[Frontiers in Bioscience 13, 2275-2289, January 1, 2008]

The role of T-regulatory cells in pregnancy and cancer

Jacek R. Wilczynski [1, 2] , Jaroslaw Kalinka[3] , Michal Radwan[4]

[1] Department of Gynecological Surgery, Polish Mother's Health Center Research Institute, Lodz, Poland, [2] 3rd Chair and Department of Obstetrics and Gynecology, Medical University, Lodz, Poland, [3] Medical and Environmental Pregnancy Health Hazards Unit, Department of Perinatology, 1st Chair of Gynecology and Obstetrics, Medical University, Lodz, Poland [4] Department of Surgical and Endoscopic Gynecology, Polish Mother's Health Center Research Institute, Lodz, Poland

TABLE OF CONTENTS

1. ABSTRACT

The acceptance of paternally-derived alloantigens during pregnancy and escape from host immunosurveillance by cancer are based on similar immunological mechanisms. Among them both natural and peripherally-induced T CD4+CD25+Foxp3+ and Tr1 regulatory cells (Tregs) play important role. Interactions of Tregs with other immunocytes including dendritic cells, mechanisms of Tregs recruitment and their suppressive properties in cancer and pregnancy have been presented in this paper. Despite the fact that mechanisms of Treg regulation are still in progress, there is a hope for use of Tregs-related immunotherapy in clinical practice, and the first attempts of such management have already been described. However, more information about the function of Tregs cells is needed to provide safe treatment devoid of potential side-effects. Resolving the secrets of Tregs cells will probably offer new options of cancer treatment and will help to improve the management of pregnancy failure.

2. INTRODUCTION

During normal pregnancy maternal immune system tolerates the fetal tissues despite of the presence of paternal alloantigens on the surface of trophoblast. It was Medawar, who first proposed that growing fetus should be viewed as allotransplant (1), and many investigations considering both physiological and pathological course of pregnancy seem to support that hypothesis (reviewed in 2). Since that time the possible mechanisms of fetal allograft escape from maternal immunosurveillance have been extensively discussed. After years of considering pregnancy as a state of systemic maternal immune suppression, the time has come to define it as a precisely regulated state of tolerance dependent on many cooperating mechanisms, of preferentially local (maternal-fetal interface) area of action. Based on both murine and human studies there were several mechanisms proposed to contribute to that tolerance: inactivation of NK cells through HLA-G expressed on trophoblast (3), depletion of

tryptophan by indoleamine 2,3-dioxygenase (IDO)-dependent mechanism (4, 5, 6), heme oxygenase (HO)-1 expression on decidual and placental tissues (7, 8), increased trophoblast-induced apoptosis of maternal effector cells (9), the presence of asymmetric blocking-antibodies (10), and an adequate Th1/Th2 cytokine cross-talk (11, 12, 13). Recent data are also strongly suggestive, that placenta-derived exosome particles present in the maternal circulation could regulate immune activation by mediating activation-induced apoptosis of maternal reactive lymphocytes (14).

Cancer developing in the host environment originates from "self" tissues, however, during development it acquires new genetic and phenotypic characteristics due to mutations. Genetic instability can produce immunogenic antigens either by the over-expression of cell proteins or by the expression of mutated proteins (15). These antigens (called tumor-associated antigens - TAA) provide a target for effector immune defense mechanisms of the host. Despite of this fact, in most situations cancer is able to escape from the host immunosurveillance and to grow and metastatize succesfully. There are several mechanisms that could be responsible for this phenomenon: total or selective loss of HLA class I molecules (16), surface expression of HLA-G antigen (17), tumor-mediated shedding of MHC class I related molecules (18), shift towards Th2-type cytokine activity produced by host immune cells or tumor itself (19), and up-regulation of the surface Fas ligand on cancer cells leading to enhanced apoptosis of effector immune cells (20). It was also shown that depletion of tryptophan by tumor-derived IDO could make tumor's cells resistant to immune attack (21). IDO-high expression was associated with a significant reduction of tumor T CD3+ infiltrating cells and correlated with the frequency of liver metastases and decreased patient survival (22). Heme oxygenase-1 protects tumor cells against hypoxic stress, showing anti-apoptotic and anti-inflammatory properties (23, 24), and controls cancer cells proliferation and tumor angiogenesis, which was indicated by studies performed on animal and human tumors (25, 26, 27). Both tumor cells and tumor-associated macrophages indicated HO-1 expression (28, 29, 30, 31). Increased HO-1 expression in tumor microenvironment could account for its progressive growth. Knockdown of HO-1 expression led to cancer growth inhibition and made the tumor cells more sensitive to chemo- and radiotherapy (32). The interplay between IDO and HO-1 produced by immunocytes or tumor itself could either stimulate or inhibit the tumor growth (33). Tumor-derived exosome-like particles released from cancer cells membranes are also capable of suppression T cell signaling in anti-tumor effector lymphocytes leading to their apoptosis (34).

An interesting issue is that both pregnancy and cancer share similar mechanisms of escape from host immunosurveillance (35). However, none of the above mentioned mechanisms could profoundly explain the observed down-regulation of maternal alloreactive immunocompetent cells or "host consent" for cancer progression. The acceptance of paternally-derived tumor cells by pregnant animals (36) suggests the possibility of systemic regulation involvement during pregnancy, which could be due to T regulatory cells (Tregs). Although tumor cells have antigens altered by mutation, antigenic similarity to self-somatic cells engages also systemic regulation of the peripheral tolerance maintained by Tregs. Treg cells belong to naturally occurring T CD4+CD25+^{high} cells, peripherally induced T CD4+CD25+^{high} population, and Tr1 IL-10+ cells. They all constitute an interesting mechanism of immunological escape which has been extensively studied in recent years both in pregnancy and cancer. Understanding of immunological phenomena which evolved in mammals in order to recognize alloantigens or changed self tissues might help to create new techniques of cancer treatment and to improve management of pregnancy complications.

3. THE ROLE OF T CD4+CD25+^{HIGH}FOXP3+ CELLS IN CANCER PROGRESSION

3.1. Mechanisms of Tregs recruitment and function in cancer

Development of cancer and poor prognosis in tumor-bearing patients is accompanied by accumulation of natural Treg cells in peripheral blood, draining lymph nodes and tumor itself (37, 38, 39, 40, 41, 42, 43, 44, 45, 46). Regulatory T Foxp3+ cells seem to be dominant bad prognostic factor even if high expression of IFN-gamma was identified (47). Regulatory T cells capable of abrogating anti-tumor reactivity are also primed peripherally together with effector T cells in the same lymphatic nodes during tumor progression. This Treg population indicates high expression of CD62L neccessary for Treg recruitment into lymphoid nodes, as well as very late antigen-4 (VLA-4) and lymphocyte function-associated antigen-1 (LFA-1) which both play role in trafficking T cells into inflammatory sites (48). Human CD4+CD25+ Tregs also preferentially move towards and accumulate in tumors. These are probably chemokine (C-C motif) ligand 22 (CCL22) or IL-16 produced by tumor cells and tumor-infiltrating macrophages which mediate trafficking of Tregs into the tumor tissue, ascites and pleural effusions (37, 49).

Acceleration of tumor growth and metastasis spreading depends on the immunosuppressive effects of T CD4+CD25+^{high} cells which can promote inhibition of antigen-specific T CD4+CD25- and cytotoxic T CD8+ cells, natural-killer T cells (NKT) and NK cells (48, 50). *In vitro* studies performed on human cells revealed that Treg cells could abrogate cytotoxicity of NK cells against target cell lines K562 and GIST882 (51) and inhibit IL-12-dependent secretion of IFN-gamma by NK cells (52). This inhibitory capability of Tregs maintains even after fixation of cells in formaldehyde suggesting an involvement of membrane-bound molecule. Using of anti-TGF-beta blocking antibodies results in restoration of NK cell cytotoxicity and IFN-gamma secretion potential (52, 53), and Treg cells isolated from TGF-beta knockout mice were unable to suppress NK cell-mediated cytotoxicity (54). Therefore, it is reasonable to conclude that membrane-bound TGF-beta on Tregs surface is responsible for NK cell inhibition probably by NKG2D downregulation (52).

Data showing that downregulation of NKG2D expression on NK cells is a bad prognostic factor in patients with colon (55) cancer seem to confirm the presented mechanism. In cancer patients higher Treg numbers could predict ineffective NK cell induction induced by immunotherapy (54). Depletion of T CD4+CD25+ regulatory cells by administration of anti-CD25 monoclonal antibody reverses tolerogenic state and enables effective tumor rejection (56, 57). The same result could be obtained by use of cyclophosphamide which selectively kills Tregs or inhibits their function (58, 59). It was shown on the murine model of leukemia (60) that depletion of Tregs before tumor inoculation augmented anti-tumor response by promoting generation of cytotoxic NK and T CD8+ cells.

3.2. Peripheral Tregs interactions in cancer

Circulating in blood Treg cells belong to thymically-derived natural T CD4+CD25+^{high} population, but those seen inside lymphatic nodes and tumor tissues result from local proliferation dependent on TGF-beta signalling (52, 61). Increased levels of cancer-derived TGF-beta or alternatively of TGF-beta cancer-induced secretion in tumor environment were found to augment tumor growth and to enhance escape from host immunosurveillance (62, 63, 64, 65). The presence of endometrial bleeding associated factor TGFB4 (ebaf), a member of TGF-beta superfamily, was confirmed in adenocarcinomas with mucinous differentiation (66). Because TGF-beta promotes Treg cell generation, it is suspected that tumor itself is capable of stimulating Treg cell activity. This possibility was confirmed by the observation that Foxp3-expressing Treg cells were increased in patients with active cancer and decreased during remission (67, 68). Another possibility concerns promotion of Tregs by TGF-beta produced by a subset of pro-tolerogenic iDCs present in tumor or in draining lymph nodes (54). It was found that progressive tumors contained increased numbers of CD4-8- tumor-infiltrating dendritic cells (TIDCs), which secreted a marked levels of TGF-beta, thus leading to immune suppression and tumor progression. In contrary, regressive tumors contained CD4+8+ and CD4+8- TIDCs which were able to express high levels of IFN-gamma and IL-6, and could stimulate Th1-type cytokine responses and effective cytotoxic lymphocyte (CTL) anti-tumor responses (69). Dendritic cells are able to present MHC class I-restricted antigens captured from apoptotic tumor cells and initiate cytotoxic T CD8+ anti-tumor responses. Therefore an impaired TIDCs function could not only stimulate Treg activity but also could be at least in part responsible for defective T CD8+ cell function. Interleukin-10 is probably responsible for augmentation of these effects, because in IL-10-knockout mice restoration of TIDCs and T CD8+ anti-tumor functions was successfuly obtained (70).

