Available online at www.sciencedirect.com
SCIENCE DIRECT®
Immunobiology 211 (2006) 127–136
Immunobiology
www.elsevier.de/imbio

REVIEW

The role of regulatory T lymphocytes in the induced immune response mediated by biological vaccines

Mercedes López[a,b], Raquel Aguilera[a], Claudio Pérez[b], Ariadna Mendoza-Naranjo[a], Cristian Pereda[a], Marcos Ramirez[a], Carlos Ferrada[b], Juan Carlos Aguillón[a], Flavio Salazar-Onfray[a,*]

[a] *Disciplinary Program of Immunology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile*
[b] *Research Support Office, University of Chile Clinical Hospital, Santiago, Chile*

Received 9 August 2005; accepted 17 November 2005

Abstract

Immunotherapy has become a novel therapeutic alternative for various kinds of tumours. Recently, we have finalized the first phase I clinical study in Chile for the treatment of advanced malignant melanoma, using dendritic cells (DCs) loaded with allogeneic melanoma cell lysate.

This study included 20 patients and the obtained results, pioneer in Latin America, showed that DC-based immunotherapy is innocuous, even provided in combination with IL-2. In addition, immunological responses were detected in 50% of the treated patients, establishing a positive correlation between the delayed type hypersensitivity (DTH) reaction, which indicates induction of in vivo immunological memory, and patients surviving. Nevertheless, objective clinical responses in vaccinated patients are still insufficient. Only sporadic objective metastasis regressions have been registered and an important proportion of the treated patients did not respond, or their responses were weak. Several strategies have been described to be used by tumours to escape from the immune response. Actually, we have demonstrated that IL-10 inhibits antigen presentation in melanoma, reducing tumour sensitivity to melanoma-specific cytotoxic T lymphocytes (CTLs). Regulation of the immunological response by inhibitory cells could be another possible cause of clinical unresponsiveness. Lately, the existence of subpopulations of regulatory T lymphocytes (RTL) able to limit the immune response in a specific form has been established, specially inhibiting the proliferation and activity of $CD4^+$ and $CD8^+$ effector T lymphocytes. These cellular subpopulations, mostly $CD4^+/CD25^+/Foxp3^+$ T lymphocytes (Treg) of thymic origin, or TR1 lymphocytes able to release IL-10, and tumour growth factor β (TGF-β) producing TH3 lymphocytes, would be accumulated in the body during tumour growth, inhibiting the immune response. In relation to RTL and cancer, evidence indicates that Treg cell numbers are increased in blood and other tissues in different types of cancer. Additionally, it has been demonstrated that in patients with refractory metastatic melanoma, the adoptive transference of anti-tumour $CD8^+$ T lymphocytes after non-myeloablative chemotherapy was able to induce important tumour regressions that would be due to elimination of RTL populations. Additionally, chemotherapeutical drugs like decarbazine, besides their effect on tumour proliferation, also have an immunosuppressive effect on T lymphocyte populations, as well as on accumulated

Abbreviations: CTLs, cytotoxic T lymphocytes; DTH, delayed type hypersensitivity; MDC, myeloid dendritic cells; PDC, plasmacytoid dendritic cells; RTL, regulatory T lymphocytes

*Corresponding author. Tel.: +56 2678 6345; fax: +56 2735 3346.
E-mail address: fsalazar@med.uchile.cl (F. Salazar-Onfray).

0171-2985/$ - see front matter
doi:10.1016/j.imbio.2005.11.003

RTL. In this article, a novel strategy for the study of RTL is proposed, including potential therapeutic innovations, which is being pioneered in current clinical trials.

Keywords: Dendritic cells; Immunotherapy; Regulatory T lymphocytes; Vaccines; Cancer

Contents

Introduction

Cancer is a disease characterized by the uncontrolled division and growth of transformed cells. These cells have the capacity to invade other tissues different from those where they originated. Tumour cells migrate through the lymph and the blood producing metastasis in distant organs. The scientific and technological advances developed during the last years have provided new knowledge about the origin, development and growth of cancer, which has translated into better treatments for the disease. However, at the present time, cancer is still the main cause of death in developed countries. In Chile, malignant tumours constitute the second cause of death in the population aged over 15 years old (Medina and Kaempffer, 2001), preceded only by cardiovascular diseases. The incidence is considered to be 220–240 per 100,000 inhabitants, which results in 36,500 new cases per year. According to the statistics from the Ministry of Health, the mortality rate from cancer in 2002 was 1122.48 per 100,000 adults, with a total of 19,146 deaths (http://deis.minsal.cl/indexmc.asp). Furthermore, melanoma is a malignant tumour of neuroectodermal origin, whose incidence has increased progressively in the last decades. In the worldwide scope, in Europe, the United States, and especially in Australia, melanoma is the type of cancer of fastest expansion and highest mortality among cutaneous tumours. In Chile, between 1992 and 2002, 2425 deaths were caused by malignant melanoma, and its incidence increased from 1.1 in 1992 to 1.7 cases per 100,000 inhabitants in 2002 (http://deis.minsal.cl/indexmc.asp). The only way to cure malignant melanoma is early detection and suitable surgical treatment. Although more than 95% of patients survive when they are diagnosed in stage I, once the tumour metastasizes it becomes highly resistant to conventional treatments such as surgery, irradiation and chemotherapy, so that less than 10% of patients experience long-term survival, with an average survival span of 4–6 months (Lawson, 2004). This fact indicates that cancer is a relevant pathology that requires basic, preclinical and clinical investigation for its understanding, and also the application of this knowledge in the elaboration of more effective and secure therapies to defeat it.

