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REVIEW – SPECIAL ISSUE

Indoleamine 2,3-Dioxygenase in Human Hematopoietic Stem Cell Transplantation

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Abstract: In recent years tryptophan metabolism and its rate limiting enzyme indoleamine 2,3-dioxygenase (IDO) have attracted increasing attention for their potential to modulate immune responses including the regulation of transplantation tolerance. The focus of this review is to discuss some features of IDO activity which particularly relate to hematopoietic stem cell transplantation (HSCT). HSCT invariably involves the establishment of some degree of a donor-derived immune system in the recipient. Thus, the outstanding feature of tolerance in HSCT is that in this type of transplantation it is not rejection, which causes the most severe problems to HSCT recipients, but the reverse, graft-versus-host (GvH) directed immune responses. We will discuss the peculiar role of IDO activity and accelerated tryptophan metabolism at the interface between immune activation and immune suppression and delineate from theoretical and experimental evidence the potential significance of IDO in mediating tolerance in HSCT. Finally, we will examine therapeutic options for exploitation of IDO activity in the generation of allo-antigen-specific tolerance, i.e. avoiding allo-reactivity while maintaining immunocompetence, in HSCT.

Keywords: indoleamine 2,3-dioxygenase, IDO, hematopoietic stem cell transplantation, HSCT, tryptophan metabolism

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Introduction

Principles of hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation (HSCT) represents a potentially curative and increasingly applied approach for several treatment objectives. These, for example, include the eradication of malignant clones in case of hematologic malignancies but also the introduction of metabolic competence in some inherited metabolic diseases (e.g. Pfaundler-Hurler disease), or the replacement of hematopoietic cells in hematopoietic stem cell disorders (e.g. aplastic anemia) and congenital immunodeficiencies (e.g. severe combined immunodeficiency). The conceptual basis of HSCT is to introduce a donor-derived hematopoiesis into the recipient with the aim to either completely replace the diseased hematopoietic system by an intact one or to substitute for a defect within the hematopoietic system. Importantly, since the immune system as a whole is an integral part of the hematopoietic system, HSCT invariably implies establishing a donor-type immune system. However, the immune system as such is not a transplantable organ but has to either expand from immune cells contained within the graft or be regenerated from the transplanted hematopoietic stem cells. In general, HSCT will result in variable degrees of coexisting recipient-type and donor-type hematopoietic and immune cells. In transplantation immunology 'chimerism' means the presence of donor-derived hematopoietic cells within a recipient. These donor-derived immune cells, in principle, are 'foreign' to the host organism. Chimerism can either be 'complete' (defined by the presence of ≥99% donor-type hematopoietic cells) or 'mixed' (any percentage of donor type hematopoietic cells ranging from >1% to <99%). Refined chimerism studies have demonstrated that the degree of donor chimerism may be different in distinct hematopoietic or immune cell populations.[1,2] Because any degree of chimerism implies the grafting of donor-derived immune cells in an allogeneic host organism, it is obvious that after HSCT the reconstitution of an intact immune system is a challenging process. While HSCT has in recent years developed into a life-saving treatment option for most of the above mentioned disease states, its final treatment success is still hampered by the profound effects on the immune system of the transplant recipient, which, though transient, may result in unpredictable complications.

Transiently disturbed immunity in the post-HSCT period (Fig. 1)

The mammalian immune system has an inherited prevalence to distinguish 'self' from 'non-self'. The peculiarity of HSCT, in contrast to solid organ transplantation, is that the immunologic recognition of 'non-self' (allo-recognition) can occur in both directions, i.e. as a host versus-graft (HvG) reaction (rejection) but also as a graft-versus-host reaction (see below). An essential principle for successful HSCT therefore is to carefully select the donor for matching the recipient within the polymorphic major histocompatibility antigen (MHC) alleles. Human HSCT is mostly done in MHC identical siblings or MHC matched unrelated donors. Despite careful MHC matching, the recipient immune system has first to be effectively suppressed to allow for engraftment and prevent the host-versus-graft (HvG) reaction. This is performed by the "conditioning regimen" (Fig. 1) which regularly comprises high-dose chemotherapy with or without total body irradiation. Most conditioning regimen cause long-term or irreversible suppression of the recipient immune system. Even in an uncomplicated post-transplant course the reconstitution of a more or less functionally intact immune system is a long-lasting process.[3,4] While the immune cell populations forming the innate part of the immune system, like natural killer cells or monocytes/macrophages, usually are rapidly reconstituted (within weeks), the adaptive part of the immune system requires months and, in rare cases, even years to be fully recovered. The cellular compartment with the slowest reconstitution kinetics is the T-cell system. The recovery of T cells is a complex process as T cells are composed of multiple subsets with different functions and with different degrees of thymic activity required for their reconstitution.[5–7] T cell reconstitution may simply occur by the expansion of residual T cells contained in the graft.[8] The expansion of such T cells is independent of residual thymic activity. However, it has been shown that these T-cells have a limited functional capacity, because they are determined in their antigen specificity and thus have a limited T-cell receptor repertoire.[8,9] The common

