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Immunotherapy with Dendritic Cells and Newcastle Disease Virus in Glioblastoma Multiforme

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1. Introduction

Malignant gliomas are the most prevalent type of primary brain tumors in adults. The most aggressive form, glioblastoma multiforme (GBM, WHO grade IV glioma), is characterized as incurable with average survivals less than 18 months (Buckner, 2003; Curran et al., 1993; De Angelis, 2001). Standard therapy consists of surgical resection to the extent that is safely feasible, followed by radiotherapy and adjuvant chemotherapy. A meta-analysis based on 12 randomized trials has supported the use of adjuvant chemotherapy treatment as compared to radiotherapy alone. A five percent increase in survival at two years, from 15 percent to 20 percent was reported (Stewart, 2002). In this context, an oral alkylating agent, temozolomide, has demonstrated antitumor activity as a single agent in the treatment of glioblastoma patients (Newlands et al., 1997; Stupp et al., 2001, 2005; Yung et al., 2000). At a median follow-up of 28 months, temozolomide treatment prolonged the median survival from 12.1 months with radiotherapy alone to 14.6 months when administered with concomitant radiotherapy (Stupp et al., 2005). Adjuvant administration of carmustine, a nitrosourea drug, has not resulted in beneficial prolongation of survival, although some long-term survivors in the chemotherapy groups have been documented in some of the non-randomized phase III trials (Chang et al., 1983; Green et al., 1983). Despite these advances in combined radio- chemotherapy treatment strategies along with surgical resection, the prognosis of patients with malignant glioma is poor and ≥ 80% of the patients survive less than 2 years. Salvage treatments upon recurrence are palliative at best and rarely provide a significant survival benefit. The minor clinical benefit of radio-chemotherapy is supposed to be mainly based on tumour stem cells, which in analogy to normal stem cells are resistant to radio- and chemotherapy (Liu et al., 2006; Singh et al., 2003). Therefore, the development of new treatment modalities is extremely important.

It is known that defects in the immune system play a crucial role in the development of cancer as well as in the host defense against cancer. Thus, specific immune therapeutic therapy approaches have been tested for a long time. Tumor-associated antigens recognized by tumor-reactive cytotoxic T lymphocytes (CTL) have been identified during the last two decades (Janeway et al., 2004). Also in patients with GBM such tumor antigen specific CD8+ T cells have been identified in the circulating blood (Tang et al., 2005). A specific cancer

immunotherapy can be based on the use of antibodies directed against specific antigens, which are expressed in tumors. However, the more recently described immunological therapy strategy for GBM with an antibody (Bevacizumab), which blocks angiogenesis by binding to the vascular endothelial growth factor also shows insufficient results (Lai et al., 2010). The more promising strategy seems to be the use of tumor specific vaccines, which induce a specific cellular immune reaction by induction of tumor specific CTL.

2. Dendritic cells in cancer therapy

A specific cellular immune response against tumors is based on the uptake and presentation of tumor antigens on MHC molecules by professional antigen presenting cells leading to the induction of antigen specific CTL, which can then attack the tumor. Dendritic cells (DC) are the most potent antigen presenting cells and have been shown to be able to induce an efficient and specific immune response (Banchereau & Steinman, 1998; Steinman, 1991; Whiteside & Odoux, 2004). In comparison to other antigen presenting cells, like B cells, which induce and activate only memory T cells, dendritic cells can stimulate naive T cells (Banchereau & Steinman, 1998; Steinman, 1991; Whiteside & Odoux, 2004). Thus, today one of the most promising immunological therapy approaches in treatment of various types of human cancer is a specific immunotherapy with autologous dendritic cells (Engleman, 2003; Gilboa, 2007; Nestle, 2000).

