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REVIEW ARTICLE

Marcus D. Säemann
Maximilian Zeyda
Thomas M. Stulnig
Georg A. Böhmig
Thomas Wekerle
Walter H. Hörl
Gerhard J. Zlabinger

Janus kinase-3 (JAK3) inhibition: a novel immunosuppressive option for allogeneic transplantation

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M. D. Säemann (✉)
G. A. Böhmig · W. H. Hörl
Department of Internal Medicine III,
Division of Nephrology and Dialysis,
Medical University of Vienna,
Vienna, Austria
E-mail:
marcus.saemann@meduniwien.ac.at
Tel.: +43-1-404004391
Fax: +43-1-404004392

M. Zeyda · T. M. Stulnig
Department of Internal Medicine III,
Clinical Division of Endocrinology and
Metabolism, Medical University of Vienna,
Vienna, Austria

T. M. Stulnig
CeMM-Centre of Molecular Medicine,
Austrian Academy of Sciences,
Vienna, Austria

T. Wekerle
Department of Surgery,
Division of Transplantation,
Medical University of Vienna,
Vienna, Austria

G. J. Zlabinger
Institute of Immunology,
Medical University of Vienna,
Vienna, Austria

Abstract Current immunosuppressive therapy in clinical organ transplantation is based on drugs that suppress various functions of immunocompetent cells but still affect cells and organ compartments other than the immune system. Hence, these drugs have considerable side effects which lead to increased morbidity and reduced life-quality of transplant recipients. A major step forward in the rationale design of clinical immunosuppression resides in the elucidation of molecular targets that play a critical role specifically within the immune system. Recently, Janus kinase 3 (JAK3) has been identified as such a molecule. Genetic absence or ablation of this tyrosine kinase is associated with defective T-cell immunity that results in severe combined immunodeficiency (SCID) without apparent changes in other organ systems. Furthermore, pharmacological inhibition has significantly prolonged allograft survival in several experimental models of organ transplantation. The present review provides an overview of the emerging role of JAK3 in the immune system and the development of JAK3-inhibiting drugs. The potential clinical application of JAK3 inhibitors in organ transplantations is discussed in the light of a recent series of successful kidney transplantations in non-human primates immunosuppressed solely with a novel JAK3 inhibitor.

Keywords Tyrosine kinase · Rational immunosuppression · Immunodeficiency · T-cell activation · Allograft

Introduction

Clinical immunosuppressive therapy is currently based on the calcineurin inhibitors cyclosporine A (CsA) or tacrolimus (FK506), in conjunction with the antimetabolite mycophenolate mofetil (MMF) and corticosteroids. Several studies have established the mTOR inhibitor, rapamycin (RAPA) as an effective immuno-

suppressive agent that can also be combined with one of the above-mentioned immunosuppressants for treating human allograft rejection [1]. Although these drugs have led to a significant improvement of immunosuppressive therapy in terms of short- and long-term graft outcome, the principal disadvantages remain. Drug-related drawbacks in organ transplantation are mainly due to: (1) a lack of specificity for immune cells, leading to side effects in other organ systems caused by affecting non-immune cells; (2) a lack of efficacy to prevent chronic allograft rejection; (3) an increased incidence of infection and tumour due to antigen-unspecific immunosuppression.

Calcineurin inhibition in non-immune cells is associated with typical side effects such as arterial hypertension due to renal vasoconstriction, ultimately leading to chronic interstitial fibrosis of the kidney in a considerable number of transplant patients [2, 3]. Further significant side effects of this therapeutic approach include glucose intolerance, hyperlipidaemia and cosmetic complications such as hypertrichosis and gingival hyperplasia [4, 5]. Presently, the most frequently used anti-metabolite, MMF, is associated with a considerable incidence of gastrointestinal side effects, such as diarrhoea and abdominal cramps, due to local accumulation in the gut [6]. The use of corticosteroids, one of the most efficient anti-inflammatory and immunosuppressive drugs, frequently leads to hypertension, post-transplantation diabetes mellitus and osteoporosis, especially when administered in the higher doses that are often necessary for combating acute allograft rejection [5, 7, 8]. Therefore, ideal clinical immunosuppression should potently suppress specific alloreactive immune cells while sparing all other tissues of the human organism.