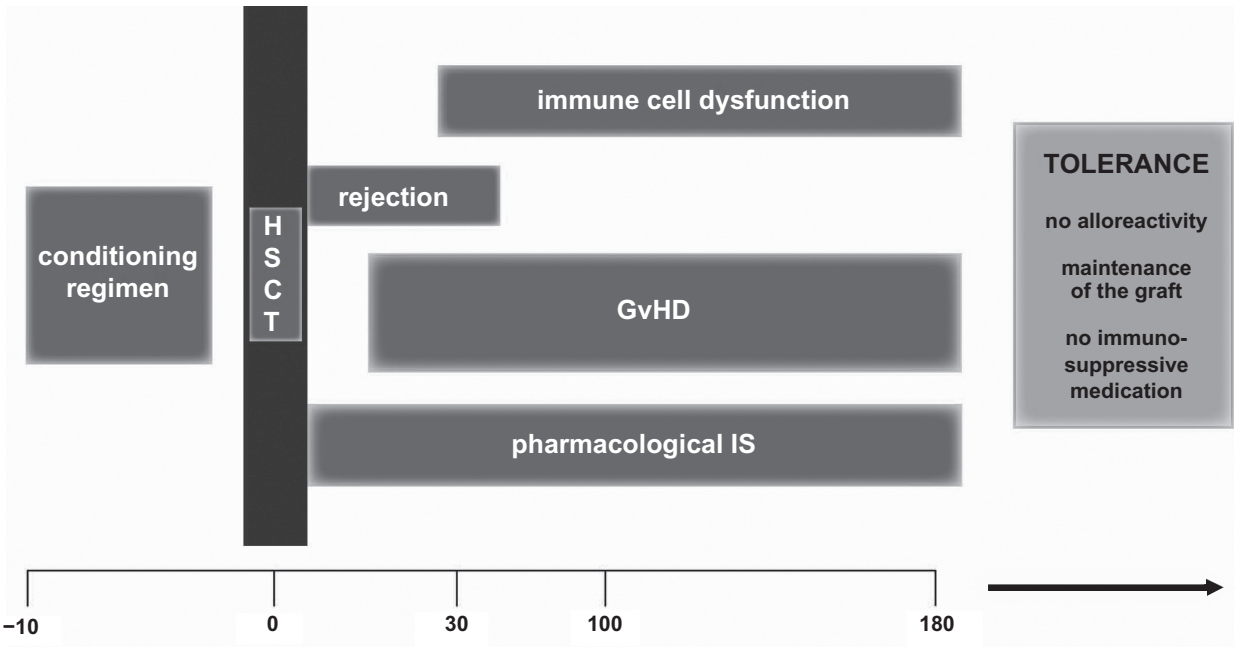

Figure 1. Immunodeficiency after HSCT. The figure comprises some important factors that invariably contribute to the immune deficiency after HSCT. The numbers of the scale indicate days after transplant. Immediately after transplantation the number of immune cells is diminishing, and the appearance of donor-derived immunocompetent cells requires some time. Therefore, the risk for GvHD increases as donor-derived immune cells reappear in the recipient.

understanding is that the reconstitution of the newly produced naïve T-cells ensures the regeneration of a well functioning T-cell system after HSCT.[7,10] The generation of new T-cells, in particular the T helper (CD4+) subsets, has been shown to be strictly dependent on residual thymic activity.[5,6] If the thymus has been surgically removed or if thymic activity is low, as in the elderly, the reconstitution of a functional CD4+ T-cell population usually is unsatisfactory. It is common knowledge that T-cells are the major executors of detrimental allo-responses.[3]

Nowadays, allo-responses in the HvG direction (rejection) can usually be successfully controlled in HSCT. However, the newly developed donor-type T-cells reappearing during the process of hematopoietic reconstitution are inclined to mount allo-responses against the recipient, i.e. to initiate an immune response directed against the organism, in which they begin to expand (graft-versus-host reaction, GvH). The GvH reaction can give rise to a clinically severe and difficult-to-treat immune disorder, graft-versus-host disease (GvHD). GvHD continues to represent a significant factor in limiting the survival and quality of life in patients receiving HSCT. GvHD can affect every organ system but mainly causes skin, liver and/or gut disease and may exacerbate into a life-threatening condition. GvHD can occur in an acute or chronic form with 100 days after HSCT being the widely accepted cut-off time point to discriminate acute from chonic GvHD.[11] The distinction between acute and chronic GvHD is clinically relevant as both, the clinical appearance as well as the pathogenesis are seemingly different and may require different treatment.[12–14] Chronic GvHD may resemble a chronic autoimmune disease rather than the acute form.

The prevailing susceptibility of HSCT recipients to develop GvHD requires long term prophylactic pharmacological immunosuppression, i.e. to be continued even after engraftment, which usually occurs by day 30 after HSCT (Fig. 1). GvHD emerging despite pharmacologic prophylaxis causes immunosuppression *per se*, but its treatment uniformly requires intensification of the immunosuppressive therapy. Taken together (i) the necessity to ablate the recipient type immune system prior to HSCT, (ii) the slow regeneration kinetics of the transplanted immune cells, particularly the T-cells, and (iii) the requirement of pharmacological immunosuppression after HSCT to prevent or to treat GvHD, leaves HSCT recipients

in an immunosuppressed state (Fig. 1). Thus, HSCT recipients are generally highly vulnerable to infections by pathogens which otherwise are either harmless or held under control by an intact immune system. Among these 'opportunistic' infections viral (e.g. by cytomegalovirus or adenovirus) or fungal infections (candida, aspergillus species)[15] are most prevalent and can be life-threatening. Thus, the HSCT related immune insufficiency is associated with a risk of transplant-related morbidity and mortality (TRM). To minimize the risk for TRM has turned into a major goal in the transplantation community.

Nevertheless, despite these immunological hazards, HSCT recipients, different from recipients of solid organ transplantation, eventually regain functional immune competence and frequently can be weaned from pharmacologic immunosuppression without re-developing allo-responses neither in the HvG nor in the GvH direction. This is compatible with a state of true immunological tolerance. Thus, a main task to be accomplished in making HSCT a safe and broadly applicable treatment approach is to bridge the period of profound immune-dysfunction which occurs after HSCT as long as tolerance is not fully achieved.