An additional mechanism of Treg regulation by DCs engages CD200/CD200-receptor pathway. Molecule CD200 is a type Ia membrane protein related to CD80/CD86 family of costimulatory molecules, exerting its suppressive action through specific CD200 receptor (CD200R). Both CD200 and CD200R is expressed on monocyte-derived dendritic cells. Stimulation of CD200R on DCs created tumor-promoting milieu by shifting cytokine secretion into Th2 profile and by induction of Treg cells. Blocking CD200/CD200R pathway using monoclonal anti-CD200 antibodies augmented Th1 responses directed against leukemic cells. Moreover, tumor itself could contribute to regulation of host defense by up-regulation of CD200 molecules on leukemic cells (71, 72). Clinical observations confirm the importance of CD200 molecule for tumor development as in multiple myeloma patients high CD200 expression on cancer cells was found to be an unfavorable prognostic factor for progression-free survival (73).

Induced Tregs could in turn modify function of DCs in tumor environment. The CTLA-4-dependent Treg-DCs interactions could effectively down-regulate anti-tumor immunity in IDO-dependent manner. Blocking of CTLA-4-dependent pathway resulted in reduced Treg activity (38). The presence of IDO-expressing DCs was confirmed in tumor-draining lymph nodes from patients with melanoma, breast, colon, lung and pancreatic cancers (74), while the number of IDO-positive cells infiltrating peri-tumoral tissues was found to correlate with poor patient survival in non-small cell lung cancer (75). As confirmation of IDO importance for anti-cancer immunity low serum median tryptophan concentrations were found in melanoma patients who subsequently died because of tumor progression (76). Apart from DCs, the cancer cells themselves could express IDO which were capable of modulating Treg-DC-effector cells interactions. In endometrial cancer high IDO expression in tumor cells was found to correlate with overall and progression-free survival (77). Moreover, prostaglandin-E2 in the tumor environment might influence IDO expression in peri-tumoral DCs (78) (Figure 1).

4. THE ROLE OF T CD4+CD25+^{HIGH}FOXP3+ CELLS IN PREGNANCY

4.1. Distribution of Tregs during pregnancy

Regulatory T cells are necessary component of maternal immune tolerance of paternal alloantigens present on trophoblast. Syngenic pregnancies in mice proceeded normally in Treg-depleted animals, but ended unfavorably in allogenically mated parents, suggesting not only that Treg cells were indispensable component of pregnancy, but also that they did not suppress female reaction directed towards male minor histocompatibility antigens (79). In fact we are still not sure what kind of antigen is recognized by Tregs in pregnancy. They might recognize maternal self antigens on semi-allogeneic trophoblast and then suppress alloreactive effector cells by mechanism of “bystander suppression”. As isolated CD4+CD25+^{high} Treg cells expressing Foxp3 antigen were capable of suppression of effectory responses to allogeneic dendritic cells (80), the possibility of direct alloantigen recognition by Tregs could not be excluded (79, 81). This supposition is confirmed by observation that transferring of CD4+CD25+ Treg cells into abortion-prone mice could prevent abortion but only when Treg cells originated from normal pregnant mice, suggesting that exclusively Treg cells exposed previously

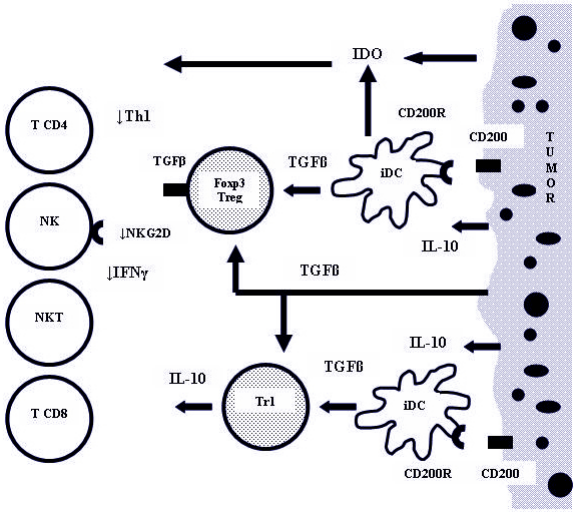

Figure 1. Participation of T CD4+CD25+Foxp3+ Treg cells and T CD4+IL-10+ Tr1 cells in tolerance induction existing locally in tumor environment. Immature dendritic cells (iDC) activated by tumor-derived CD200 molecule (via CD200R receptor) and tumor-secreted interleukin-10 (IL-10) are capable of enhancing both the T CD4+CD25+Foxp3+ Treg (Foxp3 Treg) and type 1 regulatory T (Tr1) cells through secretion of transforming growth factor-beta (TGFβ). Another source of TGF-beta is the tumor itself. The interaction between T CD4+CD25+Foxp3+ Tregs and iDCs increases indoleamine 2,3-dioxygenase (IDO) expression on iDCs. The tumor cells themselves also can express IDO. Enhanced IDO expression causes tryptophan deprivation and inhibits proliferation of host effector cells. Similarly, IL-10 secreted by Tr1 lymphocytes downregulates anti-tumor cytotoxic responses. Activated T CD4+CD25+Foxp3+ Tregs via cell-cell contact, mediated by membrane-bound TGF-beta, promote inhibition of antigen-specific T CD4+ Th1-cytokine producing cells (T CD4), cytotoxic T CD8+ cells (T CD8) and natural killer T cells (NKT). They also inhibit natural killer cells (NK) by NKG2D receptor downregulation and decrease their potential to interferon-gamma (IFNγ) production.

to paternal alloantigens could exert regulatory activity (82, 83). The treatment of female mice with an anti-CD25 antibody before mating resulted in depletion of Tregs and fetus rejection (84, 85).

In pregnant mice the proportion of CD4+CD25+ Treg cells increased significantly in the spleen, uterine-draining lymph nodes and peripheral blood, starting from the early post-implantation period. Correspondingly, in human pregnancy CD4+CD25+^{high} Treg cells contributed to 8% of peripheral blood and to 20% of decidual CD4+ T cells respectively (86, 87). In pregnant women an increase in circulating Treg cells during early pregnancy, reaching the highest level in the second trimester and then declining post partum was observed. The percentage of T CD4+CD25+^{high} cells was increased in peripheral blood of patients having normal pregnancy compared to women with spontaneous abortion and non-pregnant subjects (88). Moreover, the percentage of Tregs as well as the expression of CTLA-4 on their surface was higher inside decidua than in blood, but this was not seen in the case of spontaneous abortion (88, 89, 90). The percentage of T CD4+CD25+ regulatory cells decreases inside decidua of pregnant women with spontaneous vaginal delivery compared to patients having elective caesarean section without contractions (91) suggesting their possible role not only in sustaining the pregnancy but also in the regulation of labour.

Treg cells could be preferentially recruited or alternatively induced locally inside maternal-fetal interface (82). Preferential recruitment of Treg cells into maternal-fetal interface may depend on expression of chemokine receptor CCR4 on their surface, as it was confirmed that the chemotactic factor for CCR4, thymus and activation-regulated chemokine (TARC) is expressed on trophoblast, endometrial cells and uterine epithelial cells (92). Another candidate for regulator of Tregs recruitment into maternal-fetal interface was chemokine receptor CCR5. Regulatory T CCR5+ cells preferentially accumulated inside gravid uterus where they were enhanced by contact with paternally-derived alloantigens (93). The most important sites of Treg-mediated suppression in pregnancy are probably both uterine-draining lymph nodes and decidua (79, 85).

4.2. Regulation of Tregs function in pregnancy

Expansion of Tregs during pregnancy probably depends on hormonal changes, alterations in the expression of co-stimulatory molecules or alterations of DC function (79). It was shown that estradiol was able to enhance the proliferation of T CD4+CD25+ cells in response to CD3/CD28 activation *in vitro*, which was dependent on the presence of estrogen receptor-alpha (ER-alpha) on Treg cells surface (94). Estradiol together with TCR stimulation amplified Foxp3 expression on T CD4+CD25- cells, converting them into Treg phenotype. Pregnancy causes similar level of Foxp3 expression as *in vitro* treatment of T CD4+CD25- cells with estradiol, suggesting that high estrogen levels during pregnancy may help to maintain fetal tolerance (95). Moreover, there is evidence that 17-beta-estradiol could directly induce expression of IDO on monocyte-derived DCs, thus limiting T cell proliferation (96). It was also found that both estradiol and progesterone could influence cytokine production by DCs, converting them into Th2 cytokine profile represented by secretion of IL-10, and thus influencing pro-suppressor interactions with Tregs (97, 98, 99). Suppressor T CD8+ lymphocytes possessing progesterone receptor and secreting IL-10 and progesterone-induced blocking factor (PIBF), which low urine concentration is connected to early pregnancy failure (100), were shown to be another population of hormonally-dependent modulatory cells (101), however , their role in interactions with CD4+CD25+^{high} Tregs needs to be elucidated.

The importance of CD80/CD28 and CD80/CTLA-4 interactions on T cell suppressor activity was confirmed in animal studies performed on abortion prone CBA/J x DBA/2 mouse matings. Compared to

normal pregnant mice abortion-prone CBA/J females showed increased frequency of IFNγ-producing anti-paternal T cells in decidua and diminished number of T CD4+/CD25+ as well as IL-10+ Treg cells in thymus (102). Maternal tolerance for the paternal alloantigens could be successfully achieved either by administration of anti-CD86 monoclonal antibodies to CBA/J females or the adoptive transfer of Tregs from normal pregnant mice (102, 103, 104, 105). This management resulted in up-regulated expression of IL-10 and CTLA-4, and down-regulated expression of IFN-gamma, IL-2 and CD28 on recipient's T cells, confirming that CTLA-4/CD80 interactions in the absence of CD86 expression could preferentially restrict T cell activation and Th1 cytokine secretion (104, 105). Therefore, the level of expression of co-stimulatory molecules, especially CTLA-4, could influence the maternal immune response towards the fetus (103).

