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Th1/Th2 balance in cancer, transplantation and pregnancy

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I. INTRODUCTION

The carefully orchestrated events that regulate homeostasis of the immune system and the development of a protective immune response are coordinated to a large extent by cytokines produced by Th1, Th2, and the nominal Th3 lymphocyte subsets. An imbalance of Th1 and Th2 may be responsible for both the occurrence as well as the progression of several diseases and their resultant complications. Patients with advanced cancer often have impaired cell-mediated immunity associated with a switch from Th1 to Th2. On the other hand, shifting from one cytokine pattern to another may be highly beneficial in certain physiological conditions. For instance, IL-10, a Th2 type cytokine, may play a role in pregnancy-associated immune tolerance through the establishment of a Th2 cytokine bias at the maternal-fetal interface. Graft-versus-host disease (GVHD) is the major complication after allogeneic bone marrow transplantation (BMT) and is initiated by Th1 cytokines and resultant dysregulation of the cytokine network. The balance between type 1 and type 2 cytokines governs the extent to which a cell-mediated immune response and a systemic inflammatory response develop after allogeneic BMT. Successful interventions to regulate Th1/Th2 balance and modify the immune response may thus decrease the risk of development or relapse of malignancy, avoid impairment of donor cell engraftment, and allow successful fetal maturation.

II. TH1/TH2 BALANCE IN CANCER

It is generally thought that cellular immune responses are induced and maintained by regulatory CD4+ Th1 cells secreting IL-2 and IFN-γ. The induction of effective systemic antitumor immunity, for example, involves priming of both CD4+ and CD8+ cells specific for tumor-associated antigens. The role of Th cells is attributed to providing regulatory signals required for priming of MHC class I restricted CD8+ cytotoxic T lymphocytes (CTL). Activated CTL serve predominantly as immune effectors that induce tumor cell apoptotic death. Thus, tumor immunity is usually mediated by CTL whose activation and stimulation is supported by Th1 type cytokines. The survival of Th1 cells and CTL is in turn supported by local dendritic cells, providind a sourse of costimulatory molecules and cytokines such as IL-12, IL-15 and IL-18.

Murine studies

Several phenotypic and functional abnormalities within the immune system in tumor-bearing hosts have been identified and suggest interaction between tumor cells and host immune effectors, which results in impaired antitumor immunity. It has been recently demonstrated in a murine B cell leukemia/lymphoma model that animals susceptible to tumor challenge developed a Th2-dominant response, whereas resistant hosts developed a Th1 dominant response [70]. Thus, tumor susceptibility does not necessary presume the absence of an antitumor immune response. Rather, the nature of the antitumor immune response is critical in determining clinical outcome. Similar data were obtained using a murine melanoma model as well [48]. The data suggest that the failure to generate therapeutic T cells was not due to an inability to recognize tumor antigens per se, but, rather, to the induction of an immune response that was ineffective in mediating tumor regression, i.e., immune deviation. Furthermore, evaluating murine renal cell carcinoma and colon adenocarcinoma models in mice, Ghosh et al. demonstrated a gradual loss of Th1 populations and increase in Th2 cytokine profile during progressive tumor growth [37]. Thus, these studies on experimental animals collectively point to the possibility that a shift from Th1 to Th2 type of T cell response may play an important role in the development and progression of cancer. The possibility that tumor growth could be associated with cytokine-induced qualitative alterations in the immune

response has also been analyzed in human neoplastic diseases and have been observed to be present in a plethora of different cancers.

Clinical studies

Most clinical studies support the finding of abnormal Th1/Th2 ratio in cancer patients. The flow cytometric analysis of peripheral blood mononuclear cells (PBMC) obtained from patients with advanced cancer indicated that an imbalance of Th1 and Th2 was found not only in the frequency of the subsets in PBMC, but also in the capacity for cytokine production [103]. For instance, tumor-infiltrating lymphocytes (TIL) in non-small cell lung cancer patients express high levels of IL-10 and IL-4, but not IL-2. PBMC isolated from the same patients also expressed high levels of IL-10 mRNA. Alterations of Th1 or Th2 cytokine profile were usually characterized by decreased Th1/Th2 ratio and were described in virtually all tested patients with cancer including those with glioblastoma [3], lung cancer [13], non-Hodgkin's lymphoma [20], breast cancer [97], urinary bladder, renal cell and prostate cancer [30], head and neck cancer [91], cutaneous T cell lymphoma [46], basal cell carcinoma [134], and other tumor types (Table 1). The expansion of a peculiar subset of 'Th2-like' cells with increased IL-4 production was also found in patients with B cell chronic lymphocytic leukemia (CLL) [25].

