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Vol. 7(22), pp. 1426-1437, 15 June, 2013
DOI 10.5897/AJPPX12.004
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Review

Differentiation of stem and progenitor cells from bone marrow in activated dendritic cells and lymphocytes with anti-malignant properties

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Accepted 24 April, 2013

Dendritic cells (DCs) have been characterized as powerful antigen-presenting cells (APCs), which possess the abilities for immune modulation and are used in composition of anti-malignant vaccines and gene-engineered products. By appropriate cultivation, modifications of DCs have shown abilities for an enhanced expression of specific effector molecules. Studies on their biology are focused on their role as main immune response modulators. These properties characterize them as promising candidates for construction of novel safe vaccines and gene-engineered products. In this direction, attention is directed to development of methods and techniques for transduction of *in vitro*- and/or *ex vivo*-cultivated DCs with previously designed recombinant viral vectors with inserted genes, coding respective malignant antigens. Studies on the biology of lymphocytes are mainly focused on their role in cellular and humoral immune response. Their cultivation and differentiation in the presence of appropriate antigens, on one hand, and by appropriate modifications, on the other hand, have shown the abilities for an enhanced expression of specific effective molecules. These properties have characterized them as promising candidates for construction of novel safe vaccines and gene-engineering products.

Key words: Stem/progenitor cells, dendritic cells, lymphocytes, cell differentiation, recombinant viral vectors/gene constructs, malignant disorders, immunity.

INTRODUCTION

Dendritic cells (DCs) have been found to play a pivotal role in initiating the immune response, including powerful antigen-presenting cells (APCs) (Arthur et al., 1997; Avigan, 2004; Bonini et al., 2001; Bubenik, 2001; Caux et al., 1992; Clark et al., 1992; Gong et al., 1997; Hassan et al., 2000; Inaba et al., 1992; Kaplan et al., 1999; Kim et al., 1994; Reid et al., 1992; Ribas et al., 1997; Siena et al., 1995; Yongqing et al., 2002). In the light of their unique properties, these cells have been proposed as powerful immunomodulation agents, including in the composition of novel vaccines and gene-engineering products for treatment of malignant disorders.

Primitive hematopoietic stem cells (HSCs) have been found to be hierarchically ordered on the basis of quiescence, the most primitive of these, characterized by their Rh/Ho (dull) phenotype and their capacity for long-term hematopoietic reconstitution, are not dormant, but they have been established to cycle slowly in normal steady-state bone marrow (Ballas et al., 2002; Bradford et al., 1997; Bradley et al., 2002; Cheng et al, 1998; Gunechea et al., 2000; Koido et al., 2007; Lai and Kondo, 2007; Le Blannk and Ringden, 2005). Unrelated human MSCs have not been found to elicit T-cell activation *in vitro* and to suppress T-cell activation by tuberculin and unrelated

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allogeneic lymphocytes in a dose-dependent manner. It has been reported that the Lin-IL-7R+Thy-1-Sca-1loc-Kit(lo) population from adult mouse bone marrow possess a rapid lymphoid-restricted (T-, B-lymphocytes, as well as natural killer (NK) cells) reconstitution capacity *in vivo*, but completely lacked myeloid differentiation potential both *in vivo* and/or *in vitro* (Bovia et al., 2003; Kobari et al., 2001). A single Lin-IL-7R+Thy-1-Sca-1loc-Kit(lo) cell has been established to be able to generate at least both T- and B-cells (Amirayan et al., 1995; Bovia et al., 2003; Bregenholt et al., 1996; Dallas et al., 2007; Gray-Parkin et al., 2002; Green et al., 1992; Ikuta et al., 1990; Jackson and Bell, 1990; Kondo et al., 1997). These data have provided direct evidence for the existence of common lymphoid progenitors in sites of early hematopoiesis. Interferons (IFNs) and protein-kinases (PKRs) have demonstrated abilities to sensitize cells to apoptosis predominantly through the FADD/caspase-8 pathway.

Complex mechanisms, which include molecular, genetic and cellular components, such as *Wnt*-, BMP- and *Notch/Delta*-signaling pathways, have been found to underlie differentiation and functions of stem cells (Caux et al., 1992; Ribas et al., 1997; Terskikh et al., 2006; Vogelstein and Kinzler, 2004). By use of real time poly-merase chain reaction (RT-PCR), an ability for initiation of erythroid (β-globin) and/or myeloid (myeloperoxidase) gene expression programs by the same cell prior to exclusive commitment to the erythroid, myeloid lineages for it has been shown (Bonini et al., 2001; Caux et al., 1992; Curti et al., 2001; Davis et al., 1996; Inaba et al., 1992; Reid et al., 1992; Siena et al., 1995).