Present immunosuppressive drug therapy

Current immunosuppressants mainly target different phases of T-cell activation to disrupt alloantigen-specific immune responses. Immediately after the T-cell receptor (TCR) recognizes allopeptides that are presented by specialized antigen-presenting cells (APCs) in the context of efficient co-stimulation, calcineurin inhibition potently interferes with early T-cell signalling events, leading to impaired cytokine production (Fig. 1). After completion of early TCR signalling, calcineurin inhibitors are no longer able to suppress T-cell activation. In this second phase, activated lymphocytes are sensitive to RAPA, since cytokine signals transduced to the T cell are partially abolished by disrupting mTOR activity (Fig. 1) [9]. Furthermore, when cytokine receptors, such as the IL-2-receptor, are upregulated, monoclonal antibodies can be successfully used to block the interaction of T-cell growth factors with their receptors. Later phases are subject to interference by anti-metabolites such as MMF, causing inhibition of DNA synthesis and, hence, abolishing cell cycle progression of alloreactive T cells [10]. Importantly, most stages of the T-cell activation process are susceptible to the inhibitory actions of corticosteroids (Fig. 1). Immunosuppressive effects seem to be predominantly mediated by modulating gene expression via the glucocorticoid receptor, but non-transcriptional mechanisms contribute as well [11, 12]. Furthermore, corticosteroids suppress cytokine responsiveness of T cells by inhibiting cytokine-receptor-associated tyrosine kinase activity [13]. Thus, currently applied immunosuppressive drugs effectively disrupt different stages of the T-cell activation process while sparing other phases of the activation process [14].

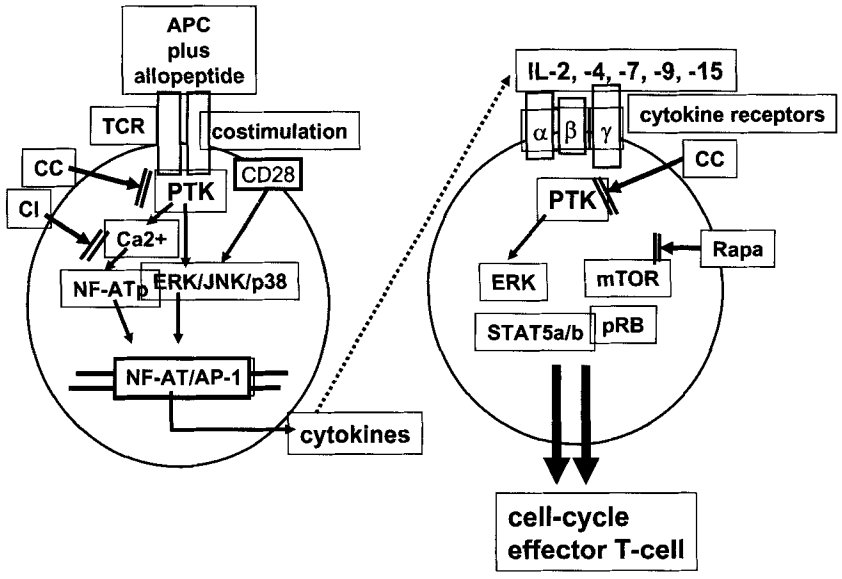

Fig. 1 Principal targets of current immunosuppressive drugs in T cells. During the very early phase of T-cell activation current immunosuppressants are able to interfere with cytokine production, while in the later phase of this process some, such as calcineurin inhibitors, may become ineffective. Note that, throughout the complete T-cell activation process, corticosteroids are able to suppress distinct activation signals early after receptor engagement. *Large arrows* indicate activation of distinct signal transduction pathways by the respective drugs. *CC* corticosteroids, *CI* calcineurin inhibitors

