Dendritic cells in Melanoma

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Chapter II

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Dendritic cell-mediated immunosuppression in malignant melanoma

Review Article

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Abbreviations: Antigen Presenting Cell, (APC); Cluster of Differentiation, (CD); Cytotoxic T Lymphocytes, (CTL); Dendritic cells, (DC); Dinitrophenyl, (DNP); Delayed Type Hypersensitivity, (DTH); Fas Ligand, (FasL); Granulocyte-Macrophage Colony Stimulating Factor, (GMCSF); Human Leukocyte Antigen, (HLA); Heat Shock Protein, (HSP); Interferon, (IFN); Interleukin, (IL); Keyhole Limpet Hemocyanin, (KLH); Langerhance Cells, (LC); Natural Killer Cells, (NK); Peripheral Blood Lymphocytes, (PBL); Tumor Associated Antigen, (TAA); T cell receptor, (TCR); Tumor Growth factor, (TGF); Tumor Necrosis Factor, (TNF); World Health Organisation, (WHO)

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Summary

Melanomas are immunogenic tumors, presenting a range of tumor-associated antigens (TAA), and cases of spontaneous tumor regression indicate that immune control over melanoma growth can be achieved. Evasion of the immune response is a critical part of tumor development and melanomas can avoid recognition by a variety of mechanisms such as impaired expression of HLA molecules, shedding TAA or secretion of immunosuppressive factors. To enhance antigen presentation and prime effector T lymphocytes, a range of dendritic cell (DC) based melanoma vaccines have been developed. A variety of strategies have been employed using autologous DC to stimulate tumor specific immune responses. Although all of these were apparently successful *in vitro*, when used in patients the responses were disappointing and they ultimately failed to destroy the tumor in the majority of patients. This may reflect observations that melanoma cells suppress immune responses in vitro, and may prevent the generation of effector cells following DC vaccination. Mature DC are normally potent activators of immune responses. However, when immature, they are often immunosuppressive. The DC found in melanoma and in the sentinel lymph nodes invaded by tumor are of an immature phenotype and therefore may suppress the anti-tumor immune response. We suggest, that a successful vaccine for melanoma must include either mechanisms to reverse *in situ* DC suppression or increase immune stimulation.

I. Introduction

A great deal of effort has been put into developing a vaccine for the treatment of melanoma. However none of the current approaches have addressed immune suppression at the tumor site. It is possible that unless this melanoma-derived immune suppression is reversed immunotherapy will be unsuccessful. Melanomas, unlike most other tumors, can be immunogenic, and can present a range of tumor-associated antigens (TAA). Cases of spontaneous tumor regression have been reported even in very advanced disease (Szekeres and Daroczy, 1981; Ralfkiaer et al, 1987; Tefany et al, 1991), and these reports have encouraged efforts towards anti-tumor immunotherapy. Despite the presence of potent anti-tumor immune cells in their blood, more than 95% of patients gain no benefit from anti-tumor immune therapy. The co-existence of anti-tumor immunity and tumor progression in the same individual remains one of the major paradoxes of melanoma immunology.

II. Escaping immune surveillance

It has been recognised for some time that the immune system plays a crucial role in the removal of malignancies arising through somatic mutation. Successful malignancies must survive this surveillance and are therefore subject to selection pressure resulting in

the evolution of escape variants, that can no longer be recognised by either T lymphocytes or NK cells (Burnet, 1970; Festenstein and Garrido, 1986). Since the recognition is based on antigen presentation, the loss of HLA molecules and impaired antigen presentation are the most obvious mechanisms of escape from destruction by cytotoxic T lymphocytes (CTL). Alterations in HLA expression are ubiquitous among tumors, but are also highly variable. So far seven different major modifications of HLA class I phenotypes have been described in different tumor types. These include complete loss of any HLA allele, significant down-regulation of one or more alleles, expression of altered HLA alleles or immunosuppressive HLA alleles, and altered responsiveness to activation signals such as type I interferons (Adrian Cabestre et al, 1999).

