Tolerance Signaling Molecules and Pregnancy: IDO, Galectins, and the Renaissance of Regulatory T Cells

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Problem
Is the concept of maternal tolerance preventing rejection of the semi-allogeneic 'fetal allograft' still valid?

Method of study
Compilation of expert reviews of literature and recent advances in research on indoleamine-2,3 dioxygenase (IDO), regulatory T cells and galectin-1.

Results and Conclusion
A role for IDO in pregnancy success remains speculative, but solid data exist to support a role for Treg cells, and for galectin-1 in induction and action of Treg cells. Just as several signals may need to be simultaneously present to induce Th1 cytokine-triggered abortions, more than 1 signal may need to be simultaneously present to prevent rejection and ensure success. Both complement and coagulation pathways appear necessary for embryo execution.

Introduction (DA Clark)

Rejection of the successfully implanted semi-allogeneic embryo differs in certain respects from rejection of allografts of histoincompatible paternal tissue. In the CBAxDBA/2 model of spontaneous abortion, NK lineage cells but not classical $CD4^+$ or $CD8^+$ effector cells are responsible, and NKT cells, most likely with γδ TCR rather than αβ TCR have been implicated.[1] NKαβ T-cell activation can also trigger loss, and possibly these cells also express CD4.[2] In the CBAxDBA/2 model, it has recently been shown that complement activation is a critical event, and it has been proposed that properdin + factor B from T cells and macrophages enable enhanced autoactivation of C3 by the alternate (tick-over) pathway.[3,4] On the other hand, effector cell secretion of the Th1-cytokines IFN-γ and TNF-α plus a TLR signal (to up-regulate receptors of the cytokines) has also been proposed to cause abortion by activating coagulation.[1,5,6] In addition to generating fibrin, thrombin is able to stimulate neutrophil recruitment via endothelial cell activation, and can generate C5a, which activates neutrophils that are an important contributor to embryonic demise.[6] Blocking either the coagulation pathway or the complement pathway prevents abortions.[3,6,7] The need for two pathways provides an element of safety. Similarly, IFN-γ + TNF-α + a TLR signal all need to be present, which is also argued as representing a safety mechanism.[5] Successful mammalian pregnancy requires adequate levels of estradiol and progesterone, and in certain

models of abortion triggered by innate immune system activation, deficient ovarian hormone production may cause pregnancy loss.[8]

Whilst deficient progesterone can cause collapse of maternal uterine deciduas needed to sustain pregnancy, progesterone has also been implicated in stimulating release of a progesterone-induced blocking factor that can inhibit NK lineage cells.[1] However, there are several proposed mechanisms of antagonizing the rejection pathways that participate in embryo execution. There are within-pathway inhibitors, e.g. complement inhibitors, and mice deficient in the cry inhibitor have a high rate of loss, even in syngeneic matings.[3,4] There are also inhibitors of the coagulation pathway where deficiency of an inhibitor predisposes to embryo loss. With respect to countering a pro-inflammatory Th1 environment, Th2,3 cytokines are believed to restore 'balance'; a pro-inflammatory Th1 environment appears to be inherent in implantation and early pregnancy, but too much may be deleterious.[1,7] Similarly, too much Th2/3 cytokines may not be healthy, particularly as activated TGF-βs (Th3 cytokines) can inhibit trophoblast growth and invasion necessary for placentation.[9,10] If the cytokines that matter are produced by maternal lymphomyeloid cells in deciduas, what controls their activity? There are several mechanisms that have been proposed, and these will be described and discussed below. Is one mechanism sufficient, or must one have >1 regulatory mechanism to prevent rejection? Each of the contributors below addresses a distinct regulatory pathway in the context of metaphorical construct of the embryo as an allograft that must be tolerated by maternal immune defences for pregnancy to succeed. Indeed, Tafuri et al.[11] demonstrated conclusively in an MHC and specific TcR transgenic model that classical alloantigen-specific T cells could be suppressed or deleted systemically during pregnancy, and similar events might occur locally at the materno-fetal interface of non-transgenic models where the quantity of paternal alloantigen is less.

Role of indoleamine 2,3-dioxygenase in preventing embryo rejection (P Terness)

Immune tolerance against the foetal antigens during pregnancy is often accredited to antigen presenting cells (APCs). Nowadays, it is clear that at least three populations of APCs, dendritic cells (DCs), macrophages and immature, monocyte-derived APC, can be found in the deciduas of the pregnant uterus.[12] DCs are not only able to stimulate but also to inhibit the immune response.[13] One molecule suspected to mediate immunosuppression in the placenta is indoleamine-2,3 dioxygenase (IDO).[14] We focus our attention on IDO-producing DCs.

Mechanisms of IDO-Induced Suppression

The IDO is an enzyme, which initiates the catabolism of tryptophan.[15] Although a couple of hypotheses have been advanced to explain the mechanism of IDO-induced suppression[16], the tryptophan catabolism hypothesis is supported by many observations and thus merits special attention. According to this hypothesis, depletion of tryptophan deprives the lymphocytes of an essential amino acid, thus compromising their ability to proliferate. It was reported that to suppress T cells, the tryptophan concentration should decrease below 0.5–1 μM.[17] Frumento et al. did not observe inhibition of T-cell proliferation even if the culture medium was completely devoid of tryptophan.[18] Under cell culture conditions, i.e. in a limited volume, complete degradation of tryptophan by IDO is conceivable. The *in vivo* situation, however, is different, as we have an open system in which a local decrease of tryptophan is rapidly compensated by diffusion from surrounding tissues and from plasma where the concentration lies in the range of 50–100 μM. At the site of inflammation, dead cells provide an additional source of tryptophan by releasing their intracellular stocks. Therefore, it is difficult to conceive that the extremely low tryptophan concentrations required for inhibition of lymphocyte proliferation can be achieved and maintained in the extracellular environment *in vivo*. Based on findings showing that certain tryptophan metabolites inhibit T-cell proliferation *in vitro*, an alternate mechanism was proposed in which not low tryptophan concentrations themselves, but the resulting metabolites are the key players.[18,19] Among the tryptophan metabolites, 3-OH-kynurenine and 3-OH-anthranilic acid, and to a lesser extent kynurenine and picolinic acid, were shown to be the components, which inhibit T-cell proliferation.[18,19] Arguments for an *in vivo* effect of metabolites were provided by rat experiments, in which skin allograft survival was prolonged by treatment of recipients with tryptophan metabolites.[20] These findings are supported by the observation that 3-OH-anthranilic and quinolinic acid, when injected into mice, cause a depletion of specific thymocyte subsets in a fashion

