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TOPIC PAPER

**Bernhard Frankenberger · Sybille Regn
Christiane Geiger · Elfriede Noessner
Christine S. Falk · Heike Pohla · Miran Javorovic
Tobias Silberzahn · Susanne Wilde
Alexander Buchner · Michael Siebels
Ralph Oberneder · Gerald Willimsky
Antonio Pezzutto · Thomas Blankenstein
Dolores J. Schendel**

Cell-based vaccines for renal cell carcinoma: genetically-engineered tumor cells and monocyte-derived dendritic cells

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Abstract Initial vaccine developments for renal cell carcinoma (RCC) have concentrated on cell-based approaches in which tumor cells themselves provide mixtures of unknown tumor-associated antigens as immunizing agents. Antigens derived from autologous tumors can direct responses to molecular composites characteristic of individual tumors, whereas antigens derived from allogeneic tumor cells must be commonly shared by RCC. Three types of cell-based vaccine for RCC have been investigated: isolated tumor cell suspensions, gene modified tumor cells and dendritic cells (DCs) expressing RCC-associated antigens. Approaches using genetic modification of autologous RCC have included ex vivo modification of tumor cells or modification of tumors in vivo. We have used gene-modification of allogeneic tumor cell lines to create generic RCC vaccines. More recently, emphasis has shifted to the use of DCs as cell-based vaccines for RCC. DCs have moved to a position of central interest because of their excellent stimulatory capacity, combined with their ability to process and present antigens to both naive CD4 and CD8 cells. The long impasse in identifying molecular targets for specific immunotherapy of RCC is now rapidly being overcome through the use of tools and information emerging from human genome research. Identification of candidate molecules expressed by RCC using cDNA arrays, combined with protein arrays and identification of peptides presented by MHC molecules, allow specific vaccines to be tailored to the antigenic profile of individual tumors, providing the basis for development of patient-specific vaccines.

Keywords Immunotherapy · Gene therapy · Tumor cell vaccines · Dendritic cells · Renal cell carcinoma

B. Frankenberger and S. Regn made equal contributions to these studies

B. Frankenberger (✉) · S. Regn · C. Geiger · E. Noessner
C. S. Falk · H. Pohla · M. Javorovic · T. Silberzahn · S. Wilde
D. J. Schendel
Institute of Molecular Immunology, GSF-National Research Center for Environment and Health, Marchioninistrasse 25, 81377 Munich, Germany
E-mail: b.frankenberger@gsf.de
Tel.: +49-89-7099301
Fax: +49-89-7099300

H. Pohla · M. Javorovic · A. Buchner · M. Siebels · R. Oberneder
Laboratory for Tumor Immunology, Department of Urology, Ludwig-Maximilians University, Munich, Germany

R. Oberneder
Urology Clinic, Munich-Planegg, Germany

G. Willimsky · A. Pezzutto · T. Blankenstein
Max Delbrück Center for Molecular Medicine, Berlin, Germany

G. Willimsky · T. Blankenstein
Institute of Immunology, Charité-University Medicine Berlin, Campus Benjamin Franklin, Berlin, Germany

A. Pezzutto
Department of Hematology, Oncology and Tumorimmunology, Charité-University Medicine Berlin, Campus Berlin Buch, Berlin Germany

Cell-based vaccines for renal cell carcinoma

Although renal cell carcinomas (RCC) are thought to express tumor-associated antigens (TAA) that serve as rejection antigens following recognition by cells of the immune system, progress on the identification of molecules that can be used for antigen-specific vaccines to target these tumors has been slow. For this reason, initial vaccine developments for RCC have concentrated

on cell-based approaches in which tumor cells themselves provide mixtures of unknown TAA as immunizing agents. When autologous tumor cells are employed there is the possibility of engaging T cells recognizing a unique set of epitopes that result from mutations in individual tumors. Although such epitopes are derived from normal self-proteins against which high affinity T cells have been negatively selected, the presence of mutations in peptide ligand sequences allow such epitopes to behave like foreign antigens that induce strong T cell responses [1]. If allogeneic tumor cells are utilized, development of specific T cell-mediated immunity relies on the presence of target molecules that are shared among various RCC. CTL recognition studies [2–6], as well as molecular antigen identification [3, 6–10], support the validity of this contention for RCC. On this basis, three types of cell-based vaccine for RCC have been pursued: direct application of devitalized autologous tumor cells, gene modified tumor cell-based vaccines and dendritic cells (DCs) expressing RCC-derived antigens.

