Current Status of Dendritic Cell-Based Tumor Vaccination

J. Dannull a, T. Cerny b, D.K. Ackermann c, M. Groettrup a

a Laborforshungsabteilung,

b Abteilung für Onkologie,

c Abteilung für Urologie, Kantonsspital St. Gallen

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Summary
For several decades, approaches utilizing nonspecific immune stimulants have provided evidence that the immune system, when properly activated, may eradicate cancer cells. However, it was only after the identification of the first human tumor-associated antigen, less than a decade ago, that development of specific vaccination procedures for cancer patients became feasible. Recent insights into the pivotal role of dendritic cells (DCs) for initiation and regulation of immune responses have allowed the design of DC-based tumor vaccination trials. In addition, the development of methods to raise large numbers of DCs from peripheral blood monocytes has paved the way for their clinical application. Tumor-specific vaccination utilizing antigen-loaded autologous DCs, has become practical and applicable to patients and may lead to vigorous antitumor responses. This review outlines recent progress, obstacles still to be overcome, and the future potential of DC-based vaccination.

Generating a Potent Antitumor Response

A prerequisite for establishing a potent and durable antitumor immune response is the simultaneous activation of antigen-presenting cells (APCs) including B cells, macrophages and dendritic cells (DCs), CD4+ helper T cells (Th cells), cytolytic CD8+ T cells (CTLs), and antibody-secreting B cells. Upon activation, Th cells secrete cytokines which, in turn, stimulate CTLs and B cells and, furthermore, augment the killing activity of natural killer cells (NK) as well as the phagocytic capacity of macrophages. Appropriately activated, CTLs are capable of directly killing tumor cells. APCs play a pivotal role in this scenario as they are able to bridge the innate, cellular and humoral arms of the immune system. APCs ingest parts of tumor cells or antigens and process and display them as peptide epitopes with a length of 9–20 amino acids on the major histocompatibility complex (MHC) class I and class II proteins. Th cells recognize their cognate antigens in the context of MHC class II molecules which are only found on APCs, whereas CTLs do so in the context of MHC class I which is expressed on all somatic cells except testis, placenta, and the majority of neurons. Each T-cell receptor (TCR) on naive T cells binds
Dendritic Cells

DCs represent a unique system of cells [3] that induce, sustain, and regulate immune responses (for an electron microscopic image of a dermal DC see fig. 2). DCs originate in the bone marrow from pluripotent CD34+ stem cells and migrate to peripheral tissues through the blood. They are distributed in most tissues and, in particular, in tissues that interface with the external environment. Here, they perform sentinel function for incoming pathogens. Langerhans cells, the first DCs described, are widely distributed in skin, esophagus, cervix, and buccal epithelia. Interstitial DCs are present in the dermis as well as the interstitium of virtually all tissues, except the brain. Furthermore, veiled DCs may be found in the afferent lymph, and interdigitating DCs reside in the cortical zone of the lymph nodes and in the spleen. Th DCs in peripheral tissues are immature but capable of actively taking up antigens by three major pathways: Phagocytosis allows uptake of soluble extracellular antigens. In addition, phagocytosis or receptor-mediated endocytosis may be initiated by direct, nonopsonic, interaction between pathogen, apoptotic cells, or effete body cells and DCs. As depicted in figure 3a, receptor-mediated uptake may occur via DEC-205 (multiligand receptor on DCs and thymic epithelial cells, the human homolog being gp200M R6), the mannose receptors, collectins (collagen-like lectins), toll-like receptors (TLR 2,4), and the scavenger receptors. Alternatively, antibodies or complement can act as bridging molecules between pathogen and Fc-type (FcγR I–II) or complement-type receptors, thus leading to opsonic uptake. Following uptake of antigens, DCs induce leukocyte recruitment to the site of inflammation through production of chemokines and inflammatory cytokines. Subsequently, DCs undergo a switch in the expression of chemokine receptors which allows them to leave the inflamed tissue and to migrate to draining lymphoid organs, in particular the T-cell areas of lymph nodes. Here, they undergo a process of maturation and acquire the ability to attract T cells by expression of M DC (macrophage-derived chemokine) and ELC (EBV-induced molecule 1 ligand chemokine) as well as to initiate T-cell responses. Maturation of DCs is accompanied by a decrease of receptors involved in uptake of antigen and an increase in the expression of MHC class I and class II molecules. Furthermore, upregulation of costimulatory molecules and of adhesion molecules (ICAM-1, ICAM-3, and LFA-3) which are essential for antigen-independent binding occurs in mature DCs. The mature stage of DCs ends by apoptotic cell death in the lymph node which is greatly enhanced by immunoinhibitory cytokines such as IL-10. The understanding and consideration of these properties of DCs is imperative for the design of tumor vaccines and will be discussed below.