similar to dexamethasone.[21] The same metabolites were shown to induce selective apoptosis *in vitro* of murine thymocytes and Th1 but not Th2 cells.[21] Further support for tryptophan metabolites as mediators of suppression came from the experiments of Platten et al. showing that these compounds suppress proliferation of myelin-specific T cells and skew the cytokine profile from Th1 to Th2.[22] Moreover, a synthetic derivative of the tryptophan metabolite anthranilic acid was able to reverse paralysis in mice with experimental autoimmune encephalomyelitis, a model of multiple sclerosis.[22] Of course, tryptophan depletion and generation of inhibitory metabolites as mechanisms of suppression are not mutually exclusive. While according to our observations tryptophan deficiency *per se* cannot be the cause of T-cell suppression, it is conceivable that it amplifies the inhibitory effect of tryptophan catabolites. Studies on murine cells support this view.[23].

Contradictory Functions of IDO-Producing DCs

The enzymatic activity of IDO depends on the presence of factors such as the haeme group, superoxide, redox potential of the medium, or nitric oxide (=NO). The presence of superoxide, for instance, is an absolute pre-requisite for tryptophan breakdown induced by IDO.[24] This also implies that the redox potential of the microenvironment in which IDO acts, can enhance or diminish its effect. Another factor which merits attention is NO, a biomolecule shown to abolish the IDO activity.[25] Taking into consideration the complex regulation of IDO function, it is not surprising that DCs were detected which express IDO but do not provide enzymatic activity (IDO-defective DCs) (Table I). In mice for example, in addition to $CD8\alpha^+$ DCs, which express IDO, break down tryptophan and induce apoptosis of Th1 cells, $CD8\alpha^-$ DCs were described, which express similar amounts of IDO but are not able to catabolize tryptophan and do not affect T-cell functions.[21] Based on cell culture experiments, Munn et al.[26] came to the conclusion that functional IDO of human DCs requires ligation of B7-1/B7-2 by CTLA4/CD28 expressed on T cells. When this interaction is disrupted, IDO remains in an inactive state and DCs are unable to inhibit T-cell proliferation.

Whereas the finding of IDO-defective DCs can be explained by the complex regulation of enzymatic activity, other observations are more difficult to interpret. In a series of experiments, we induced IDO production in human DCs by treating the cells with IFN-γ, a cytokine which has been shown to strongly stimulate the IDO activity.[15] The DCs expressed IDO and catabolized tryptophan, but to our surprise, did not or only marginally inhibited T cells.[27] Our findings that DCs express active IDO without suppressing the T cells (non-suppressive IDO-DCs) contradicted previous reports on suppressive IDO-DCs[28,29], but is supported by a recent paper by Lob et al.[30] (Table I).

Table I Various Functions of Human IDO-Producing Dendritic Cells

	IDO	Tryptophan degradation	T-cell suppression	References
IDO-defective DCs	+	–	–	26,28
Non-suppressive IDO-DCs	+	+	–	27,30
Suppressive IDO-DCs	+	+	+	28,29

IDO, indoleamine 2,3-dioxygenase; DC, dendritic cells.

Regardless of whether tryptophan deprivation, kynurenine-mediated inhibition, or a combination of both is responsible for IDO-mediated immunosuppression, it is clear that sufficient amounts of IDO must be generated to induce an inhibitory effect. We were therefore concerned that too little IDO activity may have been generated in our cultures of IFN-γ stimulated DCs. However, the obtained IDO activity per DC was comparable to that noted following IDO trans-gene expression and corresponded to the activities described by others in similar experiments.[17,28] Thus, too little IDO was not the reason for lacking suppression.

There are examples showing that identical biomolecules expressed under different conditions can have different effects. Perhaps the best example is that of MHC molecules which can either stimulate T cells – if they are expressed on cells with costimulatory signals, or anergize T cells – if they are expressed on cells lacking such stimuli. The same might apply to IDO, a biomolecule, which may or may not suppress T cells depending on the concomitant secretion of inhibitory or stimulatory agents. Activated DCs produce biomolecules which strongly stimulate lymphocytes and thus have the potential to override the action of suppressive compounds induced by

IDO. It has been shown that the immunostimulatory capacity of DCs can be further augmented by IFN-γ, the cytokine, which has been used by us and others to induce IDO synthesis.[17,29] This effect may counteract the T-cell inhibitory action of IDO-induced metabolites.

