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Cancer Escape from Immune Surveillance: How Can it be Overcome by Gene Transfer?

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INTRODUCTION

IT IS generally accepted that the spontaneous generation of cancer cells is a common event, and that the immune system assures a strict surveillance: the detection of a mutant cell leads to its rapid elimination by immune mechanisms, and prevents its progression to clinically detectable disease. A tumour thus develops only when a mutant cell escapes the immune surveillance [1, 2]. T lymphocytes are critical in controlling such anti-tumour immune responses. Recently, a better understanding of the molecular events of antigen presentation and lymphocyte activation in animal models has led to the identification of epitopes on tumour cells that can be recognised by T-cells. It now appears unequivocal that spontaneous human tumours also express tumour-specific antigens that are recognised by antibodies and/or by T-cells. Many of these are encoded by normal cellular genes and are recognised because of over-expression or aberrant expression [3]. Tumour antigens may also be encoded either by mutated cellular genes, or by viral genes. Such antigens may be weak rejection antigens failing to induce effective T-cell responses, either because they share a high homology with self epitope, or because the local environment surrounding the tumour cell may fail to support an immune response or be immunosuppressive. Tumour cells can, in fact, escape or fail to elicit tumour-specific immune responses by various mechanisms.

HOST T-CELL IMPAIRMENT

Even when cancer cells express an antigenic molecule, the host may not respond to the tumour because of a selective deletion or suppression of some T-cell populations. For instance, selective deletion of mature peripheral Vβ2+ T-cells (mostly CD4+) was observed in Balb/C mice inoculated with preneoplastic and neoplastic mammary carcinomas [4]. This phenomenon, similar to that reported for superantigens of either bacterial or viral origin, was shown by adoptive transfer experiments, to be mediated by host cells and to be related to the presence of a mouse mammary tumour virus (MMTV). Likewise, a phenomenon of T-cell unresponsiveness or anergy in a human tumour microenvironment exists, but has not yet been extensively investigated. It is well established that many human tumours are infiltrated by T-cells that can express activation antigens, such as major histocompatibility complex (MHC) class II antigens (HLA-DR) and interleukin (IL)-2 receptors [5]. Surprisingly, however, when fresh tumour-infiltrating lymphocytes (TIL) are isolated from human solid tumours and tested *in vitro* for antitumour function, they show poor cytotoxicity against autologous tumour or other tumoral targets, and fail to proliferate in response to T-cell mitogens. This anergic state seems to vary between tumours, and is reversible upon isolation of TIL and culture in the presence of exogenous IL-2 [6–8]. Therefore, although this possibility cannot be definitively ruled out, it seems unlikely that the lack of immune competence of TIL results from a selective deletion of certain subsets of tumour antigen-reactive T-cells. Rather, the T-cell unresponsiveness would mainly be due to a tumour-induced suppression, operating perhaps through a downregulatory network of T-cells and cytokines.

The requirement of both CD4+ and CD8+ T-cells for the induction of immune control on neoplastic growth was demonstrated by the work of Flamand and colleagues [9]. Using selective depletions of lymphocyte subsets *in vivo*, they showed that both CD3+CD4+ and CD3+CD8+ T cells are involved in immune responses to the class I-positive, class II-negative mouse mastocytoma P815. This work highlighted the central role played by CD4+ T-lymphocytes in immune surveillance [10, 11]. Since these cells are activated by exogenous proteins presented in association with class II histocompatibility antigens, sensitisation protocols based on the use of purified tumour antigens may prove useful for increasing the immune response against tumour. This strategy is illustrated by the work of Kündig and colleagues [12], who investigated the induction of immunity to an artificial tumour-associated antigen, the

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nucleoprotein (NP) of vesicular stomatitis virus (VSV), transfected into the EL-4 thymoma tumour cell line, syngeneic of C57B1/6 mice. The EL-4-transfected cells were lysed *in vitro* by NP-specific CTL, and could effectively restimulate them. *In vivo*, however, EL-4/NP cells grew like the EL-4 control cells and failed to induce a specific cytolytic response. When mice were pre-immunised against the native NP antigen by infection with VSV or VSV-vaccinia recombinant virus, they were protected against lethal tumour growth of EL-4/NP cells by CD8+ CTL, but not by CD4+ T-cells. This study suggests that effective anti-tumoral immune responses can be mounted by previously immunising the host with cells modified by tumour antigen gene transfer.

