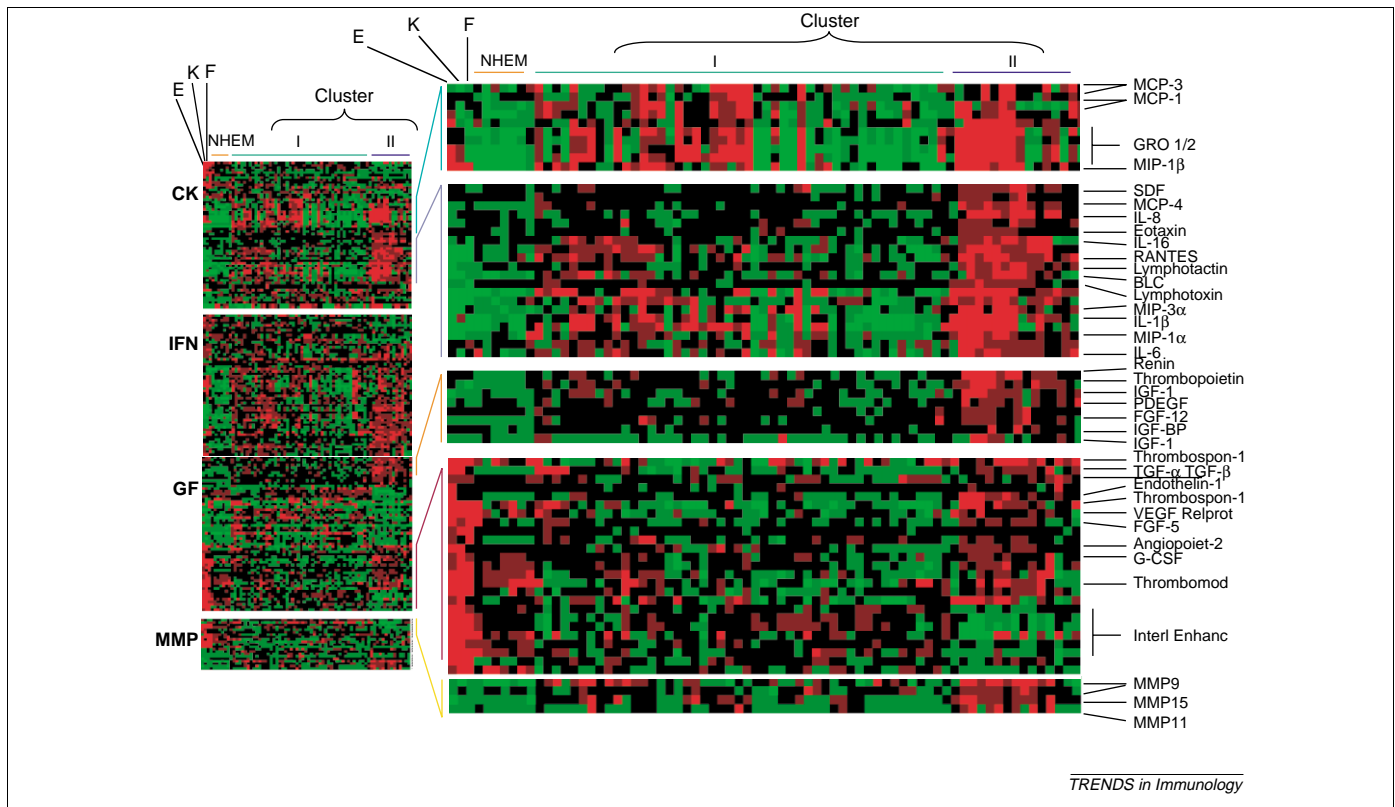
Tumor cells have the intrinsic potential of reversing to a primordial stage, where they can functionally mimic stem cells with strong modulatory effects on the surrounding environment. Indeed, the literature suggests an ever-growing number of mechanisms that might mediate tumor–host interactions [36]. The identification of individual mechanisms capable of modulating tumor–host interactions has met with many difficulties because of the genetic polymorphism of humans and the heterogeneity of their diseases. The complexity of the several, perhaps redundant, molecular pathways responsible for the natural and/or treatment-induced behavior of tumor cells could be analyzed with the microarray technology that can capture the physiology of diseases by simultaneously monitoring the expression of a large number of human genes [54]. This strategy has been applied to discover independent predictors of clinical outcome, underlining the expectation that subcategories of diseases with homogeneous natural or treatment-induced behavior might be differentially susceptible to tailored anti-cancer treatments. A search for predictors of immune responsiveness in melanoma metastases from patients immunized with TA-derived peptides failed to identify subclasses associated with immune responsiveness. However, ranking of individual genes identified 30 transcripts whose expression pattern was predictive of immune responsiveness [53]. The immune-modulatory properties of approximately half of these genes suggest that responsiveness of melanoma metastases to immunotherapy is pre-determined within an environment conducive to immune modulation or inflammation. Whether the distinctive phenotypes identified in immune-responsive lesions reflect genetic or acquired characteristics of the tumor-bearing individual, or intrinsic biological characteristics of the tumors, is not known. We favor the latter hypothesis because of the relatively frequent mixed responses to immunotherapy whereby some lesions dramatically shrink yet others continue their growth undisturbed in a patient. Given the systemic immune response as a constant in those circumstances, events occurring within the tumor microenvironment are more likely to be responsible for these divergent behaviors. A recent study identified IL-10 among genes with immunoregulatory function constitutively secreted by tumor cells [36]. IL-10 was found to have significantly increased expression before treatment in immune-responsive lesions [55]. This finding is not totally surprising because an anti-tumor role has been documented for this pleiotropic cytokine, whose dual role in the immune modulation of cancer has been recently reviewed [56].