Generation of DCs for Vaccination Purposes in vitro

Two standard methods for in vitro generation of human DCs have been described [4, 5]. The first of which utilizes hematopoietic CD34+ precursor cells which are harvested from bone marrow, umbilical cord or peripheral blood. These cells are cultured ex vivo in the presence of GM-CSF (granulocyte-macrophage colony-stimulating factor) and TNF-α (tumor necrosis factor-alpha), leading to yields of 10^6 DCs per 500 ml
of peripheral blood and about $2 \times 10^6$ DCs per 1 ml of bone marrow. A different and more practical approach utilizes CD 14+ monocytes from peripheral blood cells which differentiate to DCs in the presence of GM-CSF and IL-4. Using this method, $10^5$ DCs can be obtained from 10 ml of peripheral blood. The safety of DCs can be further increased up to 5-10-fold by pretreatment of donors with recombinant GM-CSF or Flt-3 ligand (c-fms-like tyrosine kinase), a method which is currently under clinical investigation. Following ex vivo generation and loading with tumor antigen, autologous DCs can be reinjected into patients. This autoadaptive transfer can be performed with immature DCs or with DCs that have been matured in vitro by a standard procedure employing prostaglandin E2, IL-1β, IL-6, and TNF-α [6]. Importantly, methods have been developed to cryopreserve mature DCs which greatly facilitates their clinical application. In sum, ex vivo generation of DCs for vaccination approaches has proved to be practical and safe, and, additionally, allows to circumvent immunosuppressive conditions observed in cancer tissues.

**Identification of Tumor-Associated Antigens**

Disappointing results from clinical vaccination trials may give the wrong impression that tumors are not sufficiently distinct from normal tissue to activate the immune system and would, therefore, be nonimmunogenic. However, there is strong evidence that lack of immunogenicity can be due to the tumor’s ability to actively evade recognition by the immune system. Several mechanisms by which tumors are capable of blunting an immune response have been demonstrated. These include downregulation of MHC class I expression and β2-microglobulin (and/or loss of transporter associated with antigen presentation (TAP)), overexpression of immunoinhibitory cytokines such as TGF-β1 and IL-10 and induction of Fas-mediated apoptosis of T cells via expression of Fas ligand (FasL) by a variety of malignancies. However, there is solid evidence that unaltered self-antigens aberrantly expressed in tumors or expressed in a tissue-specific fashion can be recognized by T cells from cancer patients. Accordingly, autoreactive T cells, even though they might display low avidity, escape thymic deletion and reach the periphery where they could be involved in antitumor responses if properly activated. The isolation of the first human tumor-associated antigens (TAAs) recognized by CTL from melanoma patients represents a milestone of contemporary immunotherapy [7]. This seminal work for identification of TAAs is based on the recognition of appropriate target cells (transfected with cDNA libraries prepared from tumor tissue) by autologous tumor-specific CTL clones in vitro.

Aiso, the analysis of serological responses to tumors combined with molecular cloning techniques, known as SEREX (serological analysis of autologous tumor antigens by recombinant expression cloning), is a promising means of identifying novel antigens [8]. It allows an unbiased search for an antibody response and the direct molecular identification of immunogenic tumor proteins based on their reactivity with autologous patient sera. SEREX analyses have led to the identification of a variety of novel antigens whose clinical potential is currently under investigation.