Although the T-cell-mediated acute rejection is effectively controlled by different immunosuppressants, rejection still occurs in the long run. Chronic and repeated sub-clinical rejection, as a multifactorial process, is far from being completely understood. During the "life of the allograft", even in completely MHC-matched individuals, continuous presentation of minor histocompatibility antigens leads to perpetuation of the rejection process ("epitope spreading") [15, 16, 17]. Alloreactive effector T cells, which are similar to classic memory cells, develop over time. These cells are resistant towards calcineurin inhibition and low-dose corticosteroids, possibly due to a signal dependency that relies more on cytokines such as IL-15 [18] (Fig. 2). A beneficial effect of MMF on this process is still a matter of debate, but recent data suggest that its effect is rather modest [19]. In conclusion, more effective immunosuppressants are needed to prevent chronic rejection by inhibiting the development of alloreactive effector T cells [20, 21].

Given the distinct requirements for the novel immunosuppressive agents considered above, an ideal molecular target should be expressed selectively in the immune system and should be required for essential and non-redundant functions in immune cells to guarantee strong immunosuppressive potency in the case of its ablation or inhibition. The protein tyrosine kinase (PTK) JAK3 seems to meet this criterion since (1) its expression is restricted to cells of the haematopoietic compartment, and (2) genetic absence of this PTK leads to SCID in afflicted individuals showing only a few mature T cells, if any, and no NK-cells, indicating an essential role of this tyrosine kinase within the lymphoid compartment.

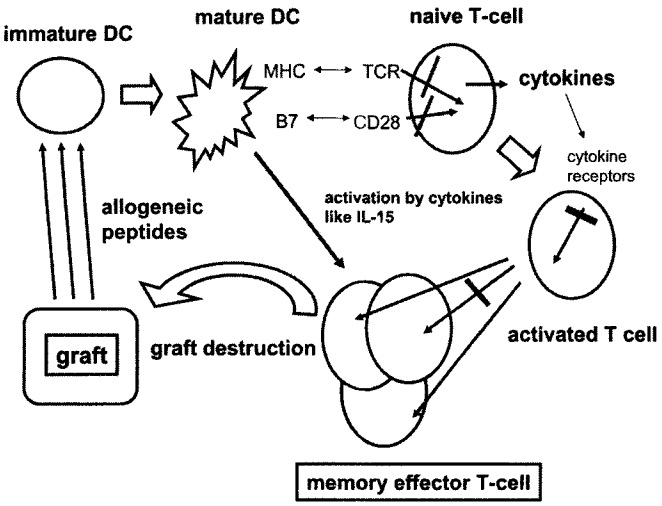

Fig. 2 Impact of current immunosuppressive drug therapy on the evolution of chronic rejection. While in the initial post-transplantation period alloantigen-specific T cells are typically activated by TCR plus co-stimulatory molecule engagement via potent APCs; later in the course of the transplant more T cells become activated by the persistence of the alloantigenic reservoir ("epitope spreading") and become memory-like effector cells that can be activated solely by cytokines. *Black arrows* indicate activation of distinct signal transduction pathways by immunosuppressive drug therapy. Notably, late in the allogeneic T-cell response, immunosuppressive drugs only incompletely inhibit lymphocyte proliferation. *DC* dendritic cells

Expression and function of JAK3 in the normal immune system: implications for rational immunosuppression

JAKs are cytoplasmic tyrosine kinases that participate in the signalling of a broad range of cell-surface receptors and, in particular, members of the cytokine-receptor superfamily, which lack intrinsic tyrosine kinase activity [22]. The four mammalian JAKs, JAK 1, 2, and 3 and Tyk2, contain recognition motifs that associate with the membrane-proximal region of cytokine receptors. Ligand-receptor-induced activation of JAKs leads to the phosphorylation of the receptor, creating docking sites for specific signalling proteins including STATs, which are phosphorylated by JAKs on a conserved tyrosine residue at their C-terminus (Fig. 3). Thereby, STATs are transactivated to direct the transcription of target genes by binding to specific regulatory sequences. First indications for the role of JAK3 in the immune system came from the analysis of patients suffering from a particular autosomal recessive form of SCID and who phenotypically lack T and NK cells, but not B cells ($T^-B^+NK^-$ phenotype) in the peripheral blood at birth [23].