Together, these data indicate the existence of a local and peripheral Th2 type cytokine pattern in most studied cancer patients. Alterations of Th1/Th2 ratio in cancer patients is a common feature of a malignant process and could result from (i) a malfunction in Th1 cells, (ii) activation of Th2 lymphocytes, or (iii) both phenomena. Further analysis of type 1 and type 2 cytokines in cancer patients and tumor-bearing animals clearly demonstrated the relationship between the stage of the disease and the relative proportion and number of Th1/Th2 cells. For instance, experimental results suggested that the extent of pulmonary metastatic disease in mice receiving B16 melanoma cells was strongly promoted by IL-4 released from tumor-associated Th2 lymphocytes [63]. Onishi et al. assessed the cytokine production in patients with renal cell carcinoma (RCC), who had undergone nephrectomy. They found not only significantly elevated levels of IL-4, IL-5, IL-6, and IL-10 production, but also demonstrated that the production of these cytokines was closely related to the stage and grade of malignancy [84]. Similarly, Th1/Th2 ratios corresponded with hematological changes observed in patients with thymoma. Specifically, Th2 type lymphocytes were dominant during hematopoietic suppression and elevation of the Th1/Th2 ratio was concomitant with hematological improvement after thymectomy [36]. It seems that this dysregulation increases as the disease progresses through to more advanced stages. This in turn suggests that it could be an important mechanism which allows the tumor to implant and progress in the host. Based on the above findings, the a logical question is whether treatment of cancer patients might result in partial or complete normalization of altered cytokine production. Several studies evaluated this possibility and demonstrated a secondary shift from Th2 to Th1 type of cytokine production in cancer patients after successful therapy or surgery.

Post-therapy changes in Th1/Th2

Tabata et al. compared Th1/Th2 cytokine production in 50 patients with digestive cancer using FACScan analysis [119]. They found that the percentage of CD4+ T cells producing IL-4, IL-6, and IL-10 were significantly higher in cancer patients than in healthy controls. Importantly, the proportion of Th2-producing lymphocytes decreased significantly 1 month after surgical excision of the tumor. Similar data were obtained in patients with ovarian cancer treated with an anti-idiotype vaccine. There was a substantial increase in intracellular IFN-γ and IL-2 characteristic of a Th1 cell type immune response after treatment with a vaccine [95]. Immunotherapy of bladder cancer

patients with BCG + IFN-α resulted in polarization of the immune response toward the cellular immune pathway promoting Th1 cytokine expression and in turn reducing Th2 cytokine expression [75]. Immunotherapy of patients with advanced prostate cancer also caused a switch from predominant production of IL-4 by PBMC to IL-2, which in several patients correlated with decreased levels of PSA [47].

Using a murine T cell lymphoma model, Wong et al. have demonstrated that only TCR-based vaccines that induced a Th1 type immune response favored protection from tumor [132]. Some chemotherapeutic agents appear to have a similar mechanism of action. Treatment of mice bearing the MethA fibrosarcoma with cyclophosphamide lead to tumor regression in part through a alteration of the host's immune status. Within the tumor, a shift of cytokine expression from Th2 to Th1 type is noted [51]. Similarly favorable Th1-dominant pathway is induced in the tumor tissue of operable patients with lung carcinoma [52]. However, a shift from Th1 to Th2 is noted with progression of tumor.

An interesting observation was recently published by Berghella et al. analyzing the levels of Th1 and Th2 type cytokines and soluble IL-2R (sIL-2R) in the serum of colon cancer patients and healthy subjects. They demonstrated that the levels of sIL-2R in patient's serum were higher than in healthy donors and correlated with the stage of tumor. Moreover, while in healthy subjects the serum level of sIL-2R was not significantly correlated with other parameters, in patients it was positively related with IL-4, IL-6, and IL-10 serum levels, and negatively correlated with serum levels of IL-2 and IFN-γ [6]. This suggests that the increase of sIL-2R in cancer patients may be associated with promotion of the Th2 immune response.

Th1/Th2 imbalance is not uniformly noted in the setting of cancer

It is important to note that not all authors observed a marked imbalance in cytokine production in cancer patients. For instance, Punnonen et al. evaluated the profiles of cytokines in the peritoneal fluids in 111 patients with ovarian, cervical, or endometrial carcinomas or benign ovarian or uterine tumors. The authors reported that with the exception of IL-6, the cytokine synthesis profiles of patients with benign and malignant gynecologic tumors were similar. These results suggest that cytokine production in these patients is a result of non-specific inflammation rather than a specific antitumor response and that skewing of cytokine synthesis toward either Th1 or Th2 phenotype is not the underlying mechanism resulting in the malignant process in these patients [92]. In contrast, al-Saleh et al. reported significant decreases in IL2+ and significantly higher densities of IL4+ and IL6+ cells within squamous intraepithelial lesions than in histologically normal tissues taken from adjacent ectocervical regions [2]. Cytokine production by TIL and PBMC obtained from Kaposi's sarcomas indicate that patients with Kaposi's sarcoma express a Th1 phenotype with marked IFN-γ production [109]. Similar data were reported after analysis of cytokine profile in low grade B cell gastric lymphoma of the MALT type [45]. Both Th2 and Th1 type patterns were detected in Hodgkin's disease and reactive lymph nodes [107], as well as in TIL freshly isolated from primary renal cell carcinoma specimens [128].

Since IL-4 directs Th2 development and has been shown to inhibit Th1 responses, IL-4 could be considered a CTL inhibitory cytokine. Surprisingly, however, it was recently shown that the development of tumor immunity requires IL-4 in the priming phase for generation of Th1-associated, tumor specific CTL [106]. In addition, the recently described stimulatory effect of IL-10 on CD8+ T cells was confirmed in other studies by the ability of IL-10 transgenic mice to limit tumor growth by a CTL-mediated mechanism [40]. Thus, mechanisms involved in the abnormal immune responses in tumor-bearing hosts are more complex than a simple Th1/Th2 misbalance and require more careful evaluation.