Loss of HLA class I is often attributable to structural alterations in the proteins involved in antigen processing leading to impaired HLA loading, and therefore surface antigen presentation (Seliger et al, 2001). Melanomas can also express HLA class II proteins, whose expression is generally restricted to APC and activated T cells. This ability does not enhance tumor immune sensitivity, but on the contrary interferes with normal T helper function due to the absence of co-stimulatory molecules such as B7 on the tumor (Becker et al, 1991; Hersey et al, 1994; Becker and Brocker, 1995; Denfeld et al, 1995). Antigen recognition and a successful immune reaction is additionally impeded by heterogeneity in surface protein expression, even within the same tumor (Dalerba et al, 1998). Moreover melanoma cells can shed antigens, which may abrogate anti-tumor cytotoxic cell function or express and release FasL, which causes apoptosis of T lymphocytes and secrete immunosuppressive cytokines (Becker et al, 1991; Ekmekcioglu et al, 1999; Gray et al, 2002; Redondo et al, 2002, 2003; Sombroek et al, 2002; Wolfl et al, 2002; Peguet-Navarro et al, 2003).

III. Dendritic cell based immune vaccines

As the generation of successful anti-tumor immune responses would greatly benefit patients with this aggressive tumor, a number of approaches have been taken to initiate protective immunity. Many of these exploit function of dendritic cells, which act as potent immune response stimulators. Dendritic cells migrate from blood to nearly every tissue in the body, take up antigens and process them. They then migrate to spleen and lymph nodes and deliver the antigens for presentation to lymphocytes. As professional APC they express both HLA class I and II, and can additionally therefore activate both helper and cytotoxic T lymphocytes. They can cross-process antigens between these two pathways and in this way switch the immune response type and evoke cytotoxic reactions against endogenous tumor antigens (Albert et al, 1998a, 1998b; Banchereau and Steinman, 1998; Inaba et al, 1998) (**Figure 1**). Numerous vaccine strategies have either utilised DC directly or used a variety of mechanisms to stimulate them.

To find suitable antigens for vaccine purposes, melanoma proteins have been screened in search of peptides with potent immunostimulatory characteristics, presented by both HLA class I and II, to activate both cytotoxic and helper T lymphocytes. Studies have identified HLA class I binding peptides, and several peptides presented in the context of multiple HLA-DR alleles and recognisable by CD4+ T cells (**Table 1**).

Peptides derived from known melanoma associated antigens have been used to load DC generated in vitro from blood or bone marrow precursors or monocytes from both melanoma patients and healthy donors. In order to enhance antigen presentation several peptide modifications have been tested. For example, the proteins were fused with TAP targeting sequence to facilitate antigen processing (Minev et al, 2000) or with heat shock protein to assist antigen delivery into dendritic cells. (Noessner et al, 2002). In one particularly successful approach, fusion of melanoma derived antigen with recombinant HIV trans-activating fusion proteins allowed enhancement of the protein incorporation rate to 95% (**Table 2**).

One of the most important disadvantages of peptide-based vaccines is the lack of non-self antigens shared by all melanoma cells. A single epitope is seldom sufficient for the induction of a potent immune response, therefore an ideal vaccine should contain a variety of epitopes and proteins. One way of avoiding these difficulties is to use whole tumor cells, or vesicles secreted by them, as a source of antigen. It has been shown that dendritic cells can phagocytose necrotic (Abdel-Wahab et al, 1998) and apoptotic (Soruri et al, 2001) tumor cells, however in the latter case DC maturation strongly depends on the presence of pro-inflammatory cytokines in the environment (Jenne et al, 2000; Labarriere et al, 2002). One approach to increase vaccine immunogenity exploits the technique of cell fusion. Hybridomas of melanoma cells and dendritic cells have been shown to preserve the features of dendritic cells vital for their function (expression of HLA-A, B, C; HLA-DR; CD40, CD54, CD80, CD83, CD86, and the pro-inflammatory cytokine interleukin-12) and expression of melanoma-associated antigens (Holmes et al, 2001; Soruri et al, 2001; Jantscheff et al, 2002).

Endogenous expression of antigen by DC offers the potential advantage of prolonged presentation of antigens in the context of both HLA classes, and potentially extends the repertoire of immune stimulation. Nucleic acid-based immunization provides an attractive method for the delivery of protein antigens and adjuvants, without the need to know the sequence of immunogenic epitopes in advance. Additionally it allows the function of multiple restriction elements for the presentation of the same antigen Kim et al, 1997 and the generation of CD8(+) T cells against multiple class I-restricted epitopes within the antigen (Alijagic et al, 1995; Lapointe et al, 2001; Larregina et al, 2001).