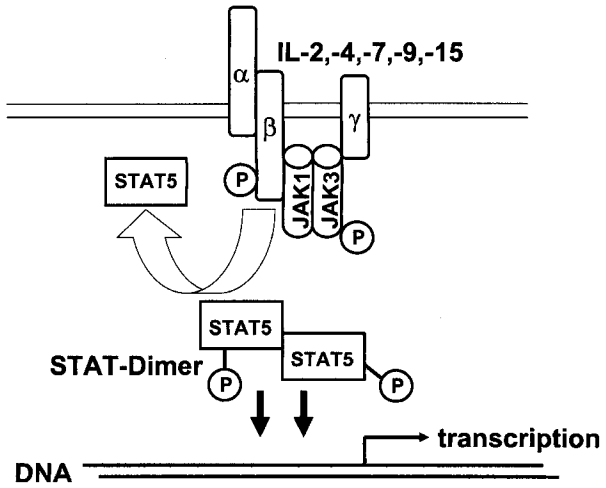

Fig. 3 A schematic representation of the JAK-STAT signalling pathway in mature T cells. A variety of cytokines is able to use the cγ-chain, which pushes T cells into the cell cycle, finally leading to optimum effector function. Furthermore, in the absence of cγ-chain dependent cytokines, T cells are committed to activation-induced cell death. Each cytokine uses a distinct specific alpha-chain of the cytokine receptor. The activation of JAK3 after cytokine stimulation results in the phosphorylation of STATs, which then dimerize and translocate to the nucleus to activate gene transcription

Focusing on the common gamma chain (cγ) of cytokine receptors, which is an essential integral component of the receptors for the T-cell growth factors IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, mutations in the cγ-associated JAK3 were identified [24, 25, 26]. Subsequently, JAK3 deficiency was shown to be the most common cause of autosomal recessive $T^-B^+NK^-$ SCID in humans. Likewise, JAK3 knockout mice exhibit a profound immunodeficiency, with markedly reduced numbers of functional T cells, and also exhibit a failure in negative selection of thymocytes [27, 28]. Although patients with JAK3 mutations are severely immuno-compromised, bone marrow transplantation is an effective means for reconstituting T-cell immunity in this defect [29]. Furthermore, if this procedure is successful, the patients are immunocompetent and do not have any significant defects or perturbations in other organs/organ compartments outside the immune system [29]. As one, but not exclusive, final intracellular effector molecule of JAK3, STAT5a/b is phosphorylated and, subsequently, transactivates critical cell cycle-regulatory molecules of T cells [30, 31]. These data indicate a non-redundant role for JAK3 in the establishment and function of the normal immune system (Fig. 3).

Traditionally, JAK3 has been associated with sensitivity towards cytokine signalling due to its physical association with the cγ-chain of cytokine receptors. Hence, $JAK3^{-/-}$ cells do not respond towards cγ-dependent cytokines, such as IL-2 and IL-15, finally leading to apoptosis induction presumably due to growth factor deprival [32] or to direct activation of anti-apoptotic genes [33]. Recently, a more complex immunoregulatory role for JAK3 has been considered, as JAK3 may be involved in early T-cell activation process: naive TCR-triggered JAK3-deficient T cells have distinct defects in early signalling cascades such as defective early tyrosine phosphorylation and calcium mobilization, along with reduced levels of IL-2 synthesis [34, 35]. Strikingly, recent data have shown that JAK3 is immediately phosphorylated after TCR engagement, since JAK3 is directly associated with the CD3-ζ chain of the TCR complex [35]. The association of JAK3 with the TCR/CD3 machinery as well as the IL-2R complex suggests a crucial role of this kinase in the regulation of both early T-cell activation and cytokine-driven cell growth at two distinct time points.

