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REVIEW

Report of the joint ESOT and TTS basic science meeting 2013: current concepts and discoveries in translational transplantation

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Summary

A joint meeting organized by the European (ESOT) and The Transplantation (TTS) Societies for basic science research was organized in Paris, France, on November 7–9, 2013. Focused on new ideas and concepts in translational transplantation, the meeting served as a venue for state-of-the-art developments in basic transplantation immunology, such as the potential for tolerance induction through regulation of T-cell signaling. This meeting report summarizes important insights which were presented in Paris. It not only offers an overview of established aspects, such as the role of Tregs in transplantation, presented by Nobel laureate Rolf Zinkernagel, but also highlights novel facets in the field of transplantation, that is cell-therapy-based immunosuppression or composite tissue transplantation as presented by the emotional story given by Vasyly Rohovyy, who received two hand transplants. The ESOT/TTS joint meeting was an overall productive and enjoyable platform for basic science research in translational transplantation and fulfilled all expectations by giving a promising outlook for the future of research in the field of immunological transplantation research.

The role of regulatory T cells

As induction of tolerance – enabling indefinite allograft survival without the need for life-long immunosuppression (IS) – is a major goal in transplantation, the meeting was opened with a lecture given by Herman Waldmann (UK) summarizing insights about natural regulatory T cells (nTregs) and peripheral regulatory T cells (pTregs). Both the transcription factor FoxP3 and TGFß signaling in T cells appear to be essential for transplant tolerance [1,2]. Furthermore, tolerance through co-receptor blockade is dominant and "infectious" as $CD4^+$ T cells from tolerant mice have the capacity to prevent graft rejection through naïve lymphocytes [3]. Another important issue addressed by Dr. Waldmann is the role of the tolerated tissue itself. T-cell-mediated suppression of graft rejection appears to be an active process that operates beyond secondary lymphoid tissue as tolerated grafts contain allogeneic cells kept under control by the persistent presence of regulatory T cells (Tregs) at the site of the tolerated transplant [4,5]. However, tolerance can be easily overridden by anti-TGFß, anti-IL9, anti-CTLA-4, and anti-GM-CSF. To translate research findings into the clinic, Dr. Waldmann introduced the concept of Physician Aided Reconstitution of the Immune System (PARIS), addressing the therapeutic approach in which T-cell depletion is followed by the *in vivo* recruitment of Tregs thus minimizing the use of toxic immunosuppressive drugs [6]. The concept is based

on the enhancement of the patient's own Tregs either within the patient or by *ex vivo* expansion and reinjection. Major aim of PARIS is to achieve selective inhibition of reconstituting "memory"-like T cells, to enhance nTregs and to control homeostatic expansion of effector cells so the graft further engages nTregs and induces pTregs to take over control.

Alternatively, Bernhard Vanhove's (France) presentation focused on the immune regulatory function of myeloid-derived suppressive cells (MDSC). Although an upregulation of Tregs was observed in a rat kidney transplantation model, his team could show that CD3-NKRP1$^+$ cells, that are MDSC, are responsible for suppression of immune response [7]. In addition, MDSC may participate in the NO-dependent maintenance phase of tolerance and suppress immune responses either directly by interacting with effector T cells or indirectly by promoting Tregs under the control of CCL5 [8]. Increased frequencies of circulating MDSC have also been identified in renal transplant recipients [9], and their induction appears to be associated with better kidney function. However, Dr. Vanhove concluded that if MDSC should be used therapeutically, additional mechanistic studies are required to better define the potential of MDSC and their interaction with immunosuppressive drugs.

In the next presentation, Kathryn Wood, Joanna Hester, and Fadi Issa (UK) described the mechanisms of Treg regulation in transplantation. In a humanized mouse model, they demonstrated that islet rejection is prevented via suppression of cytokine signaling (IFNg and IL-6) and lymphocyte proliferation [10]. In contrast, Hans Koenen (the Netherlands) investigated the effect of *ex vivo* expanded CD4$^+$CD25$^+$CD127^{low} human Tregs on the inflammatory response of human skin allograft in a humanized-SCID mouse model and observed that *ex vivo* expanded Tregs can reduce but do not fully prevent human PBMC induced skin inflammation *in vivo*.