These data have supported a model of hematopoietic lineage specification, in which unilineage commitment has been prefaced by a “promiscuous” phase of multilineage locus activation (Bonini et al., 2001; Curti et al., 2001; Engelmayer et al., 2001; Reid et al., 1992; Siena et al., 1995). Protein BCL-6 has also been detec-table in inter- and intra-follicular CD4+ T-cells, but not in other follicular components, including B-cells, plasma cells, monocytes/macrophages and DCs. Genes poten-tially important in myeloid differentiation, such as coding granulocyte colony-stimulating factor (G-CSF) and the enzyme myeloperoxidase, have been found to be located close to the breakpoint in the *t(15;17)*, but have not been conclusively shown to be rearranged in this chromosomal translocation (Bonini et al., 2001; Engelmayer et al., 2001; Reid et al., 1992; Siena et al., 1995).

It has also been proposed that the commitment of common myeloid progenitors to either the megakaryocyte /erythrocyte or the granulocyte/ macrophage lineages are mutually exclusive events (Bonini et al., 2001; Caux et al., 1992; Curti et al., 2001; Hassan et al., 2000; Reid et al., 1992; Siena et al., 1995). It has been concluded that active cell cycling of bone marrow cells, induced by cytokine stimulation, is probably very often associated with an engraftment defect in the normal host, and derangement of these pathways within stem cells, as well as the apparent lineage and differentiation status, have been found to play an important role in the development of malignancies.

In agreement with representing a lymphoid primed progenitor, Lin-Sca-1+c-kit+CD34+Flt3+ cells have been established to display up-regulated IL-7 receptor gene expression (Amirayan et al., 1995; Bregenholt et al., 1996; Dallas et al., 2007; Gray-Parkin et al., 2002; Green et al., 1992; Ikuta et al., 1990; Jackson and Bell, 1990; Kondo et al., 1997). Based on these observations, a revised road map for adult blood lineage development has been proposed. Protein Klotho has been indicated to be able to regulate B-lymphopoiesis via its influence on the hematopoietic microenvironment. SUMO-2 and SUMO-3 have been found as localized to chromosome earlier and accumulated gradually during telophase. These findings have demonstrated that mammalian SUMO-1 shows patterns of utilization that are clearly discrete from the patterns of SUMO-2 and SUMO-3 throughout the cell cycle, arguing that it is functionally distinct and specifically regulated *in vivo*, and on the other hand, that myeloid progenitor number and developmental potential do not decline with age indicates that B-lymphopoiesis is particularly sensitive to defects that accumulate during senescence (Amirayan et al., 1995; Bregenholt et al., 1996; Dallas et al., 2007; Gray-Parkin et al., 2002; Green et al., 1992; Ikuta et al., 1990; Jackson and Bell, 1990; Kondo et al., 1997; Kobari et al., 2000; Lazarus et al., 2005). Diseases and disorders, connected with retardations in these processes, have been defined as clone HSCs malignancies, characterized by independency and/or hypersensitivity of HSCs and/or of haematopoietic precursors to numerous cytokines. In many patients with such diseases, a mutation in such gene has been established, and its presence in erythropoietin-independent erythroid colonies have demonstrated a link with growth factor hypersensitivity, which has been characterized as a key biologic feature of these disorders (Sell, 2004).

Mesenchymal stem cells (MSCs) have been indentified in bone marrow as well as in other tissues of the joint, including adipose, synovium, periosteum, perichondrium, and cartilage, as well as to modulate immune responses, exhibit healing capacities, improve angiogenesis and prevent fibrosis (Djouad et al., 2009; Maitra et al., 2004; Terskikh et al., 2006). These properties have charac-terized these cells as usable for therapeutic applications in many different diseases and disorders. MSCs have also proved abilities to support tissue repair, angiogenesis and concomitant immunomodulation and to provide tissue-specific functional biodiversity, additionally mediated by direct cell-cell communications via adhesion molecule and by exchange of cytokines, exosomes and micro-RNAs (Hass and Otte, 2012; Maitra et al., 2004; Terskikh et al., 2006 Maitra et al., 2004; Terskikh et al., 2006). These features allow MSCs be used for treatment

of various morbid and degenerative processes (Kang et al., 2012; Ra et al., 2011).

BIOLOGICAL PROPERTIES OF DENDRITIC CELLS AND THEIR ROLE IN GENERATION OF ADEQUATE IMMUNE RESPONSE

In the past years, the development of novel therapeutic strategies with DCs has become extensively investigated (Arthur et al., 1997; Bonini et al., 2001; Bubenik 2001; Caux et al., 1992; Clark et al., 1992; Gong et al., 1997; Hassan et al., 2000; Inaba et al., 1992; Kaplan et al., 1999; Kim et al., 1994; Reid et al., 1992; Ribas et al., 1997; Siena et al., 1995; Wang et al., 1995). According to many literature data, granulocyte-macrophage colony-stimulating factor (GM-CSF) mobilizes CD34+ bone marrow progenitor cells both *in vitro* and *in vivo* with an increased frequency and generation of DCs with anti-malignant properties (Banchereau et al., 2000; Caux et al., 1992; Curti et al., 2001; Inaba et al., 1992; Lehtonen et al., 2007; Reid et al., 1992; Siena et al., 1995). In DCs, but not in macrophages, basal expression of SOCS-1 has been detected (Figure 1) (Lehtonen et al., 2007).