Finally, the ‘reverse immunology’ approach is a useful method to identify TAAs. It makes use of computer-assisted identification of peptides within the sequence of candidate antigens which fulfill the consensus criteria for binding to an MHC class I molecule. The peptides are synthesized and tested for their ability to stabilize MHC class I molecules on the cell surface. Subsequently, the frequency of T cells in patient blood, which react with a given peptide epitope is evaluated to monitor the in vivo relevance of a given antigen. Aiso the sequence determination of peptides which can be eluted from class I molecules of tumor cells and are recognized by tumor-specific CTLs has resulted in the discovery of novel TAAs.

**Defined Human Tumor-Associated Antigens and Their Potential for Vaccination Approaches**

As can be seen in Table 1, a broad spectrum of TAAs has been identified [9]. Theoretically, TAAs that have arisen as a result of somatic mutations in normal gene products represent potent antigens since they are unlikely to have triggered tolerance. However, identification and isolation of TAAs from individual patients are clinically not practical and currently not an option. Viral antigens would also make excellent TAAs but are applicable only to a very limited number of malignancies with high prevalence of viral infection, such as cervix carcinoma which is associated in over 90% of cases with infection by human papilloma virus A promising target for immunotherapy are TAAs that correspond to normal gene products shared among many
patients. The cancer testis antigens MAGE, GAGE, BAGE, RAGE, SSX, and LAGE-1/NY-ESO-1 are silent in most normal adult tissues but are expressed in cancers of various histological origin. Since the expression of these genes has been observed in many different tumors, the antigens they encode are of enormous practical value for cancer immunotherapy. The only normal tissues that have been found to express these genes are testes and, in some instances, placenta, two tissues regarded as immunologically privileged due to lack of MHC class I expression.

A different group of promising antigens consists of proteins that correspond to normal tissue-specific gene products. Such antigens have been found in melanoma patients and include MART-1/Melan A, gp100, and tyrosinase. Expression of these gene products is limited to melanomas as well as melanocytes and pigment-producing cells in the retina. Prostate-specific proteins like prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), and prostatic acid phosphatase (PAP) (table 1) have also been employed as vaccines against hormone-refractory prostate carcinoma as their expression is frequently conserved in malignant prostatic tissue. Furthermore, overexpression or derepression of α-fetoprotein has been demonstrated in a majority of hepatocellular carcinomas, and carcinoembryonic antigen (CEA) and a mucin (MUC-1) are expressed by several epithelial malignancies (table 1). Additional highly promising candidates for immunotherapeutic strategies are idiotypic determinants of clonal Ig found in B-cell lymphomas.

**Antigen-Loaded Autologous DCs as Cellular Vaccines**

While there has been considerable progress in identification of tumor antigens, the traditional methods for delivering antigens seem insufficient for immunotherapy of cancer. Conventional vaccines which are composed of inactivated pathogens or their components aim to stimulate antibody and, to varying degrees, T\textsubscript{H}1-cell responses. However, in order to eradicate cancer cells, potent CTL responses are needed. Experiments in murine tumor models have shown that plain peptide vaccination often leads to poor activation or even tolerization of T cells [10], whereas the application of autologous DCs charged with the same peptides resulted in vigorous CTL activation and the elimination of tumors. This indicates that antigen-charged DCs when used as a cellular vaccine can induce stronger cytotoxic responses than conventional vaccines. In clinical trials, synthetic MHC class I-restricted peptides have been loaded ex vivo onto class I molecules of DCs along with proteins like the keyhole limpet hemocyanin (KLH) [11], tetanus toxoid, or tuberculin [12] which are very immunogenic and may contain T\textsubscript{H}1-cell epitopes but which are not expressed in the tumor itself. However, an important conclusion from mouse models is that for a potent antitumor response vaccination with both tumor-specific CTL and T\textsubscript{H}1-cell epitopes is required [13]. Hence, it would be helpful if specific MHC class I- and class II-restricted peptides of tumor antigens could be employed. Unfortunately, this approach is hampered by the
diversity of MHC class I and II alleles of individual patients and by the severe lack of defined MHC class II-restricted peptide epitopes. A way out of this dilemma would be to charge DCs with recombinant tumor antigens, assuming that they contain epitopes for presentation on both MHC class I and II molecules. Since DCs are able to process endocytosed proteins for presentation on class I and class II molecules, external loading of DCs with recombinant proteins seems useful. Several methods all of which target receptor-mediated endocytosis or phagocytosis are currently being developed. These include the glycosylation of recombinant proteins for uptake by DC lectin receptors or the administration of Ig/antigen immune complexes for uptake via Fc receptors. Interestingly, DCs can process external proteins much more efficiently for presentation on class I molecules when they are offered in particulate form of a defined size of 1–10 μM [14]. Therefore, the loading of DCs with proteins packaged in biodegradable poly(...
co-glycolide) (PLG) microparticles is currently under investigation [15, 16]. Alternatively, DCs may be efficiently loaded with exosomes from DCs or apoptotic bodies derived from tumor cells.

