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Minireview

Generation of dendritic cell-based vaccines for cancer therapy

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Dendritic cells play a major role in the generation of immunity against tumour cells. They can be grown under various culture conditions, which influence the phenotypical and functional properties of dendritic cells and thereby the consecutive immune response mainly executed by T cells. Here we discuss various conditions, which are important during generation and administration of dendritic cells to elicit a tumouricidal T cell-based immune response.
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Cytotoxic T-cells (CTL) are the most efficient cells concerning defence against tumour cells (Lanzavecchia, 1993). One of the major questions in tumour immunology is, how an efficient CTL-response can be generated to elicit an antigen-specific and protective T-cell response *in vivo*. It has been shown that the immune response to tumour antigens and other antigens is altered in patients with cancer. These alterations concern many elements of the immune response and prevent effective proliferation of tumour-antigen specific T cells and their subsequent recognition of tumour cells. In recent studies antigen-presenting cells (APC) have been shown to play a crucial role in the induction of tumour-protective immune responses by generating tumour-specific T cells (Lanzavecchia, 1993). Antigen receptors (TCR) of tumour-specific T cells recognise tumour-associated peptides that are presented in the context of HLA class-I or class-II molecules by the APC. Successful recognition of tumour – antigen by the T-cell is not only dependent on TCR-peptide-HLA-interaction, but other co-stimulatory signals must be provided to prevent anergy (Schwartz, 1990). These are mainly CD80/CD86 – CD28- or CD40 – CD40L-interactions (Bennett *et al*, 1998). These interactions do not only underline the importance of T cells, but also the significant role of dendritic cells (DC), which are the most potent antigen-presenting cells among others like monocytes, macrophages and B cells.

Several *in vitro* and *in vivo* studies showed the ability of vaccination with DC to elicit tumour-specific T-cell immunity (Schuler and Steinman, 1997). This result implies that (1) DC might be just another altered element of the immune system or (2) DC are able to overcome tumour-protective alterations in cancer patients by inducing effective CTL response or (3) both. In this context a phenotypic and functional dichotomy of DC in DC1 and DC2 appears to be of importance. DC1 and DC2 cells were found to produce different cytokines and thereby induce T_H1 and T_H2 differentiation, respectively. The lymphoid-related DC (DC2) are $CD11c^-$ and have been shown to induce a tolerating response *vs* tumour cells by activating mainly T_H2 cells, whereas myeloid-derived DC (DC1) are immunostimulatory via T_H1 cells. Development of T_H2-promoting DC2 cells is inhibited by cytokines (IL-4) produced by T_H2 cells. In contrast, development of T_H1-promoting DC1 cells is enhanced by the T_H2 cytokine IL-4 (Banchereau and Steinman, 1998; Rissoan *et al*, 1999).

Vaccination with peptide- or lysate-pulsed DC showed the clinical efficiency in the induction of a curative tumour-specific therapy in metastatic melanoma and other malignancies (Table 1; Thurner *et al*, 1999a). In a study performed with peptide- and lysate-pulsed DC five out of 16 patients showed at least a partial remission, two of them a complete remission (Nestle *et al*, 1998). Monocytes drawn from peripheral blood were grown in the presence of a cytokine cocktail (GM – CSF, IL-4, IL-1β, IL-6, TNF-α, PgE_2) to DC, which display mainly the DC1-phenotype. Subsequently, DC were pulsed with peptides or autologous tumour-lysate and injected back into the patient with different clinical outcome.

Several factors seem to influence a successful vaccination by peptide- or lysate-pulsed DC: (1) Generation of DC; (2) Selection of tumour-antigen pulsing of DC; (3) Transfection into DC; and (4) Route of application of DC.

Generation of DC

Physiologically, human DC are mainly localised in tissue and represent only a small portion of less than 0.5% of peripheral blood leukocytes. For therapeutical purposes large numbers of DC are needed.