Regulation of Th1/Th2 balance in the tumor microenvironment

Tumor cells themselves have been shown to produce a variety of factors which may alter the level of Th1 or Th2 cytokines by either a direct effect on T cells or subsequent interaction with other immune cells including dendritic cells (DC) or macrophages (Fig.1). These factors include TGF-β, IL-10, prostaglandines, gangliosides and others [4, 14, 58, 60]. For instance, tumor-derived TGF-β induces overproduction of IL-10 and, in turn, promotes a shift in the Th1/Th2 balance toward a Th2 response and inhibiting the Th1 response [77].

An initial commitment to the production of Th1- or Th2-type cytokines occurs in a population of lymph-node-based naïve Th cells at the time of their priming [124]. DC, homing to lymph nodes, are a source of not only antigenic and costimulatory signals but also of early Th1- and Th2-polarizing signals. It is well established that antigen-presenting cells with different abilities to produce IL-12, such as monocytes, macrophages and DC, induce altered cytokine patterns in responding Th cells [1, 76]. Since IL-12 has been shown to play a crucial role in Th0 differentiation, DC are probably the most important regulators of Th1/Th2 balance due to the high levels of IL-12 synthesis and release. Thus, suppression of IL-12 production by DC in the tumor microenvironment may play an important role in the polarization of Th cells. There are two main mechanisms involved in the tumor-mediated inhibition of IL-12 production in DC. First, tumor-derived factors could induce apoptosis in DC and eliminate them or their functionall activity from the tumor microenvironment [32, 108]. Physical depletion of DC by tumor may account for the decreased levels of IL-12 and diminished Th1/Th2 ratio. Secondly, IL-10, TGF-β and PgE released by tumor cells could markedly inhibit DC IL-12 production by altering DC polarization during their differentiation. Third, recent observations demonstrate that profound differences in IL-12 producing capacity and in Th1 versus Th2 inducing potential, are also present among different DC subsets.

It has been suggested that similar to the ability of a single naive Th cell to develop into stable Th1 or Th2 effector/memory subsets, immature DC within a single DC lineage, depending on the microenvironment could develop into distinct subsets of polarized effector DC with different capacities to induce Th1 and Th2 responses. In the tumor microenvironment, both activating and suppressive factors, derived from tumor cells or infiltrating lymphocytes and macrophages, may play an important role in DC.

Cytokines within the tumor microenvironment

IFN-γ, a product of activated NK and T cells, PgE_2, an inflammatory mediator produced by fibroblasts, epithelia, macrophages and tumor cells, and IL-10, all serve as examples of DC-modulating factors. While IFN-γ does not interfere with changes in surface phenotype and stimulatory potential of maturing DC, it may stimulate them to produce up to 100 fold more IL-12 after subsequent CD40 mediated stimulation [55, 56]. Such, so-called type-1 polarized effector DC induce a vigorous Th1-type responses in naive Th cells. On the other hand, both PgE_2 and IL-10 induce a dose-dependent inhibition of the ability of DC to produce IL-12. For instance, PgE_2 promotes the formation of type-2 polarized subset of effector DC that produce low levels of IL-12 and induces a bias to Th2 cytokine production in naive Th cells [55]. In contrast to PgE_2, IL-10 inhibits not only the ability of DC to produce IL-12 [24] but also their stimulatory potential, promoting the development of a type 3-polarized, tolerogenic DC subset [113]. Despite being primarily suppressive, IL-10-exposed DC also promote Th2 function [72]. This suggests that various factors in the tumor microenvironment may affect the development of immature DC into type-1, type-2, and type- 3 polarized effector DC subsets and transmit an early polarizing signal to naive Th cells.

In fact, decreased production of IL-12 by PBMC was repeatedly detected in cancer patients [26, 31, 111]. Furthermore, the ratio of IL-12/IL-10 levels was significantly lower in patients with squamous intraepithelial lesions of the uterine cervix as compared with control groups. A lower IL-12/IL-10 ratio in women with squamous intraepithelial lesions was also observed when PBMC culture supernatants and plasma samples were analyzed [53]. Evaluating the cytokine patterns in a murine mastocytoma model, Grohmann et al. not only confirmed an IL-12 requirement in promoting antitumor reactivity, but also established IL-10 as an important cytokine in permitting optimal expression of this reactivity, which apparently develops in the absence of a strong bias toward a type 1 or type 2 cytokine response [39].

It is likely that tumor-derived factors are directly responsible for decreased IL-12 production by macrophages and DC from tumor bearers. For example, it has been demonstrated that macrophages isolated from tumor-bearing mice produce significantly lower levels of IL-12 [43]. It has been suggested that this defect in macrophages, rather than a shift in T cell subsets, is an important cause of the down-regulation of IFN-γ production. On the other hand, it is also possible that tumor-derived factors indirectly modify DC or macrophages and change their ability to produce IL-12. In fact, Th1 cells up-regulate IL-12 production by DC via IFN-γ-independent cognate interaction, whereas this is inhibited by Th2-derived IL-10 [96]. The inhibition of Th1-induced IL-12 production by Th2 cells with the same antigen specificity represents a novel mechanism driving the polarization of CD4+ T cell responses.

Thus, it is possible that additional mechanisms, as yet unidentified, are responsible for the abnormal regulation of cytokine production by DC, macrophages and involving T cells subsets in the tumor microenvironment. Identification of these mechanisms further supports the development of effective immunotherapies and might significantly improve diagnostics and prophylactics approaches in a population with increased risk of tumor development.