The immune system and cancer

The utilization of the immune system for the treatment of cancer is denominated immunotherapy. This therapeutic approach is based on the control of tumour growth through the activation of the immunological system. The narrow link between immunity and cancer has been known for more than a century, when the scientist William Coley obtained sporadic antitumour responses, injecting patients with bacteria extracts (Coley, 1991). In addition, experiences in animal models opened interesting perspectives for vaccine design against diverse human cancers (Jaffee and Pardoll, 1996). Nowadays, it is known that T lymphocytes have highly specific receptors that are able to recognize tumour-specific antigens (TSA) or tumour-associated antigens (TAA), in the context of the major histocompatibility complex (MHC) and mediate the destruction of cancer cells (Boon et al., 1994). The interrelation between the immune system and tumour cells was explained in the context of the tumour immune

surveillance theory proposed by Burnet in 1970. There, it was established that one of the main functions of the immune system is to recognize and destroy tumour cells. At the present time, the original concept has been adapted to observations demonstrating that lethal tumours are able to appear in immunocompetent individuals although immune-deficient individuals truly have a major predisposition to develop some kinds of tumours. For example, patients affected by AIDS develop lymphomas and sarcomas, and immunosuppressed transplanted patients can develop scamous cell carcinoma, confirming a role for the immune system in the control of cancer (Dal Maso et al., 2005).

Dendritic cells: the main professional antigen presenting cells (APCs)

The capacity to activate cytotoxic T lymphocytes (CTLs) against tumour cells in vivo requires a previous antigen presentation in a special cell context given by professional APCs, which include macrophages, B cells and dendritic cells (DCs) (Alvarez et al., 2004). DCs are characterized for having a great functional plasticity against the antigen, determining the type of immune response that should be generated (Bell et al., 1999). DCs are bone marrow-derived mononuclear cells located in most peripheral tissues in the body as resident DCs, where they perform a sentinel function. DCs capture invading pathogens and receive inflammatory signals generated in the inflammatory process, acquiring antigens, starting a maturation process and migrating toward the secondary lymphoid organs. There, DCs provide activation signals to T cells, inducing their proliferation, cytokine release and migration to peripheral tissues, where they carry out their effector functions. DCs also express many co-stimulatory molecules, which provide crucial signals that guarantee an effective immune response mediated by T cells (Bell et al., 1999). Based on phenotypic and functional properties, human DCs are commonly divided into two populations, myeloid DCs (MDCs) and plasmocytoid DCs (PDCs) (Table 1). Both populations differ in many ways, including their cytokine production, tissue distribution and growth requirements. PDCs are $CD11c^{-}CD123^{hi}$, and are mostly found in blood and lymphoid organs. PDCs are involved in immune responses against viruses, producing large amounts of interferon α, whereas MDCs are $CD11c^{+}CD123^{lo}$ and include Langerhans cells, resident in the epidermis, and interstitial DCs located in the peripheral tissues. The latter population has the capacity to secrete large amounts of IL-12 in response to components of bacterial wall, and also express the surface adhesion cellular receptor CD209 (DC-SIGN) and the macrophage mannose receptor, CD206 (Rossi and Young, 2005). These antecedents would indicate that the different populations of DCs are specialized in the recognition of distinct types of pathogens and, as a consequence, to carry out different innate functions.

Table 1. Phenotypic characterization of different dendritic cell subtypes

Marker	MDCs		PDCs
	Interstitial DCs	Langerhans cells	Plasmocytoid DCs
CD1a	+	+	−
CD1d	+	−	?
CD11b	+	−	−
CD11c	+	+	−
CD52	−	−	±
CD83	+	+	+
e-cadherin	−	+	−
CD207, langerin	−	+	−
CD208, DC-LAMP	+	+	−
CD123	+	+	++
BDCA-2,4	?	?	+

Dendritic cells as melanoma vaccines

The capacity of DCs to induce a primary immune response has made them the best candidates to be used in vaccine protocols in cancer (Ardavin et al., 2004). In fact, DCs adequately loaded with TAA have induced tumour elimination and an efficient protective immune response in animal models (Porgador and Gilboa, 1995). Clinical trials have shown the possibility to supply DCs loaded with antigenic peptides to induce T cell peptide-specific responses in patients with lymphoma, malignant melanoma and prostatic carcinoma (Svane et al., 2003). Clinical studies based on DC vaccination have been possible because of the development of ex vivo methods that permit obtaining a high number of DCs. Currently, relatively high amounts of immature DCs can be obtained from $CD14^{+}$ monocytes isolated from peripheral blood by leukapheresis, density gradients, centrifugation and plastic adhesion (Svane et al., 2003), and then stimulated with GM-CSF and interleukin-4 (IL-4) for 5–7 days (Sallusto and Lanzavecchia, 1994). The immature DCs can be differentiated into mature DCs by adding maturation stimuli, like pro-inflammatory cytokines such as TNF-α, or other factors such as, Flt-3 ligand (Flt3L), LPS or CD40L (Banchereau and Steiman, 1998). The maturation process allows for immature DCs with a high capacity to capture antigens to be