Older as well as more recent studies have applied irradiated or freeze-thawed autologous tumor cells as vaccines following their pretreatment with Bacillus Calmette-Guerin [11] or interferon-gamma (IFN-γ) [12]. Unmodified tumor cells were also applied together with systemic IL-2 [13]. All three studies demonstrated feasibility, and limited toxicity was ascribed to the tumor cells themselves. In addition, some bioactivity was noted, but long-term application of these types of vaccine was not possible because of limitations in the number of tumor cells available from individual patients. Interestingly, in the phase III trial using vaccination with autologous tumor cells in an adjuvant setting, improved 5 year and 70 month progress-free survival rates were demonstrated [12].

Gene-modified tumor cell vaccines

Tumor cell-based vaccines have been studied which utilize autologous RCC cells that have been genetically engineered to enhance their immunogenicity. Here, genes encoding immunostimulatory cytokines or positive costimulatory molecules of the B7 family have been analyzed for their impact on the induction of tumor-specific immune responses in vitro, and several phase I trials have been completed [14]. One of the first trials for RCC utilized autologous tumor cells that were genetically modified to express granulocyte-macrophage colony-stimulating factor (GM-CSF) [15]. The capacity of GM-CSF-secreting tumor cells to induce antitumor responses was attributed to their ability to induce inflammatory reactions at the vaccination site, eventually recruiting antigen-presenting cells that could utilize injected tumor cells as sources of TAA for the sensitization of T lymphocytes. Alternatively, the modification of tumor cells to express CD80 or CD86 molecules may allow such cells to directly initiate immune responses [16–20]. A clinical trial using autologous RCC modified to express CD80, in combination with systemic IL-2, was shown to be safe, and toxicity was similar to that found with application of IL-2 alone. Immunological and clinical responses were observed in some patients [21].

There are several disadvantages to autologous tumor cell vaccine strategies. First, tumor material must be available and prepared under good manufacturing practice (GMP) standards from each patient. Differences in the natural immunogenicity of individual tumors may impact on their ability to induce antitumor immunity while variations in transgene expression also influence vaccine effects. Furthermore, long-term vaccination cannot be provided because of limitations in tumor material.

Genetically modified allogeneic tumor cells as generic RCC vaccines

An alternative strategy to overcome several of the disadvantages of autologous tumor cell vaccines is to use established RCC lines in an allogeneic setting. As a prelude to developing a generic allogeneic RCC-based vaccine for MHC-selected patients, we established a model system to explore lymphocyte interactions with various gene-modified tumor cells. A parental tumor cell line (RCC-26) was cultured from the primary tumor of a patient with stage I disease (T1N0M0) in whom a single brain metastasis appeared 9 years after primary tumor nephrectomy. A tumor antigen-specific CTL line was expanded from the tumor-infiltrating lymphocyte population (TIL-26) and was demonstrated to recognize an RCC-associated determinant presented by HLA-A*0201-encoded molecules. This peptide-major histocompatibility complex (pMHC) ligand is expressed by RCC-26 cells but not by autologous normal kidney parenchyma [2]. The TIL-26 line contained two dominant CTL clones that expressed highly conserved Vα20 T cell receptors (TCR), which enabled them to be traced and quantified on the basis of their characteristic third complementary determining region (CDR3) sequences [22]. T cells bearing Vα20 TCR were prevalent in situ in the primary tumor of patient 26 and they circulated in small numbers among the peripheral blood mononuclear cells (PBMC) of this patient up to 48 months post-surgery [22]. Since HLA-A2-restricted TIL derived from several unrelated RCC patients also recognized RCC-26 cells, these tumor cells obviously displayed several distinct, but common, peptide ligands expressed by other HLA-A*0201-positive RCC [2, 22, 23]. RCC-26 cells also activated allogeneic natural killer (NK) cells and non-MHC-restricted (NK-like) T cells, revealing that they interacted well with cells of the innate immune system [24, 25]. Thus, the RCC-26 tumor line displayed a striking natural immunogenicity, allowing it to be seen by three distinct types of effector lymphocyte, even when the lymphocytes were obtained from allogeneic donors who were only matched for HLA-A*0201 alleles.

