Minireview

Immunotherapy and immunoselection – tumour escape as the final hurdle

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Abstract Tumours are immunogenic and are commonly infiltrated by anti-cancer effector cells. Why, then, are they not completely rejected by the host? Unfortunately, tumours are Darwinian paragons, winning the battle against the forces of natural immune selection. Some of the latter can even act as double-edged swords, actually being subverted to become pro-tumorigenic. Prevention or reversal of tumour escape from the immune response therefore offers the possibility of reconstituting effective anti-tumour immunity and remains the major challenge for 21st century tumour immunology.

Keywords: Immunosurveillance; Tumour immunology; Immune escape; Immunotherapy of cancer

1. Introduction

Tumours are immunogenic. They possess antigens either not expressed or expressed at low levels by normal tissues. These are recognizable by the immune system. The former include products of chromosomal translocations or mutations, the latter transcription factors, surface receptors or foetal antigens. Moreover, tumours are commonly infiltrated by tumour antigen-specific T cells. Why, then, are they not completely rejected by the host? The explanation currently favoured is that tumours are Darwinian paragons commonly winning the battle against the forces of natural immune selection. There is also a growing appreciation that many responses defined as anti-tumour effector mechanisms can act as double-edged swords, becoming not only ineffective but even pro-tumorigenic depending on the evolving tumour–host relationship. Examples of such potentially double-edged swords are interleukin (IL) 2 (pro-apoptotic for activated T cells), interferon (IFN)-γ (induces ligands for T and natural killer (NK) cell inhibitory receptors), angiogenesis inhibition (causes hypoxia-mediated induction of growth factors promoting metastasis), macrophage free-radical-mediated cytotoxicity (inhibits T cells), etc. Immune selection pressure itself, resulting in outgrowth of resistant tumour variants, could also be viewed in this light. On the other hand, knowledge of the many tumour escape pathways offers the theoretical possibility of reconstituting anti-tumour immunity [1]. For many years now, clinical vaccination trials have sought to trigger or enhance anti-tumour immunity, but thus far, on careful analysis, with rather disappointing results. Many explanations could account for the unimpressive success rates, from the classical concept of immunoselective pressure giving rise to resistant variants (now often termed "immunoediting"), to the more recent realization that tumour-induced alterations to the patient's immune system may subvert anti-cancer responses and even encourage tumour growth. These escape mechanisms may be classified into at least the following major groups: alteration of MHC class I and tumour antigen expression by tumour cells [2]; dysregulated expression of adhesion/accessory molecules by tumour and/or antigen presenting cells [3]; tumour utilization of products of stimulated leukocytes, i.e., immunostimulation of cancer [4]; secretion of immunosuppressive soluble factors either by tumour cells or infiltrating T cells or both [5]; and, as treated below, induction of immune non-responsiveness via anergy induction or clonal deletion of responding T cells, or induction of suppressor cells; and changes in T cell signal transduction molecules.

Tumour escape from immunosurveillance represents the last series of hurdles to be overcome in formulating truly effective cancer immunotherapy [1], but given the immense plasticity of the tumour cell, and the complex balance between pro- and anti-tumour activity of the very same effector pathways, this remains a major challenge for the 21st century. I have recently reviewed progress in our understanding of tumour escape mechanisms [6] and therefore in the present article I focus on alterations to T cell immunity in cancer patients and how these might be manipulated to rejuvenate anti-cancer effector mechanisms.

2. Induction of T cell unresponsiveness

2.1. T cell destruction

Clearly, were the tumour able to simply destroy any anti-cancer T cells, this would be the most direct and effective protective mechanism. There is some evidence that this may occur via the classical pathway of central tolerance induction

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Abbreviations: IFN, Interferon; IL, interleukin; NK, natural killer; TCR, T cell receptor

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[7], but of course, this applies only to newly generated T cells, not those already present before tumour formation. Perhaps more importantly, peripheral tolerance induction may be mediated by tumour-induced T cell death. One major mechanism therefore fas/fas-ligand interactions, still remains controversial. Shortly after activation, T cells begin to express fas (CD95). These CD95+ T cells may become susceptible to fas ligand-mediated cell death, although other outcomes are also possible (in fact, in the context of tumour escape, the non-apoptotic consequences of fas signalling have been relatively neglected (for review, see [8]). Following the first report several years ago, many types of tumour have been reported to express fas-ligand (for review see [9]). Therefore, either as a consequence of the interaction of fas-ligand on the tumour with CD95 on the T cells, or as a consequence of "fratricide" (i.e., fas/fas-ligand interactions between T cells [10]), anti-tumour T cells are killed. Fas-ligand expression by tumour cells may also negatively affect non-T cell components of anti-cancer immunity [11]. These important facets of tumour–host interaction continue to be discussed in a lively manner, but a consensus is clearly emerging that fas/fas-ligand interactions are indeed important for tumour escape [9,12–14]. Protecting T cells against this fas-mediated apoptosis may therefore enhance anti-tumour immunity. This might be achieved by selectively interfering with T cell apoptosis pathways or blocking fas-ligand expression.