4.3. Tregs - DCs interactions in pregnancy

Alterations of DCs function in the course of pregnancy are also considered as a strong candidate for regulator of Tregs activity. Morphometric analysis performed during mouse pregnancy confirmed the presence of DCs inside decidua and indicated that the average density of decidual DCs at early pregnancy was significantly higher compared to mid-pregnancy and late gestation or to non-pregnant state. At early pregnancy DCs were concentrated adjacent to the luminal epithelium, whereas later on, were randomly distributed in the stroma (106). Differences of DCs localization could reflect their functional importance for pregnancy maintenance. Comparison between immature vs. mature DCs showed that in mice, the number of mature DCs found inside decidua at the time of implantation is decreased, however, in pregnancies in abortion-prone matings exposed to stress an increase of mature DCs was observed. Immature DCs through decreased intercellular adhesion molecule-1 (ICAM-1)/LFA-1 interactions were capable of suppressing effector T cells, and by increasing IDO and IL-10 secretion might in turn favour Treg cells (107). During maturation DCs strongly up-regulated co-stimulatory molecules CD80 and ICAM-1, which by interaction with their ligands CD28 and LFA-1 on T cells provided crucial signal for T cell activation. Changed ICAM-1/LFA-1 cross talk was responsible for increased recruitment of pro-inflammatory cells to implantation site and subsequent Th1 polarization and pregnancy failure (107). Recent data demonstrated that IDO expression on both peripheral blood and decidual DCs after treatment with CTLA-4/Fc fusion protein or IFN-gamma is up-regulated in normal pregnancy, whereas decreased in spontaneous abortion (108). Defective CD86 expression on DCs and inefficient IFN-gamma secretion by DCs activated by CTLA-4/Fc protein were observed and could account for pregnancy failure (108). Immature DC cells have been described inside decidua during normal early pregnancy in humans (109). Immature IL-10-producing DCs are capable of suppressing T cells by IDO expression, Treg up-regulation and induction of Th2 cytokines production in the mechanism of "bystander suppression" (107, 110). Autoregulation of DCs by IDO stimulates their regulatory function by enhancement of secretion of IL-10 and TGF-beta (4). Decreased levels of IDO were observed in mice with increased fetal losses (111) and administration of IDO inhibitor (1-methyl-tryptophan) to pregnant mice provoked abortion (4). In humans expression of IDO was found to be 10-fold higher in DCs of pregnants when compared to controls (112). The presence of IDO expression in human invasive extravillous trophoblast suggested that it could participate in protection of the fetus by down-regulating local maternal T responses (113). However, the precise role of IDO during pregnancy should be clarified as allogenically mated IDO-knockout mice did not show a decrease in the size of pups (114). Similarly, like in cancer, CD200/CD200R pathway could influence Treg function inside maternal-fetal interface. Decidual DCs were shown to bear CD200R molecules, and could mediate Treg function in IDO-dependent mechanism, thus preventing abortions (115, 116). They were also capable of modulating function of other T cell populations as it was shown that male lymphocyte immunization of aborting mice stimulated female γδT CD4 cells for secretion of Th2 cytokines through the mediation of pro-tolerance molecule CD200. Its role was confirmed in investigations which showed that loss of CD200 from stored overnight lymphocytes resulted in ineffective immunotherapy (117).

5. THE ROLE OF Tr1 CELLS IN PREGNANCY AND CANCER

5.1. Tr1 cells in pregnancy

The data concerning the role of type 1 regulatory T cells (Tr1) in the maintenance of pregnancy are scarce, however, such connections have been suggested by studies performed both on animals and humans. Compared to abortion-prone mice, normal pregnant mice presented with increased populations of IL-10+ Treg cells capable of inhibiting proliferation and IFNγ secretion of effector cells (102). In normal pregnant women decidual gamma/delta T cells expressed IL-10 and TGF-beta, and thus had the potential to induce the differentiation of Th0 TCRαβ+ cells into regulatory/suppressor Tr1 and Th3 cells (118). This mechanism did not operate inside maternal-fetal interface of recurrent aborters, due to improper activation of cytotoxic NK cells that could unfavourably influence gamma/delta T cell function (119). Contradictory data indicating that lowered levels of IL-10 were reported in peripheral blood lymphocytes of recurrent abortion patients (120), while in IL-4/IL-10 knockout mice pregnancy resulted in healthy normal pups (121), rise the questions concerning the true role of IL-10 in pregnancy success as well as the role of IL-10-producing Tr1 cells. The function of Tr1 cells in pregnancy awaits precise elucidation.

5.2. Tr1 cells in cancer

The role of Tr1-mediated activity for progression of cancer has been proved by many studies. Regulatory Tr1 cells can be isolated from tumor-infiltrating lymphocytes in the B16 melanoma model. Transfer of this cells facilitated tumor growth in syngenic mice and injection of IL-10 in the tumor site promoted tumor progression (reviewed in 122). Subcutaneous inoculation of melanoma cells in murine model of anti-cancer immunity resulted in accumulation of large number of CD4+ Th3 and CD4+ Tr1

regulatory cells producing TGF-beta and IL-10 and thus collapsing local anti-tumor response represented by cytotoxic T CD8+ and NK cells (123). It was suggested that both DCs and gamma/delta T cells could be involved in recruitment and proliferation of Tr1 and Th3 Tregs. Removal of these Treg cell populations from tumor environment might be conceivable way to augment anti-tumor defense (123). The tumor cells themselves are capable of directing host immunity into tolerogenic state. It was shown that myeloma cell lysates affected DCs function by forcing them to production of large amounts of IL-10, but no IL-12. Interleukin-10 inhibits the stimulatory capacity of DCs through down regulation of MHC class II antigens and the costimulatory CD80/CD86 molecules, thus inhibiting DC maturation and polarizing DCs to tolerogenic rather than stimulatory activity (124). Such stimulated DCs generated further IL-10-producing T cells of Tr1 profile (125). Murine studies proved that immature TGF-beta-secreting CD4-CD8- immature DCs promoted expansion of Tr1 regulatory cells which in turn, when transferred into other mice, could enhance tumor growth. Using of IL-10 -/- T cells or depleting Tr1 cell population restored anti-tumor immunity (126). It has been also revealed that exposure to an adenoviral vector expressing a prostate specific antigen (PSA) (127) or COX-2-overexpressing tumors induce a Tr1 response, which is mediated by tumor-exposed, IL-10-enhanced DCs (128). Interleukin-10 secretion by both malignant and reactive cells is thought to be important in the pathogenesis of Hodgkin lymphoma (HL) (129). HL-infiltrating lymphocytes contained large populations of IL-10-secreting CD4+ Tr1, CD8+ Tr1 and CD4+CD25+ regulatory T cells which provided a profoundly immunosuppressive environment and were highly responsible for the ineffective immune clearance of HL cells (130). The importance of Tr1-dependent regulation was supported by observation that neutralization of IL-10 ameliorated HL progression (131). Recently bladder carcinoma was found to be a Tr1 dominated tumor, as was shown by the presence of TGF-beta and IL-10 mRNA copies (132). Tr1-derived interleukin-10 could play an important role in the immunosuppression of anti-cancer immunity (133). Elevated levels of IL-10 correlated with different types of tumors as well as with an unfavorable prognosis in patients (134). However, it seems that situation differs depending on tumor type, and in some hematological malignancies like chronic lymphatic leukemia (CLL), expression of IL-10 has been associated with a favorable outcome (135). Similarly, studies on murine glioma model indicated that IL-10 may have protective role against tumor by enhancing CTL or NK cell function (reviewed in 122). Furthermore, transgenic mice constitutively expressing high levels of IL-10 are also not impaired in their ability to clear allogenic tumors (136). Therefore, the precise role of Tr1 cells and IL-10 in creating pro-tumoral environment needs further investigations (Figure 1.).

6. TREG-RELATED IMMUNOTHERAPY IN PREGNANCY AND CANCER

6.1. Treg-related immunotherapy in pregnancy

The role of Treg cells in maintaining pregnancy and promoting the tumor growth investigated both in mice and humans gives rise to the question concerning the possibilities of manipulating Treg function for treatment of pregnancy failures and in anti-cancer therapy.

The attempts of immunotherapy influencing Tregs during pregnancy concentrated either on transferring Tregs into abortion-prone animals or on modulating and potentiation of the function of existing Tregs by DCs. The adoptive transfer of Treg cells from normal pregnant mice into abortion-prone females was proved to significantly diminish the abortion rate (82) by expanding both the peripheral and thymic Treg populations (83). The precise mechanism of such treatment is still obscure, however, it does not seem that it is based simply on inhibition of maternal cytotoxic and Th1 responses, as decidual levels of IFN-gamma and TNF-alpha were not diminished after therapy. Instead of inhibition, the creation of immuno-tolerant environment represented by up-regulation of leukemia inhibitory factor, TGF-beta and HO-1 at the fetal-maternal interface was confirmed (83). Several reports suggested that both murine and human CD4+CD25+Foxp3+ Tregs could be multiplied *in vitro* from CD4+CD25- T cells cultured in the presence of IL-2 (137, 138) or by retroviral transduction of CD4+CD25- T cells with Foxp3 (139, 140). Obtained during such *in vitro* manipulation Tregs could be successfully used *in vivo*. Alternative way of Treg-mediated immunotherapy investigated in abortion prone CBA/J x DBA/2 matings used transfer of anti-CD80 or anti-CD86-treated paternal T cells from DBA/2 males into CBA/J females. Modified paternal T cells had suppressive properties directed towards maternal effector T lymphocytes suggesting improved Treg activity (103). Therapy employing DCs also proved to diminish the pregnancy failure rate in animal CBA/J x DBA/2 abortion model. It was shown that syngeneic DC therapy resulted in an increase in T CD8 suppressors and gammadelta T cells as well as in up-regulation of TGF-beta and PIBF expression at the maternal-fetal interface (141). However, another contradictory results indicated that IDO and IL-10 co-expressing DCs were not capable of suppressing allogeneic T cell responses (142). Therefore, DC-mediated immune modulation in pregnancy is still the matter of future, similarly like pharmacologically targeted therapies to modify metabolic pathways of Foxp3 expression (143). The connection between therapeutic use of intravenous immunoglobulins (IVIG) or paternal lymphocyte immunotherapy in women suffering from recurrent spontaneous abortions and Treg cells function also awaits for solution.