Apart from competing immunostimulatory molecules, it is known that the function of tryptophan metabolites is influenced by the redox potential of the microenvironment.[31] We have previously shown that among the tryptophan metabolites generated by IDO, 3-OH-kynurenine and 3-OH-anthranilic acid are the most important mediators of immunosuppression.[19] Both compounds are good electron donors that reduce cytochrome c and are readily oxidized under aerobic conditions.[32] Their oxidation leads to the generation of quinone-imines, which oxidatively modify various amino acid side chains of proteins.[33] Thus, 3-OH-kynurenine and 3-OH-anthranilic acid, two reducing molecules, are the immediate precursors of potentially oxidizing agents '*in vivo*', contributing to oxidation stress. Not surprisingly, the '*in vivo*' pro- and anti-oxidant properties and hence the biological activities of these species depend on other redox agents present in the microenvironment.[32] DCs have been shown to generate such redox active substances, an example being the production of cysteine and thioredoxin.[34,35] The IDO-activator IFN-γ also influences the redox potential of DCs and therefore might interfere with IDO effector functions. In addition to the presence of immunostimulatory molecules, the redox activity is another factor by which DCs can influence the T-cell regulatory effect of IDO-induced metabolites.

Via della Conciliazione?

As already mentioned, in our hands IDO transgene-expressing DCs were suppressive, whereas IFN-γ-generated IDO-DCs were not[19,27], although both cells produced comparable amounts of active IDO as measured by the concentration of the resulting tryptophan metabolites. Evidently, the cause for the different behavior of these cells cannot be factors regulating IDO activity but those influencing the function of suppressive mediators.

These findings led us to speculate that IDO is suppressive, when acting in a non-inflammatory microenvironment, but not when acting under inflammatory conditions. By treating the DCs with IFN-γ the inflammatory machinery is activated.[36] This is not the case when IDO is expressed as a transgene in DCs. Conditions modified by inflammation are the redox potential of the medium, the production of stimulatory or IDO-inhibitory molecules, etc. These and other factors are plausible candidates for annihilating the immunosuppressive function of IDO.

The IDO activity was described first as a mechanism for stopping the growth of microorganisms.[37] Later on, it was speculated that the same mechanism suppresses the immune response.[14] From a teleological standpoint, this does not make sense because in order to fight the aggressor efficiently, a strong immune response is required at the site of infection. For controlling the maternal immune response to the foetus or auto-reactive lymphocytes, processes usually take place under non-inflammatory conditions, however, an inhibitory function of IDO is required. Therefore, a functional dichotomy of IDO, depending on the presence or absence of inflammation, is biologically justified.

Of course, the statement that IDO is suppressive only in a non-inflammatory environment is not a proven phenomenon but one of several hypothetical scenarios. Further research is required to elucidate the reasons for the discrepant findings regarding the immuno-regulatory function of IDO-producing DCs.

A difficult birth: the role of regulatory T cells in maternal tolerance (M Kallikourdes & A Betz)

In 1953, Sir Peter Medawar recognized that the maternal immune system should recognize the fetus to be foreign due to paternally derived antigens. Yet, it does not attack the fetus despite the long gestation time.[38] He suggested that this paradox might be explained by (i) the anatomical separation of blood circulation of the mother and the fetus, (ii) the antigenic immaturity of the fetus, and (iii) an inertness of maternal immune system. Medawar himself recognized that the separation between mother and fetus is at best incomplete and not long after, cell transfer between the mother and fetus was shown to occur.[39,40] The hypothesis that the fetus was antigenically immature was laid to rest 1958 when Woodruff demonstrated that fetal tissue transplanted to non-uterine tissue of the mother had immuno-reactivity.[41] This led to the proposal that the gravid uterus might be an immunoprivileged site such as the eyes and the testes.[42] Since the maternal immune system has been

shown to retain the potential to react to paternally derived antigens throughout gestation,[11] with the benefit of hindsight, it is clear that Medawar's proposals were insufficient to explain the absence of an immune attack by the maternal immune system against the fetus.

Around the same time, Medawar demonstrated that by transplanting third party embryonic tissue into a developing embryo the host could be made tolerant to the donor.[43] This seminal work led to the establishment of the field of immune tolerance, which still occupies immunologists to this day. In the 20 years that ensued most of the work in transplantation immunology focused on antigen-induced tolerance.

In 1970, Gershon described the existence of a naturally occurring immunoregulatory T-cell population, that he termed suppressor T cells.[44,45] The suppressor T cells were shown to block alloreactive antibody-mediated immune responses, and soon became the subject of extensive studies. The possibility that an immunosuppressive cell population could be actively blocking a maternal anti-fetal response did not escape the attention of reproductive immunologists, who were studying the tolerizing potential of the maternal immune system in mice.[46] T cells from pregnant females were shown to suppress the rejection of paternal grafts[47,48], in a dose-dependent manner.[48] The maternal cells displayed reduced cytotoxic activity against paternally derived targets[49], which was also identified in samples from human pregnancies.[50] The suppressor T cells were found in the spleen[47,49,50], placenta[48], uterine blood, and deciduas[52] as well as the draining lymph nodes of the uterus.[53] They were thought to express the surface marker Ly-2 (CD8α) and in some experiments exhibited allo-antigen specificity.[54,55,56]

Many of these studies, both in mouse and human, uncovered the immunosuppressive properties of the various cell populations in mixed lymphocyte reactions (MLR).[51,55,57] Amongst the various *in vivo* approaches used in an attempt to validate the *in vitro* findings only one is still used to date. The crossing of particular inbred mouse strains (CBA/J female mice with DBA/2/J males) exhibits a high rate of abortions and was used as a model for the study of maternal-fetal tolerance.[57,58] The physiological relevance of the various models used was actively questioned and extensively debated by the researchers themselves.[59,60] Some of the issues have yet to be resolved. Amongst them are the possible role of bacterial triggers to the loss of maternal-fetal tolerance[60], the exact nature of the antigen presented to the maternal immune system[61] and the balance of immunosuppressive and pro-inflammatory factors at the maternal-fetal interface.[62]