Specific protein inhibitors from the SOCS family have been proven as modulators of the activated DCs by IL-4 and GM-CSF cytokine signaling via the JAK/STAT pathway. Similarly, the addition of GM-CSF plus tumor necrosis factor-α (TNF-α), has been found to induce development of DCs from purified CD34+ cells of bone marrow, cord blood and peripheral blood (Caux et al., 1992; Reid et al., 1992; Siena et al., 1995). The critical role of TNF-α for the differentiation of DCs has been supported by the demonstration that this cytokine induces the expression of molecule CD40 on CD34+ cells (Curti et al., 2001; Reid et al., 1992; Siena et al., 1995). Besides that, CD34+/CD40+ cells have been found to express only myeloid markers, significantly increase alloantigen presenting function, compared with total CD34+ cells, and have also given rise to high numbers of DCs. Modulation of DCs differentiation from these bipotent CD34+/CD40+ cells during the later stages of their cultivation, has also been shown by cytokine interleukin-4 (IL-4). On the other hand, appropriate modifications of DCs to express tumor-antigens by *in vitro* and/or *ex vivo*-transfer of genes, coding respective antibodies, has been suggested. Taken together, these data have revealed abilities for development of different therapeutic strategies of DCs for immunotherapy of malignant diseases.

DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES WITH DENDRITIC CELLS

The antigen-presenting functions of DCs, could theo-retically be exploited as a new therapeutic tool in cancer therapy in order to amplify immune responses against tumor-specific antigens (Arthur et al., 1997; Avigan, 2004; Bennett et al., 2001; Bonini et al., 2001; Bubenik, 2001; Caux et al., 1992; Clark et al., 1992; Gong et al., 1997; Hassan et al., 2000; Inaba et al., 1992; Kaplan et al., 1999; Kim et al., 1994; Reid et al., 1992; Ribas et al., 1997; Siena et al., 1995). Promising results from clinical trials in patients with malignant lymphoma, melanoma and prostate cancer, have suggested that immuno-therapeutic strategies that take advantage of the antigen presenting properties of DCs, might ultimately prove both efficacious and widely applicable against human malignancies (Fong and Engelman, 2000). Besides that, genetically-modified cells have been widely tested in pre-clinical studies, including anti-malignant agents (Arthur et al., 1997; Avigan, 2004; Bennett et al., 2001; Bonini et al., 2001; Borysievicz et al., 1996; Engelmayer et al., 2001; Eo et al., 2001; Frasca et al., 2006; Gong et al., 1997; Kaplan et al., 1999; Panicali and Paoletti, 1982; Reid et al., 1992; Ribas et al., 1997; Siena et al., 1995; Wang et al., 1995; Wildner and Morris, 2000; Yongqing et al., 2002).

DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES WITH HYBRID CELLS, PREPARED BY FUSION OF DENDRITIC AND MALIGNANT CELLS

As alternative method for delivery into DCs, their fusion with tumor cells has been utilized, as well as the hybrid cell-based vaccines have shown high therapeutic activity, even in patients with malignant diseases (Avigan, 2004; Gong et al., 2000; Hiraoka et al., 2004; Walden, 2000; Yongqing et al., 2002). Induced by vaccination with dendritic/tumor fusion cells, antitumor immunity has reacted differently to injected malignant cells and autochthonous malignancies (Xia et al., 2003). It has also been shown that immunization with such fusion cells induces rejection of established metastases (Gong et al., 1997). The observed greatly reduced number of established pulmonary metastases both with and without *in vivo*-administration of IL-2 adoptive transfer of T-cells derived from B16/DC vaccine-primed lymph nodes into B16 tumor-bearing mice has suggested a role for the cells, developed by fusion of malignant cells with DCs hybrid products, as effective cellular vaccines for eliciting T-cell-mediated anti-malignant immunity (Wang et al., 1998). Hybrid cells, developed by fusion between DCs and tumor cells, have been found to express both major histocompatibility complex (MHC) class I- and class II-restricted tumor-associated epitopes and might, therefore, be useful for the induction of tumor-reactive CD8+ and CD4+ T-lymphocytes both *in vitro* and in human vaccination trials (Parkhurst et al., 2003). It has also been demonstrated that immunization with such vaccines, developed by fusion of DCs with mouse 4TOO plasmacytoma cells FC/4TOO hybrid fusion cells plus IL 12 potentiates anti-malignant immunity and the treatment of murine multiple myeloma (Gong et al., 2000; 2002).

Findings about fusions of ovarian cancer cells to autologous or allogeneic DC induced cytolytic T-cell activity and lysis of autologous malignant cells by a MHC class I-restricted mechanism, have suggested that fusions of such malignant cells and DCs activate T-cell responses against autologous malignancies, and the fusions are probably functional, when they are generated with either autologous or allogeneic DCs. It has also been demonstrated that sequential stimulation with hybrid cells, derived by fusion of DCs with breast carcinoma fusion cells and anti-CD3/CD28, results in a marked expansion of activated tumor-specific T-lymphocytes, which has suggested these fusion cells are probably effective antigen-presenting cells (APCs), which stimulate inhibitory T-cells that limit vaccine efficacy (Vasiri et al., 2008). On the other hand, the results, according to which the *ex vivo*-exposure of DCs to TGF-β has not appeared to lessen the efficacy of DCs vaccines, suggest that tumor-derived TGF-β probably reduces the efficacy of DC/malignant cell hybrid fusion vaccine via an *in vivo*-mechanism, and the neutralization produced by the fusion cells TGF-β might enhance the effectiveness of DCs-based immunotherapy (Kao et al., 2003). The immunogenicity of a DCs/malignant cells fusion hybrid cells-based vaccines has been increased by heat-treated tumor cells (Koido et al., 2007).

DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES WITH CELLS BY INSERTION OF ADDITIONAL CYTOKINE GENES AND ANTIGENS BY TRANSFECTION WITH RECOMBINANT GENE CONSTRUCTS

It has been demonstrated that initial materials for gene-engineering manipulations could be used by both DNA- and RNA-viruses, as well as bacterial plasmids and yeast's genomes (Arthur et al., 1997; Avigan, 2004; Bennett et al., 2001; Bonini et al., 2001; Borysievicz et al., 1996; Bubenik, 2001; Chen et al., 1997; Chun et al., 1999; Domi and Moss, 1995; Engelmayer et al., 2001; Eo et al., 2001; Frasca et al., 2006; Gambotto et al., 1999; Gong et al., 1997; Guenechea et al., 2000; Hass et al., 2000; Kaplan et al., 1999; Kauffman et al., 2001; Martinet et al., 1997; Palese and Roizman, 1996; Panicali and Paoletti, 1982; Reid et al., 1992; Ribas et al., 1997; Siena et al., 1995; Wang et al., 1995; Wildner and Morris, 2000; Yongqing et al., 2002). This indicates eventual existence of a possibility for insertion of genes, coding cell receptors, cytokines, enzymes, complement components, apoptosis activators and/or inhibitors, surface antigens, as well as markers for malignancy. Many studies have suggested that hybrid viral vector systems could improve the suicide gene therapy of tumors. These results would have significant implications for the improvement of clinical gene therapy in HIV/AIDS and malignantdiseases.Inasimilarway,theobservedelimination of the protective effect in depletion of CD8+ T-lymphocytes, but not of CD4+ T-cells *in vivo* from animals, vaccinated with vaccines, developed on the basis of *adenoviral* genome recombinant gene construct *Ad2CMV-gp100* mice, containing gene for glycoprotein (*gp*) from *cytomegalovirus* (*CMV*) genome, has suggested that these virus recombinants, encoding tumor antigens, could probably be useful as vaccines to induce specific T-cell immunity for therapy of malignant diseases.

DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES WITH DENDRITIC CELLS, CONTAINING ADDITIONAL CYTOKINE GENES AND ANTIGENS BY TRANSFECTION WITH RECOMBINANT GENE CONSTRUCTS

In investigation on the role of intra-tumor treatmet on the survival of tumor-bearing experimental animals with herpes-simplex virus (HSV), expression of IL-4 has been found to prolong their survival, whereas expression of IL-10 to reduce it (Bennett et al., 2001; Hassan et al., 2000; Liu et al., 2005; Panicali and Paoletti, 1982; Wildner and Morris, 2000). These finding have suggested further investigation on the improvement in the combination of oncolytic viral therapy and immunomodulatory strategies. They have also supported the full use of recombinant *adenovirus*-transduced DCs for *in vivo*-immunization against tumor-associated antigens. In laboratory conditions, human DCs, transduced with recombinant virus gene construct *AdV/IL-10*, have been shown to inhibit mixed leukocyte culture, reduced cell surface expression of co-stimulatory molecules CD80/CD86, as well as exhibited inability for production of the potent allo-stimulatory cytokine IL-12 (Coates et al., 2001). In investigation on the *in vivo*-properties of the so modified DCs, skin transplantation of humanized immunodeficient non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice, engrafted with human skin, reconstituted via intra-peritoneal injection with allogeneic mononuclear cells (MNCs) mixed with 1×10^6 autologous to the skin donor DCs, transduced with either recombinant virus gene construct *AdV/IL-10* or of recombinant viral vector *AdV/MX-17*, a reduced skin graft rejection, characterized by reduced infiltration with mononuclear cells and less dermo-epidermal junction destruction in comparison with the animals with inoculation of DCs, modified with the control virus alone, has been observed. Transduced by appropriate *adenoviral* gene constructs, immature DCs have shown the abilities to differentiate in different directions in respective appropriate conditions of cultivation (Addison et al., 1995; Chen et al., 1997; Dietz and Vuk-Pavlović, 1998; Gambotto et al., 1999; Lu et al., 1998). For example, in the presence of monocyte-conditioned medium, they have indicated ability to express the surface markers

of mature DCs, such as CD25, CD83, high levels of molecules CD86 and HLA-DR, or to secrete of IL-12. Their ability to induce T-cell growth has also been enhanced. Similarly, in transfer of bacterial gene *lacZ*, coding the enzyme beta-galactosidase by *recombinant retrovirus* gene constructs in human DCs, increased expression levels of MHC class I and II molecules, as well as of CD1a, CD80, CD86, CD13, CD33, CD40 and CD54 has been demonstrated (Aicher et al., 1997). So modified DCs have also shown high stimulatory activity in both allogeneic and autologous mixed lymphocyte reaction (MLR). These data have also supported the efficiency of the recombinant viral vectors in studies on the biology of DCs, including the expression of specific antigens for active immune therapy (Aicher et al., 1997; Dietz and Vuk-Pavlović, 1998; Lu et al., 1998).