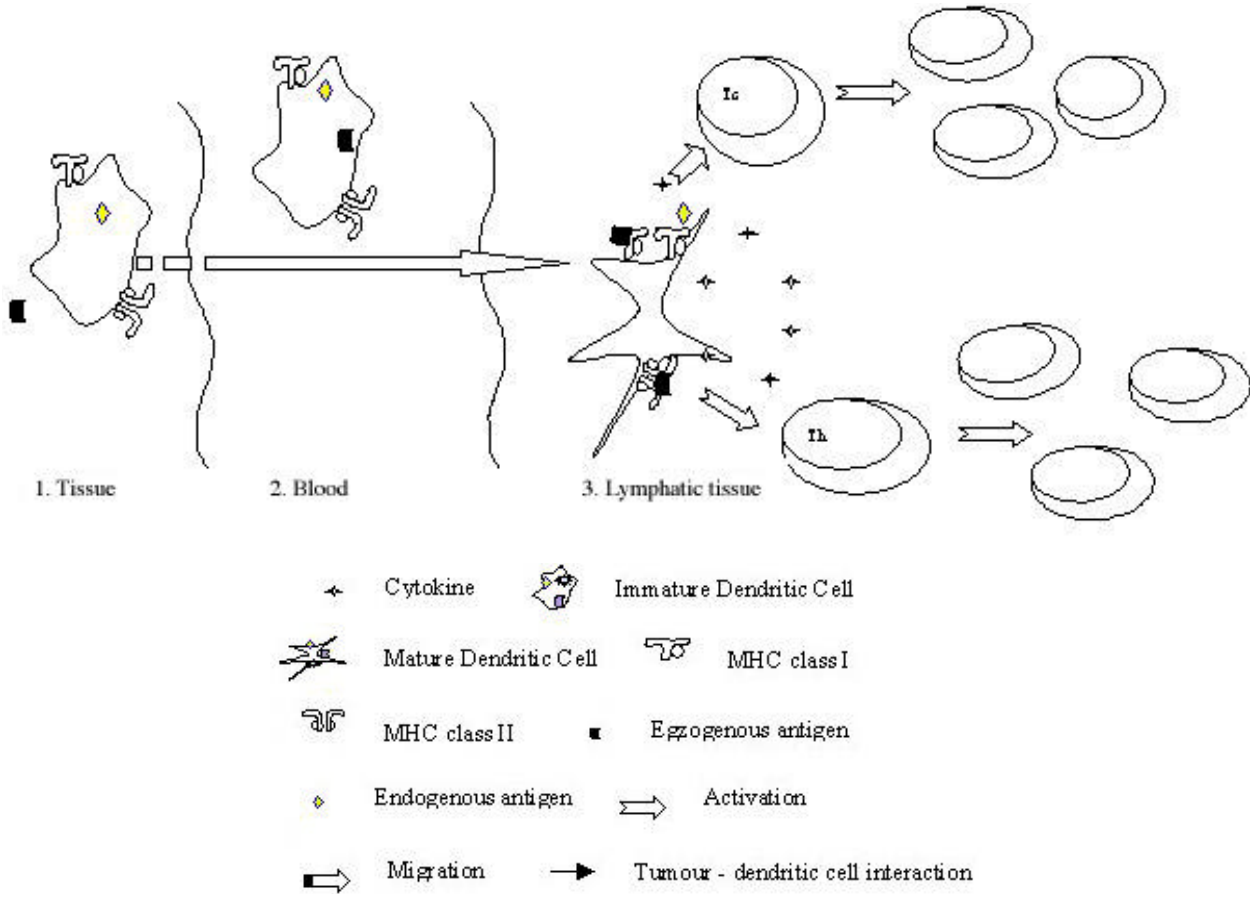

Figure 1 Role of dendritic cells in immune system activation. Dendritic cells reside in the majority of tissues, and continuously acquire and process the antigens form the environment (1). The antigen uptake gives them a primary signal for maturation. They start production of proteins necessary for antigen presentation and they migrate via blood to deliver the antigens to spleen and lymph nodes (2). Being antigen-presenting cells, they express both HLA class I and II, and can therefore activate both helper and cytotoxic T lymphocytes. Furthermore, they can cross-process antigens between these two pathways and thus switch the immune response type and evoke cytotoxic reactions against endogenous tumour antigens presented in their HLA proteins. Providing the secondary activation signal (accessory molecules and cytokines), they prevent lymphocyte anergy, resulting in the generation of an army of sensitised cytotoxic and helper, effector and memory lymphocytes (3).

Table 1. Examples of defined epitopes suitable for anti-melanoma immune vaccine therapy.