Some mechanisms of allo-recognition

Establishing a donor-derived immune system in a host, i.e. in a 'non-self' organism, is a complex process. A paradigm of mammalian immunity predicts that the recognition of non-self will lead to stimulation of an immune response irrespective of whether or not a microbial antigen is involved. It is a unique feature of allo-reactivity in the mammalian immune system that the precursor frequency of T-cells recognizing allo-antigens is two to three logs higher than the precursor frequency for cognate antigens.[16,17] Thus, the initiation of allo-responses is predominant after HSCT.

The recognition of allo-antigens primarily involves disparate major histocompatibility (MHC) molecules (reviewed in[18]). MHC class I molecules are expressed on cells throughout the body, while MHC class II molecules are restricted to antigen presenting cells (APC). Both classes of MHC molecules can serve as targets for allo-recognition. It has been proposed that the T-cell receptor (TCR) complex has an inherent affinity for the MHC surface and thus, allo-responses can occur towards polymorphisms of the MHC—including the secondary and even tertiary structure of the MHC complexes—as well as to the peptide presented in the context of MHC complexes.[16] In case of an MHC matched HSCT only the MHC-bound peptides can serve as target allo-antigens. For example, non-microbial peptides derived from polymorphic proteins differently encoded in the host and the donor, collectively called minor histocompatibility antigens (miHags),[19] can elicit strong allo-immune responses,[20] even if these differences are minimal, e.g. caused by single nucleotide polymorphisms (SNP).[21] Of note, different from conventional antigen presentation the presentation of allo-antigens is a limitless process, in other words the antigen cannot be eliminated and cannot be made innocuous.[18] The only way for the donor immune system to attenuate the allo-antigen targeted immune response is to become tolerant. To understand the mechanisms of the development of transplantation tolerance has become a center of attention in solid organ and hematopoietic transplantation.

In the context of HSCT the recognition of allo-antigens can occur in two ways. One is that donor T-cells recognize recipient-derived APCs as foreign, "direct antigen recognition". Alternatively, donor-derived APCs can present recipient-derived antigens to donor T-cells; this process is called "indirect antigen recognition". According to a recently presented concept[18] the initiation phase of GvHD involves direct antigen recognition of miHags, while the evolution of overt GvHD may preferentially involve indirect allo-antigen recognition. The individual risk of HSCT recipients to develop GvHD in humans has been described to involve the universe of the immune reactions starting from genetic polymorphisms of cytokine genes[22] to affecting multiple immune cell subsets to finally resulting in the multifaceted clinical forms of GvHD. On the cellular level, the development of allo-antigen directed tolerance appears to require two complementary steps, the elimination and the regulation of allo-reactive cells.[23] What becomes clear from the current knowledge of GvHD is that the processes involved in its development are multifaceted and that targeting a single step may not be a promising approach. However, as stated above, HSCT is a procedure which in most cases results in clinical tolerance induction, i.e. HSCT recipients can be weaned from pharmacological immunosuppression

while maintaining the donor-type immune cells and regaining a state of immunocompetence.

Multiple scientific efforts have been undertaken to understand the basic principles of tolerance induction in transplantation in general. In HSCT, in particular, the ultimate goal to improve the final outcome in HSCT is to efficiently strengthen the immunocompetence of the recipient in the defense of pathogens while avoiding the risk of promoting GvHD. In other words, donor-derived T-cells must be made tolerant to the host but retain their immuncompetence against microbial pathogens (allo-antigen-specific tolerance) (Fig. 2).

Tolerogenesis by Tryptophan Metabolism

Among the numerous approaches to tolerance induction, the role of tryptophan metabolism has attracted increasing interest in the past ten years. This interest is based on research which unravelled accelerated tryptophan catabolism as a critical factor in modulation of immune responses. The basic details of the biochemistry and biology of tryptophan and tryptophan metabolism have recently been excellently reviewed by Chen Y and Guillemin GJ in this journal.[24] For the purpose of understanding the role of tryptophan and its metabolism in human HSCT it is worthwhile to emphasize the following: 1) Tryptophan is an essential aminoacid, i.e. it cannot be newly synthesized but has to be taken up by diet, and thus is of limited availability for the organism. Human T-cells, once being stimulated to proliferate, essentially depend on the availability of tryptophan to synthesize new proteins. 2) Tryptophan breakdown occurs mainly along the kynurenine pathway. Two enzymes act as rate-limiting compounds, tryptophan 2,3-dioxygenase (TDO), whose activity is restricted to the liver, and indoleamine 2,3-dioxygenase (IDO), which is active throughout the body. Both, TDO and IDO initiate tryptophan breakdown by catabolising the oxidative cleavage of the indole-ring into N-formylkynurenine and kynurenine. Thus, IDO serves as the main tryptophan-degrading enzyme in the human body. 3) An accelerated activity of IDO results in tryptophan depletion and accumulation of biologically active tryptophan downstream metabolites, e.g. 3-hydroxy-kynurenine, 3-hydroxy-anthranilic acid and quinolinic acid. 4) A controversy still exists which of the two metabolic effects of IDO activity are predominant in mediating immunoregulation, tryptophan depletion