According to their phenotype and functional properties dendritic cells can be classified as immature and mature type (Banchereau & Steinman, 1998). Immature dendritic cells are able to capture and internalize particles and soluble antigens from the environment (Banchereau & Steinman, 1998). Upon being internalized, antigens are processed and split into peptides, which are then presented on the cell surface in association with MHC molecules (Banchereau & Steinman, 1998). In response to micro-environmental signals immature DC switch to a mature phenotype with T cell stimulatory capacity (Vieira et al., 2000; Whiteside & Odoux, 2004). Mature dendritic cells express higher levels of major histocompatibility complex class I and class II molecules as well as co-stimulatory molecules like CD80 and CD86 (Dietz et al., 2000; Whiteside & Odoux, 2004). During the maturation process DC lose their ability to take up and process antigens (Dietz et al., 2000; Whiteside & Odoux, 2004). Mature dendritic cells are characterised by a distinct cytokine expression profile and their increased ability to produce Interleukin 12 (IL-12) in response to environmental signals, a cytokine known to be necessary for induction of TH1-T cell response leading to production and proliferation of CTL (Whiteside & Odoux, 2004, Rouard et al., 2002).

Until now several methods have been published to generate dendritic cells in vitro from monocytes isolated from the peripheral blood of volunteers or cancer patients, called monocytes-derived dendritic cells (MoDC) in the presence of GM-CSF and IL-4 (Morse et al., 2002; Peters et al., 1996; Romani et al., 1996, Sallusto & Lanzavecchia 1994, Zhou & Tedder, 1996). Immature MoDC can be loaded either with tumor-cell-lysate, known tumor specific peptides or with specific RNA or DNA. Upon exposure to various maturation stimuli the antigen loaded immature MoDC can be matured and are able to induce a clinical antitumor response in patients with various types of tumors through induction of tumor specific CTL, which can then attack the tumor (Engleman, 2003; Gilboa, 2007; Morse et al., 2002; Nestle, 2000; Romani et al., 1996, Zhou & Tedder, 1996).

2.1 Dendritic cell based immunotherapy in patients with GBM

Although the tumor is hidden behind the blood brain barrier immune cells can be recruited into the central nervous system (Tambuyzer et al., 2009; Vauleon et al., 2010; Wilson, et al., 2010). Macrophages and dendritic cells are found in perivacscular zones, the choid plexus and the meninges (Vauleon et al., 2010). In gliomas infiltration of CD8+ as well as CD4+ T cells have been reported (Barcia et al., 2009; Mittelbronn et al, 2007). Thus, several types of immune reactions take place in primary brain tumors. In this context Ueda and coworkers have found T cells directed against two glioma antigens (IL13Ra2 and EphA2) within the peripheral blood mononuclear cells from a long surviving patient with an anaplastic astrocytoma (Ueda et al., 2007), showing that an efficient immune reaction in brain tumors is generally possible. Hoverer, the tumor itself shows variety of so-called "tumor escape mechanisms" which are able to dampen tumor antigen-specific immune responses. These include an immunosuppressive environment by secretion of immunosuppressive cytokines and factors such as transforming growth factor beta (TGFβ) (Schneider et al., 2006) and vascular endothelial growth factor (Naumov et al., 2006), which increase the number of tumor infiltrating regulatory T cells and lower the number of cytotoxic killer cells and others. Moreover, the high expression of HLA-4 on gliomas, upon binding to NKG2A7B has a negative effect on T and NK cell activity (Wischhusen et al., 2005). Thus, in GBM the immune reactions are mostly not effective as shown by the unfavorable course of the disease and the high level of recurrences. A specific immunotherapy based on ex vivo generated antigen loaded monocyte-derived dendritic cells may overcome this inhibition leading to a re-induction of an efficient immune reaction against GBM. Such immune responses require antigen recognition. Several groups demonstrated an over-expression of several antigens including a mutated form of the Epidermal Growth Factor Receptor (EGFRvIII), GP 100 and MAGE 1 (Okada et al., 2009; Saikali et al., 2007), which could be used as targets for induction of an antigen specific immune response by priming the MoDC with peptides from these antigens. However, using the whole tumor cell lysate as well as tumor RNA as antigen source may probably reduce the risk of an immune evasion due to clonal expansion of antigen-loss tumor cell variants (Jäger et al, 1996, Vauleon et al., 2010).