Protein	HLA-I binding peptides	HLA-II binding peptides	Authors
Cancer-testis antigens			
NY-ESO-1		+ 119-143 119-143	Jager et al, 2000; Zarour et al, 2002, 2000b
MAGE-3	 EVDPIGHLY	TQHFVQENYLEY	Schultz et al, 2000, Schultz et al, 2001
Melanocyte differentiation antigens			
gp-100	gp100[9(87)] gp100[10(86)		Kawashima et al, 1998 Cochlovius et al, 1999 Cochlovius et al, 2000 Kierstead et al, 2001
MelanA/MART-1	GILTVILGV ALMDKSLHV	 51-73	van Elsas et al, 1996 Zarour et al, 2000a
Widely expressed antigens			
HER2/neu		776-788	Sotiriadou et al, 2001

Table 2. *In vitro* immune response mediated by melanoma antigens loaded dendritic cells.

Antigen source	Antigen presentation	Inducted cells	Cytotoxic reactivity anti:	References
Defined peptide (MAA)	+++	CTL, NK, Th lymphocytes	- Peptide loaded target cells - Tumor cell lines - Normal melanocytes - Autologous tumor cells	Bakker et al., 1995; Saeterdal et al., 1998 Tjandrawan et al., 1998 Abdel-Wahab et al., 1998 Dhodapkar et al., 2000; Kikuchi et al., 2001; Minev et al., 2000; Yang et al., 2002 Noessner et al., 2002 Tanaka et al., 2003
Melanoma cell lysates	+++ cross-presentation	CTL, Th lymphocytes	- Autologous tumor cells	Abdel-Wahab et al., 1998 Soruri et al., 1998 Imro et al., 1999 Berard et al., 2000 Nouri-Shirazi et al., 2000 Jenne et al., 2000; Labarriere et al., 2002 Whiteside et al., 2002 Bateman et al., 2002 Russo et al., 2000 Andre et al., 2002
Hybridomas				Holmes et al., 2001 Soruri et al., 2001 Jantscheff et al., 2002
Genetically modified cells	+++	CTL, Th lymphocytes (1 study)	- Cells presenting MAA antigens - Autologous tumor cells	Reeves et al., 1996 Bettinotti et al., 1998; Tuting et al., 1998 Chinnasamy et al., 2000 Yang et al., 2000 Kim et al., 1998 Drexler et al., 1999 Linette et al., 2000 Motta et al., 2001; Philip et al., 2000 Lapointe et al., 2001 Larregina et al., 2001; Smith et al., 2001 Firat et al., 2002 Prabakaran et al., 2002 Temme et al., 2002 Sumimoto et al., 2002

IV. In vitro efficacy of dendritic cells vaccines

Overall, the majority of the in vitro approaches described have been successful, with regards to antigen incorporation/transfection rate, protein production and presentation, and T-lymphocyte activation. Experiments in vitro have proved that dendritic cells are able to process and present melanoma-specific antigens derived from whole melanoma cells, synthesised or purified peptides or when they are genetically modified to produce tumor antigens (**Table 2**). Moreover, synergistic effects of viral transfection and DC maturation have been observed (Rea et al, 2001; Temme et al, 2002). Transfected DC synthesised the desired product, and the antigen expression remained detectable for at least 7 days. Also DC loaded with killed tumor cells can induce HLA class I- and class II-restricted proliferation of autologous CD8+ and CD4+ T cells, and are therefore able to cross-present tumor cell-derived antigens. In all cases they presented a broad range of tumor antigen epitopes in the context of multiple HLA alleles and stimulated several types of lymphocytes reactive against multiple melanoma antigens.

In the vast majority of studies both proliferative and cytotoxic responses were reported. Lymphocytes co-cultured with genetically modified DC produced Th1 type cytokines and showed multiple antigen specific cytotoxic responses, against melanoma cell lines, HLA-matched B cell lines pulsed with peptide and, most importantly, autologous tumor (**Table 2**). Induction of not only cytotoxic and helper lymphocytes, but also clones of NK cells have been reported. Tumor-specific T cells with NK activity are potentially of great clinical significance as they provide a mechanism for lysis of tumor cells that have lost HLA expression (Saeterdal et al, 1998)

It is the ability of in vitro expanded lymphocytes to recognize naturally processed and presented epitopes that illustrates the potential use of dendritic cells for vaccination in human cancer. Unfortunate therefore that, despite these encouraging results, so far none of these strategies has found direct effective translation to patient care.