transformed into a prominently APC, expressing high levels of MHC class I and class II and co-stimulatory molecules. Additionally, this process can induce the expression of chemokine receptors, like CCR7, allowing DC migration to the lymph nodes for encountering naive T cells (Banchereau and Steiman, 1998). Many of the pioneer vaccination studies with DCs have been carried out in melanoma, due to the well-defined TAA expressed in this tumour (Shuler et al., 2003). The first of these studies, carried out by Nestle et al. (1998), used DCs loaded with tumour peptides or autologous tumour cell lysate in the presence of keyhole limpet hemocyanin (KLH) as adjuvant, supplied intranodally to patients with advanced melanoma. This study demonstrated objective clinical responses in 5 of 16 evaluated patients (two complete responses and three partial responses). A later report related to the same study established that the observed clinical response has been maintained for some years (Nestle et al., 2005). We have recently finished in Chile the first Phase I clinical trial for the treatment of advanced malignant melanoma, using DC vaccines loaded with an allogeneic tumour cell lysate (Escobar et al., 2005). The study, pioneer in Latin America, was previously approved by the University of Chile Bioethical Committee for Human Studies. The DC production and vaccination scheme took approximately 2 months. In this protocol, 20 patients with advanced melanoma were included, and those results have been recently published (Escobar et al., 2005). In general, this study demonstrated that DC vaccines loaded with tumour cell lysate are innocuous, even when these are supplied in combination with IL-2. In addition, we have detected a positive immune response in 50% of the treated patients. The delayed type hypersensitivity (DTH) reaction is indicative of in vivo immunological memory induction. The use of a tumour lysate obtained from allogeneic melanoma cell lines provides a standardized and widely applicable source of melanoma specific antigens for clinical use, independently of the patient MHC haplotype. In the mentioned study, we established a direct correlation between the DTH response against tumour antigens and the median time to disease progression (TTP), as well as to patient survival (Fig. 1; Escobar et al., 2005). Our results and those reported for other authors demonstrated that vaccines based on modified DCs can induce a significant immunological response in diseases like malignant melanoma, non-Hodgkin's lymphoma, prostatic carcinoma and other cancers, in phases of the illness where effective alternative therapies do not exist. However, and despite the advances achieved, the induced immune responses in vaccinated patients have not been optimal. An important percentage of patients did not show clinical responses or these responses are weak. In our study, almost 50% of the treated patients did not show immunological positive responses using ELISPOT or DTH assays. The utilization of IL-2 as adjuvant did not enhance the obtained immune responses. Moreover, those patients who gave negative DTH for the tumour lysate have progressive and more aggressive disease than those that showed positive reactions (Escobar et al., 2005). There are several explanations for these results. For example, it has been established that tumour cells are able to use some strategies to evade the immune response, such as the inhibition of antigen presentation or the production of anti-inflammatory cytokines, like IL-10 (Salazar-Onfray, 1999). In this line, we have demonstrated that murine tumour cells treated with recombinant IL-10 (rIL-10) or transfected with the IL-10 gene showed a changed phenotype, characterized by

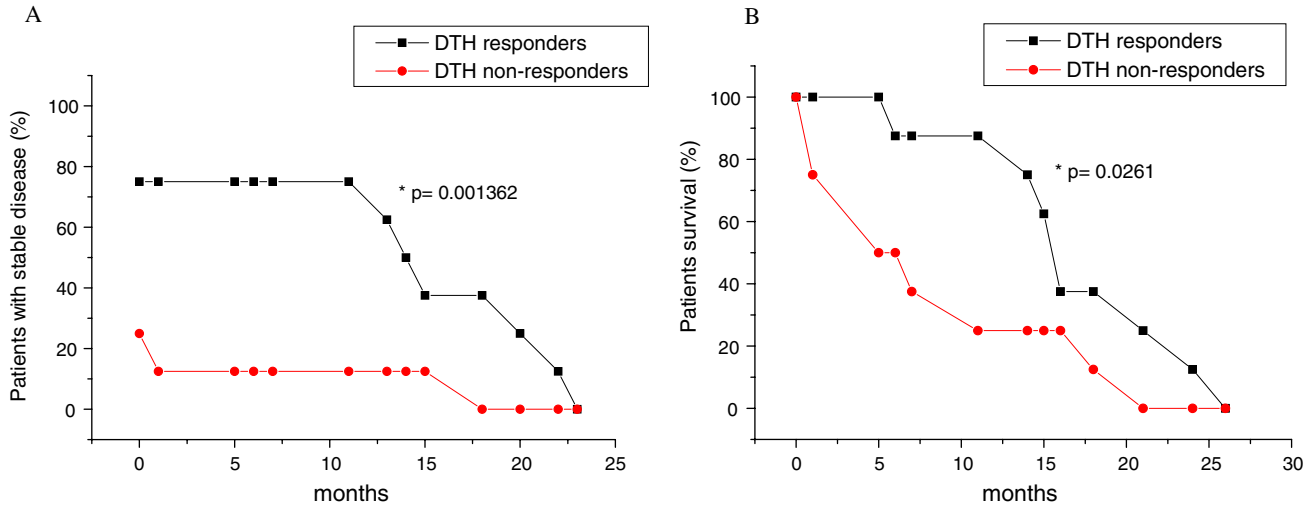

Fig. 1. Patients who showed in vivo immunological response after DC vaccination had longer stability of the disease and major survival. Correlation between delayed hypersensitivity reaction type IV (DTH) to melanoma cell lysate after vaccination with cell lysate loaded DCs and (A) time to progression (stable disease) and (B) post-vaccination patient survival.

a down-regulation in MHC class I expression, resistance to lysis by tumour-specific CTLs, and increased sensitivity to lysis by NK cells. These observations were related to an inhibition in the function and expression of TAP molecules (Petersson et al., 1998; Salazar-Onfray et al., 1995, 1997). We confirmed these findings in human melanoma cells, where rIL-10 turned melanoma cell lines less sensitive to tumour-specific CTLs associated to a significant reduction of MHC class I expression (Matsuda et al., 1994). Moreover, we recently published that a nanomeric peptide, homologous to the C-terminal of the human IL-10 sequence, can mimic several described effects of the cytokine, including down-regulation of MHC I and TAP1/TAP2 expression in human melanomas, related to an increase of tumour sensitivity to NK cell mediated lysis (Kurte et al., 2004).

On the other hand, other tumour escape mechanisms have recently been described, such as the existence of T cell sub-populations, capable to inhibit, in a specific way, the immune response against chronic infections or tumour growth (Schwartz, 2005).