As discussed previously, any functional impairment in the pathway of CD4+ T-cell activation may represent a central aspect of immune dysfunction in the tumour-bearing state. A paper recently published by Schoof and colleagues [13] supports this hypothesis. They studied the characteristics of T-cells isolated and cloned from the tumour site in patients with renal cell carcinoma (RCC). Among these cells, the predominant lymphocytic population consisted of non-cytolytic helper CD4+ T-cells capable of secreting IL-4, but not IL-2, and only low levels of interferon-γ (IFNγ) following *in vitro* activation. These results suggest that the immune failure observed in RCC could be related to a tumour infiltration with a T-cell subset, resembling murine TH2 lymphocytes, which can neither produce IL-2 nor provide T-cell help for the generation of effective CTL responses.

Downregulation of T-cell functions by tumour-related activation of lymphoid suppressor cells has also been proposed as a major mechanism responsible for depressed immunocompetence in patients with cancer. Mukherji and collaborators have extensively studied this phenomenon of cell-mediated suppression and its contribution to regulating T-cell responses to autologous tumours, especially in melanoma and renal cell carcinoma [14, 15]. Their analysis of T-cell clones that react against autologous human tumours supports the existence of a CD4+ T-cell-mediated suppression of anti-tumour cytotoxic response that is antigen-specific. This suppression seemed to result from a cytokine synthesised by these CD4+ suppressor T-cell clones, which were none of the following regulatory cytokines: IL-4, IFNγ, tumour necrosis factor (TNF) α, TNFβ or transforming growth factor (TGF) β.

However, tumour cells themselves have the capacity to blunt T-cell responses by the secretion of ill-defined substances. This was illustrated in an *in vivo* model of murine colon adenocarcinoma MCA38, weakly immunogenic in C57B1/6 mice [16, 17]. Splenic T-cells obtained from late tumour-bearing mice showed a marked decrease in therapeutic efficacy when adoptively transferred compared to those obtained from normal mice or from early tumour-bearing mice. This decrease was related to a decreased lytic function of CD8+ T lymphocytes associated with a reduced expression of mRNA for TNF-α and with a decreased ability to mediate an antitumour response *in vivo*. In this setting, proliferation, lymphokine production and up-regulation of lymphokine receptor in CD4+ T-cells were comparable in normal and tumour-bearing mice. Suppressive signals originate from the tumour itself. Decreased CD8+ T-cell function was associated with structural changes in the chains of CD3 complex and with impaired T-cell signalling, probably related to a decreased expression of some tyrosine kinases. The same authors observed similar abnormalities among T-cells from Balb/C mice bearing a Renca renal cell adenocarcinoma and among peripheral blood T-lymphocytes isolated from cancer-bearing patients.

IMPAIRMENT OF CO-STIMULATORY SIGNALS

T-cells in tumour-bearing hosts are not systematically anergic, but can fail to reject the tumour because of a lack of 'help' at the site of tumour growth. This was illustrated in a model of skin tumour in C3H mice. In this model, animals bearing malignant grafts failed to reject the tumour cells, but rejected normal transgenic grafted cells modified by transfer of the gene coding for the same rejection antigen, namely the highly immunogenic MHC class I antigen K216. Effective stimulation of antigen-specific CTL was indeed obtained after expression of the antigen into non-malignant cells, but failed to induce rejection of the established tumours. Additional manipulations will be required to break the state of immunological unresponsiveness of the tumour-bearing mice [18].