The role of memory cells

In the next session, the focus was put on the role of memory cells in transplantation. Antonio Lanzavecchia (Switzerland) gave an overview of memory B cells, neutralizing antibodies and the role of somatic mutations. As new approaches have been developed to investigate human memory B cells and plasma cells in order to isolate neutralizing antiviral antibodies against highly variable pathogens such as HIV-1 and influenza virus, Dr. Lanzavecchia nicely showed that a few somatic mutations are sufficient for high affinity binding and generation of variants with broader specificity anticipating virus escape [11,12]. These antibodies not only provide new tools for prophylaxis and therapy for viral diseases but also identify conserved epitopes that may be used to design new vaccines capable of conferring broader protection. However, memory cells have a low activation threshold, high T-cell stimulatory capacity and may break T-cell tolerance [13].

Another recurring theme was elucidated by Fadi Lakkis (USA) who showed that graft derived endothelial cells and bone marrow-derived dendritic cells (DCs) can present cognate antigens that are necessary for the firm adhesion and transendothelial migration of antigen-specific CD8$^+$ effector T cells, independent of Gαi-coupled chemokine receptors on T cells. In contrast, the adhesion and transmigration of nonspecific effector T cells (bystander T cells) remain dependent on Gαi but require the presence of antigen-specific effector T cells. The detection of this pathway of effector T-cell entry into vascularized organ transplants sheds new light on the pathogenesis and treatment of rejection [14].

The session was closed with a presentation by Stefan Tullius (USA) on the impact of aging on allograft outcome [15]. His group observed prolonged graft survival and impaired T-cell activation and proliferation in aged recipients [16] and could demonstrate that immunosuppressants have age-dependent efficacies. Donor age further impacts allograft outcome as transplantation of aged organs leads to an acceleration of acute rejection possibly because of organ-derived passenger leukocytes such as DCs [17].

To be regulatory or not to be regulatory?

A highlight of the meeting was the keynote lecture given by the Nobel laureate Rolf Zinkernagel addressing the exact role of Tregs. In his point of view, chosen experimental setups normally fail to demonstrate the functional importance and relevance of Tregs, as the interpretation of results is highly dependent on individual experimental conditions. He raised the question of how the scientific community can possibly accept the enormous number of postulated pathways in the regulatory circuits involving Treg actions – as it appears to be almost impossible to analyze two independent variables within such a complex interaction such as allograft rejection. Dr. Zinkernagel's conclusion was based on experimental observations demonstrating that an allogeneic ß-cell graft, transplanted directly under the kidney capsule, is accepted for >200 days whereas rejection occurs when mice are previously or simultaneously challenged with allogeneic cells. Moreover, once accepted, the allograft is highly resistant to rejection although the mice are transplanted with an allogeneic skin graft. Whereas the skin graft is rejected in a primary fashion, this does not apply to the ß-cell graft showing that a healed-in, strictly peripheral allogeneic cell graft is largely ignored by the immune system for a long period of time, obviously completely independent of Tregs [18]. It appears that the localization of an

allograft in or strictly outside of secondary lymphatic organs determines whether a $CD8^+$ T-cell-mediated alloresponse from the recipient is induced or not. According to Dr. Zinkernagel's conclusion, immunity is mainly driven by protection against infection and antigen presentation is the most important immune response regulator.

Innate immunity and transplantation

Opening the session, Hergen Spits (the Netherlands) presented an overview of innate lymphoid cell subsets and explained possible implications for how the plasticity of these recently discovered members of the lymphocyte family may have an effect on graft versus host disease. The following talk, given by Michelle Miller (US), picked up on the subject of T-cell-mediated tolerance. Her group showed that mice, which reject long-term accepted primary heart grafts upon infection with Listeria monocytogenes, accept secondary cardiac allografts after injection without the need for immunosuppression. These findings may lead to a better understanding of auto-immune diseases, as well as lay the foundation for successful tolerance induction in transplant recipients.

A further hot topic in innate immunity is the impact of age. With the number of potential organ recipients on the rise and the initiation of expanded criteria donors, the age of tissue has become an important fact in the outcome of graft survival. Karoline Edtinger (USA) and Rupert Oberhuber (USA/Austria) independently touched on the subject of senescence in their talks. Karoline Edtinger and her group used a mouse model of aged and young mice to investigate the effects of rapamycin and co-stimulatory blockade via CTLA4-Ig therapy and found immunosenescent significance, suggesting the importance of age-adapted immunosuppressive therapy. Rupert Oberhuber and his team on the other hand, focused on the impact of the age of the organ itself. By depleting cardiac DCs ($CD11c^+$), they could show that IL-17 mRNA levels were significantly reduced in old allografts, thus abolishing donor age-specific survival deterioration.