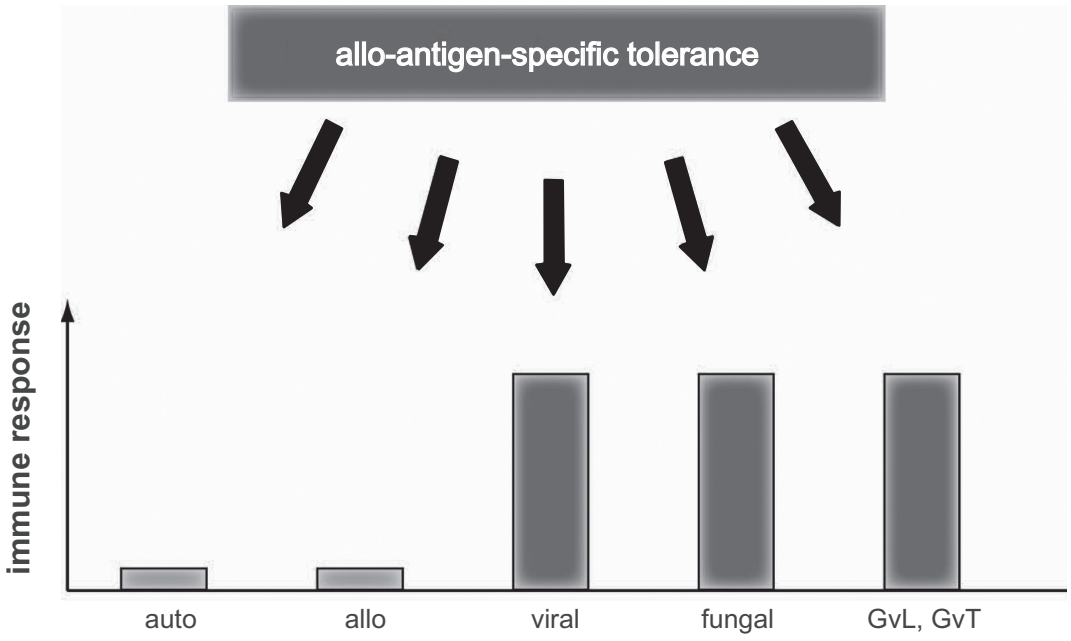

Figure 2. Allo-antigen-specific tolerance. The post-transplant immune system, which will contain some degree of donor-chimerism (see text), is required to specifically be devoid of allo-reactivity in both directions, HvG and GvH, but to protect from microbial infections and provide immune reactivity against neoplastic cells (graft-versus-leukemia, GvL, and graft-versus-tumor, GvT).

or the accumulation of tryptophan metabolites. Yet, in a comprehensive view, it seems that, especially in vivo, these effects cannot be clearly separated; rather both of them eventually will affect immune responses in a complementary fashion (see below).

The current concept of the immunologic consequences of IDO activity holds that IDO regulates immune responses by an alteration of the composition of the soluble intercellular microenvironment in which T-cells encounter antigen (Fig. 3). T-cells themselves have not been observed to express IDO. Instead, IDO expression and activity, hereafter termed IDO competence, has thus far been demonstrated in multiple cell types, among which are tumor cells,[25] fibroblasts[26] bronchial epithelial cells[27] and eosinophils,[28] and, most prominently, in the classical APC populations.[29] In the human body the essential APC populations comprise monocytes, macrophages, and the dendritic cells (DCs). These cell populations are equipped with an intracellular machinery to process antigen and present antigenic peptides in the context of an MHC molecule to T-cells by getting in close contact with the responding T-cells within the immunologic synapse. In fact, T-cells for mounting an immune response are dependent on recognizing processed antigen on the surface of APC in the context of self MHC complexes. T-cells, as they comprise the major fraction of the effector arm of the immune system, when being deprived from the access to tryptophan and exposed to tryptophan metabolites are disabled to mount a full effector immune response. The T-cell inhibitory mechanisms include cell-cycle arrest in the mid G1 phase[30] and initiation of a stress response indicated by up-regulation of the general control non-depressible-2 (GCN2) kinase.[31] Tryptophan metabolites have been shown to act as pro-apoptotic agents particularly affecting activated murine[32,33] and human[34] T-cells. In addition, IDO activity supports the differentiation of T-cell regulatory activity (Treg) activity, i.e. IDO has been shown to endow T-cells with the ability to suppress other T-cell responses.[35–38]

The molecular pathways by which IDO induces tolerogenic capability of DCs include the interaction of the programmed death receptor ligand 1 (PD-L1) and PD-2L with its receptor PD-1, which is expressed on T-cells.[39] In addition, the IDO induction in DCs is accompanied by the expression of the high-affinity IL-2