V. Response of patients to DC vaccination

Despite dendritic cells being increasingly used for the immunotherapy of melanoma only a few tumor remissions due to vaccination have been reported (**Table 3**). Several phase I/II clinical studies have shown that DC vaccines are non-toxic (no grade 3 or 4 WHO scale toxicities), that vaccine injections are well tolerated, and that DC derived in vitro are viable after injection and can mediate biologic activity in situ (**Table 3**). Both adjuvant therapies and dendritic cell based vaccines caused infiltration of immune cells (both dendritic cells and lymphocytes as well as numerous other types) into the site of vaccination, and the cytotoxic tests on patients immune cells obtained after one or several courses of vaccine administration have given encouraging results. Peripheral blood lymphocytes from patients recognised melanoma cells in vitro, produced pro-inflammatory cytokines and

Table 3. Clinical outcome of DC based vaccines

	No Patients	DC infiltration	T lymphocytes infiltration	Objective response rate – overall percentage			References
				Complete response	Partial response	Disease stabilisa-tion	
DC targeted adjuvants							
GM-CSF	72	5/5 studies	4/5 studies	1%	16.7%		Nasi et al., 1999 Kusumoto et al., 2001 Zehntner et al., 1999 Chang et al., 2000; Soiffer et al., 1998
GM-CSF + Other adjuvants	51	1/2 studies		22,5%	27%		Janik et al., 1999; Schachter et al., 1998
Antigen modified dendritic cells							
Autologous DC injected into tumor site	7	1/1 studies	1/1 studies	0	57%		Triozzi et al., 2000
Peptide loaded	172	3/13 studies	4/13 studies	2%	12%	3%	Lotze et al., 1997; Thurner et al., 1999 Lotze et al., 2000 Mackensen et al., 2000 Panelli et al., 2000 Schuler-Thurner et al., 2000 Andersen et al., 2001 Banchereau et al., 2001 Lau et al., 2001 Thomas et al., 2001 Toungouz et al., 2001 Schuler-Thurner et al., 2002 Smithers et al., 2003
Melanoma lysates loaded	66		2/4 studies	12%	13.6%	1.5%	Nestle, 2000 Chang et al., 2002 Krause et al., 2002 O'Rourke et al., 2003
Genetically modified	14	1/3 studies	1/3 studies	0%	0%	0%	Housseau et al., 2002 Nair et al., 2002 Tsao et al., 2002

even killed melanoma cells from cell lines or autologous tumors. Nevertheless, the vaccination was ultimately unsuccessful in most cases, since the melanoma survived and the patient died (**Table 3**).

A. Adjuvants

Adjuvants stimulate DC and in this way enhance immune response. An early attempt to induce clinical inflammatory response in vivo using the dinitrophenyl (DNP) -conjugated melanoma cell immunization of DNP-pre-sensitised patients resulted in cutaneous DTH. In half of the patients the inflammatory reaction was confirmed and caused regression of metastases within 2-4 months. The inflammatory response was associated with the infiltration of CD8+ T cells, enhanced expression of ICAM-1 and HLA-DR by melanoma cells and the presence of numerous HLA-DR+, CD4+, CD1-, Leu-1-dendritic cells (Murphy et al, 1993).

The cytokine GM-CSF stimulates DC maturation in vitro and has been used to stimulate DC activation in vivo. (Mortarini et al, 1997; Chen et al, 2001). Direct sub- or intra-cutaneous injections (Schachter et al, 1998; Janik et al, 1999; Nasi et al, 1999) and the use of genetically modified melanoma cells (Soiffer et al, 1998; Zehntner et al, 1999; Chang et al, 2000; Kusumoto et al, 2001) have been used for vaccine administration either alone or in combination with other cytokines (Janik et al, 1999) and cytotoxic agents (Schachter et al, 1998). Vaccine evaluation was based on immunohistochemical staining of vaccine site biopsies, peripheral blood analysis and functional tests in vitro, as well as clinical outcome. In most cases, despite DC and lymphocyte influx into metastatic tumor sites and the successful specific activation of anti-tumor T lymphocytes, the clinical outcome was far from satisfactory. In two studies achieved remission in only one patient (Chang et al, 2000; Kusumoto et al, 2001), and in three, no anti-tumor effects were observed. In the study performed by Soiffer and colleagues, extensive tumor destruction was observed, but no durable complete remission was reported. (Soiffer et al, 1998). The adjuvant vaccine worked well however when combined with chemotherapy, giving a response rate over 50%. Nevertheless the drug regimen, including cytokines,

was very toxic, and this strategy has not been explored further (Schachter et al, 1998). These results are consistent with adjuvant clinical trial studies, where IL2, IFN g, and GM-CSF did not result in an improved clinical outcome (McClay, 2002).