6.2. Treg-related immunotherapy in cancer

Modifying the Treg number and function for establishing regression of cancer has been studied more extensively than in pregnancy. Depletion of CD4+CD25+ Tregs promotes the generation of anti-tumor immune responses and tumor rejection in murine model (144). Combined with IL-12 therapy it enhanced the NKG2D pathway of NK cell activation and promoted NK cell-mediated tumor suppression in mice (52). Enhanced anti-tumor effects could be also obtained using Treg elimination with administration of IFN-gamma (145). Because proliferation and maintenace of CD4+CD25+ Tregs is IL-2

dependent, administration of anti-IL-2 antibodies together with IL-15 (which stimulates effector anti-tumor responses without stimulating Treg activity) enhanced anti-tumor immunity in mice (146). Analogically in patients with colorectal cancer depletion of peripheral blood Tregs reversed tolerogenic state and increased effector T cell responses and IFN-gamma release directed against tumor-associated antigens (41). Elimination of Treg cells, including lymph node resident Tregs, was also performed by the use of cyclophosphamide, anti-CD25 or anti-CTLA-4 antibodies, IL-2-toxin chimeric proteins or GITR ligands (38, 48, 68, 147). Therefore the reasonable suggestion is that manipulation with the number of Tregs before vaccination with tumor-associated antigens could effectively augment anti-cancer immune response (39). Another strategy consists in elimination of Tregs combined with genetic modification of tumor cells which makes them immunologically recognizable to effector T cells (147, 148). Simultaneous removal of Th3/Tr1 regulatory cells may be another conceivable way to treat progressive tumors (123). Experimental depletion of Tregs augmented also the response to DC-based immunotherapy (149), which argues for the possibility that modification of Treg-DCs interactions depending on IDO and possibly HO-1 activity might improve the efficacy of anti-cancer treatment. Strategies to inhibit the IDO pathway might help to break tolerance to tumor tissues (150). It was found that D-1-methyl-tryptophan isomer capable of blocking IDO activity is effective in abrogating the suppression of T cells created by IDO-expressing DCs isolated from tumor-draining lymph nodes. Its potential clinical usefulness was confirmed in immunopotentiation of chemotherapy regimens using cyclophosphamide, paclitaxel or gemcitabine, when tested in mouse models of melanoma and breast cancer (151). Similarly, using of zinc protoporphyrin IX, an HO-1 inhibitor, resulted in significant anti-tumor effects in mouse lung cancer (152). Pharmacological activation of gammadelta T cells *in vivo* or adoptive cell therapy with *in vitro* expanded gammadelta T cells are considered as a novel immunotherapy in certain types of cancer (153, 154, 155, 156). However, we do not know if combining these protocols with depletion of Tregs may improve outcome in cancer patients.

Regulatory T-cell-based immunotherapy of cancer meets many obstacles. Manipulation of Treg number could be unnecessary technique in some haematological malignancies, as it was shown that high T Foxp3+ cells were correlated with improved survival in B-cell derived Hodgkin's lymphomas (157). Therefore, careful consideration should be taken before implementation of Treg-depletion therapy, and differences between haematological and epithelial cancers should be taken into regard (158). Depletion of Tregs with use of anti-CD25 antibodies has short-time effects because unintentionally accelerates conversion of T CD4+CD25- cells into Tregs and might cause decrease of T cell effectors (159, 160). Therapeutic use of synthetic oligonucleotides containing unmethylated CpG motifs (CpG-ODNs) which are known stimulators of innate and adaptive immunity could induce IDO up-regulation in DCs and promote pro-tumoral immune response (161, 162). Similarly, immuno-vaccines containing prostaglandin E2 for sensitizing DCs to CCR7 ligands which direct their migration to the draining lymph nodes could unintentionally stimulate IDO expression on DCs (163).

7. PERSPECTIVES

According to the recently proposed hypothesis (79), naturally occurring Tregs have evolved to modulate maternal response directed towards paternally-derived fetal alloantigens in placental vertebrates. Similarly to trophoblast possessing both maternal and paternal antigens, cancer is also a chimera consisting of cells presenting both self and changed through mutations antigens. Therefore, mechanisms engaging Tregs and their regulation might have important influence on the course of cancer growth and spreading in this group of animals, including humans. This is the reason why investigations performed on apparently distant fields like pregnancy and cancer could have close connections. Despite the fact that mechanisms of Treg regulation are still in progress, there is a hope for their broad use in the everyday clinical practice, and the first attempts of such management have already been described. However, much more information about interactions of Tregs with other cell populations is needed to provide safe treatment and reduction of potential side-effects. The most probable directions of investigations will cover pathways of cytokine signaling between Tregs, DCs and trophoblast and cancer tissues, as well as molecules responsible for recruitment of Tregs into maternal-fetal interface and tumor sites. There are also strong suggestions that other immunocytes, like uNK, γδT cells or neutrophils have regulatory potential in certain conditions, and their connections with Tregs need to be precisely elucidated. The knowledge concerning intracellular pathways of signal transmission in Treg cells should be intensively studied in order to introduce methods of genetic manipulation of Tregs activity. In the future the methods of genomics will also bring the possibility of increase tumor immunogenicity. There is also burning need to learn how to obtain and expand Tregs having high activity directed against antigen-specified effectors. The failures of pregnancy, especially multiple failures after IVF-ET techniques, recurrent abortions and pre-eclampsia, have all great emotional burden for suffering patients and still wait for safe and effective therapeutic options, one of which could be manipulation with Tregs.

8. REFERENCES

1. P. B. Medawar: Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symp Soc Exp Biol* 7, 320-338 (1953)

2. J. R. Wilczynski: Immunological analogy between allograft rejection, recurrent abortion and pre-eclampsia - the same basic mechanism? *Hum Immunol* 67(7), 492-511 (2006)

3. N. Rouas-Freiss, R. M. Goncalves, C. Menier, J. Dausset, E. D. Carosella: Direct evidence to support the

role of HLA-G in protecting fetus from maternal uterine natural killer cytolysis. *Proc Natl Acad Sci USA* 94, 11520-11525 (1997)

4. A. L. Mellor, D. H. Munn: IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 4, 762-774 (2004)

5. D. A. Clark, S. Blois, J. Kandil, B. Handjiski, J. Manuel, P. C. Arck: Reduced uterine indoleamine 2,3-dioxygenase versus increased Th1/Th2 cytokine ratios as a basis for occult and clinical pregnancy failure in mice and humans. *Am J Reprod Immunol* 54, 203-216 (2005)

6. D. Munn, M. Zhou, J. Attwood, I. Bondarev, S. Conway, B. Marshall, C. Brown, A. Mellor: Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 281, 1191-1193 (1998)

7. A. Sollwedel, A. Z. Bertoja, M. L. Zenclussen, K. Gerlof, U. Lisewski, P. Wafula, B. Sawitzki, C. Woiciechowski, H. D. Volk, A.C. Zenclussen: Protection from abortion by heme oxygenase-1 up-regulation is associated with increased levels of Bag-1 and neuropilin-1 at the fetal-maternal interface. *J Immunol* 175, 4875-4885 (2005)

8. A.C. Zenclussen, E. Lim, S. Knoeller, M. Knackstedt, K. Hertwig, E. Hagen, B. F. Klapp, P. C. Arck: Heme Oxygenases in pregnancy II: HO-2 is downregulated in human pathologic pregnancies. *Am J Reprod Immunol* 50, 66-76 (2003)

9. G. Reinhard, A. Noll, H. Schlebusch, P. Mallmann, A. V. Ruecker: Shifts in the Th1/Th2 balance during human pregnancy correlate with apoptotic changes. *Biochem Biophys Res Commun* 245, 933-938 (1998)

10. S. Blois, A .C. Zenclussen, M. E. Roux, S. Olmos, J. di Conza, P. C. Arck, R. A. Margni: Asymmetric antibodies (AAb) in the female reproductive tract. *J Reprod Immunol* 64, 31-43 (2004)

11. J. R. Wilczynski. Th1/Th2 cytokines balance - yin and yang of reproductive immunology. *Eur J Obstet Gynecol Reprod Biol* 122, 136-143 (2005)

12. N. Ledee-Bataille, K. Bonnet-Chea, G. Hosny, S. Dubanchet, R. Frydman, G. Chaouat: Role of the endometrial tripod interleukin-18, -15, and -12 in inadequate uterine receptivity in patients with history of repeated *in vitro* fertilization-embryo transfer failure. *Fertil Steril* 83, 598-605 (2005)

13. T. G. Wegmann, H. Lin, L. Guilbert, T. R. Mosmann: Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol Today* 14, 353-356 (1993)

14. D.D. Taylor, S. Akyol, C. Gercel-Taylor: Pregnancy-associated exosomes and their modulation of T cell signaling. *J Immunol* 176, 1534-1542 (2006).