The modulation of tumor immune responsiveness by a microenvironment receptive to the systemic effects of exogenous cytokine administration or TA-specific immunization is also supported by the tumor regression

observed following the administration of normal or malignant cells engineered to secrete immunomodulatory cytokines. This finding, which was originally postulated to be caused by enhancement of TA immunogenicity *in vivo* [57], is likely to have been caused by a broader inflammatory process induced by the high level of cytokine released in the microenvironment. This interpretation fits well with the ‘danger’ model postulated by Matzinger [41]. Indeed, the microenvironment of melanoma metastases is naturally rich in expression of cytokines, growth factors (GFs) and molecules capable of modulating an immune response of a host by providing a ‘self-inflicted danger signal’ (Fig. 3). One might hypothesize that, during their progression, tumors produce factors conducive to proliferation and invasion that include growth and angiogenic factors and metalloproteinases (MMPs). Because of their concomitant inflammatory properties, many of these factors might trigger a dormant host immune system otherwise tolerant of the poorly immunogenic tumor cells. When the level of immunostimulatory molecules within the tumor microenvironment reaches the threshold required to induce an immune response, tumors spontaneously regress, as observed with relatively high frequency in melanoma and renal cancer patients. If, however, the level of immune or inflammatory stimulation is below the threshold required for immune rejection, the balance struck between the host immune system and cancer enables their co-existence. Under these circumstances, systemic cytokine administration and/or TA-specific immunization might shift the balance in favor of the host by enhancing the ongoing immune or inflammatory response. As a result, the tumor is rejected. This hypothesis provides an explanation for similar clinical responses yielded by several distinct immunomodulatory therapies in one malignancy and a similar frequency of clinical responses to a type of immunotherapy in some types of cancer in spite of distinct immunological characteristics. An example of this phenomenon is represented by the similar tendency of melanoma and renal-cell carcinoma to respond to systemic cytokine administration, although their immune background appears divergent [58].

### Complexity of the tumor microenvironment

Analysis of gene expression in FNA biopsies of melanoma metastases demonstrates an eclectic production of cytokines, GFs and MMPs (Fig. 3). In addition, comparison of the expression pattern between early- (cluster I) and late- (cluster II) progression metastases suggest an evolving upregulation of cytokine, GF and MMP expression with tumor progression. Some cytokines might have a dual role by promoting tumor growth and inducing immune regulation, such as for growth-related oncogene (GRO) 1/2 [59]. Several cytokines have chemotactic properties, including B-lymphocyte chemoattractant (BLC), eotaxin, IL-1, IL-8, IL-16, lymphotactin, monocyte chemoattractant protein-1 (MCP-1), MCP-3, MCP-4, RANTES, stromal-derived growth factor (SDF), IL-6, macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ), MIP-1 $\beta$ , MIP-2 $\alpha$  (GRO 1/2) and tumor necrosis factor- $\gamma$  (TNF- $\gamma$ ) (lymphotoxin- $\beta$ ). Interestingly, IFN regulatory or responsive genes are coordinately expressed with cytokines, suggesting that



**Fig. 3.** Differential expression of secreted factors within the tumor microenvironment. Eisen's hierarchical clustering dendrogram of fine-needle aspiration samples applied to four categories of genes clustered according to co-ordinate expression and function. The four groups include cytokine (CK); interferon (IFN) regulatory and responsive elements; growth factors (GFs) and metalloproteinases (MMPs). Gene expression is compared in early- (cluster I) and late- (cluster II) phase metastases for normal human epithelial melanocytes (NHEMs), two fibroblast strains (F) (one derived from a melanoma metastasis and one from normal neonatal foreskin), one endothelial cell strain (E) and one keratinocyte strain (K). Gene expression is portrayed as a color-transformed image of the ratio of fluorescent intensity between Cy5: Cy3 (red:green) representing test and reference samples, respectively. Reference samples consisted of amplified antisense RNA derived from peripheral-blood mononuclear cells. The right panels demonstrate details of the dendrogram presented in the left panel. Each region is color coded. The genes identified include B-lymphocyte chemoattractant (BLC), fibroblast growth factor (FGF), granulocyte colony-stimulating growth factor (G-CSF), growth-related oncogene (GRO), insulin-like growth factor (IGF), IGF-binding protein (IGF-BP), interleukin (IL), IL enhancer binding factor (Interl Enhanc), lymphotactin (Lymphotact), monocyte chemoattractant protein (MCP), macrophage inflammatory protein (MIP), MMPs, platelet-derived endothelial growth factor (PDEGF), stromal-derived growth factor (SDF), transforming growth factor (TGF), thrombomodulin (Thrombomod) and vascular endothelium growth factor-related protein (VEGF Relprot).