Table 1 Cytokine production of RCC-26 tumor cells: pg/ml released from 3×10^4 cells/well/200 µl/24 h

≤ 20 pg/ml*	20–100 pg/ml	> 100 pg/ml
IL-1β	IL-17	IL-6
IL-2	IFN-γ	IL-8
IL-4	GM-CSF	MCP-1
IL-5		
IL-7		
IL-10		
IL-12		
Il-13		
TNF-α		
MIP-1β		
G-CSF		

To better understand how RCC-26 cells could successfully engage different types of immune lymphocytes, we assessed its cytokine production and surface phenotype. The tumor line secreted a variety of factors at intermediate to high levels. In particular, it produced very high levels of the pro-inflammatory cytokines, IL-6, IL-8 and MCP-1, as well as several other immunomodulatory cytokines at low to intermediate levels (Table 1). At the same time, it released the inhibitory molecules TGFβ and VEGF (data not shown). Apparently, the net impact of this complex mixture of factors had a more positive than negative impact on eliciting specific antitumor immune responses in vitro.

RCC-26 cells also displayed a surface immunophenotype that is highly supportive of lymphocyte interactions (Fig. 1). The cells strongly expressed the adhesion molecules CD54 (ICAM-1) and CD58 (LFA-3), which foster initial contacts with T lymphocytes [26] and also influence susceptibility to NK cells [27, 28]. RCC-26 cells had good levels of MHC class I expression, including HLA-A2 molecules, enabling them to interact with autologous and allogeneic HLA-A2-restricted TIL [2, 23]. Despite strong class I expression, RCC-26 cells were still sensitive to NK killing, indicating that they had good expression of ligands that interact with the activating receptors on NK cells, enabling them to overcome inhibitory signals delivered by class I molecules [29–31]. It was possible to identify ULBP-3 and MICA on RCC-26 cells (data not shown); these molecules function as activating ligands for the NKG2D receptors of NK cells [32]. Interestingly, when RCC-26 cells were pretreated with IFN-γ, expression of these activating ligands was not increased, but rather the tumor cells lost sensitivity to both NK and LAK-derived T cells through a resistance mechanism associated with the upregulation of HLA-C and HLA-E molecules [24]. These two HLA allotypes are well-established ligands of inhibitory receptors expressed by NK and NK-like T cells [33, 34]. This strong inhibition of NK and LAK cell recognition was not caused by the pretreatment of RCC-26 cells with IFN-alpha (IFN-α) [35]. Thus, the natural state of MHC class I expression on RCC-26 cells was appropriately balanced with respect to different MHC class Ia and Ib molecules, thereby allowing these tumor cells to interact with antigen-specific lymphocytes of the adaptive immune system through their high expression of pMHC ligands presented by some HLA molecules, like HLA-A2, while avoiding inactivation of non-MHC-restricted cells of the innate immune system through their low expression of other HLA molecules, such as HLA-C and HLA-E.

RCC-26 cells did not express MHC class II molecules but expression was induced by IFN-γ [36]. In addition, the tumor cells expressed CD40 molecules, as shown previously for several other tumor types [37]. Critically missing were costimulatory molecules of the B7 family, such as CD80 or CD86. Stimulation of RCC-26 cells with CD40-activating antibodies or with cells expressing CD40-ligand did not induce CD80 or CD86 surface expression and they were also not induced by IFN-α or IFN-γ stimulation (data not shown). To partially correct this B7 deficit, we introduced cDNA encoding CD80 into RCC-26 cells by retroviral transduction [19]. Sublines that were selected for high expression of CD80 retained the same surface phenotype as the unmodified parental cells with respect to all other surface markers (data not shown).

TIL-26 cells recognized and killed unmodified RCC-26 cells and RCC-26/CD80 cells at comparable levels, revealing that CD80 signals were not required to activate the function of effector-memory CTL (data not shown). The impact of CD80 modification on the stimulatory capacity of RCC-26 cells was analyzed in mixed lymphocyte tumor cultures (MLTC) using autologous peripheral blood mononuclear cells (PBMC-26) as responding cells and RCC-26/CD80 cells for stimula-