15. S. A. Rosenberg: Progress in human tumour immunology and immunotherapy. *Nature* 411, 380-384 (2001)

16. A. Garcia-Lora, I. Algarra, F. Garrido: MHC class I antigens, immune surveillance and tumor immune escape. *J Cell Physiol* 195, 346-355 (2003)

17. C. C. Chang, S. P. Murphy, S. Ferrone: Differential *in vivo* and *in vitro* HLA-G expression in melanoma cells: potential mechanisms. *Hum Immunol* 64, 1057-1063 (2003)

18. J. D. Wu, L. M. Higgins, A. Steinle, D. Cosman, H. Haugk, S. R. Plymate: Prevalent expression of the immunostimulatory MHC class I-related molecule is counteracted by shedding in prostate cancer. *J Clin Invest* 114, 560-568 (2004)

19. M. A. Frassanito, A. Cusmai, F. Dammacco: Deregulated cytokine network and defective Th1 immune response in multiple myeloma. *Clin Exp Immunol* 125, 190-197 (2001)

20. S. N. Byrne, G. M. Halliday: High levels of Fas ligand and MHC class II in the absence of CD80 or CD86 expression and a decreased CD4+ T cell infiltration enables murine skin tumours to progress. *Cancer Immunol Immunother* 52, 396-402 (2003)

21. C. Uyttenhove, L. Pilotte, I. Theate, V. Stroobant, D. Colau, N. Parmentier, T. Boon, B. J. Van der Eynde: Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nat Med* 9, 1269-1274 (2003)

22. G. Brandacher, A. Parathoner, R. Ladumer, S. Schneeberger, P. Obrist, C. Winkler, E. R. Werner, G. Werner-Felmeyer, H. G. Weiss, G. Göbel, R. Margreiter, A. Königsrainer, D. Fuchs, A. Amberger: Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. *Clin Cancer Res* 12, 1144-1151 (2006)

23. L. E. Otterbein, F. H. Bach, J. Alam, M. Soares, H. Tao Lu, M. Wysk, R. J. Davis, R. A. Flavell, A. M. Choi: Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med* 6, 422-428 (2000)

24. J. Fang, T. Akaike, H. Maeda: Antiapoptotic role of heme oxygenase (HO) and the potential of HO as a target in anticancer treatment. *Apoptosis* 9, 27-35 (2004)

25. J. Fang, T. Sawa, T. Akaike, T. Akuta, S. K. Sahoo, G. Khaled, A. Hamada, H. Maeda: *In vivo* antitumor activity of pegylated zinc protoporphyrin: targeted inhibition of heme oxygenase in solid tumor. *Cancer Res* 63, 3567-3574 (2003)

26. S. Tanaka, T. Akaike, J. Fang, T. Beppu, M. Ogawa, F. Tamura, Y. Miyamoto, H. Maeda: Antiapoptotic effect of

haem oxygenase-1 induced by nitric oxide in experimental solid tumour. *Br J Cancer* 88, 902-909 (2003)

27. M. Sunamura, D. G. Duda, M. H. Ghattas, L. Lozonschi, F. Motoi, J. Yamauchi, S. Matsuno, S. Shibahara, N. G. Abraham: Heme oxygenase accelerates tumor angiogenesis of human pancreatic cancer. *Angiogenesis* 6, 15-24 (2003)

28. A. Nishie, M. Ono, T. Shono, J. Fukushi, M. Otsubo, H. Onoue, Y. Ito, T. Inamura, K. Ikezaki, M. Fukui, T. Iwaki, M. Kuwano: Macrophage infiltration and heme oxygenase-1 expression correlate with angiogenesis in human gliomas. *Clin Cancer Res* 5, 1107-1113 (1999)

29. H. Torisu-Itakura, M. Furue, M. Kuwano, M. Ono: Coexpression of thymidine phosphorylase and heme oxygenase-1 in macrophages in human malignant vertical growth melanomas. *Jpn J Cancer Res* 91, 906-910 (2000)

30. A. I. Goodman, M. Choudhury, J. L. da Silva, M. L. Schwartzman, N. G. Abraham: Overexpression of the heme oxygenase gene in renal cell carcinoma. *Proc Soc Exp Biol Med* 214, 54-61 (1997)

31. M. D. Maines, P. A. Abrahamsson: Expression of heme oxygenase-1 (HSP32) in human prostate: normal, hyperplastic and tumor tissue distribution. *Urology* 47, 727-733 (1996)

32. P. O. Berberat, Z. Dambrauskas, A. Gulbinas, T. Giese, N. Giese, B. Künzli, F. Autschbach, S. Meuer, M. W. Büchler, H. Friess: Inhibition of heme oxygenase-1 increases responsiveness of pancreatic cancer cells to anticancer treatment. *Clin Cancer Res* 11, 3790-3798 (2005)

33. M. Hill, V. Pereira, C. Chauveau, R. Zagani, S. Remy, L. Tesson, D. Mazal, L. Ubillos, R. Brion, K. Ashgar, M. F. Mashreghi, K. Kotsch, J. Moffett, C. Doebis, M. Seifert, J. Boczkowski, E. Osinaga, I. Anegon: Heme oxygenase-1 inhibits rat and human breast cancer cell proliferation: mutual cross inhibition with indoleamine 2,3-dioxygenase. *FASEB J* 19, 1957-1968 (2005)

34. D.D. Taylor, C. Gercel-Taylor: Tumor-derived exosomes and their role in cancer-associated T-cell signaling defects. *Br J Cancer* 92, 305-311 (2005).

35. J. R. Wilczynski: Cancer and pregnancy share similar mechanisms of immunological escape. *Chemotherapy* 52(3), 107-110 (2006)

36. A. Tafuri, J. Alferink, P. Moller, G. Hammerling, B. Arnold: T cell awareness of paternal alloantigens during pregnancy. *Science* 270, 630-633 (1995)

37. T. J. Curiel, G. Coukos, L. Zou, X. Alvarez, P. Cheng, M. Mottram, J. R. Evdemon-Hogan, L. Conejo-Garcia, M Zhang, Y. Burow, Y. Zhu, S. Wei, I. Kryczek, B. Daniel, A. Gordon, L. Myers, A. Lackner, M. L. Disis, K. L. Knutson, L. Chen, W. Zou: Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 10, 942-949 (2004)

38. Y. Q. Chen, H. Z. Shi, X. J. Qin, W. N. Mo, X. D. Liang, Z. X. Huang, H. B. Yang, C. Wu: CD4+CD25+ regulatory T lymphocytes in malignant pleural effusion. *Am J Respir Crit Care Med* 172, 1434-1439 (2005)

39. C. P. Gray, P. Arosio, P. Hersey: Association of increased levels of heavy-chain ferritin with increased CD4+CD25+ regulatory T-cell levels in patients with melanoma. *Clin Cancer Res* 9, 2551-2559 (2003)

40. U. K. Liyanage, T. T. Moore, H.G. Joo, Y. Tanaka, V. Herrmann, G. Doherty, J. A. Drebin, S. M. Strasberg, T. J. Eberlein, P. S. Goedegebuure, D . C. Linehan: Prevalence of regulatory T cells is increased in peripheral blood and tumor environment of patients with pancreas or breast adenocarcinoma. *J Immunol* 169, 2756-2761 (2002)

41. S . L. Clarke, G. J. Betts, A. Plant, K. L. Wright, T. M. El-Shanawany, R. Harrop, J. Torkington, B. I. Rees, G. T. Wiliams, A. M. Gallimore, A. J. Godkin: CD4+CD25+FOXP3+ regulatory T cells suppress anti-tumor immune responses in patients with colorectal cancer. PLOS ONE 1(1): e129.doi: 10.1371/journal.pone.0000129 (2006).

42. A. M. Wolf, D. Wolf, M. Steurer, G. Gastl, E. Gunsilius, B. Grubeck-Loebenstein: Increase of regulatory T cells in the peripheral blood of cancer patients. *Clin Cancer Res* 9, 606-612 (2003)

43. F. Ichihara, K. Kono, A. Takahashi, H. Kawaida, H. Sugai, H. Fujii: Increased populations of regulatory T cells in peripheral blood and tumor-infiltrating lymphocytes in patients with gastric and esophageal cancers. *Clin Cancer Res* 9, 4404-4408 (2003)

44. M. Viguier, F. Lemaitre, O. Verola, M. S. Cho, G. Gorochov, L. Dubertret, H. Bachelez, P. Kourilsky, L. Ferradini: FOXP3 expressing CD4+CD25(high) regulatory T cells are overrepresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells. *J Immunol* 173, 1444-1453 (2004)

45. C. Loddenkemper, M. Schernus, M. Noutsias, H. Stein, E. Thiel, D. Nagorsen: *In situ* analysis of FOXP3 regulatory T cells in human colorectal cancer. *J Transl Med* 4:52 doi: 10.1186/1479-5876-4-52 (2006)

46. E. Sato, S. H. Olson, J. Ahn, B. Bundy, H. Nishikawa, F. Qian, A. A. Jungbluth, D. Frosina, S. Gnjatic, C. Ambrosone, J. Kepner, T. Odunsi, G. Ritter, S. Lele, Y. T. Chen, H. Ohtani, L. J. Old, K. Odunsi: Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *PNAS* 102, 18538-18543 (2005)

47. D. Wolf, A. M. Wolf, H. Rumpold, H. Fiegl, A. G. Zeimet, E. Muller-Holzner, M. Deibl, G. Gastl, E. Gunsilius, C. Marth: The expression of the regulatory T cell-specific forkhead box transcription factor FoxP3 is associated with poor prognosis in ovarian cancer. *Clin Cancer Res* 11, 8326-8331 (2005)

48. T. Hiura, H. Kagamu, S. Miura, A. Ishida, H. Tanaka, J. Tanaka, F. Gejyo, H. Yoshizawa: Both regulatory T cells and antitumor effector T cells are primed in the same draining lymph nodes during tumor progression. *J Immunol* 175, 5058-5066 (2005)

49. X. J. Qin, H. Z. Shi, Z. X. Huang, L. F. Kang, W. N. Mo, C. Wu: Interleukin-16 in tuberculous and malignant pleural effusions. *Eur Respir J* 25, 605-611 (2005)

50. H. Nishikawa, T. Kato, I. Tawara, H. Ikeda, K. Kuribayashi, P. M. Allen, R.D. Schreiber, L. J. Old, H. Shiku: IFN-γ controls the generation/activation of CD4+CD25+ regulatory T cells in antitumor immune response. *J Immunol* 175, 4433-4440 (2005)

51. F. Ghiringhelli, C. Menard, F. Martin, L. Zitvogel: The role or regulatory T cells in the control of natural killer cells: relevance during tumor progression. *Immunol Rev* 212, 229-238 (2006)

52. F. Ghiringhelli, C. Menard, M. Terme, C. Flament, J. Taieb, N. Chaput, P. E. Puig, S. Novault, B. Escudier, E. Vivier, A. Lecesne, C. Robert, J. Y. Blay, J. Bernard, S. Caillat-Zucman, A. Freitas, T. Tursz, O. Wagner-Ballon, C. Capron, W. Vainchencker, F. Martin, L. Zitvogel: CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-β-dependent manner. *J Exp Med* 202, 1075-1085 (2005)

53. M. J. Smyth, M. W. L. Teng, J. Swann, K. Kyparissoudis, D. I. Godfrey, Y. Hayakawa: CD4+CD25+ T regulatory cells suppress NK cell-mediated immunotherapy of cancer. *J Immunol* 176, 1582-1587 (2006)

54. F. Ghiringhelli, P. E. Puig, S. Roux, A. Parcellier, E. Schmitt, E. Solary, G. Kroemer, F. Martin, B. Chauffert, L. Zitvogel: Tumor cells convert immature myeloid dendritic cells into TGF-beta-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. *J Exp Med* 202, 919-929 (2005)

