Chapter 1

Immunotherapy of Cancer — Some Up-To-Date Approaches

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Abstract

Treatment of cancer is currently based on three main modalities: surgery, radiotherapy and chemotherapy. Most solid tumours can only be cured at an early stage, due to the lack of effective systemic treatment. Surgery and radiotherapy are highly effective for eradicating localized tumours, but unfortunately cannot target disseminated disease. Chemotherapy represents systemic treatment, but the clinical use of current drugs is to a large extent hampered by their limited specificity.

Over the last two decades, immunotherapy has emerged as an interesting novel approach. Contrary to the traditional treatment modalities, the immune system combines inherent specificity with a systemic range of action.

The term "vaccine" is traditionally used to describe substances that protect against the development of infectious diseases. In cancer therapy, this term refers to both therapeutic and prophylactic approaches for eliciting immunological and anti-tumour responses.

Prophylactic vaccines have only been developed in a few cancer forms, mainly cancers related to viral infection, such as cervical and hepatocellular carcinoma. Therapeutic vaccines, given to patients after the development of the disease, are however investigated in a number of cancer forms. Some of the therapeutic vaccines may also be used for prophylaxis, particularly in patients with increased risk of cancer process.

This paper throws light and depicts the international experience of a number of distinguished researchers in the field of development and testing of vaccines against some tumours, mainly malignant melanoma and prostate cancer.

Keywords: Immunotherapy, anti-tumour vaccine, malignant melanoma, prostate cancer

1. Introduction

The author analyzes the experience and the research projects worked out in the Dept. Cell Therapy of the University Hospital "Radium" (Inst. Cancer Research, Oslo, Norway) by the team of Prof. Gunnar Kvalheim, Prof. Jon Kyte, Prof. Jahn Nesland, the Bulgarian immunologist M.Sc. Biol. Paula Lazarova, and the author himself, for the last several years.

The colleagues from Radium have utilized gene-transfer technology for developing vaccine therapy with dendritic cells transfected with tumour-mRNA. These vaccines are designed to combine the immunostimulatory capacity of dendritic cells with the antigen repertoire of tumour cells.

There are two approaches to the project: preclinical/experimental evaluation and clinical trials on patients.

2. The first glance throws light on

2.1. Metastatic malignant melanoma – Prognosis and treatment

Malignant melanoma (MM) is among the most common cancers in the developed countries and the incidence has increased substantially over the last decades. Surgery is frequently curative at an early stage, but the prognosis for patients with disseminated disease is generally bleak, with a medium survival of 6-10 months and a 5-year survival of about 5% only.

Decarbazin is extensively used for treatment of metastatic melanoma and has been reported to induce objective tumour responses in 5-29% of all patients. A number of studies have compared other single agents or multi-drug regimes to Decarbazin, without demonstrating a superior effect. To date, no randomized controlled trials have demonstrated improved survival after treatment with Decarbazin or any other drug.

Furthermore, there have been numerous reports of spontaneous immune responses [1] in melanoma patients, to some extent associated with a favourable clinical development. This has prompted the development of various vaccines [1] to target defined or undefined melanoma antigens.

Immunological responses against vaccine antigens have been demonstrated in a number of studies [2, 3, 4, 5, 6, 7, 8]**, but there is limited evidence of clinical effect**

With regard to non-specific immunostimulation, high-dose interleukin-2 (IL-2) has been shown to induce complete remission in about 16% of melanoma patients, but is associated with considerable adverse effects. IL-2, Interferon-alpha (INF-alpha) and other cytokines are also investigated in combination with conventional chemotherapy. Adjuvant therapy with IFN-alpha is believed to prolong the disease-free period, but most studies do not indicate improved survival. Taken together, there is an urgent need for improved therapy of metastatic malignant melanoma.

2.2. The second glance throws light on: Metastatic prostate cancer – Prognosis and treatment

Prostate cancer is the most commonly diagnosed cancer in the male population of the developed countries world-wide. Though the majority of patients eventually die of other causes, prostate cancer is also among the most common cause of cancer death among males in Europe, North America and Japan.

Metastatic prostate cancer is usually treated with bilateral orchiectomy and/or androgen suppressive drugs. The resulting androgen deprivation frequently induces tumour regression and has a palliative effect. The treatment is also considered to give prolonged survival for subsets of patients. However, after a transient response period (median 12-24 months), virtually all patients develop progressive cancer refractory to hormone therapy.