Soluble mediators of immunosuppression ('suppressor factors' at the time, 'contact-independent suppression' nowdays) were identified.[63] One such suppressor factor, derived from the decidua of allogeneically pregnant females, and capable of blocking IL-2 dependent processes was later found to be a TGFβ-related protein.[63,64] A different line of investigation uncovered T-cell mediated, tolerogenic effects of the pregnancy-related hormone human chorionic gonadotropin (HCG).[65] Research was not only focused on T-cell-mediated suppression; non-T cell populations with an immunosuppressive function were identified[66,67], leading to the belief that different cell populations may be contributing towards tolerance at different stages of gestation.[68] However, the presence of suppressor T cells was confirmed throughout all stages of pregnancy.[69]

Naturally occurring conditions associated with impaired pregnancy, such as recurring abortions and pre-eclampsia in women attracted the attention of investigators. Functional MLR studies found a lack of immunosuppressive effects in samples from pre-eclamptic women.[70] However, attempts to link the cases of pre-eclampsia[71] or recurrent spontaneous abortion[72] to a change in the peripheral blood ratios of different T-cell subpopulations did not yield useful results. At that time suppressor T cells were thought to be $CD8^+$ in contrast to the $CD4^+$ helper T cells. In fact, many experiments might be explained by the cytotoxic nature of $CD8^+$ cells rather than a suppressive effect. In retrospect, this was clearly a misconceived view of the role of the various T-cell populations. Unsurprisingly, a multitude of similar analyses in healthy pregnant or non-pregnant women were equally inconclusive.[73,74]

The field of suppressor T cells itself started to collapse around 1988 mainly because of the discrepancy between experimental findings and the increasingly complex interpretations suggested by many researchers who 'jumped onto the bandwagon' of suppressor T cells.[75,76] The main criticisms at the time were that $CD8^+$ suppressor T cells could not be differentiated from $CD8^+$ cytotoxic T cells[75,76] and that the genetic marker I-J used to identify them did not exist.[77] Meanwhile, multi-level 'circuits' of suppression were being invoked to interpret the increasingly complex experimental results.[78]

It was inevitable that the collapse of the suppressor T-cell field would affect the study of their role in maternal-fetal tolerance, as it ailed from the same faults, the use of CD8 as a marker for suppressor T cells[55,56], suppressor cell circuits[79] and I-J as a marker.[80] Thus it is not surprising, that papers challenging the role of T cells in the control of anti-fetal responses soon appeared.[81,82] In the subsequent decade, most researchers turned their back to suppressor T cells, ironically at a time when work emerged that led to the re-birth of the field of suppressor T cells under the guise of regulatory T cells.

It is indicative of the cyclical nature of scientific focus that many of the techniques and experimental models that were used at that time re-surfaced many years later in refined form after the field of suppressor/regulatory T cells had undergone collapse and re-birth. The original studies were hindered by the lack of reliable cellular and genetic markers and restricted by the limited molecular biology tools available at the time. However, it is clear that the basic observations of the experiments were correct.

Sakaguchi had been working on the immunoregulatory properties of neonatal thymocytes since the early eighties. He had found that the Ly-1$^+$ T subpopulation of the neonatal thymocytes was able to reverse the autoimmunity induced by neonatal thymectomy.[83] In 1995, his group further identified this population to be CD4$^+$ CD25$^+$ cells.[84] Powrie demonstrated an anti-inflammatory role for CD4$^+$ CD45RB^{lo} in a colitis model[85], while Shevach and co-workers identified the specificity requirements for the immunoregulatory function of the CD4$^+$ CD25$^+$ cells[86]. The field of regulatory T cells had been re-born. Importantly, a conceptual difference was that this time the suppressive cells were associated with the control of autoimmunity. The finding that Foxp3 was sufficient to confer immunosuppressive function to naturally occurring regulatory T cells provided a genetic handle for regulatory T cells.[87–89] Thus, after three decades, suppressor cells finally could be identified by a reliable molecular marker.

Our own foray into the function of regulatory T cells in pregnancy followed a winding path. Whilst setting out to characterize the chemokines required to initiate an adaptive immune response, we discovered that the chemokine CCL4 was important for the recruitment and/or retention of an immunosuppressive population of CD4$^+$ CD25$^+$ T cells towards activated APCs. Interference with this mechanism led to the rapid induction of autoimmunity. This suggested that we had interfered with recruitment/retention of regulatory T cells.[90] Our newly found interest in regulatory T cells sparked us to speculate that the role of regulatory T cells extends beyond autoimmunity to the prevention of deleterious albeit legitimate immune responses. The most fascinating scenario was a possible role in maternal-fetal tolerance. Indeed, we found that during pregnancy, the regulatory T cells underwent a systemic expansion in all lymphoid compartments and accumulated in the gravid uterus. This was observed in both syngeneic and allogeneic pregnancies. The regulatory T cells had potent immunosuppressive function against paternal alloantigens. Reconstitution of T-cell-deficient *nu/nu* mice with physiological numbers of T cells depleted of regulatory T cells led to a failure of allogeneic but not syngeneic pregnancies. Importantly, *nu/nu* mice reconstituted with 'total' T cells displayed normal pregnancy outcome. From this, we concluded that regulatory T cells are required for the sustenance of the semi-allogeneic fetus.[91]

As it has been shown that antibody-mediated depletion of CD25$^+$ cells in a mouse system has the same effect on allogeneic but not syngeneic pregnancy.[92] Interestingly, a number of groups picked up on the abortion prone-mouse model (CBAxDBA/2 model) established by Clark et al.[93] and Chaouat et al.[94], during the suppressor T-cell era. Zenclussen et al. found that the phenotype can be rescued by transfer of CD4$^+$ CD25$^+$ cells from the thymus + spleens of CBA/J females pregnant by BALB/c males.[94]