One possible mechanism by which tumour cells fail to elicit an effective immune response may be the lack of co-stimulatory signals necessary to activate T-cells. Indeed, two signals are required for the activation of a T-lymphocyte: first, an antigen-specific signal delivered through the interaction of antigen and T-cell receptor (TCR) and, second, an antigen non-specific or co-stimulatory signal delivered through the interaction between some recpetors on T-cells and ligands expressed on antigen-presenting cells. In the absence of such signalling, antigen–MHC complex interaction may lead to T-cell anergy or deletion. Several molecules are capable of providing co-stimulatory signals. Among them is the interesting molecule B7/BB1, which is expressed on activated B-cells, dendritic cells and macrophages and which interacts with its receptor CD28/CTLA-4 on T-cells, providing a key signal for the generation of T-cell immunity.

Considering that most carcinomas express MHC class I antigens but not B7, different groups of investigators reported that transfection of the B7 gene into tumour cells increases their immunogenicity. Townsend and colleagues [19] and Chen and colleagues [20] used the same mouse model, the melanoma cell line K1735, which is poorly immunogenic and B7-negative. They found that B7-positive variants were rejected by immunocompetent mice, and that such mice develop a systemic immune response against the B7-negative parental cell line. This rejection was mediated by CD8+ T-cells which can function, in this setting, in the absence of help from CD4+ cells. However, in the first study, the expression of B7 alone was sufficient to induce tumour rejection, whereas in the second, the K1735 cells also had to express a foreign viral antigen for rejection to occur. In fact, it seems that animal tumours which express detectable rejection antigens will not grow in immunocompetent mice following the transfer of the B7 gene, whereas tumours which fail to induce an immune response still grow in immunocompetent mice, even after overexpression of B7 [21]. Moreover, the fact that many B-cell lymphomas not only express potential rejection antigens, but also high B7 levels, suggests that the presence of this molecule alone on tumour cells does not necessarily lead to elimination, and that still unknown factors limit the effectiveness of B7 co-stimulation of antitumour immunity [22].

In vitro experiments suggest that IL-10 interfers with the co-stimulatory properties of antigen-presenting cells, thereby inhibiting their ability to induce T-cell activation. The fact that IL-10 inhibits B7 upregulation on mouse macrophages [23] and decreases both ICAM-1 and B7 expression on human monocytes [24] might contribute to its immunosuppressive properties, especially in antitumoral immune responses as discussed below.

Engineering tumour cells to secrete cytokines could provide the second signal needed to convert these cells into effective antigen-presenting cells. Among the cytokines investigated to date, granulocyte–macrophage colony-stimulating factor (GM-CSF), first identified as a growth factor for haemotopoietic progenitors, might be one of the most powerful cytokines for this purpose. In a mouse B16 melanoma model, comparing the ability of 10 different molecules to enhance the immunogeneticity of tumour cells, irradiated B16 cells transduced with the GM-CSF gene were indeed found to stimulate potent, long-lasting and specific anti-tumour immune responses involving both CD4+ and CD8+ cells [25]. The effect of GM-CSF may be related to its capacity to induce the differentiation of haematopoietic precursors into macrophages and/or into dendritic cells which are the most potent antigen-presenting cells for T helper cells [26].

Other studies have focused on boosting anti-tumour immune responses by providing 'help' in the form of lymphokines normally produced by CD4+ T cells [27]. For example, Fearon and colleagues [28] showed that transfection of poorly immunogenic murine tumour cells (the colon cancer CT26 or the melanoma B16 cell lines) with the IL-2 gene elicits a stong cytolytic response, which is protective against subsequent challenge with the parental tumour cell line. Providing IL-2 locally by the tumour cell bypasses CD4+ helper function in the generation of an anti-tumour response. Similarly, a spontaneously arising murine renal cell tumour engineered, by gene transfer, to secrete IL-4 at high local concentrations can generate tumour-specific CD8+ T-cells *in vivo*, capable of producing systemic protective immunity. Thus, mice immunised with Renca-IL-4 tumours efficiently reject subsequent challenges of parental non-transfected Renca cells at distant sites. Moreover, this IL-4 vaccine can, under precise conditions, cure animals bearing established parental tumours. The predominant infiltrate in the IL-4 transfected tumours consists of activated macrophages and eosinophils. IL-4 thus seems to play a role in the recruitment of antigen-presenting cells and probably in the enhancement of tumour antigen presentation [29].