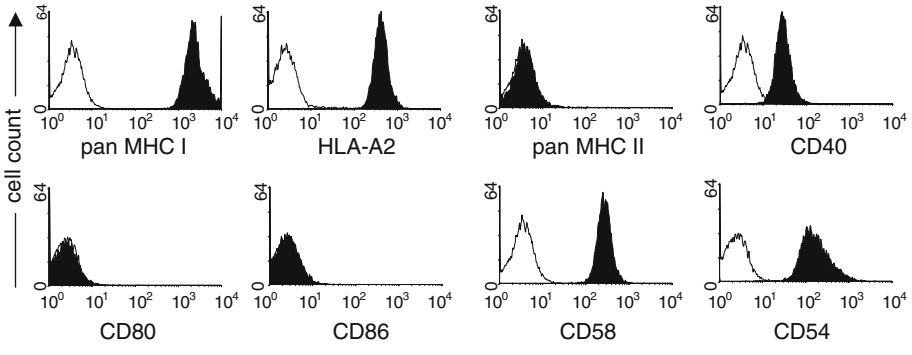

Fig. 1 Surface immunophenotype of RCC-26 tumor cells. Cells, as indicated, were stained with monoclonal antibodies to pan MHC I, HLA-A2, pan MHC II, CD40, CD80, CD86, CD58 and CD54 (*solid black histograms*). Isotype-matched antibodies were used for control staining (*solid line histograms*). Gates were set on viable propidium iodide-negative cells

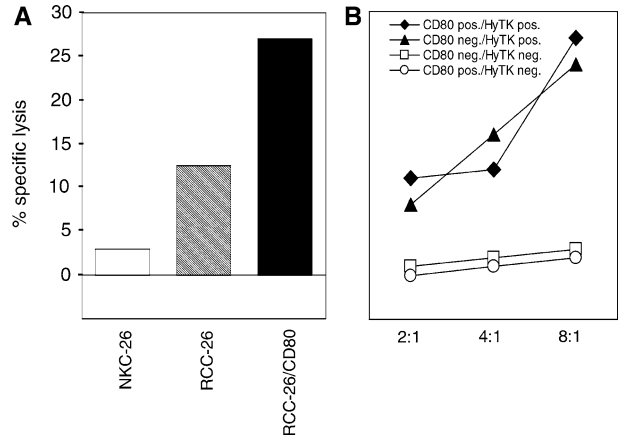

Fig. 2 Cytotoxic activity of autologous effector-memory cells induced in mixed lymphocyte tumor cell culture using autologous peripheral blood mononuclear cells (PBMC-26) as responding cells and RCC-26/CD80 cells for stimulation. **A** shows different levels of killing (effector to target cell ratio was 13:1) of unmodified RCC-26 cells and RCC-26/CD80 cells compared with normal autologous kidney cells (*NKC-26*). **B** shows that some CTL activity was directed against epitopes of the hygromycin-thymidine kinase (*HyTK*) fusion protein that served for selection of transgenic cells

tion. Cytotoxic cells were induced which showed different levels of killing of unmodified RCC-26 cells and RCC-26/CD80 cells (Fig. 2). This difference suggested that a cytotoxic response directed either against CD80 molecules or some component of the viral vector may also have been induced in the MLTC. When additional tumor lines were analyzed, it became clear that some CTL activity was directed against epitopes of the hygromycin-thymidine kinase (HyTK) fusion protein that served for the selection of transgenic cells, since modified lines carrying this marker were recognized by the CTL regardless of their expression of CD80, whereas cells transduced with vectors lacking HyTK were not killed even if they expressed CD80. Importantly, the finding that RCC-26/CD80 cells could induce HyTK-specific CTL revealed that these tumor cells could not only process and present intracellular antigens but they could also effectively prime naive T cells de novo. HyTK was shown previously to be a target antigen in other gene-modified RCC cells [38]. In other studies, we demonstrated that RCC-26/CD80 cells induced MHC class I allospecific CTL responses as well as RCC-associated CTL responses in naive allogeneic lymphocytes [19, 39], and that they fostered the expansion and survival of autologous effector-memory T cells ex vivo [39].