Several reports have demonstrated that specific cellular immunotherapy using dendritic cells is capable of inducing a clinical antitumor response within the immunologically privileged brain, confirming that the central nervous system (CNS) is accessible to this kind of immunotherapy. Table I gives an overview on clinical studies with dendritic cell vaccines performed for glioma patients. The published data are based on phase I and II trials as well as on case reports including in total over 200 patients with GBM and over 100 patients with anaplastic astrocytomas or other malignant brain tumors (table 1). Two studies and one case report include children or young adults (Ardon et al., 2010a; Caruso et al., 2004; De Vleeschouwer et al., 2004). The dendritic cells used in these studies were ex-vivo generated from patients blood monocytes using the described standard procedure and primed with peptides, autologous tumor cell lysates or tumor-derived RNA. In two trials dendritic cells were fused with autologous tumor cells (Kikuchi et al., 2001, 2004). Dendritic cells were further matured by exposure to various maturation stimuli (Ardon et al., 2010a, 2010b; De Vleeschouwer et al.; 2008; Kikuchi et al., 2004; Okada et al., 2007; Rutkowski et al., 2004; Yamanaka et al., 2005) or were used without further maturation signals (Caruso et al., 2004; De Vleeschouwer et al., 2004; Kikuchi et al., 2001; Liau et al., 2000, 2005; Yamanaka et al., 2003; Yu et al., 2001, 2004).

Antigen source	type of trial	responses	author
tumor cell lysate + TLR-7 ligand (children)	Phase I	median OS: 12.2 months	Ardon et al., 2010a
tumor cell lysate	Phase I/II	median OS: 24 months	Ardon et al., 2010b
tumor RNA (children, young adults)	Phase I (relapsed brain tumors)	1 PR, 4 SD	Caruso et al., 2004
tumor cell lysate (4-year old child)	case report (recurrent GBM)	CR over 2 years	De Vleeschouver et al. 2004
tumorc ell lysate	Phase I/II (recurrent GBM)	median OS: 9.6 months	De Vleeschouver et al. 2008
tumor cell lysate	case report (recurrent GBM)	median OS: 11 months	Khan & Yaquin, 2006
fusion with tumor cells	Phase I	1 MR, 4 SD, 2 SD	Kikuchi et al., 2001
fusion with tumor cells	Phase I (recurrent GBM)	1 SD in GBM	Kikuchi et al., 2004
peptides	case report (recurrent GBM)	no survival benefit	Liau et al., 2000
tumor cell lysate	Phase I de novo vs. recurrent GBM	median OS: 23.4 months vs. 18.3 months	Liau et al., 2005
tumor cell lysate or irradiated tumor cells	Phase I	2 PR irradiated tumor cells	Okada et al., 2007
tumor cell lysate	Phase I (recurrent GBM)	median OS : 10,5 months	Rutkowski et al., 2004
EGFRvIII antigen	Phase I	median OS: 22.8 months	Sampson et al., 2009
inactivated tumor cells	Phase I	12-/18-months survival: 46%/23%	Walker et al., 2008
tumor cell lysate	Phase I/II vaccine + chemo vs. vaccine alone	2-year-survival 42% vs. 8%	Wheeler et al., 2004
tumor cell lysate	Phase II (de novo vs. recurrent GBM)	median OS: 642 vs. 430 days	Wheeler et al., 2008
tumor cell lysate	Phase I/II	OS: > 200 weeks	Yamanaka et al., 2003
tumor cell lysate + TLR-4 ligand	Phase I/II (recurrent GBM)	median OS: 480 (+ TLR-4 ligand) vs. 400 days	Yamanaka et al., 2005
antigens	Phase I	median OS: 455 days	Yu et al., 2001

Abbreviations: OS: Overall Survival; PR: Partial Remission; CR: Complete Remission; SD: Stable Disease

Table 1. Clinical Studies for GBM treated with autologous DC

For all used methods of MoDC generation a clinical response was described. With the exception of one trial (Yamanaka et al., 2003), where some patients receive an intracerebral injection of dendritic cells, the vaccines were administered intradermally or subcutaneously. The reported side effects were in general low besides one case with grade IV neurotoxicity (Yamanaka et al., 2005). Such reactions are probably based on an excessive inflammatory process within the brain leading to the development of severe edema. Based on our experiences, in those cases, an immune suppressive treatment with corticosteroids has to be accompanied to modulate the highly inflammatory process, which can of course counteract the efficacy of the immune response. The reported major side effects are short term flu like symptoms like fever, headaches and chill. Thus, this treatment is well tolerable without having a negative impact on the quality of life.