In the RNA/DC-vaccine trial performed in Radium, all included patients had hormone refractory prostate cancer (HRPC).

At this advanced stage, the median survival is only 10-12 months. There has been no effective therapy for HRPC, and many physicians have thus recommended 'clinical observation'.

It is interesting that some separate trials, in other oncology centers, demonstrated prolonged survival after treatment with Doxetacel. This finding has been confirmed in subsequent studies, and Doxetacel is now considered as standard therapy for patients with HRPC.

It should be recalled, however, that the effect on mean survival is limited (2-3 months). Patients with HRPC may also to some extent benefit from different forms of palliative treatment, including certain cytotoxic drugs, bisphosphonates, second-line hormonal agents, glucocorticoids and radiation therapy.

There is, however, an evident need for improved systemic treatment, and immunotherapy may represent an attractive option. Several small-scale studies [2, 3, 5] have demonstrated promising immune responses after vaccine therapy, but there is limited evidence of clinical affect. Interestingly, a recent placebo-controlled phase III trial on HRPC patients has suggested a possible survival benefit from therapy with dendritic cells (DCs) pulsed with a fusion protein of prostatic acid phosphatase and granulocyte-macrophage colony-stimulating factor (GM-CSF).

3. General background for cancer immunotherapy

3.1. Immunosurveillance and immunoediting

A tumour-specific immune response will depend on the ability of immune cells to discriminate the tumour from normal host tissues. In contrast to infectious microbes and allogeneic human cells, the tumour cells are largely similar to normal host cells. According to the theory of immunosurveillance, as suggested by Burnet in 1970, the immune system is still able to recognize and eliminate tumour cells. This concept was severely challenged in the following years. However, there is now convincing evidence that the immune system may recognize

tumour cells due to their expression of altered antigens. The early concept of tumour surveillance has highlighted the importance of the immune system in protecting against cancer.

Recent modifications of this theory, now named immunoediting, provide increased insight into the role of the immune system in sculpting the tumour into an immunologically selected cell population. The immunoediting perspective points to a major challenge in cancer immunotherapy: how to make the immune system destroy a tumour that has already escaped the immune attack.

A solution may be found in exploiting the difference between spontaneously occurring immune activation and optimally engineered immunization. This is the reason for the developing of immune-gene-therapy with tumour-mRNA transfected dendritic cells.

3.2. Activation of T cells that recognized tumour-associated antigens

T-lymphocytes express antigen-specific T-cell receptors (TCRs) that enable them to recognize target cells expressing a particular antigen. The antigens are presented as peptides on HLA-molecules on the target cell surface, and the recognition is mediated by binding of the T-cell receptor to the HLA/peptide complex. Proper T-cell stimulation, including TCR-binding, leads to activation and clonal expression of T cells with the relevant antigen specificity. During the development of a tumour, numerous mutations result in novel antigens and altered expression of normal antigens. The resulting tumour-associated antigens are presented as peptides on HLA-molecules.

Figure 1a shows how host T cells may recognize tumour cells by binding of the TCR to the HLA/peptide complex [7]. However, TCR-binding does not necessarily lead to T-cell activation. In general, the activation of previously unstimulated T cells (‘naïve’ T cells) requires additional stimulation through co-stimulatory molecules like CD80 and CD86. If stimulated only through the TCR, the naïve T cells enter a stage of anergy and permanently lose their ability to be properly activated. The expression of co-stimulatory molecules is largely restricted to professional antigen presenting cells (APCs), including dendritic cells (DCs), macrophages and B cells [7].

Two major subsets of T cells exist. CD4+ T cells recognize peptides bound to HLA class II, while CD8+ T cells recognize peptides presented on HLA class I (Figure 1b).

Most human cells, except erythrocytes and testicular cells, express HLA class I. In contrast, HLA class II is expressed mainly by professional APCs, activated T cells and the cortical epithelium in the thymus. Thus, CD8+ T cells may be stimulated by most cells, while CD4+ T cells depend on stimulation from APCs. As it will be discussed a bit later, the activation of both T-cell subsets is probably important for an effective anti-tumour response.

Effector T cells and the Th1/Th2 delineation [7]:

Proper T-cell activation results in differentiation into effector T cells. CD8+ effector T cells are cytotoxic, i.e. capable of killing cells that express the relevant antigen (Figure 1c).