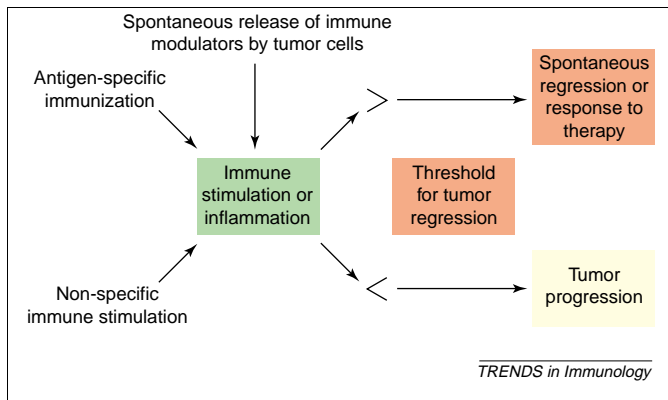
their expression in the tumor microenvironment is associated with activation of intracellular pathways in effector or target cells. GFs are also upregulated in the late-progression cluster (Fig. 3). MMPs, several angioregulatory elements, such as platelet-derived endothelial growth factor (PDEGF), renin, thrombopoietin and transforming growth factor (TGF), might condition the tumor microenvironment.

This complex picture argues against the view that few specific genes might be responsible for cancer progression. Rather, cancer appears as a complex adaptive system [60] best explained by nonlinear dynamics (chaos theory) [61,62]. In fact, the global portrait of molecular events that might take place in melanoma metastases raises more questions than it can answer. It remains unclear which cell population is responsible for the release of individual molecules, and what is their bioactivity and its functional consequence. However, this view underlines the multidimensionality of tumor-host interactions and might explain the capricious behavior of cancer cells, which are seen as powerful modulators of their surroundings rather than as independent proliferative units. This interpretation might also explain the unpredictability of tumor responsiveness to standard therapies [4]. It is possible

that, through spontaneous release of immunomodulatory molecules, tumor cells and infiltrating normal cells can strongly influence their environment (Fig. 4). In some conditions, when the constitutive immune stimulation is strong, spontaneous regression is seen [63]. However, in most instances, the inflammatory process does not overcome tumor growth. In these cases, additional proinflammatory stimuli provided by nonspecific immune stimulation, such as the systemic administration of IL-2, or by antigen-specific immunization, might shift the balance in favor of the host defense, and tumor regression occurs.

### Conclusions

In the recent past, several steps have been made towards answering the complex question of tumor immune responsiveness. In particular, the identification of shared TAs and their T-cell epitopes has led to their broad use as immunogens to induce or augment TA-specific immune responses. In clinical trials, induction of TA-specific immune responses has been remarkably successful. However, the desired clinical outcome has been below the expectations suggested by the immunological results. This discrepancy probably reflects the focus on immune



**Fig. 4.** The fine balance between immune responsiveness and immune resistance. The hypothesis proposed here suggests that 'immunogenic' tumors, such as melanoma and renal-cell carcinoma, constitutively secrete cytokines and chemokines that might induce a local inflammatory reaction. When the inflammatory reaction is strong enough, the tumor regresses spontaneously as observed relatively often in these malignancies. However, most commonly, the inflammatory reaction is not sufficient to induce spontaneous tumor regression. In these cases, response to therapy occurs when the additional inflammatory stimulus brought to the tumor site by antigen-specific and/or nonspecific therapy is above the threshold for tumor regression. By contrast, response to therapy does not occur when this threshold is not reached by the sum of the ongoing inflammatory process and the one induced by the therapeutic maneuver.

responses of circulating lymphocytes that are easily accessible, rather than on the quality and intensity of immunization-induced T-cell responses within the target tissue. Limited attention has been paid to the susceptibility of tumor cells to immune recognition and destruction. The data reviewed here emphasize the need to complement the study of the systemic effects of immunization with the analysis of the adaptation of the host immune system to immunization within the tumor microenvironment and the susceptibility of tumor cells to immune lysis. In addition, the complexity of such interactions requires a multidimensional and dynamic approach to their study to define the mechanism(s) underlying the lack of correlation between the induction of TA-specific immune responses and clinical failures. Appreciation of this complexity might elucidate why the successful induction of TA-specific systemic immune responses by immunization does not uniformly lead to tumor regression.

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### Mouse Knockout & Mutation Database

Established in 1995, the Mouse Knockout & Mutation Database (MKMD; <http://research.bmn.com/mkmd>) is BioMedNet's fully searchable database of phenotypic information related to knockout and classical mutations in mice. MKMD offers over 7000 entries and includes a new reviews section on mouse models of human diseases and up-to-date fact files for all disease reviews.