Clinical responses based on radiological findings, which were performed for a part of the treated patients (n=108 patients with GBM) consisted of complete remissions (3.7 %), partial remissions (4.6 %), stable diseases (12.9 %) and mixed responses (5.5 %). In some trials a prolongation of the overall survival compared with historical data or non randomized control groups are described (Ardon et al., 2010b; De Vleeschouwer et al., 2008; Liau et al., 2005; Sampson et al., 2009; Rutkowski et al., 2004; Wheeler et al., 2008; Yamanaka et al., 2003, 2005; Yu et al., 2001, 2004). In a pilot trial published by Ardon and coworkers 8 patients with de novo diagnosed GBM received an immunotherapy with tumor cell lysate pulsed dendritic cells after surgery and radio-chemotherapy. The median overall survival was 24 months ranging from 13 to 44 months (Ardon et al., 2010b). Comparable results with a median survival of 22.8 months are reported from Sampson and coworkers using dendritic cells pulsed with the mutated EGFRvIII antigen coupled to KLH (Sampson et al., 2009). Even in patients with relapsed GBM a dendritic cell therapy is reported to be successful. De Vleeschouwer and coworkers reported a case of a 4 year old child, who received a dendritic cell therapy after surgery of recurrence. 2 years after onset of the immune therapy the child still remains in a complete remission (De Vleeschouwer et al., 2004). In a phase I/II trial performed by the same group (De Vleeschouwer et al., 2008) 56 patients were treated with dendritic cells pulsed with tumor cell lysate after surgery of recurrences. The median overall survival was 9.6 months with a 2-year survival rate of 14.8 % after onset of the therapy.

Besides these very promising results the objective clinical responses are low and a lot of patients fail to respond to the therapy. One main reason for low clinical efficacy of MoDC-based anticancer therapies could be due to low IL-12 and high IL-10 production by the MoDC favoring a TH2 rather than a TH1 immune response. In fact, a lot of clinically approved maturation protocols mature MoDC insufficiently in a TH1-polarizing direction (Hildenbrandt et al., 2007, 2008; Mailliard et al., 2004; Zobywalski et al., 2007). Hildenbrandt and coworkers found that the used cytokine cocktail with TNF-a, IL-1ß, IL-6 and PGE2 according to Jonuleit et al. (Jonuleit et al., 1997) induce the phenotypic characteristics of fully matured MoDC expressing high levels of CD83, MHC, costimulatory molecules and CCR7, but secrete high levels of IL-10 and only low levels of IL-12p70 suggesting a preference for a TH2-polarisation (Hildenbrandt et al., 2008). For this reason, improved maturation protocols are required. In this context the activation of Toll-like receptors (TLR) by adding TLR-ligands to the MoDC cultures becomes an interesting alternative to mature monocytes-derived dendritic cells.

2.1.1 Toll-like receptor agonists in maturation protocols for dendritic cells

The expression of certain Toll-like receptors (TLR), a family yet 11 identified transmembrane receptors, expressed on dendritic cells plays a crucial role in the recognition of microbial or

viral infections (Medzhitov, 2001). TLR mediated activation of dendritic cells is supposed to be vital for the generation of a TH1 effector response (Schnare et al., 2001). Thus, TLR ligands, which are molecular signatures of microbial or viral infections, may signal the presence of an infection (danger signal), which leads to maturation of DC with a TH1 polarization profile (Akira et al., 2001; Medzhitov, 2001). Interestingly, the TLR 3, a cell surface receptor for double-stranded RNA as well as for the synthetic analogue poly I:C, is predominantly expressed on dendritic cells (Alexopoulou et al., 2001; Muzio et al., 2000). Bone marrow derived immature dendritic cells activated by poly I:C produce large amounts of IL-12 and up-regulate MHC as well as co-stimulatory molecules on their surface (Alexopoulou et al., 2001).