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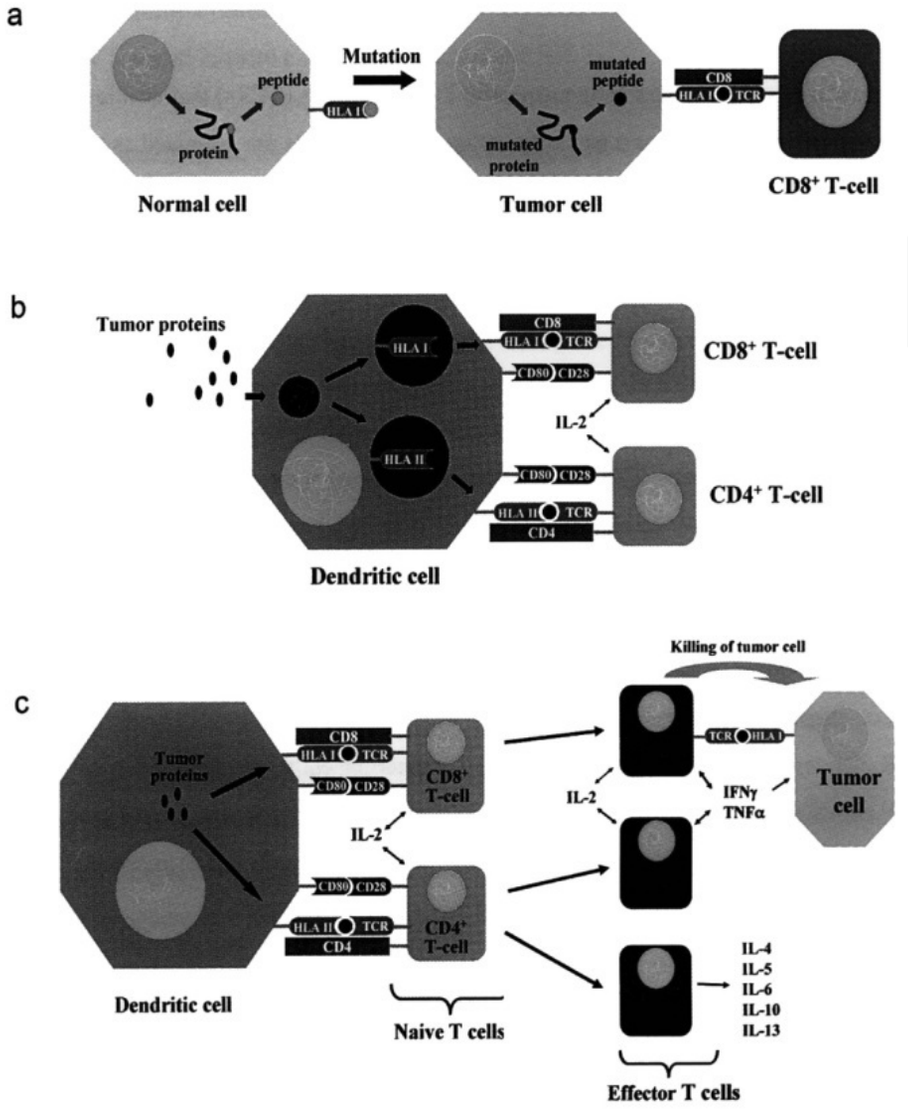

Figure 1. a) Mutations through the development of tumor lead to expression of mutated proteins. The mutated proteins are processed into peptides that are presented on HLA classI (HLA I) on the tumor cell surface. $CD8^+$ T cells specific for mutated peptides may therefore recognize tumor cells by binding of their T cell receptor (TCR) to the HLA/peptide complex. **b**) Stimulation of tumor-specific T cells by dendritic cells (DCs). Tumor proteins are engulfed by DCs and processed into peptides. The tumor-associated peptides are presented by DCs on HLA class I and HLA class II, to $CD8^+$ and $CD4^+$ T cells respectively. For proper activation, previously unstimulated ("naive") T cells also requirestimulation from co-stimulatory molecules (e.g. CD80) and IL-2. Dendritic cells constitutively express HLA class II (and I) and co-stimulatory molecules, and the expression in up-regulated upon DC mutation. T cells start producing IL-2 when stimulated through TCR and co-stimulatory molecules. **c**) Activation of $CD4^+$ and $CD8^+$ T cells induce differentiation into effector T cells. $CD8^+$ T cells differentiate into cytotoxic effector cells that specifically kill target cells expressing the relevant antigen. $CD4^+$ T cells differentiate into T-helper cells secreting high levels of cytokines. Based on their cytokine profiles, the $CD4^+$ effector cells are conventionally divided into Th1- and Th2-cells.