2.2. T cell anergy

The tumour environment may be unconducive to T cell function even if the cells themselves are not killed. Mechanisms including antigen-specific anergy-induction may be important in early tumour progression [15], whereas non-specific immunosuppression by a variety of mechanisms may be more important later. Anergy induction may occur when T cells receive activatory signals in the absence of costimulation or in the presence of over-riding negative signalling (see Table 1). The former can occur when the costimulatory receptor CD28 on T cells is either absent or not ligated, the latter when negative receptors such as CD152 or NKG2A are present and ligated on T cells. Additionally, alterations to the "quality" of the TCR-mediated signal due to the nature of the antigen recognized may contribute to anergy induction. For example, naturally occurring peptide sequences from endogenous as well as foreign proteins can act as partial agonists for the melanoma antigen MART1/Melan A (peptide positions 27–35) and anergize anti-tumour T cells [16]. Moreover, the presence of such anergy-inducing peptides on the melanoma cell surface can prevent T cell activation by immunodominant peptides [17]). Awareness of these possibilities is essential for designing appropriate tumour vaccine, to avoid potentially dangerous anergy induction by vaccination with immunogenic peptides representing tumour antigens [18]. Effector T cells identified in melanoma patients may already be anergic and unable to lyse target cells or secrete cytokines on activation [19]. Thus, not only must vaccination seek to avoid de novo anergy induction but also reverse the anergy which may already be present in the patient. This may be achieved by increasing the stimulatory environment using cytokines or transfected costimulatory molecules, as well as antibodies (as recently shown, for example, using agonistic CD137 antibody to break anergy in an animal tumour model [20]).

Table 1
T cell costimulation

Receptor	Ligand	Main effect
CD28 family – expressed on T cells only		
CD28	CD80, 86	Strong costimulation
CD152	CD80, 86	Dominant coinhibition
ICOS	B7RP-1	Accessory costimulation
PD-1	PD-L1, L2	Weak coinhibition when IL 2 limiting (blocks ICOS, not CD28)
Unidentified	B7-H3	Accessory costimulation (↑ IFN-γ) (but coinhibition also reported)
BTLA	B7x	Similar to PD-1
TNFR family – expressed mostly on T cells		
CD40	CD154	DC activation
CD137	4-1BBL	Accessory costimulation
CD134	OX-40L	Accessory costimulation
KIR family – expressed on NK cells, subpopulations of T cells		
KIR2DL, 3DL	MHC class I	Inhibition
KIR2DS	MHC class I	Stimulation
Lectin-like receptor family – expressed on NK cells, subpopulations of T cells		
NKG2A	HLA-E	Inhibition
NKG2D	HLA-E	Stimulation
KLRG-1	Unidentified	Dominant inhibition

2.3. Suppressor T cells

Anergic T cells may act as suppressor cells [21]; additionally, there may be separate lineages of regulatory cells. The recent renaissance of interest in suppressor cells has lead to the re-interpretation of many older data, which had previously been dismissed as artefacts (for review, see [22]). The realization that CD4+ CD25+ regulatory cells play an important role in many aspects of immunological tolerance has led to attention being focused on such cells also in cancer (for review, see [23]). This allows for the development of potential new treatment modalities, for example, in animal models, injection of CD25 mAb preferentially depletes CD25+ CD4 cells and can prevent tumour progression; removing "naturally" activated (i.e., CD25+) CD4+ cells has the same effect [23,24].

2.4. T cell activation blockade

Even if not killed, anergized or suppressed, T cells infiltrating the tumour may be rendered ineffective by modulation of their activatory signal transduction ability. In fact, these alterations may themselves be partially responsible for the observed outcome of cell death, anergy induction or susceptibility to suppression. Both the T cell receptor for antigen itself (TCR) and positive and negative costimulatory receptors, as alluded to above, may be involved in this inhibitory process.