Regulatory T lymphocytes

The complex network of interactions in the immune system involves molecules, cells and tissues finely regulated and whose stimulation leads to activation of diverse effector mechanisms. In addition, the normal immune system is able to be self-regulated to inhibit autoimmune responses. The generation and maintenance of functional T and B cell repertoires that are able to discriminate between own and foreign antigens are carried out in the primary lymphoid organs (central tolerance), and also in the periphery (peripheral tolerance). The presence of reactive lymphocytes against self-antigens in healthy humans and the existence of autoimmune diseases indicate that the central selection does not eliminate the totality of the self-reactive cells from the normal repertoire. In effect, T and B cells recognizing specific self-antigens can be isolated from healthy individuals (Yan and Mamula, 2002). Certain peripheral mechanisms for the control of immune responses by these cells are the generation of clonal anergy mediated by regulatory DCs and the presence of regulatory T lymphocytes (RTL) (Steinbrink et al., 2002). The idea of specific cell populations able to control the activation of T helper cells and antigen-specific effector cells preventing the development of autoimmune diseases was proposed 30 years ago for the first time (Gershon and Kondo, 1971). Nevertheless, during later years the molecular and biochemical mechanisms explaining the immune response regulation were contradictory and this theory started to be abandoned by many investigators (Moller, 1988). In the last decade, a group of $CD4^+$ T lymphocytes that constitutively express the alpha chain of the IL-2 receptor (CD25) on their surface was described to be capable of preventing the development of autoimmune diseases in experimental models (Walker, 2004). $CD4^+$ $CD25^+$ RTL (Treg) constitute 5–15% of the total population of $CD4^+$ T lymphocytes in human peripheral blood (Baecher-Allan et al., 2005), and express other markers that are useful for their characterization and enrichment like $CD5^{high}$, $GITR^{high}$, $CD45RB^{low}$, $CD25^{high}$, CD62L and the recently described Foxp3 (Sakaguchi, 2005). Mutations of Foxp3 transcriptional factor in humans and mice cause a devastating immunoproliferative syndrome associated with autoimmunity (Bennett et al., 2001). Foxp3 binds to DNA and is able to act as a repressive transcriptional factor, probably antagonizing the NFAT function, and thereafter inhibiting the production of several proinflammatory cytokines (Schubert et al., 2001). Foxp3 is highly expressed in thymocytes and peripheral $CD4^+$ $CD25^+$ T lymphocytes, but it is also expressed at lower levels in naive T cells and activated $CD4^+$ $CD25^-$ T cells (Fontenot et al., 2003). Finally, the retroviral transduction of $CD4^+$ $CD25^-$ cells with Foxp3 transform these cells into a regulatory phenotype (Hori et al., 2003) suggesting that it could act like a factor for the development of a particular population of T cells with regulatory properties.

Functional properties of RTL

Classically, it was paradoxical that either induced or congenital lymphopenia was associated with autoimmune diseases (Bennett et al., 2001), until experimental evidence demonstrated that such diseases could be prevented with specific regulatory T cell population transference, offering a immunological explanation (Malek et al., 2002). Moreover, it has been demonstrated that the transference of regulatory $CD4^+$ T cells can induce tolerance to allogeneic grafts in murine models (Benghiat et al., 2005). Although tolerance is mainly antigen specific, this condition can be actively spread to other antigens located at the same cellular microenvironment (Gershon and Kondo, 1971). The phenotypic characterization of Treg cells allowed the study of its functional properties (Baecher-Allan et al., 2005). Treg cells proliferate weakly and do not produce IL-2 in vitro; however, a protocol has been developed for the in vitro expansion of Treg cell populations after TCR and CD28 engagement in the presence of very high concentrations of IL-2 (de la Rosa et al., 2004). Adoptive transference experiments suggest that Treg cells can be activated and proliferate in vivo, because the

transfer of relatively small numbers of Treg cells can afford a long-lasting protection against autoimmunity in mice (Thornton and Shevach, 1998), inhibiting proliferation and cytokine production by effector T cells. This effect possibly will be IL-10 and soluble tumour growth factor β (TGF-β) independent (Thornton and Shevach, 1998). Nevertheless, cell surface-associated TGF-β has an important role in the contact inhibition induced by the Treg cells on effector cells (Green et al., 2003). Reverse signalling through cross-linking of B7 (CD80 and CD86) on the cell surface of APCs or activated T cells, mediated by CTLs-associated antigen 4 (CTLA-4) expressed by Treg cells, has been proposed as an additional suppression effector mechanism. The absence of these molecules markedly diminishes the regulatory activity of Treg in graft versus host models (Paust et al., 2004). Additionally, polymorphisms of the gene that codifies for CTLA-4 have been associated with alterations in the number of Treg cells in different models (Atabani et al., 2005).

Besides Treg cells, other types of RTL have the capacity to generate regulatory responses in the periphery, mainly through increasing the secretion of immunosuppressive cytokines like TGF-β and IL-10. These cells can be classified in different cellular subpopulations by surface markers and secreted cytokines (Table 2). T regulatory lymphocytes type 1 (TR1) secrete high amounts of IL-10 when they are stimulated in vitro with dexamethasone or vitamin D3. Another population of RTL is constituted by the TH3 cells that produce high amounts of TGF-β, another cytokine with suppressor properties (O'Neill et al., 2004). Other subsets of $CD4^+$ and $CD8^+$ cells, natural killer T cells, and $\gamma\delta$ T cells also have shown suppressor activity. In the periphery, suppressor T cells generated in response to environmental antigens protect their hosts from immune-mediated tissue injury by the production of immunosuppressive cytokines (O'Neill et al., 2004). Therefore, it is probable that in a physiological setting, two general mechanisms of peripheral suppression are present; on one side, the existence of naturally occurring regulatory T cells generated in the thymus, and on the other side, T cells that acquire a regulatory phenotype as a product of the dynamic balance between immunoregulatory cytokines and the cells expressing distinct levels of surface receptors.