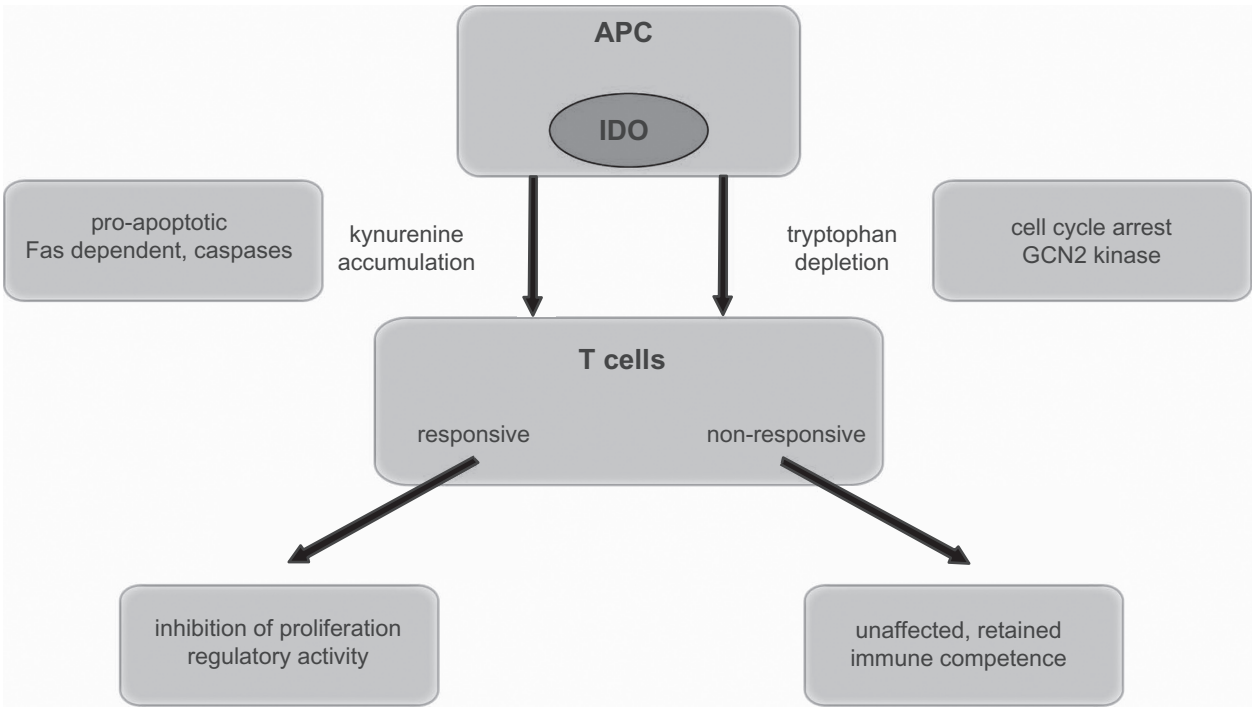

Figure 3. Hypothetical model of IDO activity in the regulation of immune responses. It is presumed that the main effects of IDO-mediated tryptophan breakdown, tryptophan depletion and kynurenine accumulation, act in concert to modulate T cell responses. Note, that according to the model T cells that are not responsive to the antigen presented are considered to remain largely unaffected by the effect of IDO.

receptor, CD25. CD25 can be released from the DC surface and this soluble CD25 molecule (sCD25) can act as a scavenger for IL-2 secreted by activated T-cells and thus limit IL-2 dependent T-cell proliferation.[40] Together, IDO activity in APCs is viewed as a critical factor to down-regulate T-cell responsiveness.

IDO as a Feedback Mechanism of Immune Activation

One significant aspect to consider for the comprehensive understanding of the biological relevance of IDO activity is its link to immune activation. Indeed, in human APCs IDO competence is usually not constitutively present but is induced upon APC activation. Multiple factors have been described to be able to activate APCs and concomitantly induce IDO. These include TLR agonists, e.g. CpG interacting with TLR9,[41] or LPS interacting with TLR4.[36] Furthermore, strong inducers of IDO competence are type I and, most prominently, type II interferons (IFN-γ) (reviewed in[42]). All these compounds have originally been associated with immune activation. In the research addressing the link of immune stimulation and the induction of immune inhibition mediated by IDO activity two important observations have been made. One is the phenomenon of 'reverse signaling'.[43] This term describes a process in which molecules, which are involved in immune activation, may transmit an inhibitory signal if they act as receptors of inhibitory signals. Such a mechanism has been proposed to be operative in murine DCs through the interaction of CTLA-4 with CD80 or CD86. CD80 and CD86, being expressed on DCs, have been recognized potent "costimulatory" signals, indispensible for stimulation of a full T-cell response, as they interact with CD28 expressed on T-cells. In the reverse setting CTLA-4 which is expressed on T-cells upon activation or present on regulatory T-cells can bind to CD80/CD86 and transmit an inhibitory signal which finally results in IDO induction and a tolerogenic activity of DCs.[44] Another example of reverse signalling is the interaction of GITR (on T-cells) with its ligand GITR-L on plasmacytoid DCs,[45] in which the inhibitory function of DCs also occurs in an IDO-dependent fashion.

The second important observation is a timely distinct kinetics of the activity of pro-inflammatory cytokines and IDO in IFN-γ activated DCs.[38] In these in vitro studies it was shown that in human DCs activated with LPS and IFN-γ, the presence of IFN-γ induced pro-inflammatory cytokine release and IDO activity in parallel, however with a timely distinct pattern. The DCs' capacity to release pro-inflammatory cytokines quickly emerged (4 hours) but ceased after 48 hours of stimulation. In contrast, IDO activity developed slowly, but the DCs' capacity to effectively metabolize tryptophan and accumulate kynurenine was maintained beyond the 48 hour stimulation period and even after the stimulants were removed. Thus, when DCs were used as stimulators of T-cells shortly after activation with IFN-γ, i.e. when cytokine production prevailed, the resulting T-cell response was polarized towards a Th-1 phenotype;[46] on the contrary, when allogeneic T-cells were encountered by DCs at a time point after which cytokine production was terminated but IDO activity continued, the T-cell response was either quantitatively down-regulated or a regulatory type of T-cell response was induced.[38] In their composite, these findings support a physiological role for IDO to act as a negative feedback response for limitation of an ongoing immune response.[47] The immune dampening effect of IDO may be of value for the host to control for the magnitude of an immune response and prevent exaggerated or misguided immune reactions which potentially result in organ damage or autoimmunity. Alternatively, overt IDO activity may become counterproductive and contribute to causing immunodeficiency. IDO-mediated immune suppression, for example, is predominant in situations of chronic immune activation, such as cancer[48] or HIV infection.[49] In fact, in the clinic the position of IDO at the crossing point of immune activation and immune dampening may make the interpretation of the presence of IDO activity difficult. An enhanced IDO activity in humans—indicated by low tryptophan and elevated kynurenines in the serum—may indicate overt immune activation, i.e. rejection,[50] or down-regulation of immune responses as shown in HIV infection[51] or beneficial immune regulation in the direction of tolerance.[52]