A highlight of the session was data on the intragraft clonal expansion and differentiation of B cells in human cardiac allograft vasculopathy, presented by Carolina G. Moore (USA). Vasculopathy (CAV) is a main cause of mortality after heart transplantation. It is therefore of great importance to further investigate this disease in order to achieve a better understanding of the mechanisms behind it. Carolina G. Moore and her group investigated rejected cardiac grafts because of CAV and found evidence of B-cell infiltration in the perivascular region of the epicardium, adjacent to the right coronary artery, as well as near the left anterior descending coronary artery. Furthermore, ca. 80% of these dense B-cell clusters corresponded to a single clone. These cells were then isolated, immortalized and reactivity was assessed using IgM, IgG, or IgA. The better understanding of CAV – of which this project is undoubtedly a major step – will hopefully lead to the development of therapeutic targets and thus the reduction of rejection in the future.

Intestinal immunity and transplantation

An interesting topic in transplantation was raised by Josbert J. Keller (the Netherlands) who spoke about fecal microbiota transplantation (FMT), for example in *Clostridium difficile* infection. This Gram-positive bacterium affects patients with disturbed bowel flora typically because of antibiotic administration, that is, after solid organ transplantation. Indeed, the success rate of restoration of colonization resistance in patients cured of the infection by FMT lies at ca. 90% [19], suggesting that these protocols are worth investigation in other diseases that result in altered and reduced microbiota diversity including irritable bowel syndrome [20] or obesity [21]. The main goal would be to develop probiotic regimens as a substitute for FMT. To shed more light on the functional relevance between commensals in the gut flora and a healthy immune system, Nadine Cerf-Bensussan (France) presented a gnotobiotic mouse model to investigate the influence of individual members of the microbiota on full maturation of the gut-associated lymphoid system (GALT). For example, an unusual symbiont called *Segmented Filamentous Bacterium* (SFB) drives the postnatal expansion of mucosal helper T cells [22]. Cerf-Bensussan also reviewed that the microbiota serves as adjuvant of the systemic innate and adaptive immune responses of T cells, macrophages, and NK cells [23–26].

The role of B cells

In his talk, Reinhold Förster (Germany) raised the question of where cells must meet to induce protective immunity against pathogens or tolerance toward harmless environmental antigens. He noted that apart from certain adhesion molecules (integrins and selectins) chemokines play an important role in guiding T cells and DCs into terminal lymphatic vessels. Especially the C-C chemokine receptor type 7 (CCR7), which is homeostatically expressed by mature B cells, various T cells, and mature DCs, is a key player for lymphatic homing. Although it is well known that memory and naïve T cells follow distinct paths into lymph nodes [27], Dr. Förster addressed the molecular mechanisms that determine the entry into the lymph node and intranodal positioning of lymph-derived cells. DCs in particular require CCR7 to leave the subcapsular sinus and to home directly into the T-cell-rich paracortex. In contrast, T cells migrate there via afferent lymphatics and the medullary sinus, only depending on CCR7 for transpositioning from the medullary sinus into

the lymph node parenchyma. This suggests that these cells sense a gradient of CCR7-ligands, such as CCRL1, specifically expressed by ceiling cells of the lymph node subcapsular sinus, in turn creating a functional gradient on nonhematopoietic cells [28]. These findings are essential for future advances in manipulating DC migration to fine-tune immune responses in clinical settings.

Taking up the subject of cell migration, Justine Durand (France) reviewed a model of cardiac tolerance in rats [29], where an accumulation of mature IgD^{-} B cells with an inhibited expression profile was observed in blood, spleen, and graft [30]. The therapeutic potential of these cells was tested in an acute rejection model and demonstrated that B cells from tolerant donors can transfer donor-specific tolerance via the TGFβ pathway. These cells migrate directly to the graft and are able to suppress TNFα secretion by T cells after anti-CD40-stimulation. Dr. Durand suggested that B cells differentiate into Granzyme-B secreting B cells with regulatory properties. Interestingly, similar cells have been detected in tolerant kidney transplant patients [31,32].