COMBINED THERAPEUTIC STRATEGIES WITH DENDRITIC CELLS

For further increase of the potency of the vaccine, a combined variation of both technologies has been applied, in which interleukin-18-transfected DCs have been used to prepare dendritic cells-tumor cells conjugates (Wen et al., 2001). Immunization with such conjugates has significantly increased the production of Th1 cytokine-producing cells, the number of antigen-specific CD8+ T-cells, as well as the anti-malignant immunity.

However, the fact that an increased Th1 cytokine production and stronger anti-malignant effect have not been observed in mice, depleted of IFN-γ, has also supported the maintenance of DCs/malignant cells conjugates as potent anti-malignant vaccines, and interleukin-18 could be additionally administered by gene transfection of cells for enhancement of this immunity, which is probably mediated mainly by IFN-γ. Immunization with gene-modified hybrid products, derived by fusion of DCs with malignant cells, received by their fusion with *IL-12* gene-transferred cancer cells, has shown an ability to elicit a previously enhanced anti-malignant effect in experimental therapeutic models *in vivo* (Suzuki et al., 2005). It has also been indicated that a hybrid vaccine, based on *GM-CSF* gene-modified DCs, might be an attractive strategy for anti-malignant immunotherapy with potentially increased therapeutic efficacy (Cao et al., 1999). Immunization of mice with engineered *DCRMAT/J558-IL-4* hybrids *in vitro* has elicited stronger J558 tumor-specific CTLs responses and has also induced more efficient protective immunity against J558 tumor challenge, and respectively, *in vivo* in comparison of hybrid vaccines *DCRMAT/J558* (Liu, 2003). Similar results have been observed in immunization of C57BL/6 mice with gene-engineered *DC/J558-IL-4* hybrids (Xia et al., 2005). It has also been demonstrated that gene-engineered fusion hybrid vaccines, could be an attractive strategy for cancer immunotherapy. These results have indicated that gene-engineered fusion hybrid vaccines, combined with gene-modified tumor and DCs vaccines, as well as their combination with Th1 gene-modified tumor with DCs, might be attractive strategies for cancer immunotherapy (Liu, 2003; Xia et al., 2004).

DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES BY LYMPHOCYTE ELIMINATION AND DIFFERENTIATION THERAPY

By understanding molecular basis, gene expression mechanisms and signaling pathways, which are involved in development and differentiation of HSCs, development of differentiation therapy and elimination therapy strategies have been developed (Cheung et al., 2003). A mutation in one or more genes, which are included in any of the mentioned cascade mechanisms, could be characterized as a probable key biologic feature of these disorders. The most common example is transplantation of allogenic HSCs with graft versus leukemia effect (Figures 1 and 2) (Amirayan et al., 1995; Bregenholt et al., 1996; Cheung et al., 2003; Dallas et al., 2007; Glymcher and Murphy, 2000; Gray-Parkin et al., 2002; Green et al., 1992; Ikuta et al., 1990; Jackson and Bell, 1990; Kondo et al., 1997).

DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES BY LYMPHOCYTE DIFFERENTIATION ON THE INFLUENCE OF RECOMBINANT VACCINES, CARRYING SPECIFIC ANTIGENS

Vaccination strategies with recombinant *poliovirus* vectors have induced protective immunity against challenge with lethal doses of ovalbumin-expressing malignant melanoma cell line (Mandl et al., 1998). Similarly, novel mechanisms, by which *vaccinia virus* has been found to interfere with the onset of host immune responses by blocking the interferon-gamma (IFN-γ-) signal cascade mechanism through the dephosphorylating activity of the viral phosphatase VH1, have been revealed. Tumor infiltrating lymphocytes isolated from metastatic melanoma patients have been characterized as predominantly cytotoxic T-lymphocytes (CTLs) with an ability to recognize and kill autologous tumor cells after their *in vitro*-cultivation (Borysiewicz et al., 1996; Hodge et al., 1997; Overwijk et al., 1999; Tsang et al., 1995). In mice, inoculated with *IL-15*-expressing *vaccinia virus* in addition to an increase in NK cells in the spleen, enhanced expression of interleukin-12 (IL-12) and IFN-γ, as well as induction of cytokines, has been established. In this way, a possibility for cytokine influence on the response mediated by human HSV therapy, has been suggested, which could probably be due to the host immune response (Andreansky et al., 1998; Liu et al., 2005). Thus, cytokine expression might be an important adjunct to tumor therapy utilizing genetically engineered