A particular role for IFN-gamma

For appreciating the role of IDO being involved in maintaining the balance between immune activation and immunosuppression it seems worthwhile to

re-consider the ambivalent role of IFN-γ. Within the immune system IFN-γ has been recognized as one of the most potent inducers of IDO,[53] particularly inducing a prolonged activity in (DCs).[38] On the other hand, IFN-γ has long been appreciated as a prototypic pro-inflammatory cytokine (reviewed in[54]). Its pro-inflammatory activity was linked to its ability 1) to enhance the antigen-presenting capacity of APCs by increasing the level of expression of class II molecules, 2) to stimulate the microbicidal activity of macrophages, 3) to drive Th-1 polarization of T cells and 4) to promote B-cell maturation and the IgG 2a production, thus, being essential in stimulating immune responses. Contrasting this pro-inflammatory effect, IFN-γ has definitively been shown to be essential in dampening immune responses in autoimmunity[55] and transplantation.[56] The pathways underlying the immunoregulatory effect of IFN-γ clearly overlap with the immune dampening effects of IDO. Like IDO activity, IFN-γ has been shown to promote apoptosis of T-cells.[57–59] The pro-apoptotic activity of IFN-γ seemingly does not affect all T-cell subsets equally, i.e. it appears to preferentially affect activated T-cells and to spare regulatory T-cells. In fact, IFN-γ signalling appeared to be essentially required for Treg activity.[60,61] In the allogeneic setting Sawitzky et al demonstrated IFN-γ release by the allo-antigen reactive Treg cell population to support their suppressive function.[62] Several studies showed that IFN-γ induces metabolic processes, which ultimately alter the intercellular microenvironment in which APCs and potentially responsive T-cells interact. These metabolic alterations include, for example, the induction of hemoxygenase-1 (HO-1),[63] or arginase-1[64] and IDO. The enzymatic activities of these enzymes are associated with down-regulation of immune response. Of note, these enzymes are closely cross-regulated and can interact with each other.[65–67] Thus far, it seems that the close alliance of IDO and IFN-γ is particularly relevant in immunomodulation. IFN-γ plays a critical role in the above mentioned model of 'reverse signalling' of CTLA-4-Ig to CD80/86 on DCs; The signal transmitted by CTLA-4 to CD80/CD86 results in the release of INF-g in a STAT-1 dependent fashion and this IFN-γ secretion acts in an autocrine loop to finally cause IDO expression and activity.[68] Likewise, as described above in detail, IFN-γ is a critical factor in the induction of strong and sustained IDO activity in human monocyte-derived DCs.[38] Furthermore, IFN-γ has been shown to be a critical factor involved in the immunosuppressive state that accompanies chronic immune activation. In an elegant study Bronstein Sitton et al reported that mice undergoing serial bacterial challenges developed a severe immune dysfunction, which was related to a chronic release of IFN-γ.[69] In this model sustained immune activation was accompanied by down-regulation of the TCR-ζ chain expression, which is strictly associated with down-regulation of T cell responsiveness. The down-regulation of TCR-ζ chain is a prominent effect of arginine depletion, but an accelerated tryptophan metabolism as induced by IDO has been shown to also exert this effect in a murine model.[35]

Altogether, these findings conceptually support that pro-inflammatory IFN-γ induces its own feedback mechanism and the IDO pathway may play a critical role in this feedback process.[47,70]

In the setting of HSCT IFN-γ has originally been suggested to be involved in the pathogenesis of GvHD.[71–73] However, more recent work provides sound evidence that IFN-γ, in particular donor-derived IFN-γ, is a critical cytokine to attenuate GvHD.[74] IFN-γ has been reported to promote allo-reactivity towards recipient hematopoietic cells but to spare host epithelial cell damage[75] and contribute to the graft-versus-leukemia (GvL) effect. In turn, the absence of IFN-g promoted the development of organ-destructive Th-17 cells.[76] However, in another study the transplantation of IFN-γ deficient bone marrow was shown to reverse subclinical GvHD and to enhance graft-versus-tumor activity.[77] These findings support the crucial but dichotomous role of IFN-γ.[78]

The post-HSCT period—a state of chronic immune activation

The immunodeficient state after HSCT has originally been understood as to result primarily from the absence of essential components of the immune system caused by the delay of the recovery of immunocompetent cells. More recent evidence, however, suggests that in addition to the quantitatively decreased number of immunocompetent cells the immunodeficient state after HSCT is also due to the dysfunction of the residual and even the newly regenerated cells.[5] To this end, our previous studies[79] of the immuno-

suppressive monocyte-mediated effect after HSCT have shown that it may not simply be the presence of, but the sensitivity to IFN-γ, which is responsible for down-regulating immune responsiveness in HSCT. Indeed, our studies suggested that after HSCT monocytes are in a state of chronic activation and display an increased sensitivity to IFN-γ and can turn into suppressor cells upon the exposure to even small amounts of IFN-γ through the initiation of IDO activity.

The Role of IDO in Human HSCT

The primary detection of IDO activity as a pivotal pathway to down-regulate immune responses included some models of allo-antigen driven immune responses (reviewed in[29,80]). In the pioneering study of Munn and Mellor, which paved the way for recognizing IDO and tryptophan metabolism as a key immunoregulatory mediator, they demonstrated an enhanced frequency of abortion upon the blockade of tryptophan metabolism, in a model of semi-allogeneic pregnancy.[81] As described above, allogeneic immune reactions represent a unique form of mammalian immunity. Since IDO activity was shown to be able to down-regulate allo-antigen driven immune responses it appeared obvious to set out for studying a role for IDO specifically in transplantation tolerance, including HSCT. As outlined above, the peculiar feature of HSCT is that it involves allogeneic reactions in both directions, HvG and GvH.