All of these characteristics supported our contention that RCC-26 cells were ideal for use as an allogeneic tumor cell-based vaccine. The natural immunogenicity of RCC-26 cells could be further improved through gene modification. We expressed a series of transgenes in RCC-26 cells and studied their impact on various immune responses (Table 2). These comparisons finally led us to select two sublines (RCC-26/CD80/IL-2 and RCC-26/CD80/IL-7) for further vaccine development. Both cell lines retained the same surface immunophenotype and cytokine secretion patterns found in unmodified RCC-26 cells, with the exception that they expressed CD80 and secreted substantial levels of IL-2 or IL-7, respectively (data not shown). Their capacity to foster proliferation, survival and function of autologous effector-memory CTL is described elsewhere [39]. We also characterized both vaccine variants for their overexpression of a series of antigens compared to normal kidney cells. HLA-A2-binding peptides derived from several of these antigens can thereby serve as molecular markers for tracking the induction of T cell responses during vaccination with RCC-26-derived vaccines [39]. A phase I/II clinical trial comparing these two lines as vaccines in HLA-A*0201-matched patients with metastatic RCC is currently in progress.

Dendritic cell-based vaccines

More recently, emphasis has shifted to the use of DCs for the development of cell-based vaccines for RCC. DCs have moved to a position of central interest because of their excellent stimulatory capacity, due to the expression of an array of costimulatory molecules and the secretion of immunostimulatory cytokines, com-

Table 2 Gene-modified RCC-26 cell lines

cDNA Modification	Impact	Reference
IL-2	Improved TIL activity Improved NK and LAK activity	[67]
IFN-γ	Increased MHC, TAP, LMP expression Improved TIL recognition Strongly diminished NK and LAK recognition	[24, 36]
IFN-α	Moderate MHC upregulation Moderately improved TIL recognition Moderately decreased NK and LAK recognition	[35]
CD80	Improved priming of antigen-specific CTL Activation of some NK cells	[19]
CD80/IL-2	Improved expansion, survival and function of effector-memory CTL Priming of RCC-associated CTL from naive lymphocytes	[39]
CD80/IL-7	No change in effector-memory CTL expansion or function Priming of RCC-associated CTL from naive lymphocytes	[39]

bined with their ability to process and present antigens to both naive CD4 and CD8 cells [40]. Furthermore, DC vaccine development has become feasible through a better understanding of how DCs can be prepared in large numbers ex vivo. The potential of DCs to induce RCC-specific T cell responses in vitro was convincingly demonstrated in several studies [3–6, 41]. To date, several phase I trials for RCC have been reported that utilized DCs that were differentiated from peripheral blood monocytes using GM-CSF and IL-4. Two phase I trials used lysates, prepared either from cultured autologous tumors or derived from an established allogeneic RCC line as sources of TAA, which were loaded onto immature DCs that were subsequently matured using tumor necrosis factor-alpha (TNF-α) alone [42] or a cytokine maturation cocktail [8]. Both trials demonstrated feasibility without adverse effects and signs of immunological reactivity were found subsequent to vaccination. Clinical responses were achieved in some patients. Interestingly, two patients who showed complete responses in one trial received DCs loaded with lysates prepared from autologous metastatic lesions, indicating that the antigenic composition of metastatic cells was perhaps better suited to stimulate clinically effective T cell responses [8]. A more recent trial used autologous tumor-derived RNA, which was naturally taken up by immature DCs, as the source of TAA [6]. Feasibility and lack of toxicity were established for this approach, but clinical responses could not be evaluated because most patients subsequently received additional treatments. Nevertheless, tumor-related mortality was unexpectedly low in this small group of patients. One widely cited trial using allogeneic DCs fused with autologous RCC cells has been retracted and merits no further consideration [43, 44]. A second study using a similar approach did not find any significant clinical responses [45].

Immune monitoring of the immune responses induced by lysate-loaded DCs and RNA-transduced DCs revealed effective stimulation of apparently polyclonal T cell responses directed against autologous and allogeneic tumor cells as well as against specific antigens, including oncofetal antigen [6, 8], human telomerase reverse transcriptase [6], and G250/CA-IX antigen [6]. The demonstration that T cells were induced in vivo against several defined molecules opens the possibility of utilizing them as specific molecular targets in future DC vaccine approaches.

Monocyte-derived DCs generated by GM-CSF and IFN-α

The ability to identify suitable targets for immune responses now opens the door to the development of antigen-specific vaccines for RCC. DCs are logical candidates for vaccine development because of their capacity to prime specific lymphocytes. To date, the few clinical trials for RCC have generated immature DCs through the culture of monocytes with GM-CSF and IL-4. We have explored an alternative method using monocytes that were isolated by plastic adherence and cultured for up to 7 days in GM-CSF and IFN-α to yield an initial population of DCs that were then exposed to TNF-α and IL-1β for an additional 48 h [46].