CD4+ effector T cells are conventionally divided into T-helper 1 (Th1) or T-helper 2 (Th2) cells, based on their cytokine secretion profiles (Figure 1c).

Interferon-γ (IFNγ), tumour-necrosis factor-α (TNFα) and IL-2 are usually designated as

Th1-cytokines, while the Th2-cytokines include IL-4, IL-5, IL-6, IL-10 and IL-13.

Th1 cells support cellular immunity, e.g. by secreting cytokines that induce up-regulation of HLA on target cells and stimulate macrophages and CD8+ T cells. Th2 cells promote antibody responses by interaction with B cells.

In cancer immunotherapy, Th1-type responses are generally believed to be desirable.

Antibody responses can only target surface antigens, whereas the CD8+ T cells are able to recognize intracellular antigens presented on HLA class I. Th1- and Th2-responses are mutually inhibitory. Th1-cytokines generally promote Th1-differentiation and inhibit Th2-differentiation, while Th2-cytokines have the opposite effects [5, 7].

Th2-cytokines may therefore suppress the development of cytotoxic anti-tumour responses.

There are considerable experimental data on the effects of individual Th1- or Th2-cytokines.

However, the validity of the Th1/Th2-delineation in humans may be questioned, which could be our next publication [8].

4. Dendritic cells – The most potent antigen presenting cells

Dendritic cells (DCs) are derived from CD34+ progenitor cells in the bone marrow, but otherwise constitute a heterogeneous cell type that is widely distributed in different tissues, including skin, mucosa, lymph nodes and spleen.

Resident DCs in the epidermis (Langerhans cells) and interstitial DCs found in other tissues [7] are believed to be derived from CD14+ myeloid precursor cells (Figure 2).

Recent studies in mice, however, have suggested that both subsets can be derived from fms-related tyrosine kinase 3 (Flt3)-expressing myeloid and lymphoid progenitors, and that plasmacytoid DCs may differentiate into myeloid DCs following viral infection.

Dendritic cells are considered to be the most potent antigen presenting cells and are instrumental in eliciting immune responses. DCs capture antigen in the tissues and then migrate to regional lymph nodes where they encounter T cells in the paracortal area.

Most DCs in the skin and other tissues have an immature phenotype. These cells are effective at engulfing antigen and constantly sample their environment, but have a limited ability to migrate and to stimulate T cells. If immature DCs reach the lymph nodes and present antigens to T cells, they are likely to induce anergy rather than activation, due to their low expression of co-stimulatory molecules. In the non-inflammatory setting, the immature DCs thus promote immunological tolerance by capturing and presenting endogenous antigens. Mature DCs, however, are potent inducers of immunity.

Maturation of DCs is commonly induced by inflammatory cytokines or by the capture of microbial ligands that stimulate Toll-like receptors (TLRs).