III. TH1/TH2 BALANCE AND TRANSPLANTATION

The immune response to organ and tissue transplants is mediated by T lymphocytes . This has been confirmed by the indefinite acceptance of allografts in T cell deficient nude mice. Helper T lymphocytes are critical mediators of acute rejection, which either enhance or modulate rejection severity, through the cytokines they secrete. Regulation of various cytokines occurring at different alloreactive phases is believed to be responsible for the fate of transplanted grafts [127]. A full understanding of the role of cytokines and T cell subpopulations in transplantation may allow development of improved strategies to prevent rejection or induce tolerance, and to improve immunological monitoring of graft recipients.

It is generally accepted that Th1 cells promote allograft rejection by enhancing the function of specific cytotoxic T lymphocytes and delayed-type hypersensitivity (DTH) responses. Th2 cells that are antagonistic to Th1 development and function, preferentially down-regulate Th1-deriven rejection responses. Many studies have demonstrated that allograft acute rejection is associated with increased Th1 reactivity, with high levels of IL-2, IFN-γ mRNA and protein being detected within the grafts [98]. In contrast, in animal models, when tolerance is induced, Th2 cytokine expression is enhanced, whereas Th1 cytokines are usually down regulated or even absent. Furthermore, the transcript levels of Th1 cytokines have been corrected with acute graft rejection severity [22], [87], [9]. One explanations for rejection induced by Th1 cells is the requirement of proinflammatory cytokines for T cell activation [79, 81]. Other than promoting the development of alloantigen-specific cytotoxic T lymphocytes and enhancing DTH responses, Th1 cells are also believed to initiate allograft rejection by facilitating IgG2a antibody switching which in turn promotes

antibody-dependent cellular mediated cytotoxicity[104]. Th1 cells can function as effectors of cell-mediated immunity. The number of graft-infiltrating $CD4^+$ cells producing IFN-γ, detected by intracellular cytokine staining, increases with time as rejection proceeds [116, 121]. However, $CD8^+$ cells are also significant cellular source of IFN-γ. It remains unclear whether IFN-γ production by $CD8^+$ cells Th1 cell is the cause of this phenomenon [116]. Taken together these data suggest that Th1 cells may facilitate cellular immune responses in allograft rejection.

Conversely, Th2 cells producing IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 stimulate humoral, allergic and mucosal responses and antagonize the effects of Th1 cells *in vivo*. Th2-type cytokine transcripts correlate with mildness or absent rejection episodes [22, 102]. Although some studies show significant levels of Th2 cytokines in graft-infiltrating cells from acutely rejecting allografts [121], many investigators reported very little, or no detectable IL-4 level in acute rejecting grafts. Furthermore, Th2 cytokines up-regulation is typically accompanied by prolongation of allograft survival and tolerance induction [120, 125, 126]. In animal models, when tolerance is induced, Th2 cytokines are up-regulated within the grafts, and Th1 cytokines are suppressed or absent [104, 121]. In addition, in a neonatal tolerance model, allograft acceptance is associated with inhibition of the Th1 response and enhancement of the Th2 response [10]. Th2 cells have also been related to tolerance induced by injection of allogeneic cells in newborn mice [90], which can be reversed by treatment with anti-IL-4 antibody during the tolerance inductive phase [27]. Immunosuppressive reagents, such CsA and glucocorticoids inhibit Th1, but spare Th2 cytokine production [82, 104]. Blocking the CD28-B7 costimulatory pathways with CTLA4 Ig resulted in a long-term allograft acceptance and associated with inhibition of intragraft IL-2 and IFN-γ, but not IL-4 and IL-10 expression [42, 98]. These observations lead to the hypothesis that Th1 and Th2 cells are the principal regulators of allograft rejection, and that induction of Th2 cytokines might inhibit Th1-mediated rejection responses, thereby promoting allograft acceptance [9, 87, 116].

Th1/Th2 paradigm hypothesis is oversimplified

Despite the theoretical attractiveness of the Th1/Th2 paradigm of transplant responses, several recent studies do not support such a simple model. Functions of Th1 and Th2 cells have been defined primarily in *in vitro* systems, and their interaction during *in vivo* alloimmune responses appear more complex than expected. The absence of strict separation of the cytokine profiles in tolerance and rejection raises questions about the validity of the Th1/Th2 paradigm in transplantation immunity. Th2 cytokines can also synergize with Th1 in effecting graft destruction. For instance, IL-10 production has been correlated with acute rejection in both renal [118] and heart allografts [116]. IL-4 and IL-10 both produced by Th2 cells have been shown to enhance CTL growth and differentiation [7, 12, 80]. Both IL-2 knockout mice and IFN-γ knockout mice can reject allografts as in normal animals [112]. Stable graft tolerance is induced in IL-4 knockout mice [117]. Several studies have also shown that Th2 cells are not protective for allograft rejection. Bile level of IL-4 and IL-10 are 10-fold higher than those observed for IL-2 and IFN-γ during liver transplant rejection episodes in humans, suggesting that overexpression of Th2 cytokines does not always protect grafts from rejection [68]. Treatment with anti-IL-12 antibodies induces Th2 cytokine expression, but fails to inhibit IFN-γ gene expression in a mouse cardiac allograft model, and graft rejection is accelerated, rather than inhibited [87]. Although IL-10 inhibits Th1 cytokine production *in vitro*, surprisingly, post transplant administration of exogenous IL-10 accelerates mouse cardiac allograft rejection [93]. The inhibitory effect of IL-10 on T cells is mainly through inhibition of accessory cells. IL-10 does not reverse antigen-presenting cell maturity, but can suppress maturation of immature cells, and then in turn inhibit T cell proliferation [5, 113]. The outcome of immune responses is influenced by these diverse and opposing regulatory effects of IL-10. These