DC can either be generated from proliferating $CD34^+$ bone marrow precursor cells (Caux *et al*, 1996) – which differentiate under a variety of different cytokines including SCF, Flt3, GM – CSF, TGF-β and TNF-α – or from non-proliferating peripheral $CD14^+$ cells (monocytes) (Sallusto and Lanzavecchia, 1994). Usually, $CD34^+$ precursors mobilised by G-CSF are isolated by leukapheresis to obtain high numbers of peripheral cells for therapeutical purposes. These cells seem to be more efficient in the activation of tumour-specific CTLs than $CD14^+$ derived DC (Mortarini *et al*, 1997). $CD34^+$ cells expand 10 – 30-fold. Yields of 5×10^6 cells per leukapheresis are typically obtained. In contrast, monocytes are abundantly present in peripheral blood and can be easily obtained by peripheral blood drawings or leukapheresis. Protocols for the

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Table 1 Various clinical trials with tumour antigen pulsed DC

Tumour	Pat.	Antigen	DC	Delivery	Specific T cell induction	Clinical response	Ref
Prostate carcinoma	39	LLHETDSAV	Immature	Intravenous	yes	yes (7/39)	(Murphy *et al*, 1996)
Prostate carcinoma	21	Xenoantigen (mouse PAP)	Immature	Intravenous, intradermal, intralymphatic	yes	yes (6/21)	(Fong *et al*, 2001)
B-cell lymphoma	4	Autologous tumour	Immature	Intravenous, id-protein pulsed DC, KLH, SAF-1	yes	yes (4/4)	(Hsu *et al*, 1996)
B-cell lymphoma	35	Autologous tumour	Immature	Intravenous, id-protein pulsed DC, KLH, SAF-1	yes	yes (24/33)	(Timmermann *et al*, 2002)
Melanoma		EADPTGHSY	Immature	Intradermal, intravenous	yes	no (3/3)	(Hu *et al*, 1996)
Melanoma	4	Autologous lysate	Immature	Intranodal, lysate pulsed DC, KLH, FCS	?	yes (2/4)	(Nestle *et al*, 1998)
Melanoma	12	MLLAVLYCL AAGIGILTV KTWGQYWQV EADPTGHSY EVDPIGHLY	Immature	Intranodal, peptide pulsed DC, KLH, FCS	?	yes (3/12)	(Nestle *et al*, 1998)
Melanoma	12	EVDPIGHLY	Mature	Subcutaneous, intradermal, intravenous, peptide pulsed DC	yes	yes (6/12)	(Thurner *et al*, 1999a)
Medullary thyroid carcinoma	7	CEA, calcitonin	Mature	Subcutaneous	yes	yes (1/7)	(Schott *et al*, 2001)
Pediatric solid tumours	15	Autologous lysate	Immature	Intradermal	yes	yes (6/15)	(Geiger *et al*, 2001)

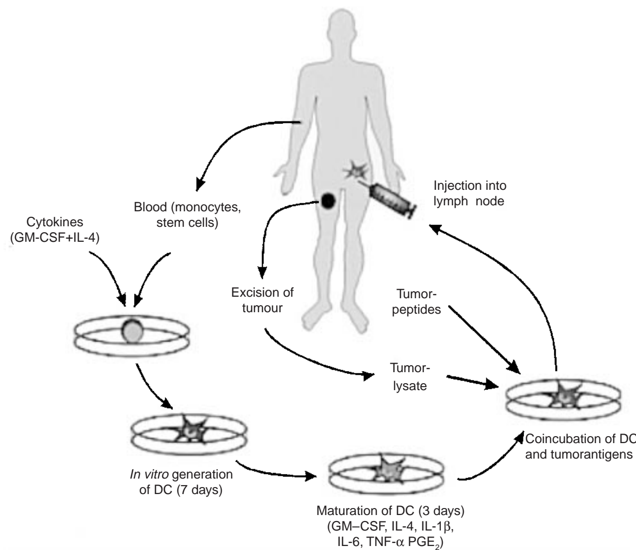

Figure 1 Schematic diagram of dendritic cell generation.