**Clinical Trials Employing DC-Based Vaccination**

Currently, DC-based immunotherapeutic approaches have just reached the stage of human clinical trial. Initial results several of which appear very promising have recently been reported (table 2). DC Immunotherapy is most advanced in melanoma due to the extensive body of knowledge about melanoma-associated antigens. Clinical trials with stage IV patients (life expectancy of about 6 months) have been performed in Erlangen (Germany) [12], Zürich (Switzerland) [11], Farmington [17] and Los Angeles (USA) [18]. These trials (except the MAGE-1 vaccination [17]) show similar clinical outcomes with response rates varying from 20 to 40% even though different antigens and routes of administration (intravenous (i.v.), intradermal as well as intranodal) had been utilized (table 2). A iso tumors which so far were considered much less immunogenic than melanoma as for instance hormone-refractory prostate carcinoma yielded clinical responses (table 2) [19]. CR = Complete response; PR = partial response; MR = mixed response; SD = stable disease.

**Table 2. DC-based vaccination trials**

<table>
<thead>
<tr>
<th>Vaccine formulation</th>
<th>Clinical response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Malignant melanoma</strong></td>
<td></td>
<td></td>
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<tr>
<td>MAGE-1 peptide + immature DCs i.v.</td>
<td>3 patients, no response</td>
<td>17</td>
</tr>
<tr>
<td>MART-1, tyrosinase, gp100 peptide + immature DCs i.v.</td>
<td>16 patients, 2 CR, 2 PR, 2 MR</td>
<td>18</td>
</tr>
<tr>
<td>Tyrosinase, MART-1, gp100, MAGE-1 and 3 peptide mix, immature DCs intranodally + KLH</td>
<td>16 patients, 2 CR, 3 PR, 1 MR</td>
<td>11</td>
</tr>
<tr>
<td>MAGE-3 peptide mature DCs intradermally + tetanus toxoid/tuberculin</td>
<td>11 patients, 6 PR</td>
<td>12</td>
</tr>
<tr>
<td><strong>Renal cell carcinoma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor lysate + mature DCs + KLH</td>
<td>7 patients, 1 PR, 5 SD</td>
<td>23, 24</td>
</tr>
<tr>
<td>DC tumor cell fusion, lethally irradiated</td>
<td>17 patients, 4 CR, 2 PR, 2 SD</td>
<td>25</td>
</tr>
<tr>
<td><strong>Pancreas carcinoma</strong></td>
<td></td>
<td></td>
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<tr>
<td>Peptide mutant p21 ras + PBMC i.v.</td>
<td>5 patients, no clinical response</td>
<td>22</td>
</tr>
<tr>
<td><strong>Non-Hodgkin lymphoma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immature DCs pulsed with Id + KLH i.v.</td>
<td>10 patients, 3 CR/PR, 6 SD</td>
<td>27</td>
</tr>
<tr>
<td><strong>Prostate carcinoma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 peptides derived from PSMA, immature DCs i.v.</td>
<td>33 patients, 2 CR, 6 PR, 1 SD</td>
<td>19</td>
</tr>
<tr>
<td><strong>Colorectal, breast, lung carcinoma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immature DCs pulsed with CEA-RNA</td>
<td>26 patients, 1 CR/PR, 2 MR, 4 SD</td>
<td>20, 21</td>
</tr>
<tr>
<td>CEA-derived peptides i.v.</td>
<td>19 patients, 1 MR, 1 SD</td>
<td>20, 21</td>
</tr>
</tbody>
</table>