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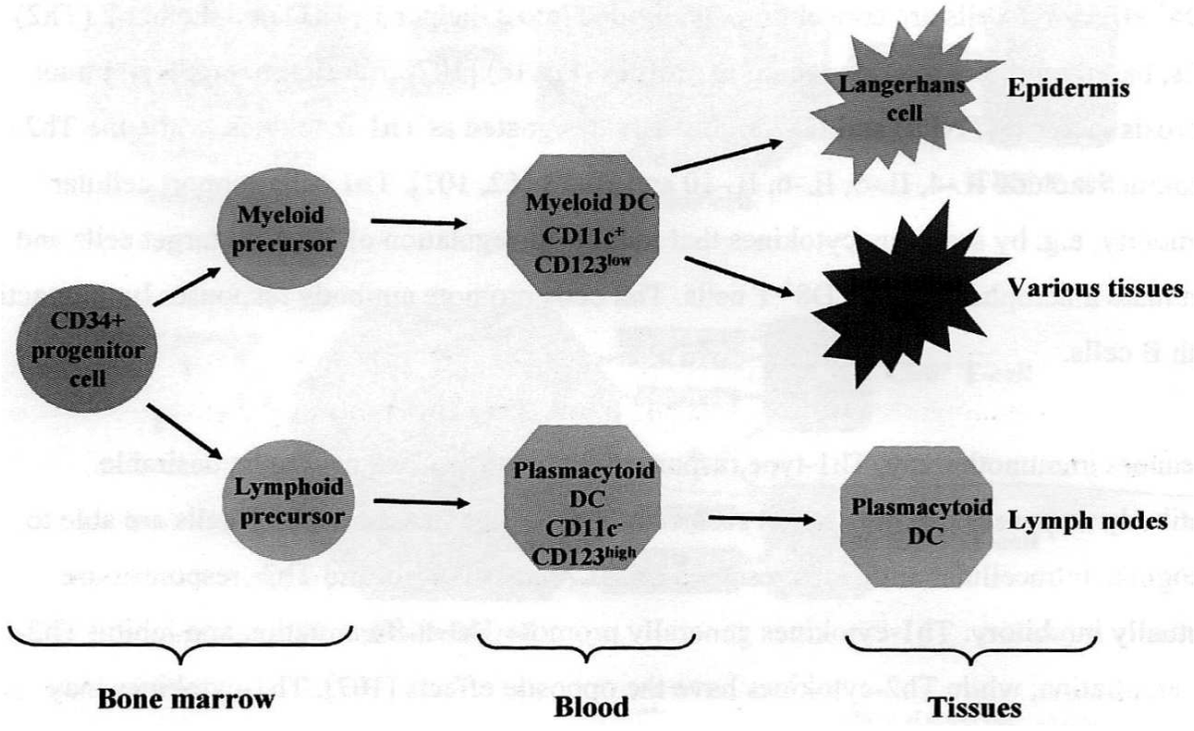

Figure 2. Subsets of dendritic cells (DCs). All DC-subsets are derived from $CD34^+$ haematopoietic profnitor cells in the bone marrow. Interstitial DCs and Langerhans cells are believed to be of myeloid origin. The plasmacytoid DCs are traditionally considered to be of lymphoid origin.

Contrary to immature DCs, the mature cells rapidly migrate to regional lymph nodes, as has been demonstrated in vivo in melanoma patients by Devries and his research group [7].

Moreover, mature DCs strongly up-regulate their expression of HLA class II and co-stimulatory molecules, and are highly effective at activating naïve CD4+.

DCs also present engulfed antigens on HLA class I and may thus directly stimulate CD8+ T cells.

Finally, the phenotype of mature DCs, including their cytokine secretion pattern, is believed to direct the differentiation of CD4+ T cells into Th1- or Th2-type cells.

5. Tumour-associated antigens

The tumour-associated antigens comprise tumour-specific antigens, overexpressed antigens, cancer-germline antigens, viral antigens and tissue-differentiation antigens. Antigens which are virtually tumour-specific may arise from genomic mutations, e.g. K-RAS or post-translational modifications, e.g. MUC-1. Other antigens used in cancer vaccines, like hTERT, survivin, and HER2/neu, are widely expressed in normal tissues, but overexpressed in tumour cells.

In contrast, the expression of cancer-germline antigens, e.g. the melanoma antigens MAGE-1 and MAGE-3, is restricted to tumour cells and normal germline cells (testis and placenta).

Viral antigens represent attractive vaccine targets for virus-induced cancers and are included in the prophylactic vaccines mentioned above for cervical carcinoma (Human papilloma virus) and hepatocellular carcinoma (Hepatitis B virus).

The differentiation antigens are tissue-specific, i.e. expressed only in normal and neoplastic cells from a particular lineage. These antigens may be utilized in cancer vaccines if an autoimmune reaction to the relevant tissue is tolerable.

For instance, prostatitis or vitiligo may represent acceptable adverse effects for patients with prostate cancer or malignant melanoma, respectively.

Several differentiation antigens are extensively used in cancer vaccines, including prostate-specific antigen (PSA) and the melanoma antigens Melan a/Mart-1, gp100 and tyrosinase [6, 7, 8].

6. Background for the present up-to-date tumour-RNA/DC vaccines

6.1. The use of dendritic cells in cancer immunotherapy

Most cancer vaccines have been based on peptides/proteins or tumour lysates that are injected intradermally. These approaches depend on uptake of vaccine antigen by immature DCs in the skin, and subsequent DC maturation and migration to lymph nodes. Alternatively, DCs may be loaded with tumour antigens ex vivo, and then injected into the patient. This strategy appears attractive, as it may result in enhanced antigen presentation and more effective T-cell stimulation. Moreover, DC-based vaccines may offer the opportunity of directing the immune response, by manipulating the DC phenotype.