2.4.1. TCR. The original observation that T cell signal transduction is compromised in tumour-bearers [25] has subsequently been confirmed and extended to a variety of human tumours, including renal, colorectal, ovarian, liver, gastric, oral, prostate, pancreatic and cervical carcinomas, glioblastomas and melanomas (for review, see [26]). Of particular interest is the repeatedly documented correlation between these alterations and disease stage in many different cancers. Loss of TCR CD3ζ chain, or abnormal association between zeta chains and other CD3 components, might help to explain the observed gradual decline of cell-mediated responses in patients

and experimental animals with progressing tumours. Several possibilities for reversal of this CD3ζ loss are being tested. In chronic myelogenous leukaemia, for example, the majority of patient's T cells were found to be CD3ζ deficient, and this could be at least partially reversed by stimulation with CD3 mAb, IL 2 and IFN-α in vitro [27]. Whether the same sort of manipulation would be effective in vivo is not yet clear. However, several studies are beginning to suggest that this might be so: normalised CD3ζ expression in patients with myeloid malignancies after successful remission induction has been reported [28] and a majority of renal cancer patients treated with IL 2, IFN-α and lymphokine-activated killer cells also showed improvement of low ζ chain expression [29]. Specific active immunotherapy itself may stimulate the immune system sufficiently to allow reconstitution of CD3ζ chain expression [30] perhaps due to local production of IL 2 which can reverse CD3ζ suppression, at least in vitro [26]. While many mechanisms may account for CD3ζ downregulation, it is encouraging that it may be potentially reversible by relatively simple manipulations.

2.4.2. Costimulation and coinhibition. A complex constellation of monomorphic positive and negative costimulatory receptors, including CD28, CD152, respectively, and many others of this type [31] modulate the signals received via the highly polymorphic antigen-specific TCR (Table 1). Hence, even though the TCR may be fully functional, or be reconstituted as described in the preceding section, T cell inhibition might still occur as a consequence of upregulated negative and/or downregulated positive costimulatory receptors. The panoply of different receptors, of which the list in Table 1 is certainly by no means complete, renders prediction of the outcome of each T cell stimulation extremely complicated and cries out for systems-biological mathematical modelling.

3. Unifying hypothesis: cause of T cell dysfunction is chronic antigenic stress

Be that as it may, it is clear that in chronic infection or inflammatory disease, clonal expansions of dysfunctional T cells are found and can be identified by their expression of these positive and negative coreceptors, and other surface molecules [32]. These cells may accumulate because the source of antigen stimulating them cannot be cleared. In the elderly, persistent herpes viruses, especially CMV, provide a reservoir of antigen and a constant stimulus to T cell-mediated immunity, and T cells of the same type can be identified. In cancer patients, chronic exposure to tumour antigens may result in a similar phenomenon. These conditions are characterized by the accumulation of apoptosis-resistant anergic CD8 cells at the expense of decreased numbers of CD4 helper cells. In the very elderly, accumulation of CMV-specific CD8 cells, decreased numbers of CD4 cells and an inverted CD4:8 ratio predict incipient mortality [33]. We suggest that a similar situation applies to cancer: chronic antigenic stress results in an accumulation of dysfunctional CD8 cells, selective clonal deletion of CD4 cells and consequently decreased immune responses to the target as well as to other antigens. We further hypothesize that dysfunctional CD8 cells accumulate because of selective loss of the CD4 helper cells, which, at least in vitro, become more susceptible to lysis on chronic stimulation [34] in contrast to CD8 cells, which become more resistant [35]. We therefore suggest that replacing the lost CD4 cells will "rejuvenate" the response. This could be approached by generating such CD4 cells in vitro and infusing them into the patient as adoptive immunotherapy or intervening in vivo to achieve this aim by targeting the involuting thymus to stimulate increased production of larger numbers of new T cells. The former aim may be the more amenable at present. Approaches to extend the lifespan of CD4 cells may include genetic manipulations to protect against damage, for example, enforced expression of heat shock proteins (because there is an age-associated decrease in expression of various stress response proteins with age in humans [36]) or proteasome ß1 or ß5 chains (because age-associated impaired proteasome function is associated with decreased stress resistance which can be reversed by gene transfection [37]). They may also target enhancing repair of damage nonetheless accumulating, as for example, is now possible in yeast [38]. Expression of the catalytic component of telomerase, hTERT, is also a candidate, although the mechanisms of its action, and the still unresolved issue of why hTERT sometimes but not always results in "immortalization" [39,40] (at least of human CD8 clones), requires further investigation. Blockade of negative receptors such as KLRG-1 and CD152 may also be beneficial, but given that the complexity and probable redundancy of signalling systems (Table 1) may be difficult to achieve. Nonetheless, in the T cell clonal model of immunosenescence [41] it is the level of expression of the major costimulatory receptor CD28 which seems dominant [42]. Therefore, controlling the level of functional CD28 expression may be paramount. This could be achieved by manipulating the cytokine microenvironment to prevent TNF-α from downregulating CD28 [43], for example, using neutralizing antibodies [42] and/or by the ability of IL 12 to upregulate CD28 expression [44]. Gene transfer to enforce ectopic expression of CD28 may also be a suitable avenue to pursue [45].

Immune escape mechanisms that are being identified in tumour immunology may be informative for microbiological escape [3]. So the means to rectify this state of affairs, which are beginning to become available, may be of enormous clinical importance not "only" for cancer but for a diversity of other intractable diseases as well.

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