Several TLR are also expressed on monocytes and immature monocytes-derived dendritic cells (Visintin et al., 2001). Moreover, it has been shown that immature MoDC have an increase in TLR 3 expression as compared to monocytes, indicating that in MoDC this TLR may play an important role (Visintin et al., 2001). At the ASCO meeting 2007 our group have show that immature MoDC from patients with GBM when activated by adding the TLR 3 ligand poly I:C switch to a mature phenotype with up-regulation of the maturation marker CD83 and of co-stimulatory molecules. After short time exposure to IFN-γ these matured MoDC were able to produce IL-12p70 at a higher level than IL-10 which clearly favours a TH1 response (Nesselhut et al., 2007). Moreover the IL-12/IL-10 ratio seems to be correlated to clinical response. The use of poly I:C is currently tested in a phase I/II trial and recent published data show that poly I:C boosted DC are safe and have clinical anti tumor activity (Okada et al., 2010). Furthermore, the authors found a correlation between the IL-12 production level and the time to progression (Okada et al, 2010). Comparable data was seen by Yamanaka and coworkers using DC matured with a ligand to TLR 4. Patients who received TLR matured DC had longer survival than those vaccinated with immature DC (Yamanaka et al., 2005). Van Gool et al. reported an improvement of the progression free survival of glioma patients, when the skin at the injection site of the mature DC was pretreated with the TLR-7 agonist Imiquimod (van Gool et al., 2009). Concordant with findings from our group Hildebrandt et al. found that the addition of INF-γ to TLR-agonists can improve the maturation status as well as the TH1 polarization of MoDC (Hildenbrandt et al., 2008). However, most TLR-agonists are not clinically approved and therefore are not allowed to be used in clinical trials, yet. In this context it is interesting, that Hildenbrandt et al. could show that IFN-γ when added to the clinical-grade cytokine cocktail (Jonuleit et al., 1997) induces the production of lower levels of IL-10 and higher levels of IL-12p70, demonstrating the TH1-prolarizing capacity of this cytokine (Hildenbrandt et al., 2008).

2.1.2 Impact of cancer induced immune suppression on the efficacy of denditic cell therapy

During the last years several investigations show an increasing evidence of a strong association between chronic infection, inflammation and cancer development as well as tumor progression (Balkwill & Mantovani, 2001; Coussens & Werb, 2002). Inflammation is a physiological process occurring after tissue damage, infections caused by microbial or viral pathogen, chemical irritation or wounding (Philipp et al., 2004.) In fact, many of the same inflammatory mediators that are secreted by wounds are found in the tumor microenvironment. Immune cells infiltrate into the developing tumor site and establish an inflammatory tumor microenvironment (Balkwill & Mantovani, 2001; Whiteside, 2008). As wound healing is self limited cancer can be described as "wounds that do not heal" (Dvorak,

1986). Meanwhile it is well established that the tumor per se can change the immunological balance into an inflammatory microenvironment by release of certain cytokines and chemokines leading not only to the promotion of tumor growth but also to the inhibition of an efficient immune response. This may be one of the reasons for an unsuccessful immunotherapy for GBM patients based on dendritic cells (Couldwell et al., 1991, Nitta et al., 1994, Hishii et al, 1995, Weller & Fontana, 1995). An example is the intratumoral expression of TGF-ß, which can suppress an adaptive antigen specific immune response. Moreover, TGF- ß expression was shown to be a predictive factor for the clinical outcome in vaccinated patients (Liau et al., 2005).

Taken together, an efficient induction of a clinical antitumor response requires both a polarization of MoDC in a TH1 direction as well as a change of the immune suppressive tumor associated microenvironment. Beside several cytokines which promote an immune suppressive microenvironment, regulatory T cells (T-reg), which are required for maintaining immunological self tolerance (Sakaguchi, 2005), play an important role in the regulation of an immune response and thus may limit an antigen specific immune response in cancer (Borsellino et al. 2007; Deaglio et al. 2007; Mandapathil et a., 2009; Nizar et al, 2009).