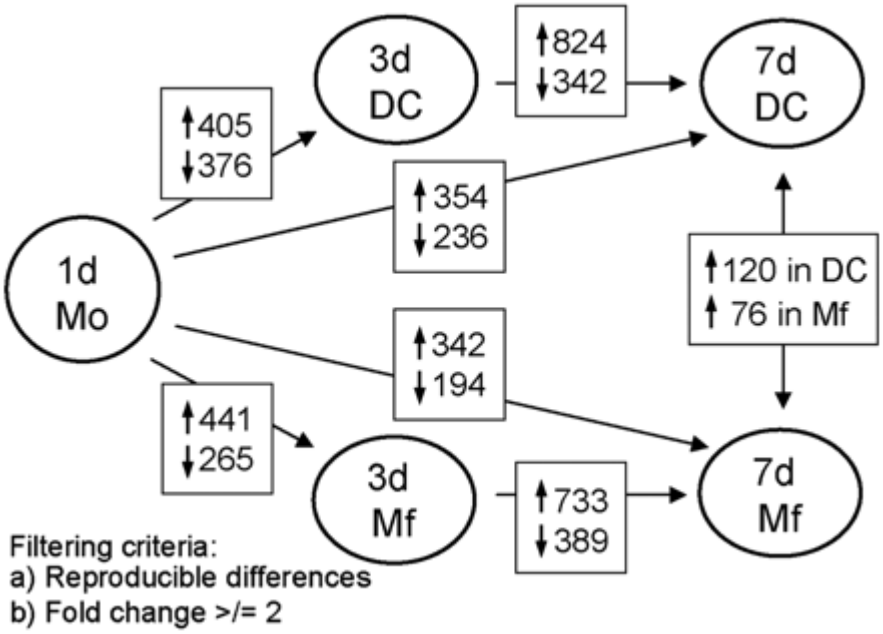

Figure 1. Experimental set-up and numbers of regulated genes during macrophage and DC differentiation (Lehtonen et al., 2007).

HSV. However, in this experimental model system, the degree of attenuation in viral virulence, attained with co-expression of IL-15, has been much less than that, achieved with co-expression of IL-2, which has suggested that the response, mediated by peripheral NK cells, is probably stronger to IL-2 than to IL-15. Immunohistochemical analyses of mouse brains after viral inoculation have shown marked accumulation of inflammatory cells, composed primarily of monocytes/macrophages/microglia, with various proportions of CD8+ and CD4+ T-cells (Figures 2 and 3) (Glymcher and Murphy, 2000), as well as few B-lymphocytes (Andreansky et al., 1998). These results have also implied that virus-encoded inhibitors of apoptosis, such as the caspase-8 inhibitor CrmA, could block the IFN-mediated apoptosis, and therefore, they are probably able to constitute an alternative family of inhibitors of IFNs in the cell.

In *CEA*-transgenic mouse model of anti-tumor effects in terms of survival, CD8+ and CD4+ responses, specific for carcino-embryonic antigen (CEA), have been observed (Figures 2 and 3) (Glymcher and Murphy, 2000; Tsang et al., 1995). The utilization of a diversified immunization scheme, using a recombinant *vaccinia virus*, followed by recombinant *avian pox virus*, has been shown to be far superior to the use of either one alone in eliciting CEA-specific T-cell responses (Hodge et al., 1997). These studies have demonstrated that the use of cytokines and diversified prime and boost regimens could be combined with the use of recombinant viral vectors, expressing multiple co-stimulatory molecules to further amplify T-cell responses. IL-2 has also been found to induce their proliferation and to augment their cytotoxic activity such that they eliminated autologous malignant cells.

The addition of GM-CSF to *rF-CEA* or *rF-CEA/TRICOM* vaccinations via the simultaneous administration of recombinant viral vector *rF-GM-CSF* has enhanced CEA-specific T-cell response (Sadegah et al., 2000). Because protein Bcl-3 has been characterized as a member of inhibitors from the subfamily the Nuclear Factor-kappaB (NFκB), a regulation mechanism on the apoptosis and survival of activated T-cells, supported by the balance in the concentration of various members of this family, has been suggested (Glymcher and Murphy, 2000; Mitchell et al., 2001). It has also been proposed that the deliberate induction of self-reactivity by use of a recombinant viral vector, could lead to tumor destruction, but CD4+ T-lymphocytes (Figure 3) are probably an integral part of this process, and hence, targeting tissue differentiation antigens vaccine strategies might be valuable in malignancies, arising from non-essential cells, tissues and organs, such as melanocytes, prostate, testis, breast and ovary (Aruga et al., 1997; Glymcher and Murphy, 2000; Grosenbach et al., 2001; Itoh et al., 1995; 1986; Kent et al., 1998; Rosenberg, 1996; Rosenberg et al., 1994; Siders et al., 1998; Wang et al., 1995; Zhai et al., 1996). Treatment of patients with metastatic melanoma with tumor-infiltrating lymphocytes (TILs) and IL-2 has resulted in objective immune responses in them.