55. E. S. Doubrovina, M. M. Doubrovin, E. Vider, R. B. Sisson, R. J. O'Reilly, B. Dupont, Y. M. Vyas: Evasion from NK cell immunity by MHC class I chain-related molecules expressing colon adenocarcinoma. *J Immunol* 171, 6891-6899 (2003)

56. S. Onizuka, I. Tawara, J. Shimizu, S. Sakaguchi, T. Fujita, E. Nayakawa: Tumor rejection by *in vivo* administration of anti-CD25 (interleukin-2 receptor α) monoclonal antibody. *Cancer Res* 59, 3128-3133 (1999)

57. R. P. Sutmuller, L. M. van Duivenvoorde, A. van Elsas, T. N. Schumacher, M. E. Wildenberg, J. P. Allison, R. E. Toes, R. Offringa, C. J. Melief: Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotxic T lymphocyte responses. *J Exp Med* 194, 823-832 (2001)

58. M. J. Turk, J. A. Guevara-Patino, A. Rizutto, M. E. Engelhorn, S. Sakaguchi, A. N. Houghton: Concominant tumor immunity to a poorly immunogenic melanoma is prevented by regulatory Tcells. *J Exp Med* 200, 771-782 (2004)

59. F. Ghiringhelli, N. Larmonier, E. Schmitt, A. Parcellier, D. Cathelin, C. Garrido, B. Chauffert, E. Solary, B. Bonnotte, F. Martin: CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors. *Eur J Immunol* 34, 336-344 (2004)

60. J. Shimizu, S. Yamazaki, S. Sakaguchi: Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. *J Immunol* 163, 5211-5218 (1999)

61. S. Huber, C. Schramm, H. A. Lehr, A. Mann, S. Schmitt, C. Becker, M. Protschka, P. R. Galle, M. F. Neurath, M. Blessing: Cutting edge: TGFbeta signalling is required for the *in vivo* expansion and immunosuppressive capacity of regulatory CD4+CD25+ T cells. *J Immunol* 173, 6526-6531 (2004)

62. T. Tada, S. Ohzeki, K. Utsumi, H. Takiuchi, M. Muramatsu, X. F. Li, J. Shimizu, H. Fujiwara, T. Hamaoka: Transforming growth factor-β-induced inhibition of T cell function. Susceptibility difference in T cells of various phenotypes and functions and its relevance to immunosuppression in the tumor-bearing state. *J Immunol* 146, 1077-1082 (1991)

63. H. L. Chang, N. Gillet, I. Figari, A. R. Lopez, M. A. Palladino, R. Derynck: Increased transforming growth factor beta expression inhibits cell proliferation *in vitro*, yet increases tumorigenicity and tumor growth of Meth A sarcoma cells. *Cancer Res* 53, 4391-4398 (1993)

64. H. Fakhrai, O. Dorigo, D. L. Shawler, H. Lin, D. Mercola, K. L. Black, I. Royston, R. E. Sobol: Eradication of established intracranial rat gliomas by transforming growth factor beta antisense gene therapy. *Proc Natl Acad Sci USA* 93, 2909-2914 (1996)

65. G. Torre-Amione, R. D. Beauchamp, H. Koeppen, B. H. Park, H. Schreiber, H. L. Moses, D. A. Rowley: A highly immunogenic tumor transfected with a murine transforming growth factor type beta 1 cDNA escapes immune surveillance. *Proc Natl Acad Sci USA* 87, 1486-1490 (1990)

66. S. Tabibzadeh, R. Kothapalli, I. Buyuksal: Distinct tumor specific expression of TGFB4 (ebaf)*, a novel human gene of the TGF-beta superfamily. *Front Biosci* 15, 18-25 (1997)

67. L. A. Ormandy, T. Hillemann, H. Wedemeyer, M. P. Manns, T. F. Greten, F. Korangy: Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Res* 65, 2457-2464 (2005)

68. M. Beyer, J. L. Schultze: Regulatory T cells in cancer. *Blood* 108, 804-811 (2006)

69. Y. Liu, X. Bi, S. Xu, J. Xiang: Tumor-infiltrating dendritic cell subsets of progressive or regressive tumors induce suppressive or protective immune responses. *Cancer Res* 65, 4955-4962 (2005)

70. C. Dercamp, K. Chemin, C. Caux, G. Trinchieri, A. P. Vicari: Distinct and overlapping roles of interleukin-10 and CD25+ regulatory T cells in the inhibition of antitumor CD8 T-cell responses. *Cancer Res* 65, 8479-8486 (2005)

71. R. M. Gorczynski, Z. Chen, J. Hu, Y. Kai, J. Lei: Evidence of a role for CD200 in regulation of immune rejection of leukaemic tumour cells in C57BL/6 mice. *Clin Exp Immunol* 126(2), 220-229 (2001)

72. J. R. McWhirter, A. Kretz-Rommel, A. Saven, T. Maruyama, K. N. Potter, C.I. Mockridge, E. P. Ravey, F. Qin, K. S. Bowdish: Antibodies selected from combinatorial libraries block a tumor antigen that plays a key role in immunomodulation. *PNAS* 103, 1041-1046 (2006)

73. J. Moreaux, D. Hose, T. Reme, E. Jourdan, M. Hundemer, E. Legouffe, P. Moine, P. Bourin, M. Moos, J. Corre, T. Mohler, J. De Vos, J. F. Rossi, H. Goldschmidt, B. Klein: CD200 is a new prognostic factor in multiple myeloma. *Blood* 108(13, 4194-4197 (2006)

74. D. H. Munn, M. D. Sharma, J. R. Lee, K. G. Jhaver, T. S. Johnson, D. B. Keskin, B. Marshall, P. Chandler, S. J. Antonia, R. Burgess, C. L. Slingluff Jr, A. L. Mellor: Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science* 297, 1867-1870 (2002)

75. S. Astigiano, B. Morandi, R. Costa, L. Mastracci, A. D'Agostino, G. B. Ratto, G. Melioli, G. Frumento: Eosinophil granulocytes account for indoleamine 2,3-dioxygenase-mediated immune escape in human non-small cell lung cancer. *Neoplasia* 7, 390-396 (2005)

76. G. Weinlich, C. Murr, L. Richardsen, C. Winkler, D. Fuchs: Decreased serum tryptophan concentration predicts poor prognosis in malignant melanoma patients. *Dermatology* 214(1), 8-14 (2007)

77. K. Ino, N. Yoshida, H. Kajiyama, K. Shibata, E. Yamamoto, K. Kidokoro, N. Takahashi, M. Terauchi, A. Nawa, S. Nomura, T. Nagasaka, O. Takikawa, F. Kikkawa: Indoleamine 2,3-dioxygenase is a novel prognostic indicator for endometrial cancer. *Br J Cancer* 95(11), 1555-1561 (2006)

78. M. S. von Bergwelt-Baildon, A. Popov, T. Saric, J. Chemnitz, S. Classen, M. S. Stoffel, F. Fiore, U. Roth, M. Beyer, S. Debey, C. Wickenhauser, F. G. Hanisch, J .L. Schultze: CD25 and indoleamine 2,3-dioxygenase are up-regulated by prostaglandin E2 and expressed by tumor-associated dendritic cells *in vivo*: additional mechanisms of T-cell inhibition. *Blood* 108(1), 228-237 (2006)

79. V. R. Aluvihare, A. G. Betz: The role of regulatory T cells in alloantigen tolerance. *Immunol Rev* 212, 330-343 (2006)

80. D. A. Somerset, Y. Zheng, M. D. Kilby, D. M. Sansom, M. T. Drayson: Normal human pregnancy is associated with an elevation in the immune suppressive CD25+CD4+ regulatory T-cell subset. *Immunology* 112, 38-43 (2004)

81. H. Waldmann, E. Adams, P. J. Fairchild, S. Cobbold: Infectious tolerance and the long-term acceptance of transplanted tissue. *Immunol Rev* 212, 301-313 (2006)

82. A. C. Zenclussen, K. Gerlof, M. L. Zenclussen, A. Sollwedel, A. Zambon Bertoja, T. Ritter, K. Kotsch, J. Leber, H. D. Volk: Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. *Am J Pathol* 166, 811-822 (2005)

83. A. C. Zenclussen, K. Gerlof, M. L. Zenclussen, S. Ritschel, A. Zambon Bertoja, S. Fest, S. Hontsu, S. Ueha, K. Matsushima, J. Leber, H. D. Volk: Regulatory T cells induce a privileged tolerant microenvironment at the fetal-maternal interface. *Eur J Immunol* 36(1), 82-94 (2006)

84. V. Aluvihare, M. Kallikourdis, A. Betz: Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 3, 266-271 (2004)

85. G. Darrasse-Jeze, D. Klatzmann, F. Charlotte, B. L. Salomon, J. L. Cohen: CD4+CD25+ regulatory/suppressor T cells prevent allogeneic fetus rejection in mice. *Immunol Lett* 102(1), 106-109 (2006)

86. S. Saito, Y. Sasaki, M. Sakai: CD4+CD25^{high} regulatory T cells in human pregnancy. *J Reprod Immunol* 65, 111-120 (2005)

87. D. A. Somerset, Y. Zheng, M. D. Kilby, D. M. Sansom, M. T. Drayson: Normal human pregnancy is associated with an elevation in the immune suppressive CD25+CD4+ regulatory T-cell subset. *Immunology* 112, 38-43 (2004)

88. Y. Sasaki, M. Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S: Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. *Mol Hum Reprod* 10, 347-353 (2004)

89. J. Heikkinen, M. Mottonen, A. Alanen, O. Lassila: Phenotypic characterization of regulatory T cells in the human decidua. *Clin Exp Immunol* 136(2), 373-378 (2004)

90. T. Tilburgs, D. L. Roelen, B. J. van der Mast, J. J. van Schip, C. Kleijburg, G. M. de Groot-Swings, H. H. Kanhai, F. H. Claas, S. A. Scherjon: Differential distribution of CD4(+)CD25(bright) and CD8(+)CD28(-) T-cells in decidua and maternal blood during human pregnancy. *Placenta* 27 suppl A, S47-53 (2006)