IMPAIRMENT OF MHC CLASS I EXPRESSION

Another way for tumour cells to escape detection by the immune system might be the failure or poor expression of MHC class I molecules [30]. This mechanism was illustrated by work on the Lewis lung carcinoma 3LL, which is a low immunogenic and highly-metastatic cell line in syngeneic C57B1/6 mice, and which expresses low levels of MHC class I antigen. Restoration of class I expression by gene transfer abrogated the metastatic competence of the transfectants, while elevating their immunogenicity [31]. Similar results were obtained in the B16-BL6 variant of a poorly immunogenic mouse melanoma of spontaneous origin which is deficient in the expression of both MHC class I and class II antigens. When these cells are transfected with a MHC class I gene, they become non-tumorigenic in syngeneic mice, and can effectively protect the inoculated mice against a subsequent challenge with highly tumorigenic class I deficient parental cells [32]. While mediating a direct anti-proliferative effect on some tumour cells, IFNγ also promotes activation and differentiation of cytotoxic T-lymphocytes, and amplifies the tumoricidal activity of nature killer (NK) cells and macrophages. Moreover, it induces or enhances the expression of MHC class I and class II antigens, of cell adhesion molecules and of transporter genes. The IFNγ gene transfer into the murine fibrosarcoma CMS-5, a weakly immunogenic and methylcholanthrene-induced tumour, abrogated the tumorigenicity of the cytokine-producing cells. This suppression was related to an increased expression of membrane class I antigens [33]. Using an identical methodology, the same group of workers found that human renal cancer cells or human melanoma cells transduced with IFNγ have an increased expression of MHC class I antigen, of β2-microglobulin and of ICAM-1 [34, 35]. Again, in the MCA 101 mouse tumour model, IFNγ expression by gene transfer resulted in an autocrine enhancement of antigen-processing and in the induction of a tumour-specific CD8+ T-cell response protective against a subsequent challenge with non-transduced tumour cells [36].

Loss of MHC class I expression can result from a variety of mechanisms, including a low expression of peptide transporter genes. At least two MHC-encoded molecules, TAP-1 and TAP-2, are required to mediate the transport of peptides from the cytoplasm to the endoplasmic reticulum where they are loaded into MHC class I molecules. In the mouse lymphoma mutant RMA-S, which has a profound decrease in MHC class I expression due to a defect in the TAP-2 transporter, TAP-2 gene transfer restores a normal expression of MHC class I molecules, a normal antigen presentation, and a sensitivity to CD8+ T-cells [37]. Restifo and colleagues [38] report the identification of human cancers deficient in antigen processing that are also characterised by low expression of transporter genes. Treatment of these cells with soluble IFNγ reversed these defects. Another way by which tumour cells can lose MHC class I expression is an altered binding of regulatory factors to MHC class I enhancer sequences. Indeed, an analysis of 23 human tumour cell lines displaying various levels of class I mRNA and surface expression showed that a combined defect in binding of NF-KB and KBF1 regulatory factors to an enhancer element (called A) was frequent among the class I negative cells lines and correlated with an absence of class I mRNA transcription [39].

Mutations of the β2-microglobulin gene may also be involved in lack of MHC class I expression. For example, lack of HLA class I antigen expression by the human SK-MEL-33 melanoma cell line is caused by a reading frameshift mutation in this gene. Since this mutation could also be demonstrated in the original melanoma tumour tissue, the molecular lesion identified in the SK-MEL-33 melanoma cell line is likely to reflect a somatic mutation which had occurred during tumour progression. Expression of normal levels of MHC class I molecules was recovered after transfer of a wild-type β2-microglobulin gene [40]. Similar abnormalities in the expression of β2-microglobulin have been described in other human cells (e.g. Daudi cells) and in mouse cell lines (e.g. in a selective mutant of the murine lymphoma EL-4 [41]). However, the loss of MHC class I expression by the mutant EL-4 cells was associated with a reduced tumorigenicity compared to the parental EL-4 cells. Surprisingly, restoration of class I expression by transfection of a β2-microglobulin gene markedly increased the tumorigenic potential. In some cases, the induction of MHC class I expression by transfection can thus lead cells to escape from lysis by NK cells *in vivo*. Similarly, the restoration of TAP-2 transporter expression in RMA-S lymphoma cells, that leads to increased levels of MHC class I expression, correlates with a loss of sensitivity of NK cell lysis and with an increase in tumorigenicity [37]. Similarly also, despite an exceptionally poor prognosis, the patient from whom the SK-MEL-33 melanoma cell line with a mutated β2-microglobulin was derived (see above), remained free from disease for more than 10 years [2, 40].