The first DC-vaccine trials in cancer patients were reported by Hsu et al. in 1996 and Nestle et al. in 1998 [7]. Promising T-cell responses were obtained, and in recent years, various approaches to DC-vaccines have been explored.

Early studies applied immature DCs, but it is now generally believed that a mature phenotype is desirable.

Targeted loading of DCs in vivo represents another strategy.

Ralph Steinman's group has explored this option by use of antibodies targeting the receptor DEC-205 on the DC surface [7]. Their data from animal model indicate that potent T- and B-cell responses may be elicited.

Alternatively, vaccine antigens might be injected and subsequently transfected into tissue DCs by use of in vivo electropermeabilization.

6.2. Large-scale generation of dendritic cells

For clinical vaccine production, large quantities of autologous DCs are required. Most studies make use of monocyte-derived DCs (Mo-DCs).

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On the other hand, DCs may be cultures from CD34+ cells obtained from bone marrow, umbilical cord blood or cytokine-mobilized peripheral blood progenitor cells.

DCs may also be purified from peripheral blood, e.g. after in vivo mobilization of DCs with Flt3-ligand.

At present, it is not clear which method results in the best DCs for cancer vaccination.

The only restriction of use of umbilical cord blood comes from the fact that the per cent of potentially active cells is rather limited and is not enough for bigger scales and clinical application.

In a study reported by Syme et al., Mo-DCs were compared to DCs generated from CD34+ cells 5, 6, 7). The results demonstrated higher expression of HLA class II and CD86 in the Mo-DCs, but no difference in the ability to elicit mixed lymphocyte reaction.

Immature DCs, with a phenotype resembling interstitial DCs, can be generated by stimulating monocytes from peripheral blood with IL-4 and GM-CSF.

The original methods made use of monocytes enriched by adherence.

However, the handling of large numbers of adherent cells is time consuming.