B. DC vaccines

Several clinical trials of DC-based anti-melanoma vaccines have been performed (**Table 3**). In a study by Triozzi and colleagues the biologic activity of dendritic cells injected directly into tumors was examined. This pilot study demonstrated that DC derived in vitro were viable after intratumoral injection and could mediate biologic activity in situ. (Triozzi et al, 2000) Whether applied intravenously or intradermally, DC can easily migrate to lymphoid organs and tumor sites (Mackensen et al, 1999; Thomas et al, 1999). Many T cell anti-tumor responses were measured, and in 7 out of 9 trials at least transient tumor-specific PBL activity was observed. (**Table 3**). When keyhole limpet hemocyanin (KLH) was administered, activation of helper T lymphocytes was reported; with DTH directed both against KHL and tumor cells (Nestle et al, 1998; Toungouz et al, 2001; Chang et al, 2002).

Unfortunately, despite the high in vitro anti-tumor activity of patients' PBL, the clinical outcome was not very successful. A maximum overall response rate of 25.6% has been reported with a12% complete response rate (**Table 3**). Interestingly, the most potent immune response was induced when autologous tumor material was used (Andersen et al, 2001; Thomas et al, 2001; Krause et al, 2002; O'Rourke et al, 2003; Smithers et al, 2003)

VI. Reasons for the failure of DC vaccination

Given that all of the strategies tested are equally successful in vitro, that their application routes in general do not differ, and that they are all based on autologous dendritic cells obtained either from patients blood, generated from CD34+ precursors ex vivo, or monocytes, the reason for the failure to eradicate the tumor is probably independent of the methods of vaccination. Since anti-tumor PBL activity has been shown, this suggests patients' immune systems are capable of producing a wide range of cytotoxic cells, potentially able to recognise tumor antigens. It appears, that although the immune response against melanoma tumors has been induced, in patients its effector phase is not carried through to completion.

There are several possible explanations for the failure of DC vaccinations to eliminate tumor. The simplest explanation would be that the modified cells or pre-sensitised CTL might have been unable to penetrate the tumor or that the antigen specificity of the CTL may have been too narrow. Studies have however confirmed the generation of potent anti-tumor CTL and their successful migration into the tumor site (**Table 3**). Alternatively, the CTL may be suppressed or killed at the site of the tumor and therefore unable to perform any anti-tumor activity. It is well known that tumors are immune privileged sites and that they create an immunosuppressive environment around themselves, preventing inflammatory responses. This is thought to be achieved by the secretion of a range of immunosuppressive cytokines, such as IL-10, IL-19, IL-6, TGF b1 and 2, macrophage migration-inhibitory factor, gangliosides, heavy chain ferritin, ICAM-1 and prostaglanoids. In addition tumors are not only resistant to TNF receptor pathway mediated apoptosis, but can also express and secrete FasL, which causes apoptosis of activated lymphocytes (Ekmekcioglu et al, 1999; Gray et al, 2002; Redondo et al, 2002; Sombroek et al, 2002; Peguet-Navarro et al, 2003; Redondo et al, 2003; Wolfl et al, 2002).

VII. Modulation of immune responses by dendritic cells

Since inappropriate immune responses can be dangerous (if e.g. induced against healthy tissue), they must be carefully regulated. DC subsets play crucial roles in the selection process in the thymus as well as regulatory roles in lymph nodes and the periphery.

One of the most characteristic features of dendritic cells is the transformation of their phenotype during maturation. DC function is highly dependent on their level of maturation, and cells in various stages of development differ not only in their morphology but also completely alter their surface antigen expression. In humans, the presence of immature DC has been reported in most organs, including liver, kidney and heart, where they tend to be associated with vascular structures (Hart, 1997; Banchereau and Steinman, 1998). An interdigitating sentinel epithelial network of DC has been described in the mucosa of the oral cavity, intestinal tract and the respiratory tract (Hart, 1997). It is increasingly believed that tissue-residing immature dendritic cells constantly incorporate and process various proteins from their environment. Under physiological conditions, they express few self-antigens on their surface for presentation to T lymphocytes. However, since the dendritic cells are immature, they do not express co-stimulatory molecules, and what results is impaired lymphocyte activation, and anergy. This simple mechanism eliminates self-reactive lymphocytes, and prevents autoimmunity (Hart, 1997; Banchereau and Steinman, 1998; Lutz and Schuler, 2002). Tissue resident immature dendritic cells can also phagocytose apoptotic bodies formed when neighbouring cells die by apoptosis. Normally this will not result in an immune response, however, if apoptosis was the result of a viral infection then additional signals at the site of infection (e.g. IFNa, HSP) induce dual activation and maturation of dendritic cells, and launch an immune reaction (Hart 1997; Banchereau and Steinman, 1998; Lutz and Schuler, 2002).