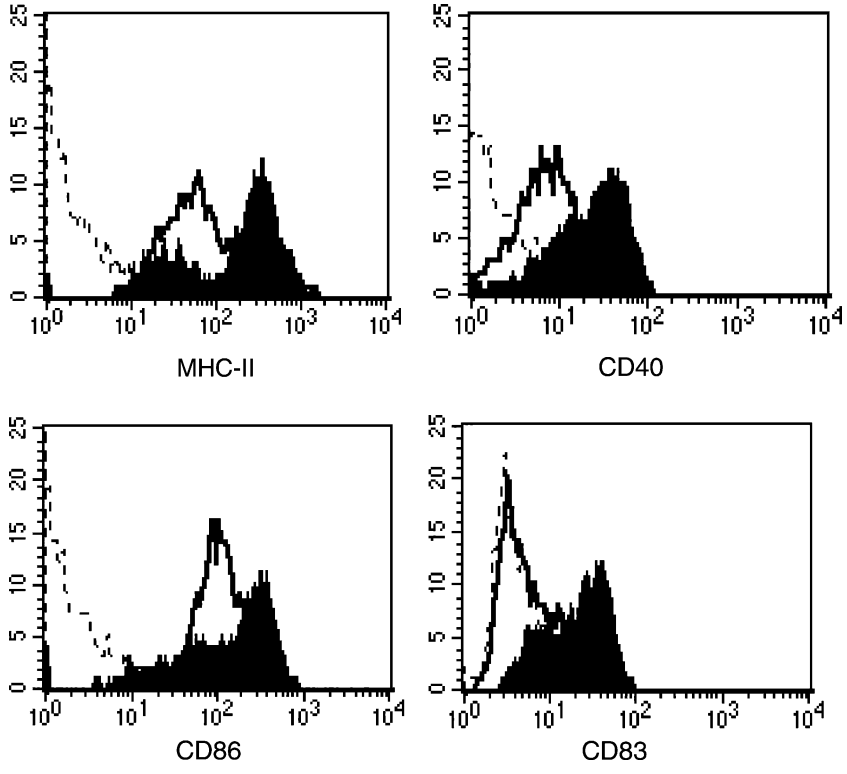

Fig. 3 Surface marker expression of two different DC populations. Semi-mature DCs (*solid line histograms*) were cultured for 7 days in GM-CSF and IFN-α while mature DCs (*solid black histograms*) were generated by exposure to TNF-α and IL-1β for an additional 48 h. While the semi-mature DCs did not display CD83, its expression was upregulated by the maturation cytokines, which also increased the levels of MHC-II, CD40 and CD86 on the mature DCs. Isotype-matched antibodies were used for control staining (*dotted line histograms*)

Analysis of the expression of various surface markers on the two DC populations revealed that the monocytes cultured with only GM-CSF/IFN-α expressed a semi-mature DC phenotype, characterized by good expression of MHC class I and II molecules and substantial levels of the costimulatory molecules CD40, CD86, and CD80 (Fig. 3 and data not shown). While the semi-mature DCs did not display CD83, its expression was upregulated by the maturation cytokines, which also increased the levels of MHC class I and II, CD40, CD80 and CD86 on the mature DCs.

DCs displaying the semi-mature phenotype still retained an excellent capacity to internalise antigens, as exemplified by their uptake of fluorescent-labeled dextran (Fig. 4). Furthermore, they were able to induce a strong proliferation of autologous lymphocytes and the activation of adenovirus-specific T cell responses following infection with recombinant virus (Fig. 5), in addition to inducing proliferation of allogeneic lymphocytes in mixed lymphocyte DC cultures. Thus, DCs produced with GM-CSF/ IFN-α retained the potential to efficiently load antigen, to express CCR7 that would support migration to lymph nodes (data not shown), and also to stimulate strong immune responses. Interestingly, this phenotype was displayed by cells after only 3 days of culture with GM-CSF/ IFN-α.