Studies on the specific T-cell responses via stimulation of peripheral blood lymphocytes with specific peptide epitopes from the CEA have demonstrated clear differences in establishment of T-cell lines post-immunization. These lines have been CD8+ and/or CD4+/CD8+, to lyse cells, transformed by *Epstein-Barr virus* (*EBV*) B-

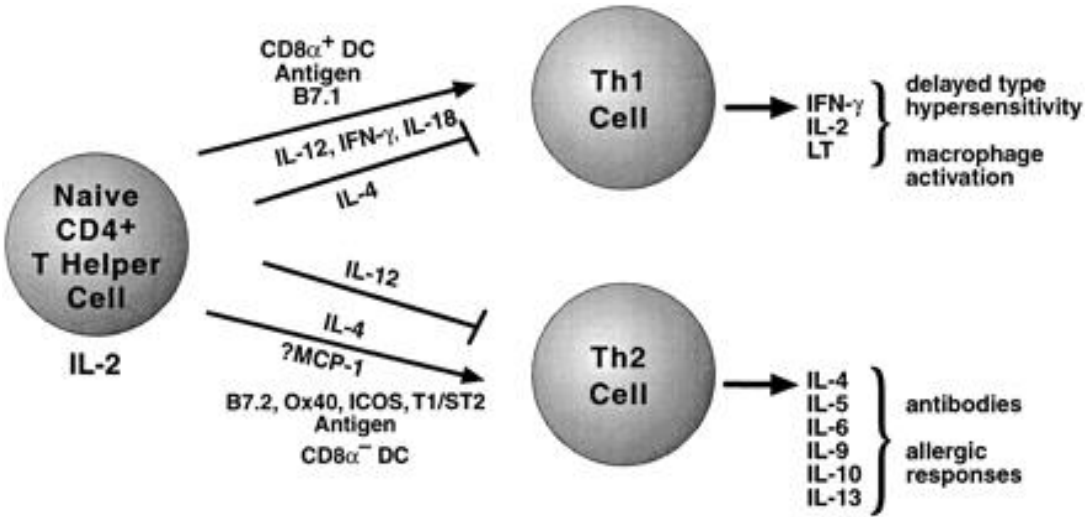

Figure 2. Signals, influencing differentiation of T-helper lymphocytes (Glymcher and Murphy, 2000).

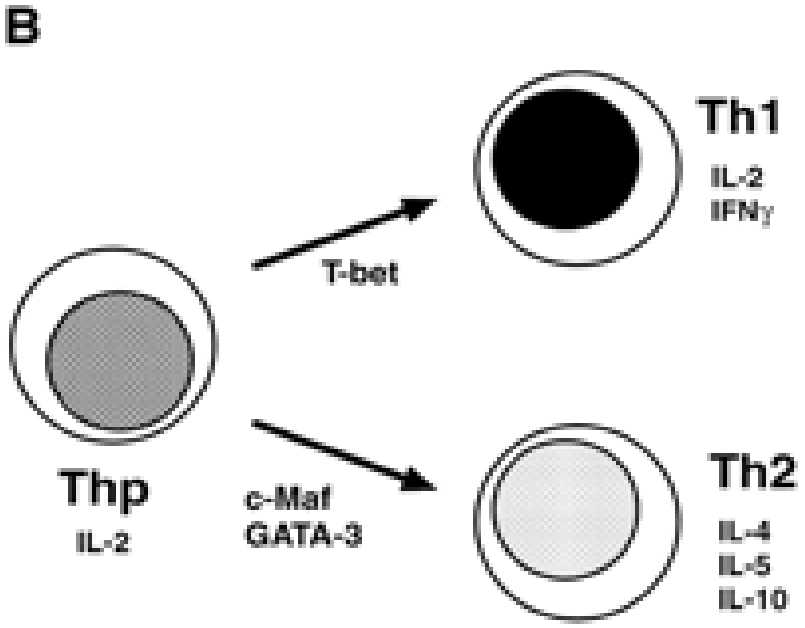

Figure 3. Transcription factors, influencing differentiation of T-helper lymphocytes (A); Tissue-specific factors that regulate $CD4^+$ T-helper cell differentiation (B) (Glymcher and Murphy, 2000).

lymphocytes and transduced with gene *CEA*, and to lyse CEA-positive carcinoma cells in a human leukocyte antigens (HLA) restricted manner (Hodge et al., 1997; Sadegah et al., 2000; Tsang et al., 1995). It has also been suggested as a model ofmetastatic growth inhibition, mediated by non-lymphocyte effective cells, including monocytes/ macrophages, neutrophils and anti-angiogenic chemokines/cytokines (Itoh et al., 1986). As a result, these effective molecules could be expressed by *HSV* in productively infected cells both *in vitro* and *in vivo*. *HSV*, expressing genes *IL-4* and/or *IL-10*, has shown an ability to infect and destroy glioma cells *in vitro* (Andreansky et al., 1998). Similarly, intra-cerebral inoculation of *HSV*, expressing either IL-4 or IL-10 into syngeneic mouse glioma GL-261 cells, implanted in the brains of immune-competent C57BL/6 mice, have been found to produce dramatically opposite physiologic responses. It has also been shown that *IL-4-HSV* significantly prolonged survival of tumor bearers, whereas tumor-bearing mice, receiving the *IL-10-HSV*, have had a median survival, identical to that of saline treated controls (Andreansky et al., 1998; Hassan et al., 2000; Liu et al.,

2005). These studies could have direct bearing on the design of vaccine clinical trials for infectious agents and/or malignancy-associated antigens, in which T-lymphocyte co-stimulatory molecules would be employed to enhance antigen-specific T-cell responses.