Recently, our group investigated whether pharmacological targeting of JAK3 with a rationally designed inhibitor has any consequences on the early phase of T-cell activation [36]. These dimethoxyquinazoline compounds were designed according to a homology model of the kinase domain of JAK3 fitting the catalytic site of JAK3 [37]. While potently inhibiting JAK3 kinase activity, they did not affect JAK1, JAK2, ZAP/SYK-, Tec- or Src -family PTKs, even at very high concentrations. Interestingly, we detected a profound inhibition of IL-2 secretion when JAK3 activation was selectively prevented. Moreover, the production of other early induced cytokines such as IFN-γ or IL-10 was similarly prevented by the disruption of JAK3 activity assessed by gene protection assays. Using a Jurkat cell line, stably transfected with an IL-2 promoter luciferase gene construct, we found that JAK3 inhibition efficiently prevented IL-2 gene transcription due to a complete failure of NF-AT and AP-1 transactivation. Further analysis of critical upstream signalling events showed defective PLC-γ1 phosphorylation and diminished Ca^{2+}-mobilization. We further observed a complete failure of JAK3-inhibited cells to undergo homo-aggregation, a β2-integrin-dependent event. Remarkably, upon TCR-independent stimulation with phorbol ester/calcium ionophore IL-2 production and T-cell homo-aggregation were completely insensitive to JAK3-inhibitor treatment, indicating an early block in TCR-mediated signal transduction [36]. These investigations extend earlier studies on the role of PTK in early T-cell activation responses and may be of fundamental importance for TCR signalling in general. As a major disadvantage, pharmacological JAK3-inhibitors, even when rationally designed, may unselectively affect several tyrosine phosphorylation events proximal to the TCR. Studies in our laboratories as well as in others, are underway to determine the significance of these findings by means of dominant-negative JAK3 constructs or immortalized T-cell lines obtained from SCID patients.

Importantly, immunocompetent cells other than T-lymphoid cells have been claimed to depend, in their functional integrity, on JAK3. B cells have been assumed, when activated via CD40 engagement, to use JAK3 and, subsequently, STAT5 for signalling [38]. However, recent data from SCID patients unequivocally showed that also in the absence of T cells, B cells rescued from the peripheral blood at birth have no significant impairment in their response to a variety of stimulatory signals [25, 39]. Furthermore, Revy et al. showed that, although CD40 is physically coupled with JAK3 in peripheral blood B-cells, no phosphorylation can be detected upon receptor ligation, and, consequently, no STAT-5 transactivation [40]. The same group provided evidence for CD40 phosphorylation and subsequent STAT5 activation in monocytes; however, the nuclear STAT5 turned out to be a truncated form of the STAT5a/b molecule [40]. Recently, our group showed that monocyte-derived dendritic cells (DCs), which belong to the most efficient APCs, cannot mature upon CD40 activation in the presence of a JAK3-inhibitor [41]. The resulting impaired immunostimulatory function of JAK3-inhibitor-treated DCs led to an allogeneic T-cell hyporesponsiveness that suggested a further immunomodulatory mode of JAK3 inhibitors. Collectively, it is now established that JAK3 works by enabling effective T-cell function, but its activity does not seem to be

essential in the B-lymphoid compartment (Fig. 3). Possible functions of JAK3 extend to distinct cells from the myeloid compartment, as also the functional integrity of mast cells has been influenced by JAK3 [42]. These data should be carefully taken into account when one is considering the immunosuppressive activity of specific JAK3 inhibitors in patients undergoing allogeneic organ transplantation.