A role of IDO and its ability to modulate GvHD has recently been addressed in several experimental murine models. By using B57BL/6 IDO knock out mice as recipients of wild type Balb/c donors Jasperson et al. showed that the capability to express IDO and efficiently metabolize tryptophan plays a critical role in protecting from GvHD lethality.[82] Interestingly, in this model, different from the general opinion, that IDO induces transplantation tolerance through the induction of regulatory T-cells, the IDO effect was not dependent on donor regulatory T-cells. In a subsequent study the same authors showed that donor T-cell derived IFN-γ was critical for the up-regulation of IDO expression by APCs and the protection from GvHD in the colon of recipients.[83] This effect seemingly was related to the effect of tryptophan metabolites rather than of tryptophan starvation. In a similar model of experimental GvHD Reddy and co-workers showed that DCs exposed to histone deacetylase inhibitors (HDAC) expressed IDO and upon adoptive transfer acquired the capacity to ameliorate experimental GvHD.[84]

One particularly interesting model was recently presented by Romani et al.[85] In this model the authors found a significant association of IDO expression in plasmacytoid DCs (pDCs) and the protection from Aspergillus infection after experimental HSCT. pDCs possess a high affinity to IDO competence in mice,[86,87] and humans.[37,88] In their model of experimental HSCT DCs obtained from B57BL/6 mice which had been stimulated by Flt3L became IDO competent. When these DCs were pulsed with *Aspergillus fumigatus* they contributed to the development of protection against fungal infection, as shown by prolonged survival time and reduced fungal growth in infected mice. In contrast, the adoptive transfer of DCs, which had been stimulated by granulocyte-macrophage colony stimulating factor (GM-CSF) only and did not develop IDO competence, failed to confer protection against fungal infection. When T-cells were co-administered with the DCs, the GM-CSF activated DCs favored the development of GvHD while Flt3L activated DCs failed to do so. Thymosin α-1 (Tα1) is a naturally occurring peptide,[89] which, by signaling through TLR 9, promotes DC activation of murine and human DCs.[90] The exposure of DCs to Tα1 led to the induction of IDO activity in either DC population, irrespective of whether the DCs were additionally stimulated by GM-CSF or Flt3L. Upon IDO induction by Tα1 either DC population acquired protective capacity against *Aspergillus fumigatus* infection. Thus, it seems that in some specific situations, such as fungal infection, which is highly relevant in HSCT (see Introduction), IDO expression by pDC may exert a dual effect, protecting from GvHD and protecting from fungal infection. This, at first sight, seems to be a paradox because IDO activity is generally viewed as being associated with immunosuppression.[91] But further in-depth studies of the same group showed that the effect of IDO in the circumstance of fungal infection was to prevent the organ-destructive activity of Th17 cells and shift the immune response towards a Th1 polarized response. Thus, fungal infection seemingly represents an example, where the attenuation of an immune response might finally be to the benefit of the host. In the setting of experimental

HSCT and *Aspergillus* infection IDO appears as a critical factor mediating such an immunomodifying effect. The effect of IDO activity was suggested to be related to the induction of IFN-γ producing Th-1 cells, and foxp3 expressing and IL-10 producing regulatory T-cells.[85]

The knowledge of IDO in human HSCT is limited. As described above, in our studies of the mechanisms of T-cell deficiency after HSCT we unexpectedly discovered that the inability of T-cells to adequately mount a proliferative response to antigens essentially involved a suppressor activity of post-HSCT monocytes. Further investigations indicated that the post-HSCT monocytes were highly sensitive to react to even low doses of IFN-γ by the activation of IDO and release of kynurenines.[79] Another study directly tested an association of IDO and GvHD in clinical HSCT.[92] These authors reported that the ability to express IDO in response to stimulation with IFN-γ was inversely correlated to grade of GvHD after allogeneic HSCT.

An interesting role for IDO may come into play when considering mesenchymal stem cells (MSCs). These cells represent a recently identified cell population, which has attracted high interest in the transplantation field because of their uniquely powerful tolerogenic effect (reviewed in[93]). In brief, MSCs are rare bone marrow derived cells, which have a stem-cell-like behavior as indicated by their ability to develop into bone, cartilage and fat tissue. Besides their stem-cell like capacity MSCs have been recognized as to potently down-regulate T-cell responses. MSCs have been found to interfere with multiple cell types, including DCs, and the interaction of DCs with natural killer cells, and T-cells. For example, MSCs have been shown to down-regulate NK function, i.e. NK cell proliferation and NK cytotoxicity, by down-regulating NK receptors NKp30, NKp44 and NKG2D in an IDO and PGE2 dependent fashion.[94] MSCs may have a direct effect on T-cells by arresting activated T-cells in the G0/G1 phase of cell cycling[95] and to induce apoptosis in activated T-cells.[96] Furthermore, MSCs have been reported to attenuate CD8-mediated cytotoxicity.[97] MSCs also indirectly modulate T-cell responses by affecting DC maturation and their development into regulatory DCs.[98] For example MSCs have been shown to interfere with the pro-inflammatory effect of myeloid DCs by inhibiting TNF-α production and to favor IL-10 production by pDCs, thus, triggering Treg cell differentiation.[99] As well, MSCs may act via bystander cells as they induce monocytes to release immunosuppressive molecules such as IL-10.[100] MSCs do not constitutively express IDO but turn into IDO competent cells by an exposure to IFN-γ[101] and IFN-γ exposed MSCs being induced to express IDO acquire the ability to suppress T-cell and NK cell proliferation.[102] IDO, though, may not be the exclusive mediator of the MSCs' immunoregulatory actitivity. Several other factors have been proposed by which MSCs exert their tolerogenic effect, including nitric oxide,[103] or HO-1.[104] It may well be that these effectors act in concert with other immunomodulators such as cyclooxygenase 1 and 2 and hepatocyte growth factor.[105] It is intriguing to note that many of these factors are induced by IFN-γ, thus, underlining the multifaceted functions of this cytokine. In the context of regulating allo-responses MSCs have been observed to affect allogeneic immune effector cells more than virus-specific responses.[106] The outstanding tolerogenic capacity attributed to this cell population has led to the introduction of MSCs in a number of clinical trials addressing the question of a beneficial effect in the control of GvHD in HSCT.[107] However, in the reverse, the potent immunoregulatory capacity of MSCs has also been associated with tumor metastasis[108] and with leukemia relapse,[109] thus pointing at the fine line of the beneficial and adverse effects of adoptive cell transfer therapies with potently immunoregulatory cell populations.