It is not only immature tissue-resident dendritic cells that anergise T lymphocytes. The presence of "semi-matured" dendritic cells circulating in the blood of healthy donors was described by Lutz and Schuler (2002). These

cells are loaded with self-antigen, and express antigens associated with a mature phenotype, but do not release cytokines, and therefore do not provide sufficient activation signals for lymphocytes. They react with CD4+ lymphocytes, inducing a subset of regulatory helper lymphocytes, which remain in the organism as memory cells, providing a mechanism that supports peripheral tolerance.

VIII. Melanoma derived, DC mediated immune control

A. Altered phenotype of lymph nodes invaded by melanoma

The induction of T lymphocyte anergy in tissues and the creation of a population of regulatory cells are two distinct pathways leading to tolerance to self-antigens. Thanks to these control mechanisms, severe autoimmune reactions can be avoided. If however dendritic cells are kept artificially immature, it creates a potential hazard for the function of immune system.

Many groups have reported alterations in cell ratio and function in lymph nodes invaded by melanoma. Several authors observed the recruitment of immature plasmacytoid dendritic cells, and T lymphocytes with a suppressive phenotype. (Fernandez-Bussy et al, 1983; Lana et al, 2001; Salio et al, 2003; Vermi et al, 2003). A comparison between sentinel and non-sentinel lymph nodes showed a quantitative reduction in dendritic cell markers, in the sentinel lymph node. This suggests a loss of mature DC and a concomitant decrease in total DC content (Essner and Kojima, 2002)

Histological studies show a profound decrease in the number of antigen-presenting cells expressing HLA class II in the epidermis above the melanoma, with zonal immune suppression in involved lymph nodes. There are decreased numbers of DC in the paracortex of the lymph node, and the majority of remaining LC and DC are phenotypically immature (Fernandez-Bussy et al, 1983; Cochran et al, 1987; Toriyama et al, 1993; Garcia-Plata et al, 1995; Barbour and Coventry, 2003) (**Figure 2**). In vitro assays confirmed the suppressed functional characteristics of cells derived from melanoma-invaded sentinel lymph nodes or exposed to conditioned supernatants from melanoma cell cultures (Hoon et al, 1987a, 1987b; Farzad et al, 1997; Chang et al, 1999).

Several studies have examined the tolerizing influence of melanoma cells on the maturation and function of DC and show that under the influence of melanoma, DC acquire an immunosuppressive phenotype and cause the generation of anergic T lymphocytes. The immunostimulatory function of DC obtained from progressing and regressing melanoma metastases show a significant difference in the abilities of each population. In addition, monocyte-derived DC exposed to tumor supernatant failed to acquire full allo-stimulatory activity and rapidly underwent apoptosis (Enk et al, 1997; Steinbrink et al, 1999; Kiertscher et al, 2000; Steinbrink et al, 2002).

In the presence of melanoma cells or tumor conditioned media, CD 80, 86 and HLA class I and II are up-regulated on the DC surface, even though expression of immature DC-related antigens like E-cadherin is retained, and the DC maturity factor, CD 83, is not expressed (Remmel et al, 2001; Padovan et al, 2002).

These cells therefore have a phenotype allowing antigen presentation by immature cells, leading to the suppression of antigen-specific immune responses.

It may be worth considering whether the characteristic dissemination of melanoma via the lymphatics and primary metastatic spread into the lymph nodes is coincidental.

B Dendritic cells as a key to immune escape

Melanoma is a tumor recognisable to the immune system and cannot grow and develop in the presence of a competent immune system. In the early stages of tumor development melanoma acquire an “invisible” phenotype following the selection pressure of the immune system. This however might be not enough to ensure further tumor cell survival. The tumor needs a more secure and permanent strategy. Lymph nodes are the nerve centres of the immune response, places where antigens are presented to lymphocytes and where decisions about immune responses are made. By invading these, melanoma creates an immune-privileged site in the centre of immune reaction. What can be harmful to infiltrating cytotoxic cells must also influence to at least the same degree any regulatory cells residing in vicinity. Dendritic cells act as the key component in immune reaction regulation. Under normal circumstances they are able to stimulate populations of lymphocytes against danger (e.g. tumor cells), however if their maturation is halted and their phenotype switched into modulatory mode, instead of immune stimulation, they will induce immune tolerance. Melanoma cells have the potential to keep dendritic cells in an immature state (**Figure 2**), impaired and suppressed, yet able to control and suppress other components of the immune response. By invading lymph nodes, melanoma acquire a potent strategy of immune evasion. The hunted transforms into the hunter – instead of escaping the immune system, in effect the melanoma takes control and deletes the tumor sensitive lymphocytes at the command centre of immune reactivity (**Figure 3**).