Efficacy of pharmacological JAK3 inhibitors in experimental allograft rejection

First indications of the efficacy of blocking cγ-dependent cytokine signals to induce permanent allograft acceptance were reported by Li et al. [43]. Mice receiving allogeneic islets under the cover of monoclonal anti-cγ-receptor antibodies permanently accepted the allografts, presumably due to activation-induced cell death of alloreactive T lymphocytes, indicating that mere ablation of cγ-signalling might be sufficient for permanent graft acceptance [43]. Owing to the recognized importance and restricted expression of JAK3, this PTK was selected as a target molecule for a selective pharmacological inhibition approach. Using a pattern-recognition algorithm program AG490, a tyrphostin family member and derivative of benzylidene malononitril inhibited both JAK3 and JAK2. However, AG490 did not disrupt other essential PTKs such as Lck, Lyn, Btk, Syk, Src, JAK1 or Tyk2 [44, 45]. As in vitro correlate of transplant rejection, mixed lymphocyte cultures (MLCs) were studied with AG490, showing potent immunosuppressive activity and, beyond this, a tolerance-inducing capability of this JAK inhibitor [46]. The mechanisms involved in this alloantigen-specific hypo-responsive state were shown to include deletion of activated T cells, which is in line with evidence obtained from JAK3-deficient lymphocytes [46]. Of note, IL-2 production was ablated by AG490 in MLCs, while essential early TCR-triggered signals such as Ca^{2+}-mobilization were not affected. When injected intraperitoneally, for 1 week, into mice receiving heterotopic heart allografts, mean survival time (MST) of the grafts was 18.0 days, compared to 8.8 ± 0.8 days in control mice [47]. PNU156804, a chemically different JAK3 inhibitor derived from the toxic compound undecylprodigiosin, displayed more selectivity towards JAK3, leaving JAK2 autophosphorylation intact. Peritoneal administration of PNU156804 for a 7-day period prolonged the MST from 6.3 ± 0.5 days in untreated animals to, maximally, 55.0 ± 21.0 days in treated mice [48, 49]. In both of these studies it would have been interesting to investigate whether prolonged or continuous delivery of the respective JAK3-inhibitors might have contributed to a further prolongation of graft survival. The Kirken group suggested that inhibition of JAK3 is analogous to prevention of signal 3 during the T-cell activation process, where signal 1 represents TCR-engagement and signal 2 the co-stimulatory signal [45, 50]. Hence, in their later studies, CsA as signal 1 inhibitor and RAPA as signal 3 inhibitor were tested in combination with the JAK3 inhibitor. The group found, in the same rat strains, that combination of the JAK3 inhibitor with RAPA led only to modest prolongation of the allograft survival, which was interpreted as an additive action of both signal 3 inhibitors [47, 48]. However, graft survival was prolonged from 12.2 ± 0.8 days to 57.6 days $\pm$ 39 days when CsA was given in combination with AG490, suggesting synergy between a signal 1 and signal 3 inhibitor. Similar results were obtained with PNU156804 in another heterotopic transplant model [50]. In all JAK3-inhibitor-treated animals histopathological analysis showed reduced graft damage and decreased infiltration of activated leukocytes. While these studies were the first to demonstrate that JAK3 is a potential target for the prolongation of allograft survival, the number of treated animals was too small and the variation of MSTs in the treated groups too high to conclude that JAK3 inhibition is a simple signal 3 inhibitor. Beyond testing "signal 3 inhibition", however, JAK3 inhibitors may be the more potent cytokine signalling inhibitors, since rapamycin, even at very high doses, is not able to suppress fully the T-cell cytokine responsiveness. In contrast, owing to the essential role of JAK3 proximal to the heterotrimeric cytokine receptor, its ablation results in a complete downregulation of the lymphocyte responsiveness to external growth factors.

Further in vivo studies were undertaken with the dimethoxyquinazoline compound WHI-P131, which was designed according to the catalytic domain of JAK3. Mice transplanted with allogeneic bone marrow did not develop significant graft-versus-host disease (GVHD) when treated with WHI-P131 for 56 days, whereas vehicle-treated mice survived for only 37 days [51]. Importantly, the combination of methotrexate, as standard anti-GVHD drug, and the JAK3 inhibitor resulted in disease-free survival for the entire 85-day observation period. In a subsequent study that used WHI-P131 in mice challenged with BCL-1 leukaemia cells, adoptive transfer experiments showed that spleens of mice with allografted bone marrow contained fewer than 0.001% leukaemic cells [52]. Furthermore, mice challenged with an otherwise invariably fatal dose of BCL-1 leukaemia exhibited a 100% survival rate after being treated with the JAK3 inhibitor plus methotrexate [52]. Interestingly, these data were corroborated by allogeneic bone marrow from $JAK3^{-/-}$ mice, which did not induce fatal GVHD [53]. These data indicated that (1) JAK3 is an essential mediator in GVHD and that (2) pharmacological JAK3 inhibition prevents GVHD while sparing the graft-versus-leukaemia function of the allografted bone marrow. The same JAK3 inhibitor was also studied in non-obese

diabetic (NOD) mice (Table 1), most of which develop overt diabetes at approximately 25 weeks. When the animals were injected daily, from week 10 to 25, the incidence of overt diabetes was 9% in JAK3-inhibitor-treated mice versus 60% in the vehicle-treated control animals [54]. Since the initiation of the disease is strictly dependent on the generation of effector T cells, the data clearly demonstrate the effectiveness of JAK3 inhibition in this disease model, apart from indicating an involvement of JAK3 in disease progression in an autoimmune model.