observations suggest that the Th1/Th2 paradigm is oversimplified to elucidate the outcome of allograft rejection or tolerance. The basis for the paradoxical observations in various correlative studies and models with targeted cytokine gene disruption remains unclear. The observation that different strain combinations affect the prominence of Th1 or Th2 responses in mice demonstrated altered genetic influence on recognition of transplant antigens and biased cytokine responses to allostimulation. It is also probably due to the redundancy of cytokine functions and to the versatility of transplant related immune effector mechanisms.

Regulation of alloreactivity by shifting of Th1 to Th2 response

The absence of clear separation of Th1/Th2 cytokine profiles in tolerance and rejection raised questions about the validity of the Th1/Th2 paradigm in transplantation immunity. It remains useful for developing strategies to promote graft acceptance by preferential induction of Th2 responses. Several approaches have been tested during the past decade.

A. Influence of Th1/Th2 balance by manipulation of cytokines. Th1 and Th2 subsets are driven from Th0 cells, which produce small amounts of almost all T cell-derived cytokines, including IL-2, IL-3, IL-4, IL-5, IL-7, IL-10, IL-13, IL-15, IFN-γ, TNF, GM-CSF and lymphotoxin [82]. Upon stimulation and maturation, the Th0 cells differentiate into Th1 or Th2 cells that produce a more restricted profile of cytokines in much greater quantities. The cytokine milieu in which the response develops may be an important factor in determining whether a response develops toward a Th1 or Th2 pattern. As we mentioned above, IL-12 appears to be a key cytokine in establishing Th1 dominance. The potential use of IL-12 antagonists as an inductive therapy to promote alloreactive Th2 and inhibit Th1 response is under evaluation. Th2 cytokine expression was induced in cardiac allograft recipients treated with either neutralizing anti-IL-12 antibodies or the IL-12 p40 subunit, which bind to the β1 component of the IL-12 receptor and serve as a competitive inhibitor for IL-12 p70 binding. However, graft rejection was accelerated rather than inhibited [87]. The experiment conducted in IL-12 deficient mice demonstrated that alloantigen-reactive Th1 cells could be developed independently of IL-12, and that IL-12 p40 homodimer may function as a regulatory cytokine with both agonistic and antagonistic activity. IL-10 is likely to be the second multifunctional regulatory cytokine. It has anti-inflammatory effects, and has been an attractive candidate for cytokine therapy in transplantation due to its anti-inflammatory properties. Short course and pre-operative treatment of recipient mice with IL-10 could prolong graft survival. However, prolonged administration of IL-10 actually has a detrimental effect on graft survival due to augmentation of CTL activity by IL-10 [74, 93]. Hence, manipulation of cytokine profiles under defined condition may influence Th1/Th2 balance. Promoting a Th1–>Th2 shift can decrease acute rejection episodes, which might also favor the prevention of chronic rejection. In addition it might decrease the requirement for immunosuppressive therapies that cause the transplant recipient to be vulnerable to opportunistic infections and cancer. It may also be an approach for tolerance induction [117].

B. Switch Th1 to Th2 by blockade of costimulatory pathway. As mentioned above, blockade of the CD28-B7 costimulatory pathway with CTLA4 Ig leads to allograft acceptance with inhibition expression of IL-2 and IFN-γ, but not IL-4 and IL-10. Simultaneously blocking both CD28-B7 and CD40L-CD40 pathways inhibits both acute and chronic rejection of allografts, associated with down-regulation of both Th1 and Th2 cytokine expression [69]. Furthermore, blockade of CD40/CD40L pathway with anti-CD40 antibody in combination with infusion of donor-derived DC progenitors resulted in long-term of heart allograft survival, which is associated with decreased splenic IL-2 production in the recipient.

C. Direct Th2 dominated response by gene engineering. Most cytokines have a short half-life *in vivo* and their ability to influence the immune responses may be reduced following delivery *in vivo.* Two approaches are currently being examined, designed to induce high levels of Th2-type cytokine expression: (i) treatment of the recipient with Th2 cytokine gene-transduced cells, including DC and (ii) genetic engineering of donor organ with Th2 type cytokine genes. IL-4-transduced cells prolong heart allograft survival, but neither IL-4-, nor IL-10-producing cells had an effect on graft survival in mouse skin graft models. There was no evidence that immune responses were converted to a Th2 dominant type. However, we have shown that graft transduction with viral IL-10 significantly prolonged heart allograft survival in a mouse vascular heart transplantation model [94].

D. Adoptive transfer of Th2 cells. Adoptive transfer of MHC class II reactive CD4+ cell clone producing both IL-4 and IL-10, but not IL-2 or IFN-γ upon stimulation with antigen into recipient mice has been demonstrated to prolog skin graft survival in an antigen-specific manner. This effect was associated with inhibition of antigen-specific CTL responses [78]. These results provide direct evidence that Th2 cells are able to contribute to the induction of antigen-specific tolerance.