generation of large amounts of monocyte-derived DC are known since 1994 (Romani *et al*, 1994; Sallusto and Lanzavecchia, 1994) and have been used for both experimental and therapeutical purposes. Here, leukocytes are prepared from peripheral blood using Ficoll–Hypaque density centrifugation. Monocytes are isolated by an adherence step and subsequently cultured in the presence of GM–CSF, IL-4 and 10% FCS or alternatively – under serum free conditions (Jonuleit *et al*, 1997; Thurner *et al*, 1999b) – with 1% autologous plasma for 7 days (Figure 1). After 1 week the yield of DC generated varies from about 25 to 50% of the starting population. Yields of $0.5-2.0\times10^6$ cells per 10 ml blood are typically obtained. Adherent cells show cytoplasmic processes typical for DC. After co-culturing with immunologic effector cells DC form typical cluster.

DC display several antigens on their cell surface, all of which are characteristic, but not specific. The most typical markers at present are HLA-class-I-, -class-II-molecules and co-stimulatory markers (CD80, CD86). Immature DC – obtained after 7 days of culture with GM–CSF and IL-4 – can be grown to mature DC by co-culturing with TNF-α, IL-6, IL-1β, and PGE2 or, alternatively, with a so-called monocyte conditioned medium (MCM) for another 3 days (maturation phase) (Thurner *et al*, 1999b). In contrast to immature DC, mature DC are much more potent in inducing T_H1 and CTL responses *in vitro* and are resistant to immunosuppressive effects of tumour-derived IL-10 (Steinbrink *et al*, 1999). Therefore, mature DC have been used in recent vaccination protocols (Thurner *et al*, 1999a). Table 2 shows major phenotypical and functional differences between immature and mature DC.

Selection of tumour-antigen pulsing of DC

Small antigenic peptides (consisting of 8–10 amino acids) are loaded directly from outside the cell on HLA-class-I-molecules, whereas tumour-lysates – as a protein or oligopeptide (>10 amino-acids) – are internalised by endocytosis into the antigen presenting cell, processed and then presented with the HLA class-II-molecule. Cross-presentation of tumour-lysate is possible, i.e. the presentation of proteins or oligopeptides with HLA class-I-molecules (Bennett *et al*, 1997). This implies that pulsing with tumour-lysate or oligopeptides is also able to activate $CD8^+$ T-cells immediately by the HLA-class-I-pathway and not only $CD4^+$ T-cells by the 'conventional' HLA-class-II-pathway. CD40/CD40L signalling via DC/$CD4^+$ T-cell-interaction is able to pre-activate DC temporarily. Subsequently these $CD4^+$ T-cell-pre-activated DC can generate cytotoxic responses in $CD8^+$ T cells (Bennett *et al*, 1998).

The selection of the peptide used for vaccination, is influenced by several factors: type of tumour, HLA class-I or -II of the patient, successful induction of CTL-response *in vitro* or *in vivo*, etc. Today, more than 50 melanoma-associated epitopes are known, which can be recognised by T cells. These epitopes are presented via different HLA class-I- and HLA class-II-molecules (e.g. HLA A1, HLA A2, HLA DR4, ...). An optimal selection of these epitopes allows the treatment of almost 100% of patients with peptide-pulsed DC for the case of malignant melanoma.