Several subpopulations of T-reg have been described (Sakaguchi, 2005; Seddiki et al., 2006). Most commonly used is the CD4+, CD25 +high, CD127-/dim phenotype with intracellular expression of the transcription factor FoxP3 (Seddiki et al., 2006). More recently, a sub-population of CD39+ T-reg was described (Borsellino et al. 2007; Deaglio et al., 2007). CD39 belongs to the family of ecto-nucleoside triphosphate-diphosphatehydrolase-1 and hydrolyses extracellular ATP. This leads to a pericellular accumulation of the nucleoside adenosine, which is known to play an important role in inhibiting effector functions of activated T cells (Borsellino et al. 2007, Huang et al., 1997). The authors propose that the immune suppressive activity of T-reg is partly due to this enzymatic activity of CD39 and thus may play a crucial role in inhibition of antigen specific T cell response. Woo et al. were the first who reported an increased percentage of T-reg cells in tumor infiltrating lymphocytes in non-small-cell-lung cancer and ovarian cancer (Woo et al., 2001). Meanwhile accumulating T-reg cells are detected in the tumor microenvironment, ascites as well as in the peripheral blood of cancer patients and have been associated with poor prognosis (Beyer & Schultze 2010; Nizar et al. 2009, Wolf et al., 2003).

Our group have shown that subpopulations of T-reg characterized by a CD4+, CD25 +high as well as CD127-/dim or CD39+ expression profile can be detected in the peripheral blood of healthy donors, tumor free patients as well as patient with tumor load (Neßelhut et al., 2009). The percentage of T-reg was significantly higher in cancer patients, including patients with GBM, as compared to healthy donors. Moreover, patients with advanced tumors show higher levels of T-reg cells as compared to patients with no evidence of disease. Several other groups reported an increase of T-reg cells in GBM patients in peripheral blood as well as within the tumor microenvironment (Fecci et al., 2006a; Grauer et al., 2007; Hussain et al., 2006, El Andaloussi et al., 2006). Measurement of T-reg cells along with an immune therapy may be useful to discriminate between non- responding and responding patients. In fact, first analysis of our group show that rising T-reg numbers are partly associated with lack of clinical response to a dendritic cell therapy in patients with GBM. Thus, the elimination of excessive T-reg cell numbers seems to be a promising approach to improve the efficacy of a dendritic cell based immune therapy.

Several chemotherapeutical agents have immune stimulatory and T-reg modulating effects when used at lower dosages with reduced toxic effects (Nizar et al., 2009). Beside this option there are some reagents which target T-reg directly through recognition of CD25 (Morse et al., 2008, Fecci 2006 b) or through CTLA-4 blockade (Ribas et al., 2009). Some of these approaches are tested in association with DC therapy in cancer (Chiringelli et al, 2004, Ribas et al. 2009, Morse et al., 2008) including GBM (Fecci ate al. 2006 b, Maes et al., 2009). Interestingly, in a rat glioma model low dose temozolamide could also decrease the number of T-reg cells. (Banissi et al, 2009). In this context it is noteworthy that Wheeler et al. show that GBM patients, who responded to DC therapy, also exhibited a better response to a following chemotherapy (Wheeler et al., 2004). In the study published by Ardon et al. immunotherapy based on dendritic cells started after radio-chemotherapy (Ardon et al., 2010b). One can suppose that the chemotherapy with temozolamide has lead to a decrease of T-reg cells and thus enhanced the immunological response causing the high 24 months median survival for the treated patients. The combination of dendritic cell therapy and adjuvant chemotherapy was also tested in malignant astrocytomas (Walker et al., 2008). The 12- and 18-months survival rates were 46% and 23%, respectively. Depletion or inhibition of T-reg may thus improve the efficacy of dendritic cell based therapy.