An increase in CD4$^+$ CD25$^+$ T cells had actually been described in the human decidua more than 10 years earlier.[95] At the time Saito et al. thought that they were dealing with T cells that had been activated in the decidua. As, the same group confirmed that these cells are indeed regulatory T cells.[96] Interestingly, they found the number of regulatory T cells in decidua samples from spontaneous abortions to be significantly lower compared to elective abortions.[96] The expansion of regulatory T cells in the periphery during human pregnancy was further confirmed by several other groups.[97,98]

Clearly, the gravid uterus is one of the sites where regulatory T cells are likely to exert their function during pregnancy. Recently, we have shown that effector regulatory T cells, which like their pro-inflammatory counterparts expresses CCR5$^+$, accu-

mulate in the gravid uterus. Interestingly, the chemokine CCL4, which originally led us to study regulatory T cells, appears to be responsible for this accumulation.[99]

We are confident that the modern tools of molecular biology will aid the field of reproductive immunology to answer many of the remaining questions. In particular, the nature of the antigen presented to the maternal immune system and the mechanism of regulatory T-cell expansion are central to our understanding of the process. In pursuing this, it would be wise to learn from the mistakes in the past, without brushing over potential gems hidden in decades of hard work.

Dissecting the role of protein-glycan interactions in the regulation of immune cell tolerance (G Rabinovich)

Protein-Glycan Interactions in the Regulation of Immune Cell Homeostasis

Immune cell processes are accompanied by changes in the glycosylation pattern of cell surface glycoconjugates orchestrated by the sequential action of a limited number of glycosyltransferases which are regulated throughout immune cell activation, differentiation, and apoptosis.[100] The responsibility for decoding the information displayed by glycan structures is assigned in part to a number of endogenous glycan-binding proteins or lectins.[101] Understanding the 'sugar code' is a major challenge for immunologists and will be critical to support the design of rational therapeutic approaches aiming at manipulating immune cell tolerance during autoimmune settings, transplantation, and failing pregnancies. In the present section, we will concentrate on the role of galectins, particularly galectin-1, in the regulation of immune cell tolerance and homeostasis.

Galectins: Glycan-Binding Proteins with Immunoregulatory Activities

Recently, experimental evidence has emerged, illuminating a novel role for galectins in the regulation immune cell homeostasis and inflammation.[101,102] Members of the galectin family are defined by a conserved carbohydrate recognition domain (CRD) with a canonical amino acid sequence and affinity for β-galactosides.[102,103] To date, 15 mammalian galectins have been identified, which can be subdivided into those that have one CRD (proto-type) and those that have two CRDs in tandem (tandem-repeat type). In addition, galectin-3, a one-CRD galectin, is unique in that it contains unusual tandem repeats of short amino acid stretches fused onto the CRD (chimera-type).[104] Many galectins bind carbohydrate moieties in a bivalent or multivalent manner. Similar to cytokines and growth factors, galectin-mediated cross-linking of cell surface glycoconjugates can trigger a cascade of transmembrane signaling and modulate processes that include proliferation, cell migration, and apoptosis.[104]

Although most mammalian galectins bind preferentially to glycoconjugates containing the ubiquitous disaccharide *N*-acetyllactosamine [Galβ1-3GlcNAc or Galβ1-4GlcNAc], binding to individual lactosamine units is of relatively low affinity (Kd∼1 mM), and arrangement of lactosamine disaccharides in repeating chains (polylactosamine) increases binding avidity. Moreover, a detailed structural analysis of the CRD suggests subtle differences in carbohydrate-binding specificities of individual members of this family.[105]

Galectin-1: A Key Regulator of Immune Cell Homeostasis

Galectin-1, a 14 kDa proto-type member of the galectin family, has been proposed to be, in general, a negative regulator of the immune response.[102] Within the immune system galectin-1 is found in activated but not resting T cells, B cells, and macrophages.[106–108] In addition, recent studies using gene expression arrays have indicated elevated levels of galectin-1 in natural regulatory T cells.[109]

Compelling evidence indicates that galectin-1 can restore immune cell tolerance in several autoimmune settings by acting as an anti-inflammatory and immunosuppressive cytokine.[110–117] Treatment with recombinant galectin-1 or its genetic delivery abrogates clinical and pathological manifestations of autoimmune disease in experimental models of myasthenia gravis[110], encephalomyelitis[111], arthritis[112], hepatitis[113], colitis[114], diabetes[115], and uveitis[116] by skewing the cytokine balance toward a Th2-mediated response. From a therapeutic standpoint, these findings suggest the potential use of galectin-1 for the selective treatment of Th1-mediated inflammatory disorders.