No data on the tolerogenic potential of JAK3 inhibitors are currently available. Whereas functional T-cell inactivation seems unlikely, deletion of activated T cells, due to the role of JAK3 for T-cell survival, as another possibility for T-cell unresponsiveness, might be possible in vivo. Furthermore, future studies will have to determine the effects of JAK3 inhibitors on the establishment of T-cell tolerance induced by monoclonal antibodies or mixed chimerism.

JAK3 inhibition in non-human primate model of kidney transplantation

Prolonged allograft survival by pharmacological JAK3 inhibition was recently observed in a large animal study. A chemical library was screened for potential inhibitors of JAK3, and the leading compound was then chemically modified [55]. The resulting JAK3 inhibitor, CP-690,550, was found to possess selectivity for JAK3, showing, in ambitious kinase assays, no inhibitory potency for essential kinases of, e.g., the TCR, including lck and some cytokine signalling pathways. The activity of JAK2, however, was affected considerably, and that of JAK1 to a lesser degree. Hence, in contrast to all previously reported JAK3 inhibitors CP-690,550 exhibited, approximately, a 1,000-times-higher potency in suppressing JAK3 activity with an IC50 of 50 ng/ml, which was chosen as the target value for the in vivo studies of CP-690,550. In in vitro assays CP-690,550 effectively inhibited IL-2-triggered, but not TCR-triggered, cell activation and, of note, also GM-CSF-induced proliferation. The apparent disparity between the genetic studies and, also, JAK3 inhibitor studies, and the effects of CP-690,550, might be resolved if the inhibitor is used in T cells activated via "weak" stimulation that mimics TCR triggering via, e.g., antigen-presenting cells, since "strong" T-cell activation might overcome early JAK3-dependent TCR-signalling [35]. In the murine model of cardiac allograft rejection, administration of CP-690,550 for 28 days led to a median graft survival time of >60 days, versus 12 days in the vehicle-treated group [55]. Analysis of the grafts showed substantially reduced chemokines and immune cell effector molecules such as granzyme B or FasL in the JAK3-inhibitor-treated cells.

In a next step, kidneys were transplanted in non-human primates, which resulted in constant rejection after 6 ± 1 days. The animals were administered CP-690,550 without any co-medication, and those with adjusted trough levels between 50 and 100 ng/ml rejected kidneys after 62 ± 6 days, while animals adjusted to 200–400 ng/ml rejected kidneys after 83 ± 6 days. Importantly, CsA had previously been used in the same animal model, resulting in a median survival time of 39 days when used as monotherapy [56].

JAK2 is known to be associated with several receptors of the haematopoietic system, such as for erythropoietin, thrombopoietin and several colony-stimulating factors [57]. Hence, a dose-related but reversible anaemia was observed in the CP-690,550-treated animals with lowest haemoglobin levels, of approximately 8 g/dl. However, results of the low-dose group without anaemia were sufficient for significant anti-rejection therapy, and, therefore, the results in the "anaemia-group" could be regarded rather as dose-escalation experiments. With respect to the analysis of peripheral blood leukocytes it is interesting to note that typical "SCID-defects" in the treated animals were not detected and included significantly decreased or clearly apoptotic numbers of CD3-positive cells. Only a modest reduction of NK-cells was found. It is not clear why rather normal numbers of CD3-positive cells were detected, given the results in SCID patients and the proposed role of JAK3 in T-cell survival. One possibility might be that JAK3 has a more important role in thymocyte generation, as has been shown in experimental studies, but "acute" inhibition of JAK3 in mature T cells does not result in significant deletion of the peripheral lymphocyte repertoire. Another possibility could be the fact that only alloreactive T cells were deleted during JAK3 inhibition and the respective cell number is, therefore, too small to be detected. However, experimental data could show a role