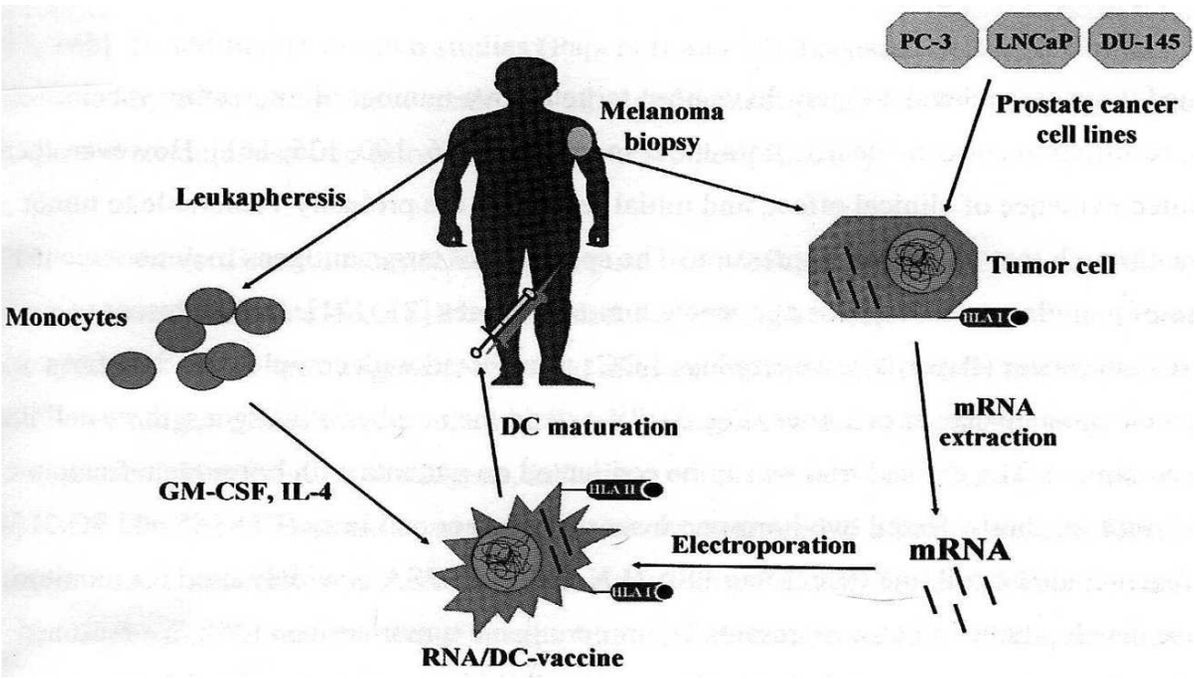

Figure 3. Production of the present RNA/DC-vaccines against malignant melanoma or prostate cancer. Tumor-mRNA was extracted from autologous melanoma biopsies (melanoma vaccine) or from three prostate cancer cell lines (PC-3, LNCaP, DU-145; prostate cancer vaccine). Autologus DCs were generated from monocytes obtained from leukapheresis products. The monocytes were cultured 5 days with IL-4 and GM CSF fro differentiation into immature DCs. Tumor mRNA was then transfected into DCs by electroporation. After transfection, the DCs were cultured for 2 days with cytokines promoting maturation and frozen in vaccine batches.

The production procedure used in the trials performed in Radium Hospital (Inst. Cancer Research, Oslo, Norway) by the group of Gunnar Kvalheim, Jon Kyte and Paula Lazarova [2, 6, 7] is outlined in Figure 3.

The research group did not make use of adherence, but isolated monocytes from leukapheresis products by immunomagnetic depletion of lymphocytes.

The monocytes were transferred to gas permeable Teflon bags that allowed the intrinsically adherent cells to stay in the suspension.

After five days' culture with IL-4 and GM-CSF, the cells were transfected with tumour-mRNA (Figure 3).

Finally, the transfected DCs were matured for 2 days ex vivo with TNFα, IL-6, IL-1β and PGE2.

Contrary to most previous studies, a serum-free culture medium was used. Thereby, unwanted antigens from bovine or human serum were excluded from the vaccine product.

The procedure for DC generation was first established at Radium Hospital by experiments on healthy donors, as reported by Mu et al. [3, 4] and Lazarova et al. [2, 6, 8], later on patients as well.

Subsequently, the generation of clinical grade DCs from patients was evaluated as part of the full-scale preclinical evaluation of the research group in Radium, namely Paula Lazarova et al. [2, 6, 8].

7. Choice of vaccine antigens

Defined tumour-associated antigens have been targeted in a number of interesting vaccine trials world-wide, resulting in antigen-specific immune responses. However, there is a limited evidence of clinical effect, and initial responses are probably vulnerable to tumour escape through loss of antigen expression. The spectrum of target antigens may be widened by use of peptide cocktails or allogeneic tumour cell lines. In the vaccine for prostate cancer, as proposed by the Radium group [2, 3, 4, 5, 6, 7, 8], the employed DCs are transfected with complete mRNA from allogeneic prostate cancer cell lines. To extend the number of antigens, three cell lines were combined.

The clinical trial was conducted on patients with hormone refractory cancer, and thus two hormone-insensitive tumour cell lines were selected (DU-145 and PC-3). A cell line expressing PSA (LN-CaP) was also included.

PSA is widely used for monitoring disease development and also represents an immunogenic tumour antigen.

It was considered that the allogeneic antigens included in the tumour cell lines may increase the risk of side effects, but may also be beneficial.

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T-cell recognizing allogeneic antigens will be primed in the same lymph nodes as the tumour-specific T cells.

The allo-reaction may therefore result in an inflammatory milieu promoting the development of effective anti-tumour responses.

It is argued that the majority of tumour antigens are probably specific to each patient and not even expressed in allogeneic cancer cell lines. The individual tumour antigens are believed to arise from numerous incidental mutations occurring during the development of tumour.

The melanoma RNA/DC-vaccine, worked out by the Radium group [2, 3, 4, 5, 6, 7, 8], represents individualized immune-gene therapy. The autologous tumour material as source of mRNA is used in the procedure (Figure 3) and thereby targets the entire spectrum of tumour antigens in each individual. Moreover, non-expressed tumour antigens are excluded.

In general, it is believed that personalized vaccines, targeting the unique spectrum of tumour antigens in each patient, may emerge as a major principle in cancer immunotherapy

The tumour-mRNA strategy is in principle applicable to any cancer form and may prove particularly useful in rarer cancer forms, where common tumour antigens have not yet been defined. Contrary to peptide vaccines, the use of cell line/tumour-mRNA bypasses requirements for defined HLA alleles and for expression of identified antigens by tumours.

