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Activation of cytotoxic T cells by solid tumours?

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Abstract. Tumour-specific cytotoxic T cells (CTLs) are among the best-defined biological anticancer weapons. Nevertheless, they often fail to control tumour growth in vivo. Many reasons for this have been evoked: tumours may actively inhibit CTLs, or may protect themselves from CTL recognition by various means. However, one does not necessarily need to postulate such active immune evasion mechanisms specifically acquired by tumour cells. In this review we argue that the failure of immune protection is due to the intrinsic inability of tumours to activate an effective immune response, and that many tumours are similar to normal tissues in this respect. It is striking to see that the majority of the so-called immune escape mechanisms are not specifically acquired by selected tumour cells, but are common mechanisms shared between solid tumours and normal, healthy tissues. Immune responses are poor because tumour antigens do not efficiently localize to lymph follicles in lymphoid tissues, and are not efficiently presented to CTLs in an immunogenic context. The fact that tumours do not induce CTLs but are often susceptible to lymphocyte-mediated cytotoxicity indicates that more intensified immunization protocols should result in improved clinical outcome.

Key words. Acute autoimmunity; chronic autoimmune disease; CTL; immunotherapy; tumour antigen; tumour immunogenicity.

Immunosurveillance

The term *immunosurveillance* is used to describe immune mechanisms that counteract uncontrolled tumour growth. There are two extreme points of view: one is that the immune system may react to tumours but this does not protect from malignomas, and the other that most malignancies never become clinically apparent because they are controlled by protective immune responses. The truth lies somewhere in between.

A major focus of current research in tumour immunology is to more clearly define tumour-specific immune mechanisms. There is accumulating evidence that the immune system can indeed protect from malignant tumours. Tumour patients with ongoing inflammation and immune responses usually have a better prognosis than patients without inflammatory activity. Another well-known observation is the increased incidence of malignant tumours in patients with disabled immune functions, for example in HIV infection or during immunosuppressive drug therapy. Finally, and as outlined below, some experimental immune therapies have successfully induced protective antitumour reactions.

Virally induced tumours have been found to be more susceptible to immune attack than other types of tumours. However, it is difficult to establish general rules or categories that allow us to predict whether or not a particular tumour will be susceptible to immunotherapy. There is a very broad range of natural immune activities to tumours. Often, localized solid tumours are not affected by the immune system, while they are rejected at distant sites, a phenomenon called 'concomitant immunity'. As a simplified rule, syngeneic tumours are frequently unaffected by the immune system, whereas most allogeneic (derived from another individual) or xenogeneic (derived from another species) tumours are readily rejected by the host's T lymphocytes. There are, however, occasional observations of growth

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of even allogeneic tumours, such as the recently reported development of a local sarcoma in the hand of a surgeon who inoculated this tumour accidentally during surgery on a patient with a sarcoma [1].

It is important to elucidate the reasons for the failure of immunosurveillance. The current knowledge on potentially responsible factors will be summarized in this review. The main focus will be on tumour-reactive cytotoxic T lymphocytes (CTLs). The involvement of T helper lymphocytes, NK and LAK cells, macrophages or antibodies in tumour protection have been reviewed elsewhere [2, and references therein] and are not part of this paper.

Tumour-rejection antigens

CTLs recognize peptides that are derived from endogenously synthesized proteins and are presented on the cell surface on major histocompatibility complex (MHC) class I molecules [3–6]. The large number of genetic alterations found in cancer cells could give rise to proteins with novel sequences and thus to tumour-specific peptide 'neoantigens' that could be recognized by CTLs when presented on MHC class I molecules [7]. Based on this concept, many researchers probed tumours for antigen expression and undertook great efforts to molecularly characterize tumour antigens recognized by CTLs [8, 9]. Because of these efforts, many antigens have become available. It is now clear that CTLs can specifically and sometimes also efficiently interact with tumour cells through recognition of MHC/tumour peptide antigen complexes by their T-cell receptors (fig. 1). Based on this knowledge, patients can now be immunized with selected antigens, opening an important field for clinical trials in oncology.