2.1.3 Newcastle disease virus in treatment of GBM; a tool for improving DC therapy

Besides dendritic cell therapy another promising approach for the treatment of malignant brain tumors is the treatment with replication-selective viruses, also called oncolytic viruses. This is based on the fact that most tumor cells are more or less unable of an effective virus defense. This approach is also known as virotherapy. The application of viruses for cancer treatment is based on reports since the beginning of the 20th century on temporary improvement of cancer following natural viral infections or vaccinations against viral diseases. (DePace 1912). Meanwhile several replication competent viruses (mainly herpes and adenoviruses) were tested in vitro, in animal models as well as in phase I/II clinical trials for treatment of malignant brain tumors (Shah et al., 2003; Rainov & Ren, 2003; Wollmann et al., 2005). However, the viruses have to be genetically modified in a way that makes sure that they selectively infect and replicate in tumor cells. Within the viruses tested for human anticancer treatment the Newcastle Disease Virus (NDV), an enveloped poultry virus with single strained RNA as genetic material, seems to be one of the most promising candidates. NDV is not a pathogen for humans, and is absolutely harmless causing only mild flu-like symptoms or conjunctivitis in the worst of cases (Lorence et al., 2001; Reichard et al., 1992). NDV shows a natural distinct tropism for cancer cells. Cancer cells infected with NDV can be killed directly by the virus within a short time after infection, whereas normal infected cells are not lysed by NDV.

As described earlier tumor cell lysate may be the better antigen source for priming of dendritc cells because it contains the whole antigen repertoire of the tumor. However, it has to be taken in mind that most of the antigens expressed in tumors are poor inducers of immune response and are often recognized by the immune system as self antigens (Vergati et al., 2010). Opposed to this adjuvant active specific immunization based on tumor cells modified with a low pathogenic strain of the NDV has been reported to achieve sustained immune responses in patients with advanced colonic cancer and liver metastasis (Lehner et al., 1990 Schulze et al., 2009). NDV can have lytic activity on tumor cells directly as well as

immune stimulating properties that affect both innate and adaptive immune responses. Infection of tumor cells with live NDV results in a potent up-regulation of cell adhesion molecules on the tumor cells surface (Lehner et al. 190; Wasburn et al., 2002). Moreover, NDV infection of tumor cells leads to an improved tumor cell / T cell interaction and an increased T cell co-stimulatory activity (Ertel et al., 1993; Termeer et al., 2000). Expression of viral proteins on the tumor cell surface and presence of virus derived pathogen-associated molecular patterns (e.g. double-stranded RNA) result in breaking of host tolerance towards the tumor in vitro (Bai et al., 2002). The T cell stimulatory action of dendritic cells pulsed with lysates of NDV infected tumor cells as well as the antitumor cytotoxicity of macrophages and monocytes is increased (Schirrmacher et al., 2000; Washburn et al., 2003; Zeng et al., 2002). Finally, NDV induces an increased production of various cytokines, e.g. Interferon-α as well as chemokines, influencing the migration, the activation status and cytotoxic activity of various immune cells (Lokuta et al., 1996; Schirrmacher 2005, Schlag et al., 1992). Clinical phase I and II studies in various tumor entities have proven the safety of active specific immunization with NDV-modified tumor cells. A detailed description of the mechanisms of action of NDV modified tumor cell vaccines and results from other studies in cancer patients were reviewed by Schirrmacher (Schirrmacher, 2005).

In malignant brain tumors case reports as well as clinical phase I/II studies have shown that treatment with intravenously applied NDV as well as with vaccines utilizing NDV modified tumor cells can induce a clinical anti-tumor response in malignant brain tumors with objective clinical responses as well as with a trend towards improvement of overall survival (Csatary & Bakacs, 1999, Csatary et al., 2004; Freeman et al., 2005; Schneider et al., 2001; Wagner et al., 2006). Recent results from our group show that a therapy with dendritic cells in combination with the NDV virotherapy may improve the clinical anti-tumor response in patients with GBM (Neßelhut et al., 2007, 2011). Patients were pre-treated with intravenously administration of NDV. Dendritic cells were primed with NDV modified tumor cells or with NDV alone in patient with tumor recurrence. When tested in vitro, NDV primed MoDC of such treated patients induce the activation of autologous CD8+ T cells with release of IFN-γ. This leads to the hypothesis that, if viral antigens are expressed on the tumor cell surface, a NDV specific dendritic cell based therapy may lead to the induction of NDV specific CD8+ T cells and thus to the induction of a specific immune response against the virus infected cancer cell (Neßelhut et al., 2011).