The mRNA can encode multiple epitopes and recruit a wide spectrum of T cell clones, including both CD4+helper and CD8+cytotoxic cells.

There is a number of cancer vaccine trials that have applied RNA-transfected DCs.

Certain parts of these studies include use of undefined antigens.

There are definite disadvantages related to the use of undefined antigens.

First, a wide array of possibly harmful autoantigens will be included, suggesting an increased risk of autoimmune side effects.

Second, the antigens recognized after vaccination will usually not be known.

If HLA-matching peptides can be obtained, T-cell responses to defined antigens may be demonstrated.

However, most antigens, including unique patient-specific targets, will remain unknown.

The T-cell responses can thus only be characterized to a limited extent.

Third, in a vaccine based on autologous tumour material, each individual will receive different vaccines.

This complicates the comparison of results from different patients. It should, however, be recalled, that in any trial on humans the inter-individual variability is immense, even though the vaccine itself may be fully standardized.

8. Loading of DCs with antigen

There are several strategies for loading DCs with antigen ex vivo. A number of important trials have applied simple co-incubation of DCs with peptides, proteins or tumour lysates. Other interesting vaccine studies have made use of tumour-DC hybrids, i.e. tumour cells that are fused with DCs. Similar to DCs loaded with tumour lysates or tumour-RNA, these hybrids may combine the antigen repertoire of tumour cells with the stimulatory capacity of DCs. However, sufficient numbers of living tumour cells are required. In most patients, clinical scale vaccine production may therefore only be feasible from allogeneic cell lines, not from autologous tumours.

DNA- or RNA-transfection represents other alternatives for DC-loading. It is accepted by the Radium group that mRNA has certain important advantages compared to DNA.

First, the use of mRNA bypasses the complex issues of transcriptional regulation.

Second, DNA requires entry into the nucleus, while mRNA has direct access to the translation machinery upon entry into cytoplasm.

In experiments with liposome-mediated loading of plasmid DNA, Saeboe-Larssen et al. found that only a minute fraction (10^{-4}) was detected in the nucleus.

Third, transfected DNA may persist in the cell and encode harmful proteins, while RNA will rapidly degrade.

The latter point is of particular relevance for the safety of using tumour-derived DNA/RNA, likely to encode proteins involved in tumour genesis.

The intrinsic instability of RNA, however, also carries a prominent obstacle to clinical use.

The tumour-mRNA may easily degrade if the tumour samples and RNA-preparations are not carefully handled right from the initial biopsy excision.

Contrary to tumour lysates or tumour/DC-hybrids, tumour–mRNA may be amplified from small tumour biopsies.

This may be of particular importance if the clinical trials are extended to patients with early-stage disease, where only small tumours will be available.

Moreover, mRNA-amplification may enable us to make vaccines from small biopsies of tumours located at difficult sites, e.g. in the brain or visceral organs.

The efficiency of RNA-transfection with conventional RNA/DC co-culture or liposome-mediated loading is limited, probably reflecting degradation of RNA both outside of the cell and in endocytic DC compartments.

The limited efficiency results in low intracellular concentrations of the transfected mRNA.

Though immune responses have still been obtained, it is believed that a higher transfection efficiency is desirable for recruiting a wider spectrum of T-cell clones.

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While some T-cell clones will respond even to low peptide concentrations on the DC surface, the low affinity clones will require higher concentrations.

Viral vectors represent an effective alternative for both, DNA- and RNA-transfection, but there are considerable safety concerns and regulatory obstacles regarding their clinical application.

The Radium group [2, 3, 4, 5, 6, 7, 8] has developed an efficient method for mRNA-transfection by square-wave electroporation, compatible with clinical use.

The electroporation procedure has been optimized for full-scale vaccine production, as worked out by the group, and applied both, in the melanoma and the prostate cancer trials in Radium. The measured transfection efficiency is substantially higher than was expected to be obtained with other methods like liposome-mediated delivery.

In addition to dendritic cells, the research group works also on Epstein-Barr-Virus-transformed cell lines, monocytes and several cancer cell lines, and the initial results indicate efficient transfection.

9. Summary

The development and evaluation of immuno-gene therapy of cancer based on tumour-mRNA transfected dendritic cells, and focused on malignant melanoma and prostate cancer give certain optimism for future successful application of anti-cancer vaccines.

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