Vaccination of tumour–patients with a single peptide can result in peptide-specific cytotoxicity. In these cases tumour escape mechanisms may be a problem, for example by the loss of tumour-associated epitopes or of essential antigen presenting molecules. This problem can be circumvented by the usage of polyvalent vaccines in a single patient, i.e. the application of tumour lysates or

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Table 2 Phenotypical and functional differences between immature and mature DC

	Immature DC	Mature DC
CD83	−	++
CD80 (B7.1), CD86 (B7.2)	+	+++
HLA-class I/HLA-class II	+	+++
CD11a, CD11b, CD11c	+	+++
Motility	+	++
Endocytosis	++	+/−
T-cell stimulation	+	+++

the application of several peptides. This phenomenon can also be observed in a subgroup of a trial conducted by Nestle *et al* (1998). Patients treated with tumour–lysate showed highest response rates (50%, two out of four) compared to patients treated with peptides alone (25%, three out of 12) (Table 1).

Tumour-associated peptides do bind with a defined affinity both to the HLA molecules (i.e. HLA class-I and HLA class-II) and the TCR. Whether this peptide is useful for therapeutical purposes or not mainly depends on the degree of its affinity. Low affinity to the HLA molecules is synonymous with low potency in CTL induction, whereas high affinity means high potency in CTL induction (Sette *et al*, 1994). Therefore, vaccination was performed with a so-called heteroclitic peptide. Original melanoma-associated peptides show a substitution of one amino acid with another at the same position. Heteroclitic peptides, that are changed at the HLA-binding motif to achieve a higher affinity between the HLA and the peptide, are potent immunogens. They are able to elicit cross-reactivity with the original peptide, because the TCR-binding motif remains unchanged. As a consequence a tumour-protective immune response against the original peptide can occur after vaccination with the heteroclitic peptide (Rosenberg *et al*, 1998).

Transfection into dendritic cells

Enhancing the immunogenicity of tumour cells is an interesting approach to cancer gene therapy (Schmidt-Wolf *et al*, 1994). Cytokine genes have been used in most instances to enhance tumour immunogenicity (Schmidt-Wolf and Schmidt-Wolf, 1996). DC are attractive targets of gene transfer since DC are easily accessable and since these cells seem to be sensitive to immunologic strategies. For further enhancement of the antigeneic presentation by DC various genes like the genes for interleukin-7 (Westermann *et al*, 1998), GM–CSF, interleukin-12, interferon-gamma and interferon-alpha (Tüting *et al*, 1998) have been transfected into DC. Up to 10% transfection efficiencies using electroporation for gene transfer into $CD83^+$ mononuclear cell derived DC were reported. Other non-viral techniques produce robust DC transfection with 17% of monocyte-derived DC using cationic peptide or report the ability of using lipofection in principle.

Higher efficiencies can be achieved using viral vectors. Adenoviral vectors seem to be the most efficient transfection method (Mulders *et al*, 1998). Fifty to 85% transfected $CD83^+$ DC generated from PBMC were reported. Thirty to 40% of precursor DC derived from human umbilical cord blood can be transduced using adenoviral vectors without cytopathic effect. With the aid of liposome-mediated infection, gene transfer into $CD83^+$ DC resulted in more than 90% of the cells transduced. Using a protocol with UV-irradiated adenoviruses similar results can be obtained without addition of liposomes (Mulders *et al*, 1998; Märten *et al*, 2001). Adenoviral vectors can also be used for transduction of $CD34^+$ cell derived DC (Bregni *et al*, 1998). For retroviral vectors a transduction efficiency of 10–30% has been reported; other groups described a resistance of DC to transduction by retroviral vectors. Recently, there were reports of using other viral vectors like fowlpox virus, lentivirus, avipoxvirus or vaccinia virus (Di Nicola *et al*, 1998).