Exploitation of IDO in HSCT

The thus far promising role for IDO activity for the development of transplantation tolerance has stimulated research for the therapeutic use of IDO activity in promoting allo-antigen-specific tolerance in HSCT. Pre-clinical models confirmed that IDO transgenic DCs do have the potential to induce tolerance in HSCT. A study of Mulley et al. showed that IDO can act in concert with other immunoregulatory pathways, such as IL-10, CTLA-4-Ig or CD40-Ig in the prevention of graft rejection in a xenogeneic transplantation model.[110] In a second study Wee et al. showed that pig-DCs transgene for the full length of human IDO were able to delay xeno-graft rejection.[111]

When considering the exploitation of IDO in human HSCT in a clinical context, it has to be emphasized that the use of this immunoregulatory pathway should

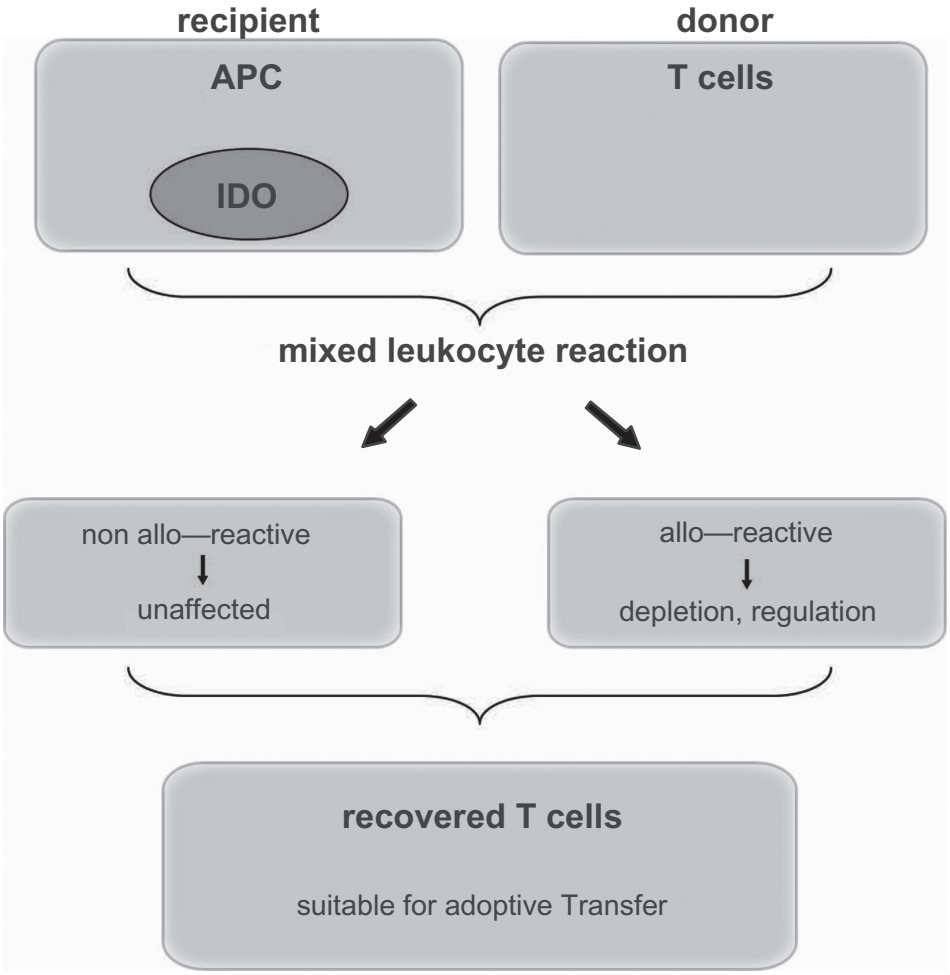

Figure 4. Exploitation of IDO activity to generate allo-antigen-specific tolerized T cells. After an ex vivo mixed leukocyte reaction of recipient IDO competent APCs and donor T cells the recovered T cells are supposed to be unable to mount allo-responses in the GvH direction due to the effects of IDO but retain the immune competence against microbial pathogens. These T cells should therefore be suitable for being adoptively transferred to the recipient to provide transient immunocompetence but avoid GvHD induction.

circumvent strategies that would finally aggravate the immunosuppressed state of the transplant recipient (see above). Thus, for example, a transfer of IDO expressing or transgenic DCs is predicted to have a generalized immunosuppressive effect, probably increasing the anyhow exceptionally enhanced susceptibility of the recipient to the same opportunistic infections and/or to a diminished anti-tumor or GvL effect as conventional immunosuppressants.

Rather it seems feasible to use IDO expressing DCs or MSCs in an ex-vivo setting for the generation of allo-antigen-specific tolerance (Fig. 4). The hypothesis of this approach is that in an MLR using the recipient type IDO competent cells (which express solely recipient type allo-antigens) and allogeneic, donor-derived T-cells (which are naïve to the presented allo-antigen), the allogeneic T-cells would become tolerant through IDO activity in the stimulating cells in an allo-antigen-specific fashion. This means that the allo-responsive T-cells are being tolerized whereas the non-allo-reactive T-cells are left unaffected. Thus, these T-cells may eventually be suitable for being used in adoptive cell transfer strategies as they would provide a source of cells with a retained ability to protect against microbial antigens while not carrying the risk of initiating GvHD.

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors report no conflicts of interest.

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