In summary, in transplantation models, the Th1 cytokine profile often associates with allograft rejection, while the Th2 profile favors the acquisition of tolerance. However, this paradigm may not be sufficient to explain the recently demonstrated *in vivo* effects of cytokine manipulation on allograft survival. For instance, although it is believed that Th1 cells promote acute graft rejection, the role of these cells in chronic rejection remains unclear. Using SCID cardiac allograft model, Picotti et al. demonstrated that the absence rather than the presence of donor-reactive Th1 is associated with chronic rejection. Th1-independent effector mechanisms are thus responsible for chronic rejection in their model [88]. Th2 cytokines may therefore not be necessary for tolerance induction, while Th1 cytokines may even be beneficial in promoting allograft survival. Such data however, should be interpreted in light of the diverse and often redundant effects displayed by cytokine networks *in vivo*. Understanding the complex interactions of cytokines in the alloimmune cascade is therefore critical for designing therapeutic strategies that abrogate allograft rejection and induce donor-specific tolerance, an elusive goal in organ transplantation [135].

IV. TH1/TH2 BALANCE AND PREGNANCY

Normal pregnancy

Pregnancy is the adaptation by which a mother carries a temporary semi-allogenic graft. The mother shares only half of the fetal MHC antigens, the other half of the fetal MHC antigens are contributed by the father. During pregnancy immunosuppression is required to maintain the fetus in an environment where as many as thirty percent of the cells present in the uterus are of bone-marrow origin. At the time of implantation the endometrial leukocytes consists mainly of large granular leukocytes (LGL), macrophages and T cells, while B cells and plasma cells are rare [8, 59, 61, 62]. DC are also present in the uterus but these cells have only been studied in the rat [57]. At implantation, 50-60 % of the leukocytes are LGL with macrophages representing 20-25% and T cells the remaining 20-25%. The leukocyte cells in similar proportions can be found in numerous other specieswhich have been examined including humans, rodents, pigs and sheep. Distinct changes in leukocyte numbers and activation are associated with decreased fertility in rodents [41, 89], and in humans, although the evidence more circumstantial [85, 115, 133]. Thus the uterine leukocytes and their contribution to the uterine cytokine environment are important in both the establishment and maintenance of pregnancy.

Wegmann et al. first proposed that pregnancy is a Th2-like state [71, 130]. In their landmark studies they demonstrated that IL-4, IL-5 and IL-10 were spontaneously produced from murine fetoplacental tissues but not from maternal splenocytes [71]. The production of these cytokines can also be demonstrated from leukocytes in the draining lymph nodes of the uterus during pregnancy. Thus the immunosuppression associated with pregnancy, while greatest in the uterus, has systemic effects. In humans there is an increase in the abundance of Th2-type cytokines, IL-4, IL-5, IL-6, and IL-10 during the secretory phase of the menstrual cycle with a further increase occurring in pregnancy [64]. These cytokines have been demonstrated to down-regulate many of the cellular immune effector functions, including cytotoxic activity and release of inflammatory cytokines.

Within the uterus are several leukocyte populations, including LGL and T cells, which have cytolytic capabilities. The LGL population has a distinct phenotype (CD56+, CD16-, CD3-) when compared with the NK cell commonly found in the peripheral circulation ($CD16+/CD56^{dim}$) [21, 114]. While the uterine LGL displays a marked decrease in the cytotoxic ability from the peripheral NK cell, non-specific up-regulation of the LGL, in a mouse model of spontaneous abortion results in greater fetal loss. The T cell population of the uterus also may be cytotoxic [131], however this effector function is absent in the secretory phase, the time that implantation occurs. The T cell in the decidua has not yet been examined for cytotoxic activity. Thus the immunosuppressive state of the uterus, provided by the Th2-type cytokine dominated environment, is necessary for fetal survival.

The production of the Th2 cytokines and immunosuppression molecules can be documented from both the maternal and fetal tissues. Table 2 shows the production of Th1, Th2 and other immunosuppressive factors from various sources during pregnancy. Cytokine and immunosuppressive factors are produced from maternal uterine epithelium and decidual leukocytes. When the balance of cytokines is disrupted, the pregnancy results in significant mortality and morbidity (Table 2). Although, the Th2 cytokines predominate in the uterus during pregnancy, Th1 cytokines, including IL-2, IFN-γ and TNF-α can also be found. The normal physiological role of these Th1 cytokines is not known. It has been suggested that they may play a role in activating cells to secrete factors necessary for the tissue remodeling that accompanies the extensive vascularization that occurs during pregnancy and in the initiation of labor at the end of pregnancy.

Pre-eclampsia

The changes that occur in pathological states of pregnancy are conditions that confirm the apparent importance of the Th1/Th2 balance in normal pregnancies. Pre-eclampsia is a condition with as yet an unknown etiology. Elevated levels of TNF-a have been documented in the serum during a pre-eclampic pregnancy and elevation of both TNF-a and IL-1 were seen in the placentas after delivery. However changes in the decidua and placenta while the patient develops pre-eclampsia cannot be studied directly. Recently the administration of IFN-γ or TFN-α to pregnant mice has been shown to have a deleterious effect on the vascular system in the uterus [15]. These cytokines induce formation of plaques that block the maternal blood vessels; similar to what has been observed in pre-eclampsia. This mouse model may help in modeling of cytokine changes that occur during pre-eclampsia and the uterine changes that occur in response to these cytokines.