Regulatory T cells and cancer

T lymphocytes that recognize TAA have been identified in blood, lymph nodes and at the tumour site of patients with advanced cancer. Expansion of these anti-tumour T cells in vitro is used to establish tumour-specific T cell lines, which have the ability to destroy tumour cells both in vitro and in vivo (Coulie and Connerotte, 2005). However, spontaneous tumour regression is rare, and although many immunotherapeutic trials have shown their ability to enhance the number of anti-tumour T cells, this strategy does not always coincide with metastasis eradication (Escobar et al., 2005; López et al., 2004; Nestle et al., 1998, 2005). Since the in vitro characterization of Treg cells by co-expression of CD4 and CD25 markers, considerable

Table 2. Phenotypic and functional characterization of regulatory T lymphocyte subpopulations

	Treg	Tr1	Th3	$CD8^+CD28^-$
Marker				
CD25	++	+	+	+
GITR	++	–	?	+
CTLA-4	+++	+	++	+
CD103	++	?	?	+
CD122	++	++	?	?
Foxp3	++	–	?	+
Nrp1	++	?	?	?
CD45 RA	++	?	?	+
CCR 7	++	?	?	–
CD28	+	?	?	–
Cytokine secretion				
IL-10	±	++++	±	+
TGF-β	+	++	++++	–
IL-4	–	–	±	–
IL-5	–	++	?	–
IL-2	–	±	–	–
IFN-γ	±	++	±	+
Differentiation factor (s)	TGF-β	IL-10, IFN-α	TGF-β, IL-10, IL-4, Anti-IL-12	IL-10

indirect evidence about their immunopathological role in the anti-tumour responses has been collected. Patients with different types of cancer have shown an increase of $CD4^+$ $CD25^+$ T cells in peripheral blood, lymph nodes, tumour ascites and tumour tissue (Nomura and Sakaguchi, 2005). Recently, a new melanoma antigen (ARTC1) has been identified as being recognized by a specific-Treg clone established from tumour-infiltrating-lymphocytes of a melanoma patient (Wang et al., 2005). In addition, studies in ovarian carcinoma affected women indicate that Treg cells, present in tumour tissue and ascites liquid, are able to inhibit the anti-tumour responses to Her2/neu-derived antigens, correlating with a reduced survival (Nomura and Sakaguchi, 2005). It is not clear yet whether this inhibitory effect is due to abnormal priming of naive T cells, or a regulatory effect on activated T cells at the tumour site (Nomura and Sakaguchi, 2005).

Recently, Rosenberg and colleagues (Dudley et al., 2005) showed that adoptive transfer of autologous tumour reactive $CD8^+$ T cells after non-myeloablative but lymph depleting chemotherapy generates objective regressions of extensive-vascularized tumour tissues in 50% of patients with refractory metastasic melanoma, including complete responses in 11% of patients. Compared with other T cell transfer therapy trials in absence of non-myeloablative treatment, this study showed significantly better anti-tumour responses. The authors suggested that previous immunoinhibition probably eliminates RTL populations, favouring a less hostile immune system for transfused T lymphocytes, leading to a remarkably improved elimination of major tumour masses (Dudley et al., 2005). Further support to this theory came from studies in murine cancer models and T cell transfer therapy plus IL-2, after immune-inhibition with radiation or chemotherapy, showing a relationship between the depletion of Treg cells and effective anti-tumour responses (Antony et al., 2005). This evidence is very important for melanoma anti-tumour immunotherapy, because it demonstrates the capacity of the immune system to eliminate considerable tumour accumulations in melanoma patients, opening the debate about the central role that the Treg cell population has in the immune-inhibition of anti-tumour responses.

Immunosuppression by drugs

Chemotherapy is a generic expression that includes many drugs that have the capacity to destroy tumour cells. In general, cancer cells are destroyed or their growth is stopped by intervention at different cell cycle checkpoints. Chemotherapy works by killing fast-growing cells, but the drugs cannot discriminate between cancer cells and other fast-growing cells. This lack of specificity generally produces adverse effects with a broad variety and intensity. For example, normal cells can be destroyed causing hair loss or hepatic dysfunction. Different drug combinations have been used in patients with melanoma stage IV; nevertheless, this systemic palliative therapy has not demonstrated a major average survival for patients (Huncharek et al., 2001). Decarbazine (DTIC-Dome®) is an alkylant agent introduced in the 1970s for the treatment of malignant melanoma and it is the only one showing certain objective tumour regressions in approximately 13–20% of treated patients (Huncharek et al., 2001). The majority of observed responses are partial, although durable complete remissions have also been observed (Huncharek et al., 2001). Although many studies use decarbazine combined with other drugs, including the biotherapeutic drugs IL-2 and IFN-γ, they do not show better effects than monotherapy with decarbazine (Huncharek et al., 2001). Recently, a new drug Temozolomide (TMZ) for oral use has been approved by the Food and Drugs Administration (FDA). This drug is converted spontaneously to the active metabolite of decarbazine and shows similar effects to the original drug (Su et al., 2004). A current retroactive analysis of several clinical studies found that an important collateral effect observed during the treatment with decarbazine was lymphopenia (60% of the patients) and the appearance of opportunistic infections, mainly Pneumocystis and Aspergillus pneumonia, indicating lymphocyte dysfunction (Su et al., 2004). In addition, cellular subpopulations showed that $CD4^+$ T lymphocytes were the most affected cellular subtype, whereas no neutropenia or thrombocytopenia was observed (Su et al., 2004). These changes were completely reversed within 3–6 months after treatment finished. Accumulated evidence indicates that palliative treatment of advanced melanoma with chemotherapeutical drugs can affect the $CD4^+$ T lymphocyte compartment, including RTL, making possible to propose the use of a chemotherapeutic protocol before vaccination with DCs loaded with tumour cell lysate for melanoma treatment. This strategy should be implemented with the purpose of eliminating RTL accumulation, creating a propitious atmosphere for the maximal development of the vaccine potential and probably increasing the beneficial effect of clinical immunization for patients (Fig. 2).