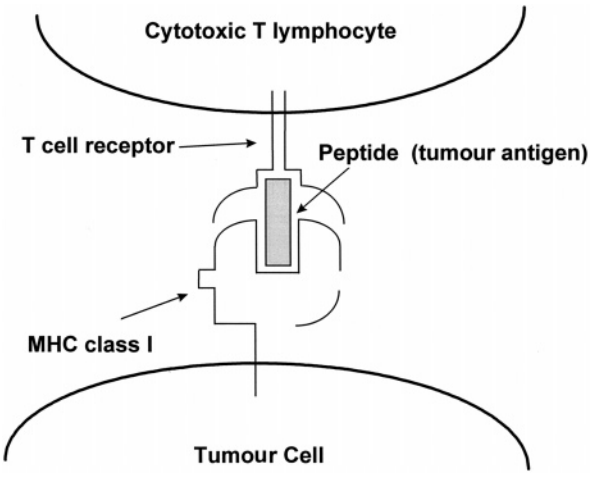

Figure 1. Antigen-specific interaction between a T cell and an antigen-bearing tumour cell. Similar to peptide antigens derived from pathogens such as viruses, peptide antigens derived from tumour cells are presented by MHC class I molecules on the cell surface. The MHC/peptide complex is recognized by the T-cell receptor.

Table 1 shows examples of tumour antigens classified into three major groups. The tissue-specific antigens represent the largest group. Since melanomas were found to be relatively well recognized by patient CTLs, this type of tumour was intensively analysed in the search for tumour CTL epitopes. Therefore, most of the available antigens are specifically expressed in cells of the melanocyte lineage [10–16].

The second group are antigens that are preferentially expressed by tumour cells (i.e. tumour-'associated'), but are also found in some healthy tissues. These antigens are derived from oncogenes [17, 18], tumour suppressor genes [19] or various other nonmutated proteins [20–25]. Finally, the third group consists of tumour-specific antigens derived from mutated genes [26–31]. From several points of view, these are the most ideal tumour antigens, since the patient's immune system is less likely to be tolerant to such antigens, and autoimmune attack to healthy tissues may not occur. However, this third group of antigens is rather small, in part because the mutation must be in an amino acid sequence stretch that can associate with an MHC product of the patient. Also, most patients have individual mutations, which would need to be identified individually, and vaccines would need to be developed accordingly. In summary,

Table 1. Tumour antigens recognized by CTLs.

	References
Tissue-specific	
lineage specific proteins	
Melan-A/MART-1, gp100	[10, 11]
tyrosinase	[12, 13]
tyrosine-related protein (TRP)-1	[14]
TRP-2	[15]
TRP-2 (murine)	[16]
Tumour-'associated'	
oncogenes	
human papillomavirus E6 and E7, HER-2/neu	[17, 18]
tumour suppressor genes	
p53	[19]
nonmutated proteins	
MAGE, BAGE, GAGE, RAGE, *N*-acetylglucosaminyltransferase type V	[20–23] [24]
P1A (murine)	[25]
Tumour-specific	
mutated proteins	
β-catenin MUM-1, CDK-4, CASP-8	[26–29]
connexin 37 (murine), p53 (murine)	[30, 31]

All antigens were characterized in humans except P1A and connexin 37, which are murine and TRP-2 and p53, which contain peptide epitopes demonstrated in both humans and mice.

Table 2. Mechanisms involved in failure of immune protection against tumours.

	References
A) Specialized mechanisms acquired by tumour cells (by mutation selection and outgrowth of immune escape mutants)	
mutated peptide antigens	[26–28]
MHC class I or peptide downregulation	[36–39]
failure of lymphocyte homing	[40]
B) Mechanisms shared by many normal tissues and tumours	
lack of MHC class II expression	[103]
lack of costimulatory and adhesion molecules	[41–46]
lack of T help	[50]
induction of lymphocyte unresponsiveness	[51, 52]
killing of lymphocytes by target tissue (FasL mediated)	[58]
prostaglandins neuropeptides	[53]
immunosuppressive cytokines (TGF-β, eventually IL-10)	[54–57]
shedding of antigens receptors	
'hiding' of target tissue cell surface (fibrin, glycocalyx)	
cellular growth kinetics (sneaking through of tumours)	[60, 61]
tissue localization	[62, 63]

The assignment of the mechanisms to group A (specialized mechanisms acquired by tumour cells), or group B (mechanisms shared by many normal tissues and tumours) are based on current knowledge and are thus in part preliminary. For many mechanisms, further evaluation is necessary before more definitive statements can be made as to whether they belong to group A or B.

for the majority of patients no tumour-specific antigens are available, and most tumour epitopes are thus also expressed by some normal healthy tissues. This issue will be discussed later in this review.