Table 1 Overview of pharmacological JAK3 inhibitors employed in vivo

JAK3 inhibitor	Chemical compound	In vivo model
PNU156804	Undecylprodigiosin derivative	Murine heart TX [48, 50]
AG490	Benzylidene malononitrile derivative	Murine heart TX [44, 45]
WHI-P131	Dimethoxyquinazoline derivative	Murine GVHD [51, 52, 53] NOD mice [54]
CP-690550	Piperidine propanenitrile derivative	Murine and NHP heart TX [55]

for JAK3 in T-cell survival, even in resting T cells, possibly due to low amounts of cγ-dependent cytokines secreted by non-activated lymphocytes in an autocrine fashion [58]. Similarly, no obvious influence was observed regarding the B-cell repertoire, which would be in accordance with recent data that indicate that JAK3 is not essential for B-cell function and survival. Clearly, long-term results on the respective JAK3-inhibitor therapy in this animal model might answer most of those questions. No typical side effects known from current immunosuppressive drug therapy, such as hypertension, diabetes mellitus or other metabolic abnormalities, were observed, which clearly indicates a lack of typical collateral toxicity by this tyrosine-kinase inhibition approach. Since JAK3 inhibition is an antigen-unspecific approach to suppress T-cell activation, interestingly, no increased incidence of infections was detected. However, the time period for the observation of infectious disorders, and, also, a lack of lymphoproliferative diseases, may be too short, so that further meticulous assessment in the same animal model, beyond 12 months, is required. Further studies are underway to determine the efficacy of JAK3 inhibition in combination with calcineurin and mTOR inhibitors. Furthermore, it would be interesting to analyse the effects of JAK3 inhibition on chronic allograft damage, since no direct nephrotoxic effects are known and JAK3 inhibition should also powerfully suppress epitope spreading. Alternatively, it might be envisaged that in future studies specific inhibitors of the innate arm of the immune system (dendritic cells, monocytes/macrophages), such as 15-deoxyspergualin, will be combined with JAK3 inhibitors to ensure optimal allograft survival.

Perspectives and outlook

Despite significant progress in the field of allogeneic transplantation, rejection processes, particularly of the chronic form, cause excessive morbidity and, finally, often graft loss. It was recently shown that during long-term calcineurin inhibitor treatment, organ toxicity is common, although its use prevents acute rejection in the early post-transplantation period [3]. Furthermore, other collateral toxicities such as arterial hypertension, metabolic derangements and myelosuppression are common in transplant recipients. Hence, a significant advance in the entire field of allogeneic transplantation would be efficient immunosuppression without any effects on other organ systems. As outlined above, the PTK JAK3, would be an ideal candidate molecule to achieve such tailored immunosuppression, due to its importance in the proper activation of T cells and its immune-cell restricted expression. Pharmacological disruption of its activity has led to dramatic improvements in allograft survival in various animal models and, finally, in a clinically relevant non-human primate model of kidney transplantation. In a short time the development from randomly selected inhibitory molecules (first-generation drugs) towards rationally designed enzyme inhibitors (second-generation drugs) has been made, and it can be envisioned that further highly selective and pharmacokinetically optimized drugs will be developed in the near future. Furthermore, tyrosine kinase inhibition has already entered clinical medicine with reasonable success in the treatment of chronic myelogenic leukaemia (CML) with the PTK inhibitor imatinib suppressing the function of the bcr-abl fusion protein [59]. Possibly, in the not-too-distant future, a combination of drugs that selectively target myeloid cells as part of the innate arm of the immune system, as well as highly selective T-cell inhibitors such as JAK3-inhibitors, will be routinely administered to allogeneic organ recipients. The next achievable goal in present research will be highly effective immunosuppressive therapy devoid of significant direct toxicity against non-immune cells, to prolong allograft survival and minimize drug-related morbidity in transplant patients.

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