91. A. P. Sindram-Trujillo, S. A. Scherjon, P. P. van Hulst-van Miert, H. H. Kanhai, D. L. Roelen, F. H. Claas: Comparison of decidual leukocytes following spontaneous vaginal delivery and elective cesarean section in uncomplicated human term pregnancy. *J Reprod Immunol* 62, 125-137 (2004)

92. H. Tsuda, T. Michimata, S. Hayakawa, K. Tanebe, M. Sakai, M. Fujimura, K. Matsushima, S. Saito: A Th2 chemokine, TARC, produced by trophoblasts and endometrial gland cells regulates the infiltration of CCR4+ T lymphocytes into human decidua at early pregnancy. *Am J Reprod Immunol* 48, 1-8 (2002)

93. M. Kallikourdis, K. G. Andersen, K. A. Welch, A. G. Betz: Alloantigen-enhanced accumulation of CCR5+ 'effector' regulatory T cells in the gravid uterus. *Proc Natl Acad Sci USA* 104(2), 594-599 (2007)

94. G. A. Prieto, Y. Rosenstein: Oestradiol potentiates the suppressive function of human CD4+CD25+ regulatory T cells by promoting their proliferation. *Immunology* 118, 58-65 (2006)

95. M. J. Polanczyk, B. D. Carson, S. Subramanian, M. Afentoulis, A. A. Vandenbark, S. F. Ziegler, H. Offner: Estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. *J Immunol* 173, 2227-2230 (2004)

96. W. H. Zhu, C. Z. Lu, Y. M. Huang, H. Link, B. G. Xiao: A putative mechanism on remission of multiple sclerosis during pregnancy: estrogen-induced indoleamine 2,3-dioxygenase by dendritic cells. *Mult Scler* 13(1), 33-40 (2007)

97. X. Dong, L. A. Bachman, R. Kumar, M. D. Griffin: Generation of antigen-specific, interleukin-10-producing T-cells using dendritic cell stimulation and steroid hormone conditioning. *Transpl Immunol* 11, 323-333 (2003)

98. C. L. Butts, S. A. Shukair, K. M. Duncan, E. Bowers, C. Horn, E. Belyavskaya, L. Tonelli, E. M. Sternberg: Progesterone inhibits mature rat dendritic cells in a receptor-mediated fashion. *Int Immunol* 19(3), 287-296 (2007)

99. B. Huck, T. Steck, M. Habersack, J. Dietl, U. Kammerer: Pregnancy associated hormones modulate the cytokine production but not the phenotype of PBMC-derived human dendritic cells. *Eur J Obstet Gynecol Reprod Biol* 122(1), 85-94 (2005)

100. J. Kalinka, J. Szekeres-Bartho: The impact of dydrogesterone supplementation on hormonal profile and progesterone-induced blocking factor concentrations in women with threatened abortion. *Am J Reprod Immunol* 53, 166-171 (2005)

101. J. Szekeres-Bartho, T. G. Wegmann: A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. *J Reprod Immunol* 31, 81-95 (1996)

102. A. C. Zenclussen, K. Gerlof, M. L. Zenclussen, A. Sollwedel, A. Zambon Bertoja, T. Ritter, K. Kotsch, J. Leber, H. D. Volk: Abnormal T-cell reactivity against paternal antigens in spontaneous abortion. Adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. *Am J Pathol* 166, 811-822 (2005)

103. L. P. Jin, D. J. Li, J. P. Zhang, M. Y. Wang, X. Y. Zhu, Y. Zhu, Y. Meng, M. M. Yuan: Adoptive transfer of paternal antigen-hyporesponsive T cells induces maternal tolerance to the allogeneic fetus in abortion-prone matings. *J Immunol* 173, 3612-3619 (2004)

104. L. P. Jin, Y. H. Zhou, M. Y. Wang, X. Y. Zhu, D. J. Li: Blockade of CD80 and CD86 at the time of implantation inhibits maternal rejection to the allogeneic fetus in abortion-prone matings. *J Reprod Immunol* 65(2), 133-146 (2005)

105. X. Y. Zhu, Y. H. Zhou, M. Y. Wang, L. P. Jin, M. M. Yuan, D. J. Li: Blockade of CD86 signaling facilitates a Th2 bias at the maternal-fetal interface and expands peripheral CD4+CD25+ regulatory T cells to rescue abortion-prone fetuses. *Biol Reprod* 72, 338-345 (2005)

106. A. H. Zarnani, S. M. Moazzeni, F. Shokri, M. Salehnia, M. Jeddi-Tehrani: Kinetics of murine decidual dendritic cells. *Reproduction* 133(1), 275-283 (2007)

107. S. Blois, M. Tometten, J. Kandil, E. Hagen, B. F. Klapp, R. A. Margni, Arck PC: Intercellular adhesion molecule-1/LFA-1 cross talk is a proximate mediator capable of disrupting immune integration and tolerance mechanism at the feto-maternal interface in murine pregnancies. *J Immunol* 174, 1820-1829 (2005)

108. N. Miwa, S. Hayakawa, S. Miyazaki, S. Myojo, Y. Sasaki, M. Sakai, O. Takikawa, S. Saito: IDO expression on decidual and peripheral blood dendritic cells and monocytes/macrophages after treatment with CTLA-4 or interferon-gamma increase in normal pregnancy but decrease in spontaneous abortion. *Mol Hum Reprod* 11(12), 865-870 (2005)

109. L. Gardner, A. Moffett: Dendritic cells in the human decidua. *Biol Reprod* 69, 1438-1446 (2003)

110. A. L. Mellor, B. Baban, P. Chandler, B. Marshall, K. Jhaver, A. Hansen, P. A. Koni, M. Iwashima, D. H. Munn: Induced indoleamine 2,3-dioxygenase expression in

dendritic cell subsets suppresses T cell clonal expansion. *J Immunol* 171, 1652-1655 (2003)

111. D. A. Clark, S. Blois, J. Kandil, B. Handjiski, J. Manuel, P. C. Arck: Reduced uterine indoleamine 2,3-dioxygenase versus increased Th1/Th2 cytokine ratios as a basis for occult and clinical pregnancy failure in mice and humans. *Am J Reprod Immunol* 54, 203-216 (2005)

112. N. K. Steckel, U. Kuhn, D. W. Beelen, A. H. Elmaagacli: Indoleamine 2,3-dioxygenase expression in patients with acute graft-versus-host disease after allogeneic stem cell transplantation and in pregnant women: association with the induction of allogeneic immune tolerance? *Scand J Immunol* 57, 185-191 (2003)

113. A. Honig, L. Rieger, M. Kapp, M. Sutterlin, J. Dietl, U. Kammerer: Indoleamine 2,3-dioxygenase (IDO) expression in invasive extravillous trophoblast supports role of the enzyme for materno-fetal tolerance. *J Reprod Immunol* 61, 79-86 (2004).

114. B. Baban, P. Chandler, D. McCool, B. Marshall, D. H. Munn, A. L. Mellor: Indoleamine 2,3-dioxygenase expression is restricted to fetal trophoblast giant cells during murine gestation and is maternal genome specific. *J Reprod Immunol* 61, 67-77 (2004)

115. D. A. Clark, C. B. Coulam, S. Daya, G. Chaouat: Unexplained sporadic and recurrent miscarriage in the new millenium: a critical analysis of immune mechanisms and treatments. *Hum Reprod Update* 7, 501-511 (2001)

116. Gorczynski RM, Hadidi S, Yu G, Clark DA: The same immunoregulatory molecules contribute to successful pregnancy transplantation. *Am J Reprod Immunol* 48, 18-26 (2002)

117. Clark DA, Chaouat G: Loss of surface CD200 on stored allogeneic leukocytes may impair anti-abortive effect *in vivo*. *Am J Reprod Immunol* 53, 13-20 (2005)

118. O. Nagaeva, L. Jonsson, L. Mincheva-Nilsson: Dominant IL-10 and TGF-beta mRNA expression in gammadeltaT cells of human early pregnancy decidua suggests immunoregulatory potential. *Am J Reprod Immunol* 48(1), 9-17 (2002)

119. M. K. Pandey, S. Thakur, S. Agrawal: Lymphocyte immunotherapy and its probable mechanism in the maintenance of pregnancy in women with recurrent spontaneous abortion. *Arch Gynecol Obstet* 269, 161 (2004)

120. M. Marzi, A. Vigano, D. Trabattoni, M. L. Villa, A. Salvaggio, E. Clerici, M. Clerici: Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clin Exp Immunol* 106, 127 (1996)

121. L. Svensson, M. Arvola, R. H. Sallstrom, R. Holmdahl, R. Mattson: The Th2 cytokines IL-4 and IL-10 are not crucial for the completion of allogeneic pregnancy in mice. *J Reprod Immunol* 51, 3 (2001)

122. M. G. Roncarolo, S. Gregori, M. Battaglia, R. Bacchetta, K. Fleischhauer, M. K. Levings: Intrleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol Rev* 212, 28-50 (2006)

123. N. Seo, S. Hayakawa, M. Takigawa, Y. Tokura: Interleukin-10 expressed at early tumor sites induces subsequent generation of CD4+ T-regulatory cells and systemic collapse of antitumor immunity. *Immunology* 103, 449-457 (2001)

124. K. E. Steinbrink, S. Graulich, J. Kubsch, A. Knop, H. Enk: Interleukin-10-treated human dendritic cells induce a melanoma-antigen-specific anergy in CD8+ T cells resulting in a failure to lyse tumor cells. *Blood* 99, 2468-2476 (2002)

125. F. Fiore, B. Nuschak, S. Peola, S. Mariani, M. Muraro, M. Foglietta, M. Coscia, B. Bruno, M. Boccadoro, M. Massaia: Exposure to myeloma cell lysates affects the immune competence of dendritic cells and favors the induction of Tr1-like regulatory T cells. *Eur J Immunol* 35(4), 1155-1163 (2005)

126. X. Zhang, H. Huang, J. Yuan, D. Sun, W. S. Hou, J. Gordon, J. Xiang. CD4-8- dendritic cells prime CD4+ T regulatory 1 cells to suppress antitumor immunity. *J Immunol* 175, 2931-2937 (2005)

127. A. Lundqvist, A. Palmborg, M. Pavlenko, J. Levitskaya, P. Pisa:. Mature dendritic cells induce tumor-specific type 1 regulatory T cells. *J Immunother* (1997). 28(3), 229-35 (2005)