Immune escape mechanisms

The large volume of data reviewed above and elsewhere [32–35] makes it clear that tumour cells express many antigens that can be recognized by CTLs. However, the immune system often fails to protect the host from malignant tumours. Tumours therefore express antigens to which the immune system can react, but there is no efficient protection. The reasons for this are only partially understood, and they need to be further elucidated in order to establish a solid scientific basis for the development of efficient tumour vaccines.

Several reasons may account for escape of tumour cells from immune protection (table 2). Tumours may mutate the peptide antigens they present on MHC class I [26–28]. Alternatively, they may reduce MHC or peptide expression [36–39]. Recently, a further strategy has been identified, since it has been demonstrated that some of the various lymphocyte homing receptors are not expressed by endothelial cells in tumour vessels [40]. The fast mutation and growth rate of tumours is likely to contribute to the establishment of immune escape mechanisms, because one can expect that the most vital tumour cells are selected and grow out to immunologically 'resistant' tumours.

One is tempted to conclude that the lack of immune protection in cancer patients is due to immune escape mechanisms which are actively acquired by tumour cells. Does this indicate that tumour cells are much more efficient in evading the immune system than the immune system is in performing antitumour activity? If this is the case, then severe doubts must be raised as to whether tumour vaccines will ever be efficient.

Tumour immunogenicity

The immune escape mechanisms outlined above (table 2A) are examples of properties that have been identified in some tumour cells. In this review we would like to introduce the novel distinction of tumour-specific mechanisms vs. mechanisms shared between tumours and normal tissue. It is striking to see that the majority of the so-called immune escape mechanisms are not specifically acquired by selected tumour cells, but are common mechanisms shared between solid tumours and normal, healthy tissues that together help maintain tolerance and avoid autoimmunity. Such common mechanisms are listed in table 2B.

In contrast to antigen-presenting cells, normal and tumour cells of solid body tissues often do not express MHC class II molecules and therefore are unable to interact with the $CD4^+$ helper T-cell subsets. Together with the lack of costimulatory and adhesion molecules, this results in poor antigen presentation and thus inefficient immune activation [41–46]. As a consequence, essentially no T-helper cell activity is induced, and CTL activation is limited [47–50]. These properties may also support the induction of lymphocyte unresponsiveness [51, 52].

Other immune escape mechanisms include local secretion of prostaglandins, neuropeptides, transforming

growth factor (TGF)-β, or interleukin (IL)-10 [53–57] which can be detected in local inflammatory reactions. Recently, it was described that Fas ligand expressing cells of various organs can kill lymphocytes [58], but the expression of Fas ligand has also been found to be associated with various other functions [59]. Further possible mechanisms contributing to immune escape may be the shedding of membrane antigens or receptors, or the 'hiding' of the target cell surface by fibrin layers or glycocalyx.

Many years ago it was observed that the cellular growth kinetics play a crucial role. Tumours with a small initial cell load were associated with absence of immune protection, a phenomenon termed 'sneaking through' of tumours [60, 61]. Subsequent studies suggested that tumour localization was of great importance. While tumour cells in the periphery did not activate immune cells, their presence in secondary lymphoid tissues caused significant and protective immune responses. The localization appears to be more important than previously thought, and it is likely that not only the few defined immunologically 'privileged sites', but most non-lymphoid organs and solid tissues are inefficient in promoting immune responses [62, 63].

Thus, in many cases, the tumour is not in an environment that enhances an antitumour response, or tumour metastasis to organized lymphatic tissues occurs too late to induce an effective antitumour response in vivo. Some degree of immune response may nevertheless be induced, but activation is usually inefficient [18, 64]. Furthermore, lymphocytes may be rendered unresponsive locally, as was recently demonstrated in an elegant study of murine insulinomas [52]. This suggests that solid tumours may tolerize CTLs, confirming earlier observations on peripheral tolerance induction [65–68]. Further studies are necessary to elucidate whether the induction of CTL unresponsiveness can occur systemically or whether this phenomenon remains restricted to localized peripheral sites.

We have performed studies to investigate tumour growth and immunosurveillance in vivo [69]. We have generated a murine model with endogenous insulinomas that develop due to the transgenic expression of an oncogene (SV40 large T antigen) in pancreatic β-cells [70]. As a model tumour antigen, a viral glycoprotein (of the lymphocytic choriomeningitis virus; LCMV-GP) was introduced by transgenic expression in pancreatic β-cells [71–73]. In this model, the LCMV-GP-specific T cells were not tolerant to the LCMV-GP self-antigen.