In examination of the clinical and environmental safety and immunogenicity in the first clinical trial of a live recombinant *vaccinia virus*, expressing proteins E6 and E7 of types 16 and 18 of *human papilloma virus* (*HPV*), in each patient has been found an anti-*vaccinia virus* antibody response and three of the eight patients have developed an *HPV*-specific antibody response (Adams et al., 2001). In one of three available patients, *HPV*-specific CTLs have also been detected. As highly effective method, with no significant toxicity in mice, gene therapy strategies for treatment of malignant disorders by using of recombinant *adenoviral* vectors, containing gene *IL-12*, have been characterized (Cordier et al., 1995; Tozola et al., 1997). In vaccination with synthetic RAS peptide, representing mutation *k-ras* in their malignant diseases, a transient *ras*-specific T-lymphocyte response has been induced (Kung et al., 2000). These results have indicated that specific T-cell responses against uniquely harbored mutations in malignant cells could be induced in cancer patients by vaccination. In this way, recombinant *adenoviral* gene constructs, encoding malignant antigens, have been proven as useful vaccines, inducing specific T-lymphocyte immunity for therapy of malignant disorders (Cordier et al., 1995; Tozola et al., 1997).

DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES WITH LYMPHOCYTES AND THEIR PRECURSORS BY INSERTION OF ADDITIONAL CYTOKINE GENES AND ANTIGENS WITH RECOMBINANT GENE CONSTRUCTS

Human T-lymphocytes have shown abilities to be efficiently transduced under clinically applicable conditions, by *adenoviral* gene constructs after 7 days of culture, in the presence of IL-2 and/or IL-7 (Di Nicola et al., 1999; Rosenberg et al., 1998; Zhang et al., 1996). A significant improvement in transduction efficiencies of mouse and human T-lymphocytes by a prolonged pre-incubation with IL-2 and by the addition of the recombinant *adenoviral* vector *Lipofectamine* has also been indicated (Di Nicola et al., 1999). In transfer of chimeric immune receptor genes, ligated into vector *pMSCVneo*, in human lymphocytes, a mediated antigen-specific non-MHC-restricted cytokine release and malignancy cytotoxicity, as well as inhibition of human xenograft engraftment in experimental mice with severe immunodeficiency, combined with non-obese diabetes (NOD/SCID), has been observed (Douglas et al., 2001). Similarly, a clinically applicable protocol that meets good clinical practice criteria regarding the gene system for transduction and expansion of primary human T-lymphocytes, in which recombinant gene construct, encoding a single chain FvG250 antibody chimeric receptor (ch-Rec), specific for a RCC-associated antigen (TAA), has been designed in preparation of a clinical phase I/II study in patients with renal cell carcinoma (RCC) (Douglas et al., 1999; Lamers et al., 2002; Wu et al., 1999; Zhou et al., 2003). According to the results from other studies, evidence of increase of the anti-malignant effect *in vivo* of lymphocytes, transduced by recombinant *herpesviral* gene construct *HSVtk-DLI*, has been provided (Burt et al., 2003). The observed enhanced cytotoxicity of TILs, transduced with recombinant *retroviral* vector with insertion of gene for TNF, has been ascribed to autocrine effects of this cytokine, which has probably included augmentation of adhesion molecules (CD2 and CD11a) and IL-2 receptor expression, as well as elevation of production of IFNs, lymphotoxin, GM-CSF, as well as their paracrine effects on target cells to facilitate them to be differentiated and genetically-modify lymphocytes (Rosenberg et al., 1990). Treatment with TILs, transduced with *retroviral*-mediated gene transduction to introduce gene, connected with neomycin resistance, plus addition of cytokine IL-2, has also been proven to mediate the regression of metastatic melanoma (Guenechea et al., 2000; Hass et al., 2000). These data have confirmed the feasibility and safety of strategies, based on recombinant *retroviral* vectors gene transfer for human gene therapy and have implications for the designing of TILs with improved anti-malignant potency, as well as for the possible use of lymphocytes for the gene therapy of other diseases (Zhang et al., 2004; Zhang et al., 2002).

Conclusion

DCs and lymphocytes have been characterized as hopeful vehicles for appropriate modulation of the immune response in the presence of appropriate growth factors, cytokines and specific antigens. In this way, they have shown abilities for differentiation in respective directions in the presence of appropriate recombinant viral gene constructs, coding respective antigens, on one hand, and as cell vaccines by gene-engineering manipulations with recombinant vectors, carrying genes for respective malignant antigens or cytokines, on the other. These properties characterize them as promising candidates for construction of novel and safe therapeutic products on their basis, by use of new technologies.

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