During the past decade many laboratories have made considerable efforts toward providing a rational basis for understanding the immunoregula-

tory activity of galectin-1. In this regard, accumulating evidence indicates that galectin-1 induces cell growth inhibition and promotes apoptosis of activated T cells (Fig. 1).[108,117,118] In addition, Dias-Baruffi and colleagues reported that galectin-1 can induce exposure of phosphatidylserine, thus favoring turnover of leukocytes without inducing cell apoptosis.[118] Different cell surface glycoconjugates appear to be primary receptors for galectin-1, such as CD45, CD43, and CD7.[119] Susceptibility to galectin-1-induced cell death can be regulated by the coordinated action of glycosyltransferases acting sequentially and determining the glycophenotype of T cells. In this regard, it has been shown that T cells lacking the core 2 β-1,6N-acetylglucosaminyltransferase I (C2GnT I), an enzyme responsible for creating branched structures on O-glycans of T-cell surface glycoproteins, are resistant to galectin-1-induced death.[119] In addition, the α2,6-sialyltransferase (ST6Gal I) can selectively modify N-glycans on CD45 and negatively regulate susceptibility to T-cell death.[120,121] Interestingly, Endharti and colleagues demonstrated that, in contrast to the pro-apoptotic role of galectin-1 on activated T cells, secretion of this protein by stromal cells is capable of supporting the survival of naïve resting T cells without promoting proliferation.[122] Thus, galectin-1 might trigger different signals (i.e. apoptosis or survival) and even different apoptosis endpoints (full apoptosis or only phosphatidylserine exposure) depending on a number of factors including the activation state of the cells and the spatiotemporal expression of specific glycosyltransferases.

In addition to the modulation of T-cell survival, we found that galectin-1 may favor the expansion of $CD4^+$ $CD25^+$ T regulatory cells.[115] Adoptive transfer of regulatory T cells obtained from galectin-1-treated mice prevented the development of autoimmune disease in naïve recipient mice.[115] In this regard, recent studies demonstrated that specific blockade of galectin-1 significantly reduces the suppressive effects of $CD4^+$ $CD25^+$ regulatory T cells.[108]

Additionally, galectin-1 has been shown to regulate T-cell activation negatively and influence cytokine production.[117,123,124] Chung and colleagues demonstrated that galectin-1 can induce partial TCR ζ-chain phosphorylation and antagonize full TCR responses including the production of IL-2.[124]

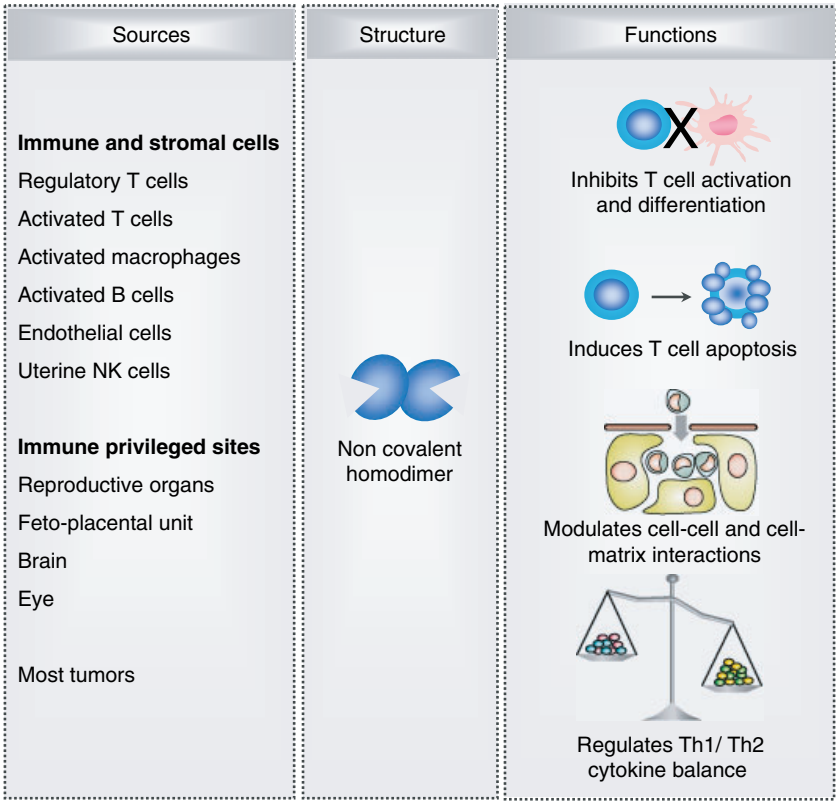

Fig. 1 Role of galectin-1 in the regulation of immune cell tolerance. Galectin 1 is synthesized by a wide variety of cells including immune cells, stromal cells, and is up-regulated in immune privileged tissues and tumors (left panel, sources). This protein is secreted as a non-covalent homoclimer composed of 14.5 kDa subunits (middle panel, structure) and interacts with poly-N-acetyl-lactosamine containing glycoconjugates. By forming galectin-1-carbohydrate lattices, this protein can modulate T cell homeostasis by interfering with T cell activation, promoting T cell apoptosis, modulating transendothelial T cell migration, and regulating Th1/Th2 cytokine balance (left panel, functions).

In addition, galectin-1 in its monomeric form inhibits T-cell adhesion to extracellular matrix and abrogates the secretion of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and IFN-γ without inducing T-cell apoptosis.[124] Furthermore, recent evidence reported a marked increase in IL-10 secretion by T cells exposed to stable dimeric galectin-1.[125]

Whereas compelling evidence has been accumulated regarding the effects of galectin-1 on T-cell fate, limited information is available on how galectin-1 may impact on cells of the myelomonocytic lineage (Fig. 1). In this regard, Fulcher et al. recently demonstrated that galectin-1 can influence the initiation of an adaptive immune response by activating a genetic program involved in dendritic cell migration through the extracellular matrix.[126] Furthermore, we have recently shown that galectin-1 can differentially control (depending on its concentration and physicochemical properties) the expression and function of critical regulatory molecules (i.e. FcγRI and MHC-II) on human monocytes and mouse macrophages through a non-apoptotic ERK1/2-mediated pathway.[127] This result together with our previous observation that galectin-1 favors arginase activity, but inhibits iNOS activity[128], suggests that this endogenous lectin might promote a state of 'alternative activation' or 'deactivation' in elicited macrophages. Finally, galectin-1 may also interfere in acute and chronic inflammatory responses by restraining the migration and extravasation of different immune cell types.[129–131]