Mice that develop insulinomas had a short life expectancy of only about 12 weeks, since the uncontrolled insulin overproduction by the tumour cells induced lethal hypoglycemia. Infection with LCMV led to the activation of LCMV-GP-specific T cells and specific infiltration of the pancreatic insulinomas expressing LCMV-GP, which prolonged the survival of the animals by 4 to 5 weeks. This demonstrated that already a single infection with a tumour antigen expressing virus can successfully treat tumours, mediated by tumour antigen-specific CTLs.

However, the immunotherapy treatment did not eradicate the tumours entirely, and the disease progressed in all cases. After immunotherapy, the islet tumours still expressed immunologically detectable levels of tumour-associated antigen and MHC class I, and memory LCMV-GP-specific CTL remained detectable and could be reactivated normally. These results demonstrate several important points. T-cell tolerance to tumour-associated antigens did not develop in vivo, even in the presence of a high tumour burden with recurring tumours. Furthermore, the islet tumours were not sufficiently immunogenic, since they did not induce an immune response and did not activate CTLs. More important, the islet tumours were even unable to maintain a functional memory CTL response in vivo. The result was that memory CTLs did not provide sufficiently long lasting protection against tumour regeneration. In addition, we have shown that a tissue-specific ligand may serve as an effective continued target for immunotherapy in vivo.

There is clear evidence that solid tissues and tumours are intrinsically inefficient to activate immune responses, supporting the notion that this may be the major mechanism of peripheral lymphocyte unresponsiveness, rather than the active induction of immune tolerance in the periphery. Interestingly, in many cases one can identify weak activation of CTLs specific for tumour antigens. This low-level activation may be insufficient to protect from tumour cells, but can be increased in strength through appropriate therapeutic means. One example is the enhancement of antitumour responses by treatment with anti-CTLA-4 antibody to block the inhibitory effect mediated through the T-cell surface molecule CTLA-4 [74].

Tumour cells may or may not induce a primarily efficient immune response. It is important to determine which of the two possibilities is the more general pathway. One may keep in mind that one of the major findings in tumour immunology during the past years was that 'most tumours are antigenic but not immunogenic', and that this was confirmed by a large number of papers. This speaks in favour of the argument that tumours are generally inefficient in activating immune response. It does not, however, exclude the possibility that induction of lymphocyte unresponsiveness may also occur. Further studies are necessary to determine which of the two possibilities are more dominant. There is an urgent need to resolve this issue in order to understand the failure of immune protection in cancer patients. In any case, current knowledge indicates that multiple and/or continuous stimulation against tumour-

associated antigens may be required to maintain tumour immunosurveillance in vivo.

Autoimmune responses

As reviewed above, the list of immune escape mechanisms which are shared by normal and tumour tissues is longer (table 2B) than the list of mechanisms specifically acquired by tumour cells (table 2A). This supports the concept that 'immune escape' is often not a specialized property of malignant cells but is a more general feature of solid body tissues. It is likely that the immune system has evolved primarily to protect from pathogenic microbes and that reactions to uninfected body (tumour) cells are limited and kept under tight control. The reason for this is probably the risk of autoimmunity. In this regard, one may operationally define two 'types' of immunity against self-antigens and tissues: first, immune responses triggered by microbes (or other pathogenic factors), which are (cross-) reactive to self-structures and usually only last until the pathogen is cleared; second, the much more aggressive type of autoimmunity where the autologous tissue itself together with unknown factors triggers continuous autospecific destruction. The former mechanism is transient and can be controlled in most cases by treating the underlying pathogenic trigger (usually an infection), whereas the latter results in continuous, aggressive and/or chronic autoimmune disease. The distinction of these two types of immunity is artificial but nevertheless useful in defining and understanding immune activity. We would like to use the term *acute autoimmunity* for the first, and *chronic autoimmune disease* for the second type of autoimmunity.

As mentioned earlier, the majority of the known tumour antigens are not entirely tumour specific but are also expressed by normal tissues (table 1). Therefore, treating cancer patients by immunization against self-antigens must take into consideration that potentially harmful autoimmune reactions may be triggered. Not only in experimental models, but also in patients, it is important to determine whether such reactions may become self-sustaining and eventually turn into chronic autoimmune disease.

In several instances, autospecific immune responses associated with cancer have been observed. Melanoma patients may have lymphocytes activated against tyrosinase and other melanocyte-specific antigens [75–77] which can cause destruction of healthy melanocytes as revealed by clinically apparent vitiligo. This has also been demonstrated in an animal model, since passive immunization with antibodies directed against the melanocyte differentiation antigen gp75 (tyrosinase-related protein 1) successfully treated murine melanomas but caused limited damage of normal skin [78].