It is premature at this stage to attempt conclusions regarding which DC-based approach to immunotherapy would be the most promising since our current level of understanding concerning pivotal issues of cancer vaccination is too preliminary. Key questions still to be resolved include the number of DCs used for vaccination which varies by 2 logs between different trials and the frequency and schedule of DC administration. We do not know for instance how long the treatment will have to be continued and in what intervals. It is also a matter of current debate which differentiation state of DCs is best and what route of administration should be used. The latter issue was addressed by a recent study which analyzed the biodistribution of in vitro-generated, antigen-loaded human DCs labeled with Indium-111 oxyquinoline after i.v., subcutaneous (s.c.) and intradermal injection [20]. While the DCs injected i.v. localized in lungs and then redistributed to liver, spleen and bone marrow, they were not detected in lymph nodes or tumors. The same applies to DCs injected s.c. Only intradermal injection led to a small percentage of DCs that migrated rapidly to the regional lymph nodes. It should be emphasized that the various parameters of DC-based vaccination in human cannot be inferred from mouse experiments, and it will be a daunting but unavoidable task to determine these important parameters in separate clinical trials in the human setting. Finally, the development of standard criteria (besides clinical response) for evaluating and comparing the efficacy of vaccine formulations is of great importance. Especially the quantitative assessment of specific CTL responses is difficult because the frequency of specific CTLs in the blood is too low to quantify them in functional assays without prior restimulation of CTLs with antigen-loaded APCs in vitro. This amplification step, however, may distort the picture which is found in the patient.
The Issue of Autoimmunity

It has recently been shown in a murine model that DC vaccination may induce autoimmune destruction of islet cells of the pancreas expressing the target antigen [26]. During the DC-based vaccination melanoma patients with melanocyte antigens, tumor regression was occasionally associated with destruction of melanocytes, resulting in depigmentation of the skin (vitiligo). Remarkably, signs of autoimmunity leading to dysfunction of other organs have not been reported in these cases. Only occasionally modest and transient elevations of antinuclear or anti-TSH (thyroid-stimulating hormone) receptor antibodies were observed after extensive vaccinations [11]. In general, vaccination with autologous DCs was very well tolerated and did not show side effects, except for local reactions (erythema, induration, pruritus) and elevation of body temperature.

Even in clinical trials in which DCs have been loaded with tumor lysates [11, 23] or total RNA of tumors, no significant signs of autoimmunity have been reported. One possible explanation for the lack of autoimmunity is that the stimulation of CTLA responses is simply too weak to induce autoagression. Alternatively, the mechanisms of inducing peripheral tolerance to antigens which are contained in tumor cells as well as other tissues may prevent activation of autoreactive T cells. At present, this issue is unresolved, and vaccination with complex and undefined protein mixtures will continue as long as clinical evidence for severe autoimmune destruction is lacking. However, if we succeed to improve the potency of antitumor responses by developing better vaccine formulations, autoimmunity may become an issue. In this case, it would be important to have defined tumor-specific antigens which are presently unknown for the majority of neoplastic diseases. These antigens should ideally be expressed in a majority of tumors but should be absent from tissues which are essential for survival. It therefore seems justified to continue the search for tumor antigens expressed by different types of malignancies.

Conclusion

DC-based tumor vaccination is a promising novel treatment modality that can augment standard treatment options for malignancies. In DC-based vaccination trials complete and partial responses in patients with malignant melanoma, renal cell carcinoma, and hormone-refractory prostate cancer have been obtained at frequencies which have not been observed previously with established modes of treatment. Although the clinical data argue that DC-based vaccination may be effective, a final proof of principle will require larger trials with randomized patient accrual to determine the statistical significance of the reported findings. The outcome of such trials will tell how effective DC-based vaccination is compared to radio- or chemotherapy and whether it will become a routine treatment in the combat against cancer.

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