Galectin-1 in the Establishment of Immune Cell Privilege

Galectin-1 is up-regulated in several types of tumors and immune privileged organs including testis, eye, placenta, and reproductive organs.[102,103] Interestingly, expression of galectin-1 in the tumor microenvironment positively correlates with the aggressiveness of different types of tumors and the acquisition of metastatic phenotype.[103] We have recently demonstrated that galectin-1 contributes to tumor-induced immunosuppression and tumor-immune privilege.[132] Blockade of the immunosuppressive and pro-apoptotic activity of galectin-1 within tumor tissue resulted in heightened T-cell mediated tumor rejection with increased survival of IFN-γ-producing Th1 cells.[132] Supporting our findings, Le and colleagues found a strong inverse correlation between galectin-1 expression and the presence of T cells in human tumor sections corresponding to head and neck squamous cell carcinoma patients.[133]. Taken together, these results support the concept that galectin-1 contributes to immune privilege of tumors by negatively regulating the survival of effector T cells and skewing the balance toward a Th2-predominant cytokine milieu. In addition, Gal-1 suppresses ocular inflammation and restores immune privilege in a model of autoimmune ocular inflammation by fostering the secreation of TGF-β and favoring the expansion of regulatory T cells.[115]

Remarkably, galectin-1 is abundant in the female reproductive tract and is significantly up-regulated in the late secretory phase endometrium and in decidual and placental tissue.[134–136] Particularly interesting, galectin-1 is markedly over expressed in human uterine NK cells.[137] These observations together with the profound effects of galectin-1 in the regulation of immune cell homeostasis, suggest that galectin-1 might regulate feto-maternal tolerance similar to other immunoregulatory mediators including PD-L1, FasL, and IDO. This attractive hypothesis is currently under thorough investigation. We anticipate that an improved understanding of the role of protein-glycan interactions in immune cell tolerance will reveal novel targets for intervention in immune-mediated pathology.

Tolerance signaling molecules and pregnancy: IDO and regulatory T cells (S Saito)

The Cross-Talk Between $CD4^+$ $CD25^+$ Treg Cells and IDO Expressing DC and Mφ

An activation signal is necessary for induction of the regulatory function of $CD4^+$ $CD25^+$ Treg cells (Fig. 2). After activation, $CD4^+$ $CD25^+$ Treg cells express surface CTLA-4 and display immunoregulation by cell-to-cell interaction or production of immunoregulatory cytokines such as TGF-β and IL-10 (Fig. 2). Decidual $CD4^+$ $CD25^{high}$ Treg cells express surface CTLA-4 in normal pregnancy, but these cells decreased to non-pregnancy level in miscarriage cases[96], suggesting that decidual $CD4^+$ $CD25^{high}$ Treg cells are stimulated by some antigens such as fetal antigens in normal pregnancy, and fetal antigen-recognized decidual $CD4^+$ $CD25^{high}$ Treg cells which express surface CTLA-4 on their surface may prevent fetal rejection. Indeed, anti-CTLA-4

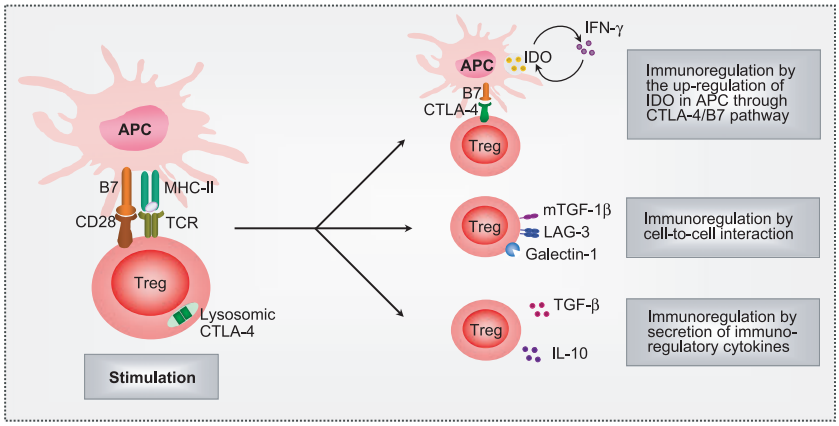

Fig. 2 Immunoregulatory mechanisms of $CD4^+$ $CD25^+$ regulatory T cells. $CD4^+$ $CD25^+$ Treg cells may act by up-regulation of IDO in antigen-presenting cells (APC), by cell-to-cell interaction through membrane TGF-β1, LAG-3, and surface galectin-1, or through immunosuppressive cytokines such as TGF-β and IL-10.