DCs in reperfusion

Osamu Yoshida (United States) presented a further lymphocyte subset that attenuates pro-inflammatory activity and liver transplant ischemia–reperfusion injury (IRI). He reviewed that CD39 expression, which strongly correlates with extracellular ATP hydrolysis that in turn acts as a damage-associated molecular pattern [33], was significantly higher in murine liver conventional myeloid dendritic cells (mDCs) compared with their splenic counterparts. Based on observations in an orthotopic liver transplantation model ($CD39^{-/-}$ to $CD39^{-/-}$ mice), Dr. Yoshida concluded that CD39 expression may promote liver DC tolerance and regulate liver IRI [34]. On the other hand, plasmacytoid DCs (pDCs), which are the principal source of type I interferon (IFN I) in the liver, play an essential role in hepatic IRI pathogenesis by upregulating the pro-apoptotic transcription factor IRF-1 in hepatocytes. Antonino Castellaneta (Bari, Italy) could show that depletion of pDCs in a warm ischemia mouse model decreased hepatic IFNα and IRF-1-expression, lowered apoptosis and reduced IRI [35].

Regulatory cells in clinical trials

Another recurring theme of the meeting was the importance of reducing adverse reactions and comorbidities of immunosuppressive agents currently in use. A possible concept under discussion, cell-therapy-based immunosuppression, is being tested in the "One Study" (Edward Geissler, Germany). The goal of the study was to develop and test various cell therapies given to the patient at transplantation to trigger self-sustaining immune regulation thus protecting the allograft [36]. The fundamental difference in this approach compared with conventional immunosuppression is that functional cellular components are "add in" to the patient. The most promising cell types are donor-specific Tregs, type 1 regultory T cells (Tr1), tolerogenic DCs, suppressive macrophages (Mregs), natural Treg, and mesenchymal stem cells (MSC) [37]. The latter were discussed as a cell-based approach by Perico *et al.* (Italy) in the setting of clinical kidney transplantation [38]. Results from the first two patients enrolled in the study indicated that MSCs did promote a pro-tolerogenic environment. However, transient renal insufficiency, associated with intragraft recruitment of neutrophils and complement C3 deposition, was observed in these patients. With these results in mind, the authors went back to the experimental setting and studied the impact of MSC therapy timing (post vs. pretransplant) on kidney graft survival. They were finally able to show that MSC given pretransplant promoted early Treg expansion and induced a significant prolongation of kidney graft survival.

Upon transplantation, patients are dependent on immunosuppressants that bear many risks. Therefore, it would be ideal to reduce or even eliminate the necessity of such toxic medication for the transplanted patient. A multicenter pilot trial (ITN029) on immunosuppression withdrawal in pediatric recipients of parental living-donor liver transplants identified 12 tolerant participants that maintained normal liver function after having been taken off immunosuppressive medication [39]. In these operationally tolerant children, withdrawal of immunosuppression was not associated with augmented allograft inflammation or liver fibrosis over a 5 year period. Although immunosuppression withdrawal has been linked to the emergence of donor-specific antibody (DSA) in several participants, C4d scores did increase over time after withdrawal and did not correlate with DSA titer.

Biomarkers in transplantation

The identification of biomarkers is an emerging field in transplantation medicine. Dr. Neil Dalton (UK) gave an excellent overview of renal biomarkers for kidney transplant outcome. An ideal biomarker should rapidly react, determine IRI dimension, mirror the benefit of therapy, and offer prognostic potential on functional outcome. A perfect example of such a specific, sensitive, and robust biomarker is the urine retinol binding protein (RBP)/creatinine ratio. Other markers including neutrophil gelatinase-associated lipocalin (NGAL) or kidney injury molecule-1 (KIM-1) have not been proven to be as sensitive or specific as RBP. The potential of newer plasma biomarkers for

example Cystatin C or symmetric dimethylarginine remain to be clarified. The search continues.

This topic was followed by Petra Reinke (Germany), who presented the current work of European transplant research networks such as RISET or BIODRIM. The insufficient classification of low and high risk patients results in inappropriate therapy for the majority of transplant recipients. To redefine these patient groups and to offer personalized, biomarker-guided immunosuppressive therapy, multiple cross-platform tests, multicenter clinical validation, and collaboration with the diagnostic industry would be necessary. In this context, the RISET/BIODRIM-Consortium is trying to answer the need for stratification of biomarkers to predict successful weaning protocols in long-term stable liver/kidney transplanted patients identified by recently established biomarker tolerance signatures [32,40,41].