Spontaneous Abortions

Spontaneous abortions, in the absence of chromosomal abnormalities, account for up to 20% of the documented fetal loss in humans. Loss of pregnancy has been associated with alterations in the leukocyte populations [41, 133], as well as in cytokines. In humans although there are distinct decidual leukocyte changes [133] it is difficult to determine if these are a secondary inflammation due to the death of the fetus or are the primary cause of the spontaneous abortion.

The murine model of spontaneous abortion, CBA x DBA/2 cross, has provided much of the information available on the cytokine changes that occur with impending fetal loss because we can observe pregnancy prior to as well as during the spontaneous abortion. IL-2, IFN-γ and TNF-α expression is much higher in the placental tissues obtained from a CBA x DBA/2 cross compared with fetuses from a CBA x BALB/c cross [122]. While one possible effect of the presence of these cytokines may be to activate the cytotoxic activity of the maternal LGL and T cells, a secondary effect of the increase in Th1 cytokines may be to modify the maternal vasculature in a manner similar to events that occur in pre-eclampsia.

Infection and Pregnancy

Infection is another way to alter immunity. In humans the presence of malaria infection results in premature birth or low birth weights, accompanied by an increase in IFN-γ and TNF-α production [35]. While we know that overwhelming infections in the mother can result in spontaneous abortion of a fetus, the events that occur within the uterus are difficult to study.

The systemic cytokine environment of a mouse and its effect on pregnancy was studied in a murine *Leishmania* model. When C57BL/6 mice are infected with *Leishmania*, they develop a Th1 immune response, characterized by high levels of IFN-γ, which leads to successful resolution of the infection. However when pregnant C57BL/6 mice, were infected with *Leishmania major* (*L. major*) they were unable to resolve the infection and this was associated with a decrease in their IFN-γ production [65]. These experiments demonstrate that pregnancy can bias the systemic immune system towards a Th2 response, sufficient to blunt expression of IFN-γ and diminish a Th1 immune response. Furthermore, if mice were first infected with *L. major* and then mated, there was a decrease in the number of implantation sites as well as an increase in fetal resorptions [66]. This decreased fertility was associated with a decrease in IL-10 production and an increase in IFN-γ production from placental tissues. Thus the strong Th1 response to *L. major* could overwhelm the protective Th2 uterine environment and compromise concurrent pregnancy. In humans, interestingly, an increase in IL-2 can be found in spontaneously aborting tissue.

In summary, pregnancy is an immunosuppressive state dominated by Th2 cytokines. When the balance of cytokines shift from Th2 towards Th1 cytokines then the pregnancy is in jeopardy. While pre-eclampsia and some infections can shift the cytokine environment toward a Th1 state, Th2 predominance allows for continuance of pregnancy. When the Th1 cytokines are able to overcome the protective uterine immune balance, the pregnancy ends as in the case of some spontaneous abortions. By studying cytokines and their production during pregnancy, it may be possible to intervene and shift the cytokine balance when a pregnancy is at risk.

V. CONCLUDING REMARKS

It is noteworthy that marked similarities were demonstrated in the basic immunological defects that favor the appearance, development and progression of infectious, inflammatory and neoplastic diseases that are etiologically unrelated. Thus, it is possible that qualitative impairment of the immune responses, exemplified by changes in type 1 to type 2 cytokine patterns, could be the *primum movens* permitting pathologic conditions to develop into disease [17]. This additionally suggests that therapeutic approaches, including antitumor therapy, should widen their focus and pay particular attention to testing and modifying the existing cytokine misbalance. The third important aspect of understanding of Th1/Th2 model in tumor immunology is the possibility of new interpretation of early misunderstand phenomena. For instance, Flexner and Jobling in 1907 first demonstrated enhancement of tumor growth in experimental animals receiving antiserum to

autologous tumor [33]. It is clear now that antibody enhancement of tumors may be a reflection of Th1 to Th2 cytokine shift that results in reduced protective cellular immune response against tumor. Reinterpretation of enhancement of tumor growth in the context of Th1/Th2 balance regulating the cellular and humoral immune responses is consistent with increasing evidence that cytokines and cellular immunity play a crucial role in tumor rejection or progression.

Table 1. Th1/Th2 cytokine patterns in cancer patients

Type of Cancer	Th1 profile		Th2 profile		References
	Up-regulation	Down-regulation	Up-regulation	Down-regulation	
Digestive cancer			IL-4, IL-6, IL-10		[119]
Renal cell carcinoma		IL-2, IFN-γ	IL-4, IL-5, IL-6, IL-10		[84]
B cell chronic lymphocytic leukemia			IL-4		[25]
Cutaneous T cell lymphoma		IL-2, IFN-γ	IL-5, IL-6, IL-10		[46]
Thymoma		IL-2	IL-4		[36]
Prostate cancer		IL-2, IFN-γ	IL-4		[47]
Head and neck cancer		IL-2, IFN-γ			[91]
Gynecologic tumors			IL-6		[92]
Non-small cell lung cancer	IFN-γ	IL-2	IL-4, IL-6, IL-10		[4]
Cervical cancer		IL-2	IL-4, IL-6, IL-10		[2, 53]
Kaposi's sarcoma	IFN-γ				[109]
Colon cancer		IL-2, IFN-γ	IL-4, IL-6, IL-10		[6]
Hodgkin's disease	IFN-γ	IFN-γ	IL-4	IL-4	[107]
Progressing melanoma Responding (regressing) melanoma	IL-2, IFN-γ		IL-10		[31] [34] [73]
Glioblastoma		IL-2			[3]
Gastric lymphoma	IFN-γ				[45]
Cutaneous T cell lymphoma		IFN-γ	IL-4		[26]
Lung cancer		IFN-γ	IL-10		[13]
Non-Hodgkin's lymphoma			IL-10		[20]
Ovarian and breast cancer	IFN-γ				[38]