The simple and time saving procedure for generating semi-mature DCs is certainly attractive in the development of GMP grade vaccines for clinical studies. Various sources of antigen may be considered for use with this type of DC, including synthetic peptides representing epitopes of RCC-associated antigens or tumor cell-derived lysates. Unfortunately, we have not been successful to date in using RNA as a source of TAA in these DCs, perhaps due to its rapid degradation through

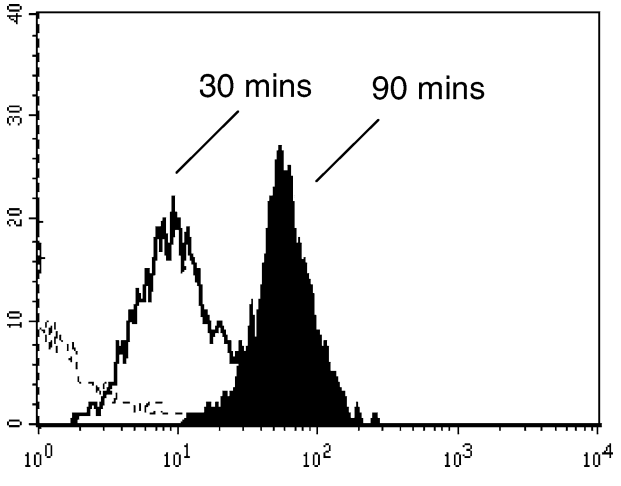

Fig. 4 Uptake of fluorescent-labeled dextran by semi-mature DCs. DCs displaying the semi-mature phenotype still retained an excellent capacity to internalise antigens, as exemplified by their uptake of FITC-dextran (*solid line histogram*: +FITC-dextran for 30 min; *solid black histogram*: +FITC-dextran for 90 min; *dotted line histogram*: control untreated DCs)

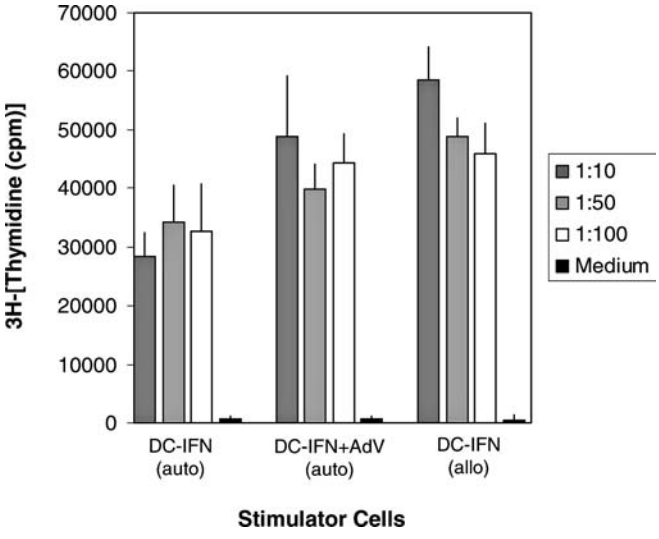

Fig. 5 Semi-mature DCs induce strong proliferation of autologous lymphocytes and activation of adenovirus-specific T cell responses following infection with recombinant virus, in addition to inducing proliferation of allogeneic lymphocytes in mixed lymphocyte DC cultures as measured by [3H] thymidine incorporation. DCs were incubated with varying numbers of responding lymphocytes, ranging from ten lymphocytes/DC to 100 lymphocytes/DC

intracellular mechanisms activated by the type I interferon (data not shown).

RCC contain substantial numbers of DCs, which may contribute to the suppression of T cell responses in situ [47–50]. Because some RCC patients show remarkable responses to immunotherapy with IFN-α and most RCC and tumor-infiltrating lymphocytes produce GM-CSF, it is intriguing to speculate that tumor-infiltrating DCs may acquire the semi-mature phenotype in patients receiving IFN-α immunotherapy. Very recent findings have demonstrated that exposure of DCs to IFN-α can allow them to cross-present antigens for the priming of CD8 T cells in the absence of CD4 help [51, 52]. Thus, tumor-infiltrating DCs that are exposed to systemic IFN-α in vivo may acquire TAA from neighboring tumor cells which they can utilize to prime $CD8^+$ T cells. These T cells, in turn, could proliferate in response to systemic IL-2, thereby generating improved antitumor immunity. It remains to be determined whether these events occur in RCC patients receiving combined IL-2 and IFN-α immunotherapy and whether DCs activated by IFN-α contribute to improved antitumor immunity and clinical response.