Preclinical models

Although the mouse is the most widely used animal model in modern immunology, lack of translation to the clinic remains a limiting factor. Fadi Issa (UK) reviewed possibilities and limitations of humanized mouse models. The use of NOD-SCID-IL2r$\gamma^{-/-}$ mice appears to be the most favorable humanized mouse model in combination with hematopoetic stem cells, fetal liver, and thymus, although authors have demonstrated that for the study of skin graft transplantation BULB/c-Rag2$^{-/-}$-c$\gamma^{-/-}$ mice (treated with human PBMCs and Tregs) displayed stable long-term human skin transplant survival versus sole PBMC treatment [42]. Interestingly, the selection of the appropriate Treg subgroup appears to be essential, as Tregs expressing the skin homing cutaneous lymphocyte antigen (CLA) were more efficient at preventing skin destruction than their CLA-deficient counterparts [43]. Limitations of humanized mouse models lie mainly in the incomplete development of the immune system and the fact that residual murine immune cells still remain in the organism and therefore influence immune response. Nevertheless, these models provide a unique possibility to evaluate human-specific therapies and can therefore guide bench to bedside therapy development.

Improvements for a further mouse model were presented by Di Santo *et al.* (France) concerning a humanized mouse model in which immune deficient recipient mouse strains are reconstituted with a human immune system (HIS), human hepatocytes (HuHEP), or both (HIS/HuHEP) [44]. Improvements included the aspects of human cytokine production, transgenic MHC expression and DC development. HIS mice can thus be used to investigate innate and adaptive human immune responses as well as to test antiviral treatment compounds *in vivo*.

As Sir John B. Gurdon and Shinya Yamanaka received the Nobel Prize in 2012, induced pluripotent stem cells (iPS) have attracted growing interest. In his talk, Foad Rouhani (UK) described their generation as well as their potential in disease modelling. Based on the technique of Yamanaka *et al.* [45] with which adult mouse fibroblasts were reprogrammed into embryonic stem cell(ES)-like cells, induced pluripotent stem cells (iPS) from α1-antitrypsin deficient fibroblasts were generated and differentiated into hepatocyte-like cells that did not show any of the pathophysiological α1-antitrypsin accumulation compared with controls. Moreover, upon transplantation of these cells into a SCID mouse, these hepatocyte-like cells proved functional by their production of human albumin. These results provide the first proof of principle that iPS could potentially be used for genetic correction with consecutive cell-based therapy [46].

The potential of regenerative medicine in transplantation as an alternative to life-long immunosuppressive therapy was further illustrated by four case studies of successful tissue-engineered trachea transplantations presented by Mark Lowdell (UK). The concept of tissue-engineered products (TEP) consists of scaffolds, either decellularized or synthetic, which are then colonized by cells of the recipient [47]. Recellularization of the trachea is a two-step procedure, the inner lining is colonized with tracheal epithelial cells, and the outer layer is colonized with chondrocytes differentiated from bone marrow-derived mesenchymal stem cells [48]. These case studies give a good example of what is possible in regenerative transplantation medicine today and by showing how far we have already come, give us hope for the future.

Tolerance in clinic

Current immunosuppressive therapy is not only expensive but can lead to a plethora of side effects for the patient. Therefore, the need for alternative means of therapy has been driving transplant research to discover and study new therapeutic approaches such as cell therapy or the use of off-label biologics. An example of such an alternative cell-based therapy was given by Manuela Battaglia (Italy) who discussed the beneficial effects of Tr1 therapy in hematopoetic stem cell transplantation (HSCT). To analyze the success of such studies, fastidious monitoring of patient progress via biomarkers and assays is essential. Birgit Sawitzki (Germany) gave an overview of the importance of the correct selection of and standardization of biomarkers for the differentiation between operational tolerance and acute rejection. The session was closed by Robert Lechler (UK) who gave an excellent summary of where we stand in tolerance in clinic to date.

The joint meeting posed to be an interesting venue for basic science research in translational transplantation and fulfilled all expectations by giving a prosperous outlook for

the future of research in the field of immunological transplantation research.

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