SECRETION OF IMMUNOSUPPRESSIVE FACTORS

As discussed above, tumours have evolved different ways of escaping immune recognition. The ability to release immunosuppressive factors directed against T-cells may also be one mechanism of such escape.

TGFβ is produced by a variety of normal cells, including activated lymphocytes, macrophages, neutrophils and platelets, and also by a number of malignant cell types. Since TGFβ inhibits T-cell responses [42], it might play a key role in the *in vivo* immunosuppression associated with tumour progression. For example, depressed immune responsiveness in patients with glioblastoma has been related to the production of TGFβ by tumour cells [43], and an enhanced expression of TGFα and β1 was observed in renal cell carcinoma compared to normal kidney [44]. Moreover, transfection of a regressor cell line derived from a highly immunogenic, C3H-derived and UV-induced fibrosarcoma, with the TGFβ gene, was found to induce its growth *in vivo*. Although retaining expression of class I MHC molecules and of a tumour-specific antigen, the TGFβ-producing tumour cells did not stimulate primary CTL responses *in vitro*, and were ineffective *in vivo* in priming for CTL responses [45]. Moreover, in a model of fibrosarcoma in Balb/C mice, Zou and colleagues [46] observed that the function of CD4+ T-cell subset was more severely suppressed than CD8+ T-cell subset. Mainly due to the activity of TGFβ produced by tumour cells, this suppression was related to the responsiveness of CD4+ T-cells themselves rather than to the APC function. Further studies have indeed demonstrated a stage-related increase in the capacity of APC to bind and present tumour antigen with a reciprocal reduction of CD4+ T-cell activity in response to tumour antigen. It is thus conceivable that TGFβ functions to inhibit the activation of CD4+ T-cells and/or to interfere with the development of CTL precursors by influencing their reactivity to lymphokines. This was also suggested in a study on MH134 hepatoma tumour, where the selective immune dysfunction of CD4+ T-cells was prevented by treatment with antibodies against TGFβ [47].

Evidence from different systems indicates that prostaglandins (PG) produced by tumours *in vivo* are able to inhibit immune-mediated tumour destruction, and to provide an advantage for the tumoral growth [48]. Indeed, in a human metastatic melanoma, a tumour-mediated inhibition of induction and expansion of tumour-infiltrating cells *in vitro* was found to be mediated, at least partly, by PGE2. High levels of this molecule were detected in the culture supernatant of tumour cells. Moreover, addition of indomethacin, which is an inhibitor of cyclo-oxygenase and of prostaglandin synthesis, partially blocked this inhibition [49].

Insulin-like growth factor-1 (IGF-1) has also been implicated as a possible mediator of tumorigenicity. Rat C6 glioma cells normally secrete IGF-1 and are tumorigenic in syngeneic BDX rats. When transfected with IGF-1 anti-sense cDNA, these cells lost tumorigenicity. Moreover, when these transduced cells are subcutaneously injected, they can prevent formation of both subcutaneous tumours and brain tumours induced by non-transfected C6 cells. These antitumour effects seem to be mediated through a glioma-specific immune response involving CD8+ T-cells accumulated at tumour sites. Blocking IGF-1 expression by antisense gene transfer may thus reverse a phenotype allowing C6 glioma cells to evade the immune system [50].

Several human tumours (e.g. neuroblastoma, melanoma and glioma) shed gangliosides or sialic acid-containing glycosphingolipids, which are a major component of the outer plasma membrane bilayer. Significant correlations have been found between the cell surface expression of particular gangliosides and tumorigenicity. For example, GM2, GM3, GD2 and GD3 gangliosides are expressed on melanoma cells and can be used as targets for active specific immunotherapy. They may enhance tumour formation by suppressing host anti-tumour immune function [51]. This hypothesis was supported by the demonstration that some of them (GM2 and GM3) are very effective inhibitors of human NK activity [52].