There is clear evidence that autoimmune responses can help control tumour growth in patients: the presence of autoantibodies has been correlated with improved prognosis [79, 80]. Furthermore, the relatively rare occurrence of vitiligo in persons with metastatic melanoma was associated with prolonged patient survival [81, 82].

Importantly, both clinical and experimental evidence suggest that autoimmune damage of healthy tissues remains limited despite effective antitumour immune activity [75–78, 81–84]. Thus, the findings do not support the 'trigger and go' concept, whereby antiself immune responses, once triggered, become self-sustaining and cannot be stopped. Rather, our current knowledge indicates that immune responses against solid tissues are generally not self-sustaining [69].

Immunotherapy

Tumour antigen-specific CTLs can frequently be isolated from the blood or tumour-infiltrating lymphocytes of patients. However, the CTLs are quiescent and not 'spontaneously' activated. Moreover, it is often difficult to activate such CTLs by specific immunization. Therefore, it is important to develop more efficient means of immunization.

Many different protocols have been evaluated for immunization. Only a few aspects are briefly mentioned here: Special antigen-delivery protocols are being developed to render tumour antigens more immunogenic and to target them to lymphoid compartments. Purified proteins or synthetic peptides are poorly immunogenic and may depend on efficient adjuvants [85–89]. Alternatively, autologous antigen-presenting cells may be administered after introduction of tumour antigens with either defective recombinant retroviruses, vaccinia or adenovirus constructs [41, 90, 91]. Recently, promising results have been obtained with dendritic cells [92–94], which present antigens loaded as proteins [95], peptides [96] or material eluted from tumours [97]. The use of genetically modified *Salmonella* bacteria or recombinant Bacillus Calmette-Guérin (BCG) is also under investigation [98, 99]. Finally, passive immunization by adoptive transfer of activated lymphocytes may successfully eliminate tumour cells [100], but this approach largely depends on the success of currently developing techniques for in vitro T-cell activation and expansion.

The currently most efficient clinical tumour immunotherapy is the Graft-versus-Leukaemia (GvL) effect, which is observed after allogeneic bone-marrow transplantation [101, 102]. We would like to outline the prominent features of GvL biology, since they clearly illustrate the hallmarks of antitumour immunity. Most data point to a common mechanism of GvL activity

and Graft-versus-Host (GvH) disease. Both are mediated by activated allospecific donor lymphocytes attacking host tissues. Since haematopoietic host cells are among the major target tissues of GvH disease, GvH activity leads to destruction of host haematopoietic and thus also leukaemic cells. GvL activity can be seen as a 'side product' of continuous lymphocyte activation by host antigen-presenting cells competent to initiate and maintain T-cell alloreactivity. This situation represents continuous lymphocyte activation leading to sustained antitumour activity. It is very likely that long-term immune tumour protection is the direct result of continuous immune stimulation.

The disadvantage of GvL activity is the obligatorily accompanying GvH disease, which is sometimes difficult to control. In this situation, immunogenic antigen-presenting cells and cytokines reside within the lymphoid organs of the patient and cannot be withdrawn. In contrast, in immunotherapy of (solid) tumours, immunogenic reagents can be administered until the desired effect has been reached or until undesired side effects become limiting.

In conclusion, there are at least two factors justifying that patients should receive not single but serial or continuous immunizations. First, tumour antigens must be present for an extended time period in an immunogenic context, and second, the danger of triggering chronic autoimmune disease is limited.

Conclusion

Immune protection may be responsible for the elimination of some types of tumours before they become clinically evident or in cases of spontaneous regression. The immune system is, however, not successful in controlling the majority of progressed malignancies. In these situations, therapeutic interventions activating lymphocytes can lead to inhibition of tumour growth or may even eradicate tumours. In recent years, a variety of tumour antigens targeted by specific CTLs have been characterized. Interestingly, most of these antigens are not exclusively expressed by tumours, but also by other tissues. Nevertheless, the specific CTLs with significant antitumour effects have not caused major damage to healthy tissues expressing antigens shared with tumours. The results emphasize the existence of many self-specific T cells, which are not activated by self-tissues or by tumours, but can exert tumour-selective activity upon therapeutic immunization. 'Immune resistance' of tumours is therefore often due to the inability of tumours to activate immune cells, rather than to immune evasion mechanisms actively acquired by tumours. This indicates that optimizing and intensifying immunization protocols is a promising strategy to improve tumour immunotherapy.

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