Therapeutic perspective

Finally, there is no data concerning the relation between DC-based immunotherapy and activation/proliferation of RTL. Immunizations with TAA, able

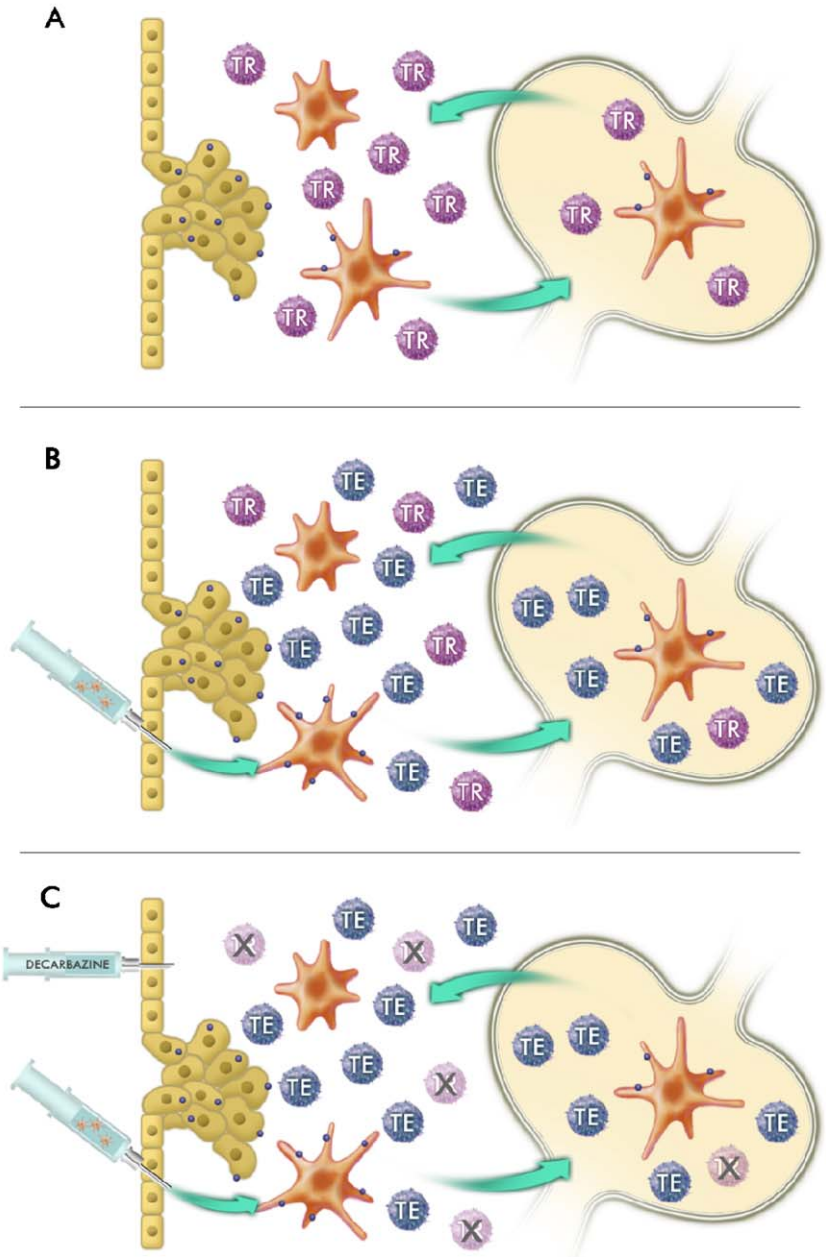

Fig. 2. Model for combination of chemotherapy and DC-based vaccines. (A) Slow tumour growth causes migration of tolerogenic DCs to the lymph nodes where T regulatory cell populations (TR) are induced, inhibiting an effective immune response. (B) DC-based vaccines placed in mature DCs elicit effector T cell (TE) populations specific for tumour antigens; however, pre-existing TR cells limit an efficient immune response. (C) Pre-treatment of patients with decarbazine, an anti-melanoma drug, may eliminate TR cell populations, allowing DC-vaccines to develop a successful immune response against tumour cells.

to induce cytotoxic activity against tumour, may be restricted by RTL populations. Thus, the clinical effectiveness of the induced immune response will be limited (Fig. 2B). Therefore, it is very important to study the presence of RTL populations, before and after vaccines in patients treated with DC-based immunotherapy. In our laboratory, we have initiated studies in this line with samples obtained from 40 treated patients. Accumulated evidence in clinical studies and murine models indicates that pre-treatment with immunoinhibitory drugs may favour the immune response induced by DC therapy, eliminating accumulated regulatory cell populations, improving immunological and clinical responses (Antony et al., 2005; Dudley et al., 2005). Studies in this field will allow us to understand better the RTL role in DC-based immunotherapy and to design therapeutic strategies that will permit us to improve the clinical effectiveness of the induced anti-tumour specific

immune response in patients with melanoma and other cancers.

Acknowledgements

Financed by FONDEF DO2I1088 and Fondecyt 1031005.

References

Alvarez, A., Sanroman, B., Carro, E., Vazquez-Martul, E., 2004. Neoplasia in solid organ transplant recipients: single-center experience. Transplant. Proc. 36, 784–786.

Antony, P.A., Piccirillo, C.A., Akpinarli, A., Finkelstein, S.E., Speiss, P.J., Surman, D.R., Palmer, D.C., Chan, C.C., Klebanoff, C.A., Overwijk, W.W., Rosenberg, S.A., Restifo, N.P., 2005. $CD8^+$ T cell immunity against a tumor/self-antigen is augmented by $CD4^+$ T helper cells and hindered by naturally occurring T regulatory cells. J. Immunol. 174, 2591–2601.