antibody treatment inhibited $CD4^+$ $CD25^+$ Treg function *in vivo*.[138] To clarify which mechanism is important for immunoregulation, cell-to-cell interaction or immunoregulatory cytokines production, Sasaki et al. examined the immunoregulatory function of $CD4^+$ $CD25^+$ Treg cells using the Transwell system.[96] If secreted TGF-β or IL-10 are important for immunoregulation, decidual $CD4^+$ $CD25^{high}$ Treg cells should inhibit the DNA synthesis of conventional T cells under Transwell-culture conditions. If cell-to-cell interaction is necessary for immunoregulation, the Transwell membrane system should completely abrogate the immunoregulatory activity of decidual $CD4^+$ $CD25^{high}$ Treg cells. They showed that cell-to-cell contact is necessary for immunoregulation.[96] As shown in Fig. 2, CTLA-4 on $CD4^+$ $CD25^{high}$ Treg cells induced the tryptophan catabolizing enzyme IDO. When surface CTLA-4 on $CD4^+$ $CD25^{high}$ Treg cells bind to the B7 complex on APCs, IFN-γ production is induced[139,140], and then the produced IFN-γ enhances the IDO expression on DC or Mφ. The expressions of CD86 on peripheral blood- and decidual-DC and Mφ are up-regulated in normal pregnancy subjects and down-regulated in miscarriage cases.[141] IFN-γ production by peripheral blood mononuclear cells and decidual leukocytes stimulated with CTLA-4/Fc in normal pregnant subjects is dramatically increased, but decreased in miscarriage cases.[141] Furthermore, IDO expression on both peripheral blood- and decidual-APCs is up-regulated during normal pregnancy. On the other hand, IDO expression on both DC and Mφ after IFN-γ treatment or CTLA-4 treatment is decreased in miscarriage cases[141], suggesting that surface CTLA-4 on $CD4^+$ $CD25^{high}$ T cells efficiently up-regulate the production of IFN-γ by APCs, and IFN-γ efficiently up-regulates IDO expression on DC or Mφ during normal pregnancy. The up-regulated IDO enzyme depletes tryptophan at the materno-fetal interface and thereby prevents T-cell activation and NK cell activation.[14,17,28,141] $CD4^+$ $CD25^+$ T and IDO-expressing DC and Mφ are thus very important in the maintenance of semiallogeneic pregnancy.

Another important molecule for cell-to-cell interaction of $CD4^+$ $CD25^+$ Treg cells is cell surface TGF-β1, which regulates T-cell activation and NK cell function[142] (Fig. 2), although $CD4^+$ $CD25^+$ Treg cells in TGF-β1 knockout mice can mediate the suppressor function.[143] As another molecule for immunoregulation, Lag-3 contributes to the suppressive function of $CD4^+$ $CD25^+$ T cells and Treg cells[144], but reversal of its suppressive effect is only modest. Recently Garin et al. performed a transcriptomic and proteomic analysis of activated $CD4^+$ $CD25^+$ T cells, and they found galectin-1 was selectively up-regulated in $CD4^+$ $CD25^+$ Treg cells[108] (Fig. 2). Blockade of galectin-1 binding reduces the inhibitory effects of human and mouse $CD4^+$ $CD25^+$ Treg cells, and reduced regulatory activity is observed in $CD4^+$ $CD25^+$ T cells obtained from gelactin-1 homozygous null mutant mice.[107] Interestingly, uterine $CD16^-$ $CD56^{bright}$ NK cells produce a lot of gelactin-1, and this production is regulated by sex hormones.[145] $CD4^+$ $CD25^+$ Treg cells and uterine NK cells may closely co-operate in the immunoregulation at the materno-fetal interface. Surface CTLA-4, membrane TGF-β1, surface LAG-3, and surface gelactin-1 co-operate in immunoregulation by $CD4^+$ $CD25^+$ Treg cells (Fig. 2).

The PD-1 receptor is a CD28 family inhibitory receptor, and it is involved in the regulation of peripheral tolerance[146], while $CD4^+$ $CD25^+$ Treg cells also express PD-1 on their surface. Interestingly, the

ligand for a PD-1, PDL-1 knockout mouse, results in dramatic abortion of allogeneic, but not of syngeneic mice.[147] In humans, PDL-1 is present on syncytiotrophoblasts, cytotrophoblasts, and extravillous trophoblasts throughout pregnancy.[148] Trophoblasts may be protected by maternal T-cell attack via the PDL-1/PD-1 system and trophoblasts might regulate $CD4^+$ $CD25^+$ Treg function by the PDL-1/PD-1 system.

Concluding comments and unanswered questions (all of the coauthors)

From Terness one may infer that proof for a role of IDO-producing APCs for prevention of rejection of intrauterine embryos is still lacking.[149] The data also suggest an alternative explanation for rejection of embryos when 1-methyltryptophan (1-MT) was given to pregnant mice by contrast to lack of rejection in IDO-knockout mice.[150] It had been concluded that rejection occurred because the protective action of IDO was abrogated by 1-MT. If IDO-generated metabolites selectively eliminate certain lymphocyte subpopulations, as discussed by Terness[19,21], it is conceivable that 1-MT is converted to a toxin that inactivates anti-abortive mechanisms, such as the Treg cells described by Kallikourdis and Betz, and by Saito in this paper. This would be an IDO-independent mechanism of embryonic rejection. Moreover, although it is known that DCs play an important role in preventing embryo rejection[151], and may act to enhance Treg development, mediation of this mechanism by IDO remains questionable.

Galectins, as discussed by Rabinovich, may represent important mediators in induction of Treg cells, and Saito suggests an additional effector role. Although uterine NK cells may express galectin-1, absent uterine NK cells do not appear to cause pregnancy failure notwithstanding lack of IFN-γ-dependent modification of maternal arterial walls.[152] However, the effect of absent uterine NK cells has not been tested in allogeneic matings, or with abortogenic stimuli, such as LPS. The tolerance co-signaling molecule CD200 (formerly OX-2), is also known to play a preventive role in abortions in the CBAxDBA/2 model, as is PD-L1.[147,152–154] Immature DC bear receptors for CD200, and these can lead to differentiation to DCs that promote Treg cell development.[155] Inhibiting either CD200 or PD-L1 increases the loss rate. Both pathways seem to be required for optimal pregnancy success. Is galectin-1 also obligatory? There may be more, as yet undiscovered ligand-receptor interactions. As mentioned in the introduction, there seems to be a requirement for >1 signal for a decision to abort to be made, and a similar bureaucracy may be required for a decision not to abort. It remains to be determined if Treg cells are a *sine qua non* for the success of 'foetal allograft'.

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