3. Conclusion

Taken together a dendritic cell therapy as well as the NDV virotherapy have shown clinical efficacy and have been proven to be safe with only minimal side-effects. Especially patients with primary resistance against temozolamide caused by the MGMT status could benefit from such alternative therapy strategies. Furthermore, new insights in the nature and biology of glioma tumor stem cells may improve an immunological concept by using tumor stem cell specific antigens as targets in dendritic cell therapy as it was recently shown (Pellegatta et al., 2006, Stupp & Hegi, , Xu et al., 2009). An immunotherapy with dendritic cells can either be used in adjuvant, curative settings to avoid or delay tumor recurrence or in palliative settings to keep the disease stable offering the patients a chance for prolonged overall survival. Based on current results it has to be supposed that patients with minor tumor burden or no evidence of disease are the best candidates for an immunotherapy.

The main reasons for a deficient anti-tumor immune response are most likely the immune suppressive tumor microenvironment and the up-regulation of immune suppressive cells. Thus, a combination with therapies, which can modulate the tumor microenvironment as well as lower the percentage of the immune suppressive T-reg cells should be considered as treatment prior to or in combination with dendritic cell based immune therapy. Additionally, glioma cells are considered to be poor antigen-presenting cells. In order to promote antigen-specific recognition and avidity of potential anti-tumor effector cells, the biology of antigens expressed by malignant glioma cells must be taken into consideration while designing protocols for programming autologous dendritic cells and defining the appropriate patient. For example, Prins and coworkers could recently shown that the gene expression profile of the individual glioma can identify a subgroup of patients, that may be more responsive to dendritic cell based immunotherapy (Prins et al., 2011). In this context the combination with NDV seems to be a promising tool to modify tumor cells in vitro as well as in vivo to a more immunogenic phenotype resulting in better immune recognition as well as in better immune response.

The encouraging results of phase I/II trials have to be confirmed in phase III studies with special emphasis on the maturation protocols for improvement of TH1-polarisation of dendritic cells, which so far are still a matter of controversial discussion. Moreover, the optimal antigen source, cell number of injected dendritic cells, frequency of vaccinations as well as the right time point for vaccination are still not fully defined. Future studies should also involve an immune monitoring of vaccinated patients. Immune monitoring methods can include quantification of CD8+ and CD4+ T cells generated in response to the antigen or measurement of the functional status of T cells analyzed by cytokine production. However, it has to be taken into account that in vitro measurement of T cell responses do not always correlate with clinical outcome (Carpentier & Meng, 2006). Thus, the best immunological monitoring strategy is still intensively debated.

Finally, clinical trials for the evaluation of immune therapeutical approaches have to be designed in a different way as for chemotherapeutical drugs. In this context the overall survival rather than the reduction of tumor burden is probably the best primary endpoint. Based on such a study design Provenge™, an immunological treatment based on antigen presenting cells, received the FDA approval for treatment of hormone refractory prostate cancer in spring 2010.

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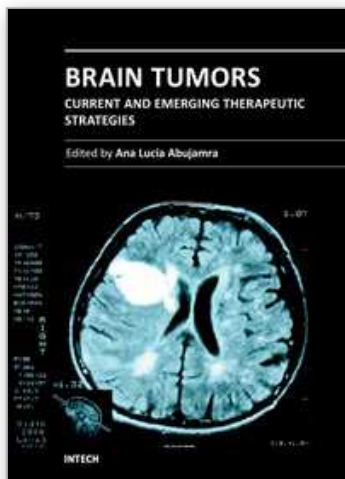
Brain Tumors - Current and Emerging Therapeutic Strategies

Edited by Dr. Ana Lucia Abujamra

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