Ardavin, C., Amigorena, S., Reis e Sousa, C., 2004. Dendritic cells: immunobiology and cancer immunotherapy. Immunity 20, 17–23.

Atabani, S.F., Thio, C.L., Divanovic, S., Trompette, A., Belkaid, Y., Thomas, D.L., Karp, C.L., 2005. Association of CTLA4 polymorphism with regulatory T cell frequency. Eur. J. Immunol. 35, 2157–2162.

Baecher-Allan, C., Wolf, E., Hafler, D.A., 2005. Functional analysis of highly defined, FACS-isolated populations of human regulatory $CD4^+$ $CD25^+$ T cells. Clin. Immunol. 115, 10–18.

Banchereau, J., Steiman, R.M., 1998. Dendritic cells and the control of immunity. Nature 392, 245–252.

Bell, D., Young, J.W., Banchereau, J., 1999. Dendritic cells. Adv. Immunol. 72, 255–324.

Benghiat, F.S., Graca, L., Braun, M.Y., Detienne, S., Moore, F., Buonocore, S., Flamand, V., Waldmann, H., Goldman, M., Le Moine, A., 2005. Critical influence of natural regulatory $CD25^+$ T cells on the fate of allografts in the absence of immunosuppression. Transplantation 79, 648–654.

Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M.E., Ferguson, P.J., Whitesell, L., Nelly, T.E., Saulsbury, F.T., Chance, P.F., Ochs, H.D., 2001. The immune dysregulation, polyendocrinopathy, enteropathy, Xlinked syndrome (IPEX) is caused by mutations of FOXP3. Nat. Genet. 27, 20–21.

Boon, T., Cerottini, J.C., Van den Eynde, B., van der Bruggen, P., Van Pel, A., 1994. Tumor antigens recognized by T lymphocytes. Annu. Rev. Immunol. 12, 337–365.

Burnet, F.M., 1970. The concept of immunological surveillance. Prog. Exp. Tumor Res. 13, 1–27.

Coley, W.B., 1991. The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. (1893). Clin. Orthop. Relat. Res. 262, 3–11.

Coulie, P.G., Connerotte, T., 2005. Human tumor-specific T lymphocytes: does function matter more than number? Curr. Opin. Immunol. 17, 320–325.

Dal Maso, L., Tirelli, U., Polesel, J., Franceschi, S., 2005. Trends in cancer incidence rates among HIV-infected patients. Clin. Infect. Dis. 41, 124–126.

de la Rosa, M., Rutz, S., Dorninger, H., Scheffold, A., 2004. Interleukin-2 is essential for $CD4^+CD25^+$ regulatory T cell function. Eur. J. Immunol. 34, 2480–2488.

Dudley, M.E., Wunderlich, J.R., Yang, J.C., Sherry, R.M., Topalian, S.L., Restifo, N.P., Royal, R.E., Kammula, U., White, D.E., Mavroukakis, S.A., Rogers, L.J., Gracia, G.J., Jones, S.A., Mangiameli, D.P., Pelletier, M.M., Gea-Banacloche, J., Robinson, M.R., Berman, D.M., Filie, A.C., Abati, A., Rosenberg, S.A., 2005. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J. Clin. Oncol. 23, 2346–2357.

Escobar, A., López, M., Serrano, A., Ramirez, M., Pérez, C., Aguirre, A., González, R., Alfaro, J., Larrondo, M., Fodor, M., Ferrada, C., Salazar-Onfray, F., 2005. Dendritic cell immunizations alone or combined with low doses interleukin-2 induce specific immune responses in melanoma patients. Clin. Exp. Immunol. 142, 555–568.

Fontenot, J.D., Gavin, M.A., Rudensky, A.Y., 2003. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. Nat. Immunol. 4, 330–336.

Gershon, R.K., Kondo, K., 1971. Infectious immunological tolerance. Immunology 21, 903–914.

Green, E.A., Gorelik, L., McGregor, C.M., Tran, E.H., Flavell, R.A., 2003. $CD4^+CD25^+$ T regulatory cells control anti-islet $CD8^+$ T cells through TGF-β–TGF-β receptor interactions in type 1 diabetes. Proc. Natl. Acad. Sci. USA 100, 10878–10883.

Hori, S., Nomura, T., Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. Science 299, 1057–1061.

Huncharek, M., Caubet, J.F., McGarry, R., 2001. Single-agent DTIC versus combination chemotherapy with or without immunotherapy in metastatic melanoma: a meta-analysis of 3273 patients from 20 randomized trials. Melanoma Res. 11, 75–81.

Jaffee, E.M., Pardoll, D.M., 1996. Murine tumor antigens: is it worth the search? Curr. Opin. Immunol. 8, 622–627.

Kurte, M., Lopez, M., Aguirre, A., Escobar, A., Aguillon, J.C., Charo, J., Larsen, C.G., Kiessling, R., Salazar-Onfray, F., 2004. A synthetic peptide homologous to functional domain of human IL-10 down-regulates expression of MHC class I and transporter associated with antigen processing 1/2 in human melanoma cells. J. Immunol. 173, 1731–1737.

Lawson, D.H., 2004. Update on the systemic treatment of malignant melanoma. Semin. Oncol. 4, 33–37.

López, M., Escobar, A., Alfaro, J., Fodor, M., Larrondo, M., Ferrada, C., Salazar-Onfray, F., 2004. Advances in cellular immunotherapy for malignant melanoma. Rev. Med. Chile 132, 1115–1126.

Malek, T.R., Yu, A., Vincek, V., Scibelli, P., Kong, L., 2002. CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rβ-deficient mice. Implications for the nonredundant function of IL-2. Immunity 17, 167–178.