Breast cancer			IL-4, IL-10		[97]
Bladder, renal cell and prostate cancer		IL-2, IFN-γ			[30]
Glioma		IL-2, IFN-γ	IL-4		[99]
Pancreatic and gastric adenocarcinoma			IL-10		[34]
Colorectal cancer		IL-2, IFN-γ	IL-4, IL-6		[86]
Non-small cell lung cancer		IL-2, IFN-γ	IL-4, IL-5, IL-10, IL-13		[49]
Bronchogenic carcinoma			IL-10		[110]
T cell leukemia and the Sezary syndrome		IL-2, IFN-γ	IL-4, IL-5, IL-10		[123] [100]
Melanoma			IL-10		[11] [67]
Basal and squamous cell carcinoma		IL-2, IFN-γ	IL-4, IL-5, IL-10		[60, 134]
Renal cell carcinoma		IL-2, IFN-γ	IL-4, IL-10		[105] [129] [83]
Hodgkin's disease		IL-2			[23] [16]
Glioma		IL-2	IL-10		[28] [50]
Intracranial tumors		IL-2			[29]

In general Th1 responses are suppressed in cancer patients.

Table 2. Cytokines in normal and pathological pregnancies

	Decidua	Serum	LGL	Macrophages	T cells	Placenta
Normal Pregnancy	↑Th2*, ↓Th1† [71] GM-CSF, M-CSF, LIF, PgE_2	ND±	GM-CSF, M-CSF, LIF, IFN-γ [54, 101]	IL-10, GM-CSF, PgE_2	IL-4, IL-10, IFN-γ [101]	Th2, GM-CSF, PgE_2
Pre-eclampsia	IL-2 [44]	TNF-α [19]	ND	ND	ND	↑TNF-α, IL-1 [18]
Spontaneous Abortion	↓Th2, ↑Th1 [122]	ND	ND	ND	ND	ND
Infection	↓Th2, ↑Th1 [35, 65]	ND	ND	ND	ND	↓Th2, ↑Th1 [35, 66]

*, Th2 = IL-4, IL-10, TGFβ-1; †, Th1 = IFN-γ and IL-2; ±, ND = not determined; LGL, large granular lymphocytes or NK cells. In general Th2 responses seem to be associated with successful pregnancies.

FIGURE LEGENDS

Figure 1. Carefully orchestrated balance between Th1 and Th2 type cytokines is a key component of immune homeostasis. Misbalance of these two arms of the immunity plays an important role in the etiology and pathogenesis of cancer and graft rejection. Understanding of the crucial events in Th1/Th2 shifts and mechanisms responsible for this dysregulation could improve immunotherapies and outcome in cancer and transplant patients.

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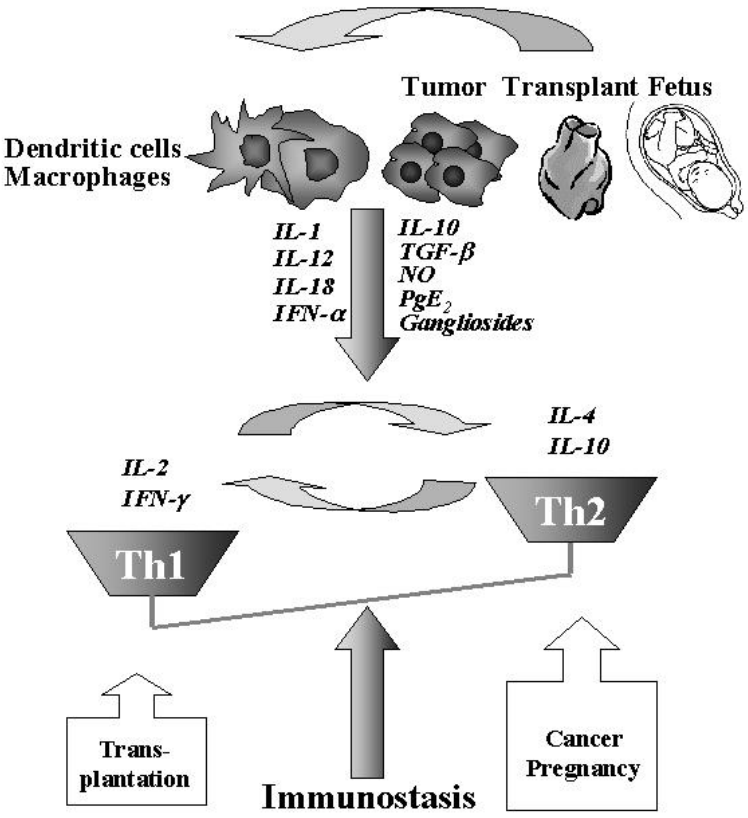
Figure 1.


Tumor
Transplant
Fetus
Dendritic cells
Macrophages
IL-1
IL-12
IL-18
IFN-α
IL-10
TGF-β
NO
PgE2
Gangliosides
IL-4
IL-10
IL-2
IFN-γ
Th2
Th1
Trans-
plantation
Immunostasis
Cancer
Pregnancy