Functional alterations in lymph nodes invaded by melanoma should be considered when attempting immune therapy. If our hypothesis is correct, any external immune intervention is unlikely to result in tumor destruction, despite the induction of immunocompetent cells. Tumor specific cytotoxic cells will migrate into the lymph nodes and instead of being activated they will be anergized and may undergo apoptosis due to the interaction with dendritic cells modulated by the melanoma. To obtain a successful anti-melanoma vaccination, the immune suppression in draining lymph nodes must be overcome.

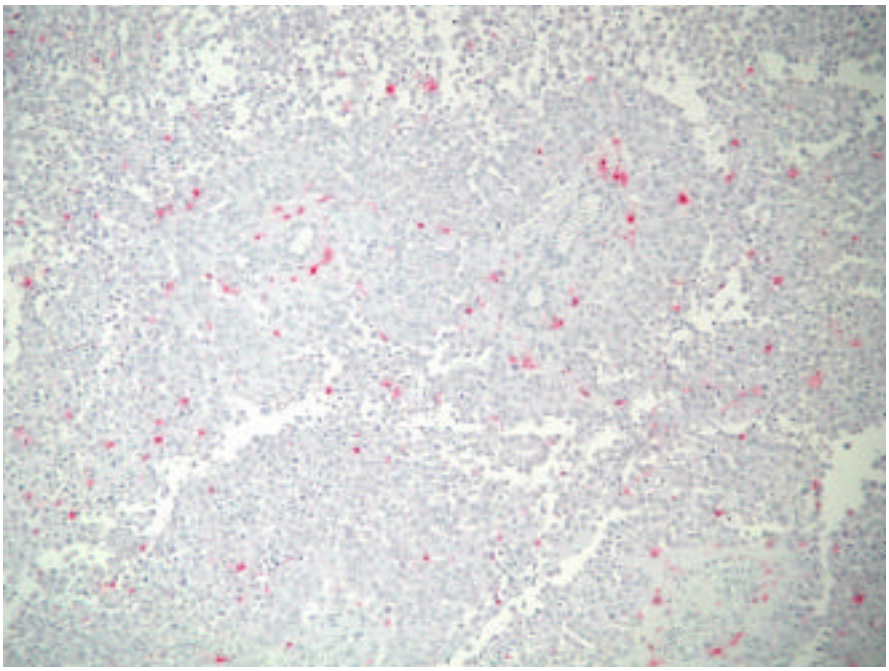

Figure 2. Presence of immature DC within the lymphoid tissue (Immunohistochemistry): FXIIIa staining of cells without protrusions.

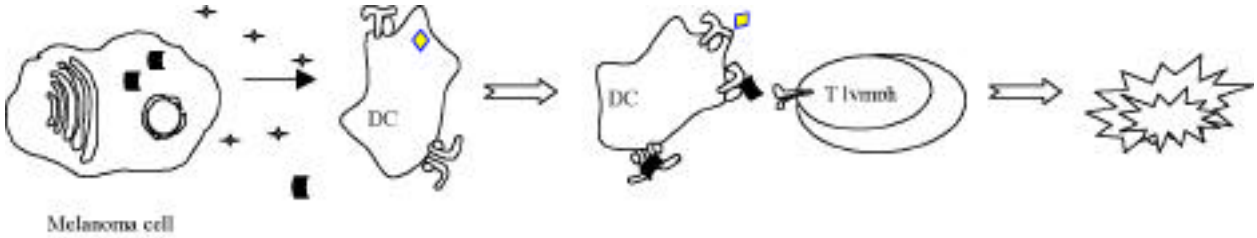

Figure 3. Suppression of immune system managed by melanoma-derived altered maturation of dendritic cells. Antigen presentation by immature dendritic cells is one of the immune control mechanisms. Dendritic cells kept in the immature state by cytokines released by melanoma are capable to modulate immune response and anergise antigen specific T lymphocytes. This mechanism can be potentially used by melanoma to avoid immune recognition and to suppress the immune reaction.

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