128. Y. Akasaki, G. Liu, N. H. Chung, M. Ehtesham, K. L. Black, J. S. Yu: Induction of a CD4+ T regulatory type 1 response by cyclooxygenase-2-overexpressing glioma. *J Immunol* 173(7):4352-4359 (2004)

129. L. R. Munro, P. W. Johnston, N. A. Marshall, S. J. Canning, S. G. Hewitt, K. Tveita, M. A. Vickers: Polymorphisms in the interleukin-10 and interferon gamma genes in Hodgkin lymphoma. *Leuk Lymphoma* 44(12), 2083-2088 (2003)

130. S. Barath, M. Aleksza, K. Keresztes, J. Toth, S. Sipka, G. Szegedi, A. Illes: Immunoregulatory T cells in the peripheral blood of patients with Hodgkin's lymphoma. *Acta Haematol* 116, 181-185 (2006)

131. N. A. Marshall, L. E. Christie, L. R. Munro, D. J. Culligan, P. W. Johnston, R. N. Barker, M. A. Vickers. Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. *Blood* 103(5), 1755-1762 (2004)

132. A. Loskog, C. Ninalga, G. Paul-Wetterberg, M. de la Torre, P. U. Malmstrom, T. H. Totterman: Human bladder

carcinoma is dominated by T-regulatory cells and Th1 inhibitory cytokines. *J Urol* 177(1), 353-358 (2007)

133. H. Jonuleit, E. Schmitt, G. Schuler, J. Knop, H. Enk: Induction of interleukin 10-producing nonproliferating CD4+ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 192, 1213-1222 (2000)

134. K. W. Moore, R. de Waal Malefyt, R. L. Coffman, A. O'Garra. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 19, 683-765 (2001)

135. J. Sjoberg, M. Aguilar-Santelises, A. M. Sjogren, E. K. Pisa, A. Ljungdahl, M. Bjorkholm, M. Jondal, H. Mellstedt, P. Pisa: Interleukin-10 mRNA expression in B-cell chronic lymphocytic leukaemia inversely correlates with progression of disease. *Br J Haematol*. 92(2), 393-400 (1996)

136. M. Rouleau, F. Cottrez, M. Bigler, S. Antonenko, J. M. Carballido, A. Zlotnik, M. G. Roncarolo, H. Groux. IL-10 transgenic mice present a defect in T cell development reminiscent of SCID patients. *J Immunol* 163(3), 1420-1427 (1999)

137. P. Hoffmann, R. Eder, L. A. Kunz-Schugart, R. Andreesen, M. Edinger: Large-scale *in vitro* expansion of polyclonal human CD4(+)CD25high regulatory T cells. *Blood* 104, 895-903 (2004)

138. K. E. Earle, Q. Tang, X. Zhou, W. Liu, S. Zhu, M. L. Bonyhadi, J. A. Bluestone: *In vitro* expanded human CD4+CD25+ regulatory T cells suppress effector T cell proliferation. *Clin Immunol* 115, 3-9 (2005)

139. E. Jaeckel, H. von Boehmer, M. P. Manns: Antigen-specific FoxP3-transduced T-cells can control established type 1 diabetes. *Diabetes* 54, 306-310 (2005)

140. S. E. Allan, L. Passerini, R. Bacchetta, N. Crellin, M. Dai, P. C. Orban, S. F. Ziegler, M. G. Roncarolo, M. K. Levings: The role of 2 FOXP3 isoforms in the generation of human CD4 Tregs. *J Clin Invest* 115, 3276-3284 (2005)

141. S. Miranda, S. Litwin, G. Barrientos, L. Szereday, E. Chuluyan, J. S. Bartho, P. C. Arck, S. M. Blois: Dendritic cells therapy confers a protective microenvironment in murine pregnancy. *Scand J Immunol* 64(5), 493-499 (2006)

142. S. Lob, S. Ebner, S. Wagner, J. Weinreich, R. Schafer, A. Konigsrainer: Are indoleamine-2,3-dioxygenase producing human dendritic cells a tool for suppression of allogeneic T-cell responses? *Transplantation* 83(4), 468-473 (2007)

143. B. Li, A. Samanta, X. Song, K. Furuuchi, K. T. Iacono, S. Kennedy, M. Katsumata, S. J. Saouaf, M. I. Greene: FOXP3 ensembles in T-cell regulation. *Immunol Rev* 212, 99-113 (2006)

144. D. Golgher, E. Jones, F. Powrie, T. Elliott, A. Gallimore: Depletion of CD25+ regulatory cells uncovers immune responses to shared murine tumor rejection antigens. *Eur J Immunol* 32, 3267-3275 (2002)

145. Q. Tang, J. Y. Adams, A. J. Tooley, M. Bi, B. T. Fife, P. Serra, P. Santamaria, R. M. Locksley, M. F. Krummel, J. A. Bluestone: Visualizing regulatory T cell control of autoimmune responses in non-obese diabetic mice. *Nat Immunol* 7, 83-92 (2006)

146. P. A. Antony, C. M. Paulos, M. Ahmadzadeh, A. Akpinarli, D. C. Palmer, N. Sato, A. Kaiser, C. Heinrichs, C. A. Klebanoff, Y. Tagaya, N. P. Restifo: Interleukin-2-dependent mechanisms of tolerance and immunity *in vivo*. *J Immunol* 176, 5255-5266 (2006)

147. G. Frumento, T. Piazza, E. Di Carlo, S. Ferrini: Targeting tumor-related immunosuppression for cancer immunotherapy. *Endocr Metab Immune Disord Drug Targets* 6(3), 233-237 (2006)

148. J. Bubenik: MHC class I down-regulation: tumour escape from immune surveillance? *Int J Oncol* 25(2), 487-491 (2004)

149. J. Steitz, J. Bruck, J. Lenz, J. Knop, T. Tuting: Depletion of CD25(+)CD4(+) T cells and treatment with tyrosinase-related protein 2-transduced dendritic cells enhance the interferon alpha-induced CD8(+) T cell-dependent immune defense of B16 melanoma. *Cancer Res* 61, 8643-8646 (2001)

150. D. H. Munn: Indoleamine 2,3-dioxygenase, tumor-induced tolerance and counter-regulation. *Curr Opin Immunol* 18(2), 220-225 (2006)

151. D. Y. Hou, A. J. Muller, M. D. Sharma, J. DuHadaway, T. Banerjee, M. Johnson, A. L. Mellor, G. C. Prendergast, D. H. Munn: Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses. *Cancer Res* 67(2), 792-801 (2007)

152. K. Hirai, T. Sasahira, H. Ohmori, K. Fujii, H. Kuniyasu: Inhibition of heme oxygenase-1 by zinc protoporphyrin IX reduces tumor growth of LL/2 lung cancer in C57BL mice. *Int J Cancer* 120(3), 500-505 (2007)

153. B. L. Guo, Z. Liu, W. A. Aldrich, R. D. Lopez: Innate anti-breast cancer immunity of apoptosis-resistant human gammadelta-T cells. *Breast Cancer Res Treat* 93(2), 169-175 (2005)

154. F. Lozupone, D. Pende, V. L. Burgio, C. Castelli, M. Spada, M. Venditti, F. Luciani, L. Lugini, C. Federici, C. Ramoni, L. Rivoltini, G. Parmiani, F. Belardelli, P. Rivera, S. Marcenaro, L. Moretta, S. Fais: Effect of human natural killer and γδ T cells on the growth of human autologous melanoma xenografts in SCID mice. *Cancer Res* 64, 378-385 (2004)

155. D. Kabelitz, D. Wesch, E. Pitters, M. Zoller: Potential of human gammadelta T lymphocytes for immunotherapy of cancer. *Int J Cancer* 112(5), 727-732 (2004)

156. D. Kabelitz, D. Wesch, W. He: Perspectives of gammadelta T cells in tumor immunology. *Cancer Res* 67(1), 5-8 (2007)

157. T. Alvaro, M. Lejeune, M. T. Salvado, R. Bosch, J. F. Garcia, J. Jaen, A. H. Banham, G. Roncador, C. Montalban, M. A. Piris: Outcome in Hodgkin's lymphoma can be predicted from the presence of accompanying cytotoxic and regulatory T cells. *Clin Cancer Res* 11, 1467-1473 (2005)

158. A. H. Banham, F. M. Powrie, E. Suri-Payer: FOXP3+ regulatory T cells: current controversies and future perspectives. *Eur J Immunol* 36, 2832-2836 (2006)

159. B. Valzasina, S. Piconese, C. Guiducci, M. P. Colombo: Tumor-induced expansion of regulatory T cells by conversion of CD4+CD25- lymphocytes is thymus and proliferation independent. *Cancer Res* 66, 4488-4495 (2006)

160. K. Ko, S. Yamazaki, K. Nakamura, T. Nishioka, K. Hirota, T. Yamaguchi, J. Shimizu, T. Nomura, T. Chiba, S. Sakaguchi: Treatment of advanced tumors with agonistic anti-GITR mAb and its effects on tumor-infiltrating Foxp3+CD25+CD4+ regulatory T cells. *J Exp Med* 202, 885-891 (2005)

161. A. L. Mellor, B. Baban, P. R. Chandler, A. Manlapat, D. L. Kahler, D. H. Munn: CpG oligonucleotides induce splenic CD19+ dendritic cells to acquire potent indoleamine 2,3-dioxygenase-dependent T cell regulatory functions via IFN type 1 signalling. *J Immunol* 175, 5601-5605 (2005)

162. D. M. Klinman: Immunotherapeutic uses of CpG oligodezoxynucleotides. *Nat Rev Immunol* 4, 249-258 (2004)

163. D. Braun, R. S. Longman, M. L. Albert: A two-step induction of indoleamine 2,3-dioxygenase (IDO) activity during dendritic-cell maturation. *Blood* 106, 2375-2381 (2005)

Key Words: $CD4^+CD25^+$T cells, $Foxp3^+$T cells, Tregs, Tr1 cells, pregnancy, cancer, Review

Send correspondence to: Jacek R. Wilczynski, MD, PhD, Department of Gynecological Surgery, Polish Mother's Health Center Research Institute, 281/289 Rzgowska Street, 93-338 Lodz, Poland, Tel: 4842271-1501, Fax: 4842271-1221, E-mail: jrwil@post.pl