Finally, as discussed previously, the local production of cytokines probably has a key role in the interaction between tumour and immune system. For example, an investigation of cytokine patterns in biopsies of human basal cell carcinoma (BCC), by reverse polymerase chain reaction technology, showed high levels of IL-4, IL-5, IL-10 and GM-CSF mRNAs. This cytokine pattern suggests a humoral response (TH2 type) with concomitant suppression of cell-mediated immune responses [53]. Another study has been investigating the transcription of genes coding for IL-2, IL-4, IL-10 and IFNγ in freshly excised renal cell carcinoma biopsies infiltrated by lymphocytes. Although a heterogeneous pattern of cytokine gene transcription was observed, the most frequently transcribed cytokine was IL-10 [54]. These observations suggest a potential role for IL-10 in tumorigenesis. This immunosuppressive cytokine is mainly acting by blocking the release of inflammatory mediators by activated macrophages, and by reducing the capacity of antigen-presenting cells to provide co-stimulatory signals to TH1 lymphocytes. By this inhibitory action on cell-mediated immune responses, IL-10 may play a major role in carcinogenesis by preventing adequate antitumoral responses against cancer cells. Some cancers are indeed associated with high levels of IL-10 expression. Gotlieb and colleagues [55] reported the presence of IL-10 in the ascites of patients with ovarian and other intra-

Table 1. Examples of gene transfer overcoming cancer escape from immune surveillance

Mechanism of escape	Corrective gene transfer
Lack of antigen presenting cell	GM-CSF gene
Low MHC expression	MHC, IFNγ gene
Mutated β2-microglobulin	β2-microglobulin gene
Low expression of tumoral antigen	Gene coding for tumour-associated antigen
Defective antigen processing	Transporter gene (TAP-1, TAP-2,...), IFNγ?
Lack of co-stimulatory signal	B7 gene, IL-2 gene
Suppressive factors: IGF-1	Antisense IGF-1
TGFβ	Antisense-TGFβ
IL-10?	Antisense IL-10, GM-CSF?

abdominal cancers. Pisa and colleagues [56] also found a selective expression of IL-10 mRNA in ovarian tumour tissue, as compared to normal ovaries and ovarian tumour cell lines. Moreover, abnormal IL-10 production was reported in multiple myeloma and in AIDS-associated B-cell lymphomas, suggesting that IL-10 might act as an autocrine factor in human B-cell malignancies [57, 58]. Finally, IL-10 production was found to be the highest and most commonly expressed cytokine among cell lines derived from human colon carcinomas, compared to cell lines established from carcinomas of kidney, breast and pancreas, from melanomas and from neuroblastomas [59]. However, the complexity of the immune responses is illustrated by the observation that transfer of the IL-10 gene in tumour cells had entirely unexpected effects. Instead of the anticipated increase, we and others [60] observed a profound decrease of tumorigenicity of the transduced tumour cells.

CONCLUSION

Gene transfer to overcome some mechanisms of cancer escape from immune surveillance is only one of several approaches aiming to manipulate the immune system for the treatment of cancer. Other attempts include the transfer of genes coding for various cytokines, in order to stimulate antitumour responses, or for foreign histocompatibility antigen, in order to activate cellular immune responses in a manner similar to the rejection observed in mismatched organ transplantation. Other approaches are also underway to identify the genes that code for tumour-associated antigens. Their transfer might be used to develop new strategies for the treatment of cancer. Future directions include the development of new gene transfer techniques, especially for targeting DNA and for *in vivo* and *in situ* gene delivery. The first human protocols using gene therapy have yielded encouraging preliminary results, and have demonstrated the safety of this new therapeutic approach. However, it is clear that we are only at the start of a new era for the genetic treatment not only of cancer, but also of other somatic diseases, such as AIDS, and of hereditary disorders, such as cystic fibrosis.

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