Molecular target antigens for RCC

The long impasse in identifying molecular targets for specific immunotherapy of RCC is being rapidly overcome through the use of tools and information emerging from human genome research. Molecular assessment of candidate molecules expressed by tumor cells using RT-PCR, combined with tissue arrays to determine protein expression, provides a systematic approach to

identifying potential target molecules for RCC, even on an individual patient basis [10]. Using this strategy, the G250/CA-IX molecule [53] was confirmed to be an attractive antigen for use in vaccines against clear cell carcinomas [10]. A fusion protein combining GM-CSF and CA-IX offers a clever means of delivering this RCC-associated antigen to DCs ex vivo and perhaps in vivo [54]. Alternatively, candidate antigens can be introduced into DCs using viral vectors or by loading cells with in vitro transcribed RNA prepared from cDNA encoding any specific antigen [5, 55]. An alternative integrated approach using cDNA arrays combined with the identification of specific peptides eluted from MHC molecules of RCC assures that candidate molecules are indeed processed and presented as peptide-ligands for T cells at the tumor cell surface [9]. This strategy identified adipophilin to be a suitable antigen to induce CTL using peptide-loaded DCs [56]. In addition, several other candidates have been defined using these reverse immunology approaches to analyze RCC [9, 57, 58].

Future vaccines may well employ pools of defined antigens in order to induce multiplex immune responses that will help to protect against immune selection of antigen-loss tumor cell variants. Use of DCs loaded with synthetic peptides derived from different proteins and presented by different MHC class I molecules would be efficient and could be tailored to individual patients, but this necessitates defining the HLA molecules of the patients and does not provide epitopes for CD4 T cell responses. The pMHC ligands generated by exogenous peptide loading of DCs are relatively short-lived, which is a substantial disadvantage. As an alternative, pools of defined RNAs can be used for DC loading, a method which allows antigens to be directed to both the class I and class II pathways for priming of CD4 and CD8 T cells. This approach does not require information on specific peptide sequences or the HLA types of the patients. Furthermore, pMHC ligands generated via RNA-loading seem to be expressed for longer periods on DCs, thereby facilitating T cell priming [59].

Antigen-specific vaccines offer an improved means of monitoring the development of immune responses, which is useful in early stages of DC vaccine development when questions need to be answered regarding what, when and how DCs vaccines can best be administered to patients [60, 61]. Ultimately, precisely defined vaccines will also be needed to reduce the potential of inducing unwanted autoimmune reactions if patients with earlier stages of the disease are to be treated.

Conclusions

These different cell-based vaccine approaches each have distinct advantages and disadvantages. Allogeneic tumor cell-based vaccines are generic reagents that can be applied to partial HLA-matched patients following GMP production, whereas DC-based vaccines must be produced individually for each patient. Tumor cells express a variety of known and unknown TAA, enabling them to potentially support a multiplex immune response. In contrast, we have recently shown that the full complement of TAA expressed by tumor cells is not efficiently transferred to DCs using RNA transfection [62]. How efficiently transfer of many TAA occurs using cell lysates or apoptotic cells remains to be determined. On the other hand, DCs selected to present well-defined antigens have a decreased risk for inducing deleterious autoimmunity but a diminished capacity to induce complex immune responses. The expression of both MHC class I and class II molecules by DCs allows them to induce both CD4 and CD8 T cell responses, whereas RCC-based vaccines predominantly stimulate the expansion of CD8 cells because the tumor cells do not constitutively express class II molecules. However, strong T cells responses directed against mismatched MHC molecules, which are induced by allogeneic tumor cell vaccines, such as gene-modified RCC-26 cells, can actively support the development of tumor-associated T cells [14, 19]. Currently, not enough information is available to determine which type of cell-based vaccine will induce immune responses that are clinically most relevant in RCC. Extensive immune monitoring of patients immunized with different types of vaccine will be important in helping to determine those approaches that may best improve the clinical outlook for patients.

In the next few years, optimal forms of cell-based vaccines for RCC will be defined. A major hurdle still to be overcome is finding the means to further modulate the immune system to enable clinically effective antitumor responses to be generated in patients whose ability to respond to optimal vaccines has been badly compromised by progressive disease [63, 64]. The recent success in obtaining remission of advanced disease in patients receiving non-myeloablative conditioning and subsequent allogeneic bone marrow transplantation provide indications that this hurdle can be overcome in RCC patients with advanced disease [65, 66].

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