Matsuda, M., Salazar-Onfray, F., Petersson, M., Masucci, G., Hansson, J., Pisa, P., Zhang, Q-J., Masucci, M.G., Kiessling, R., 1994. Interleukin-10 pre-treatment protects target cells from tumor- and allo-specific cytotoxic T-cells and down-regulates HLA class I expression. J. Exp. Med. 180, 2371–2376.

Medina, E., Kaempffer, A.M., 2001. Cancer mortality in Chile: epidemiological considerations. Rev. Med. Chile 129, 1195–1202.

Moller, G., 1988. Do suppressor T cells exist? Scand. J. Immunol. 27, 247–250.

Nestle, F.O., Alijagic, S., Gilliet, M., Sun, Y., Grabbe, S., Dummer, R., Burg, G., Schadendorf, D., 1998. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat. Med. 4, 328–332.

Nestle, F.O., Farkas, A., Conrad, C., 2005. Dendritic-cell-based therapeutic vaccination against cancer. Curr. Opin. Immunol. 17, 163–169.

Nomura, T., Sakaguchi, S., 2005. Naturally arising $CD25^+CD4^+$ regulatory T cells in tumor immunity. Curr. Top. Microbiol. Immunol. 293, 287–302.

O'Neill, E.J., Sundstedt, A., Mazza, G., Nicolson, K.S., Ponsford, M., Saurer, L., Streeter, H., Anderton, S., Wraith, D.C., 2004. Natural and induced regulatory T cells. Ann. N. Y. Acad. Sci. 1029, 180–192.

Paust, S., Lu, L., McCarty, N., Cantor, H., 2004. Engagement of B7 on effector T cells by regulatory T cells prevents autoimmune disease. Proc. Natl. Acad. Sci. USA 101, 10398–10403.

Petersson, M., Charo, J., Salazar-Onfray, F., Noffz, G., Mohaupt, M., Qin, Z., Klein, G., Blankenstein, T., Kiessling, R., 1998. Constitutive IL-10 production accounts for the high NK sensitivity, low MHC class I expression, and poor transporter associated with antigen processing (TAP)-1/2 function in the prototype NK target YAC-1. J. Immunol. 161, 2099–2105.

Porgador, A., Gilboa, E., 1995. Bone marrow-generated dendritic cells pulsed with a class I-restricted peptide are potent inducers of cytotoxic T lymphocytes. J. Exp. Med. 182, 255–260.

Rossi, M., Young, J.W., 2005. Human dendritic cells: potent antigen-presenting cells at the crossroads of innate and adaptive immunity. J. Immunol. 175, 1373–1381.

Sakaguchi, N., 2005. Naturally arising Foxp3-expressing $CD25^+CD4^+$ regulatory T cells in immunologic tolerance to self and non-self. Nat. Immunol. 6, 345–352.

Salazar-Onfray, F., 1999. Interleukin-10: a cytokine used by tumors to escape immunosurveillance. Med. Oncol. 16, 86–94.

Salazar-Onfray, F., Petersson, M., Franksson, L., Matsuda, M., Blankenstein, T., Karre, K., Kiessling, R., 1995. IL-10 converts mouse lymphoma cells to a CTL-resistant, NK-sensitive phenotype with low but peptide-inducible MHC class I expression. J. Immunol. 154, 6291–6298.

Salazar-Onfray, F., Charo, J., Petersson, M., Freland, S., Noffz, G., Qin, Z., Blankenstein, T., Ljunggren, H.G., Kiessling, R., 1997. Down-regulation of the expression and function of the transporter associated with antigen processing in murine tumor cell lines expressing IL-10. J. Immunol. 159, 3195–3202.

Sallusto, F., Lanzavecchia, A., 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. J. Exp. Med. 179, 1109–1118.

Schubert, L.A., Jeffery, E., Zhang, Y., Ramsdell, F., Ziegler, S.F., 2001. Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. J. Biol. Chem. 276, 37672–37679.

Schwartz, R.H., 2005. Natural regulatory T cells and self-tolerance. Nat. Immunol. 6, 327–330.

Shuler, G., Shuler-Thurner, B., Steinman, R.M., 2003. The use of dendritic cells in cancer immunotherapy. Curr. Opin. Immunol. 15, 138–147.

Steinbrink, K., Graulich, E., Kubsch, S., Knop, J., Enk, A., 2002. $CD4^+$ and CD8 anergic T cells induced by interleukin-10-treated human dendritic cells display antigen-specific suppressor activity. Blood 99, 2468–2476.

Su, Y.B., Sohn, S., Krown, S.E., Livingston, P.O., Wolchok, J.D., Quinn, C., Williams, L., Foster, T., Sepkowitz, K.A., Chapman, P.B., 2004. Selective $CD4^+$ lymphopenia in melanoma patients treated with temozolomide: a toxicity with therapeutic implications. J. Clin. Oncol. 22, 610–616.

Svane, I.G., Soot, M.T., Buus, S., Johnsen, H.E., 2003. Clinical application of dendritic cells in cancer vaccination therapy. APMIS 111, 818–834.

Thornton, A.M., Shevach, E.M., 1998. $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J. Exp. Med. 188, 287–296.

Walker, L.S., 2004. $CD4^+$ $CD25^+$ Treg: divide and rule? Immunology 111, 129–137.

Wang, H.Y., Peng, G., Guo, Z., Shevach, E.M., Wang, R.F., 2005. Recognition of a new ARTC1 peptide ligand uniquely expressed in tumor cells by antigen-specific $CD4^+$ regulatory T cells. J. Immunol. 174, 2661–2670.

Yan, J., Mamula, M.J., 2002. Autoreactive T cells revealed in the normal repertoire: escape from negative selection and peripheral tolerance. J. Immunol. 168, 3188–3194.