Route of application of DC

Cell-based immunotherapy strategies using peptide- or lysate-pulsed DC require interaction between DC and T cells. Physiologically, bone marrow-derived DC or their progenitors migrate to tissues of inflammation, internalise antigens and subsequently reach the paracortex of the lymph nodes (Steinman, 1991) and the periarteriolar lymphoid sheath of the spleen (PALS). Here, DC prime naive T cells. The optimal route of administration of *in vitro*-cultured DC for migration to T-cell-rich sites is unknown, particularly since migratory capacity of cultured tumour-antigen-pulsed DC may be altered. Possible routes of administration are *intradermal, subcutaneous, intranodal, intravenous* and *intraperitoneal injection* of DC. Except intraperitoneal injection, which was performed in animal studies only, all of these applications have been employed in human cell-based vaccination protocols (Table 1). To examine migration patterns of DC, they were radioactively labelled with indium-111. Subsequently, tumour antigen-pulsed DC were administered by an intravenous, subcutaneous, or intradermal injection in patients with metastatic malignancies (Morse *et al*, 1999): Three patients received intravenous injection, four patients received intradermal or subcutaneous injections simultaneously on both sides of their body. *Intravenous* injection revealed highest activity in the lungs after 1 min, which decreased continuously and redistributed after 24 h to highest spleen and liver activities. No activity was found in lymph nodes and tumour. *Subcutaneous* injection showed no activity in the lymph nodes. It could not be detected where the injected DC remained. *Intradermal* injection revealed highest activity in the draining lymph nodes after 24 h. Only 0.1–0.4% of relative activity was found here, i.e. only 4000 of 10^6 injected cells reached the lymph node in contrast to 10^6 of 10^6 cells after successful intranodal injection. In conclusion, greatest activity in lymph nodes was only found after intradermal injection. Subcutaneous injection seemed to be ineffective and intravenous injection showed accumulation in the spleen as a T-cell-rich area. Technetium-labelled immature monocyte-derived DC have also been shown to migrate rapidly to the draining lymph nodes after intradermal injection (Thomas *et al*, 1999). Nonetheless, injecting DC directly into the lymph node seemed to deliver highest numbers of DC in T-cell-rich area, although this mode of application may destroy the normal architecture of the lymph node.

Studies where antigens were continuously injected into a lymph node, for example by a pump, have yet to be performed in humans. It has been shown that route and kinetic of peptide administration determine its immunogenicity. This may also be the case for DC administration.

Adverse effects

Peptide- or tumour-lysate pulsed DC are able to induce CTL-response in patients with malignant melanoma. Peptide or tumour-lysate antigens, used for DC vaccination, are normally not restricted to tumour tissue, but can be found at least partially on healthy tissue. Therefore a risk for the development of autoimmune diseases exists, that has been shown in animal models (Ludewig *et al*, 2000). Pilot clinical studies in humans could not find clinical signs of auto-immunity except vitiligo and the occurrence of auto-antibodies (anti-TSH-receptor-Ab, ANA). Unexpectedly, vaccination with tumour-lysate pulsed DC did not show a higher incidence of auto-immunity than vaccination with peptide-pulsed DC. The occurrence of IgG, IgM and IgE antibodies to bovine serum albumin (BSA) causing anaphylaxis after vaccination with human peptide-pulsed DC was reported (Mackensen *et al*, 2000). Therefore, for therapeutical *ex vivo* applications the use of serum-free generated DC was recommended.

More frequently systemic flu-like symptoms occurred: fever or painful swelling of the injected lymph node (after intranodal injec-

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tion). After intradermal injection of the pulsed DC swelling, itching and erythema at the injection site could be detect. These reactions regressed within 48 – 72 h.

Induction of tolerance against tumour cells may be a problem, although recent studies pointed out the possible therapeutical value of DC vaccination.

Future developments

In conclusion, DC are able to increase the tumouricidal activity of immunologic effector T cells against tumour cells. This ability depends on several factors as has been discussed. Nonetheless, factors that influence effectively DC-activation of T cells against tumour cells have to be optimised. The generation of DC-subtypes that are more effective than those known today seems to be promising in inducing tumouricidal, specific immune responses not only in patients with malignant melanoma, but other malignant diseases. DC-based cell therapy will not only be conducted in patients with distant metastases, but also in patients with minimal residual disease or in adjuvant settings for high risk situations, where tumour-load is low and therefore tumours can be recognised specifically and eliminated effectively by DC-activated immunologic effector cells.

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