



Cell therapies for autoimmune diseases

3rd CELLAID Symposium

20 – 22 February 2007

Executive Summary
2007

The third CELLAID symposium on curative cellular therapies was held in Florence on February 20th-22nd 2007. The purpose of the meeting was to serve as a discussion platform for rheumatologists, haematologists and immunologists as well as industrial partners and European Commission representatives, focussing on translation of promising cell-based therapies for autoimmune diseases into clinical applications. It was a major aim to provide the infrastructure to define research demands and future activities with relevance for the 7th framework program of the EU.

The sessions:

Introducing talks presented the **CELLAID objectives**, the competence and potentials of collaboration with European associations such as EBMT (European Group for Blood and Marrow Transplantation) and the potentials of the coordination of the CELLAID working party within the ESCIR (EULAR Standing Committee for Investigative Rheumatology).

In the first scientific session '**Mechanisms in autoimmunity**' *Vivian Malmström* (Karolinska Institute, Stockholm, Sweden), *Niek de Vries* (Division of Rheumatology, AMC-University Amsterdam, Netherlands), *Arne Akbar* (University College London, UK), *Francesco Annunziato* (Excellence Center for Research, Florence, Italy), *Thomas Kamradt* (Dept. of Immunology, Medical School Jena, Germany), *Simon Jones* (Dept. of Medical Biochemistry, School of Medicine, Cardiff, Wales, UK) and *Günther Steiner* (Dept. of Rheumatology, Medical University Vienna, Austria) provided insights into the current understanding of the regulation of chronic inflammation in autoimmunity. In view of the recent attention in the field, it was not surprising that the focus of this session was on regulatory T cells (Treg). Very recent work by the *Arne Akbar* group has provided evidence that Treg have a very high turnover *in vivo* (J Clin Invest, 2006, 116: 2423). Moreover, they found extremely close TCR clonal homology between regulatory and memory CD4⁺ T cells. It is therefore unlikely that all human CD4⁺ CD25⁺ Foxp3⁺ Tregs are generated as a separate functional lineage in the thymus. Instead, the data suggest that a proportion of this regulatory population is generated from rapidly dividing, highly differentiated memory CD4⁺ T cells (reviewed in Akbar et al., Nat Imm, 2007, 7: 231). The assumption that Treg may develop as a consequence of antigen-induced CD4⁺ T cell responses was strongly supported by data from *Francesco Annunziato* and colleagues showing allergen specificity of circulating Treg. The notion that Treg may develop as a consequence of different immune reactions can have considerable implications for the therapeutic manipulation of these cells in human autoimmune diseases (AID). Understanding of signals that steer CD4⁺ T cells towards Treg differentiation is essential to progress the field towards clinical applications. *Vivianne Malmstrom* discussed that there is still a big need for markers that can reliably identify Treg *in vivo*. Usage of the currently available tools ((CD25, CD127, FoxP3) is problematic, because they typify next to Treg also recently activated CD4⁺ T cells.

Ideally, manipulation of the immune system in autoimmune diseases should result in specific tolerance for the autoantigens that stimulate chronic activation of the immune system. *Thomas Kamradt and Günter Steiner* discussed immunological and pathological phenomena observed in rodents in which arthritis was induced with glucose-6-phosphate isomerase (G6PI) and heterogeneous ribonucleoprotein (hnRNP) A2, respectively. Future studies in humans should reveal if these two novel antigens are indeed candidates for influencing the immune system in patients with rheumatoid arthritis and/or other human autoimmune diseases. For monitoring the effect of immune modulation in humans it is mandatory to have high through-put read out for changes in the distribution of antigen receptors in T and B cell populations. To meet this aim *Niek de Vries* and colleagues developed a novel micro-array based method that monitors the beta chain repertoire with a resolution of a single T-cell clone (PLoS One, 2006, 1:e55).

The session '**Cell targeting *in vivo***' covered aspects of immunotherapy and focussed on the targeting of B cells and T cells, as well as a review of the current state-of-the-art of cell therapy applying the immunomodulatory properties of mesenchymal stem cells (MSC) with presentations from *Paul Emery* (Leeds Institute of Molecular Medicine, UK), *Wietse Kuis* (Department of Pediatric Immunology and Rheumatology, UMC Wilhelmina, Utrecht, Netherlands), *Jan-Matthias Braun* (Translational Centre for Regenerative Medicine, Leipzig, Germany), *Thomas Hünig* (Institute for Virology and Immunology, University Würzburg, Germany), *Alessandro Moretta* (Dept. of Experimental Medicine, University of Genova, Italy), *Yuti Chernajavsky* (Bone and Joint Research Unit, Queen Mary's School of Medicine, University of London, UK) and *Antonio Uccelli* (Neuroimmunology Unit, Dept. of Neurosciences, University of Genova, Italy).

Paul Emery (Leeds Institute of Molecular Medicine, Leeds, UK) summarised the impressive data derived from phase III clinical trials of B cell depleting therapy with the chimeric anti-CD20 monoclonal antibody, rituximab. The striking clinical response rates are associated with apparent profound and sustained depletion of immature and mature B cell subsets, but not plasma cells. The possibility of more selective B cell targeting, e.g. of long-lived plasma cells, remains an attractive alternative, especially if it becomes possible to discriminate populations of terminally differentiated self-reactive cells producing autoantibodies from those cells that underpin the protective immunity of the host.

Wietse Kuis (Department of Pediatric Immunology and Rheumatology, UMC Wilhelmina, Utrecht, Netherlands), *Jan-Matthias Braun* (Translational Centre for Regenerative Medicine, Leipzig, Germany) and *Thomas Hünig* (Institute for Virology and Immunology, University Würzburg, Germany) updated the workshop on different aspects of T cell targeted therapies. It is clear that selective expansion of regulatory T cell subsets *in vivo* is now a realistic goal. For example, comparison of the numbers of CD4⁺CD25^{bright}Foxp3⁺ Tregs from children with different forms of inflammatory arthritis, as presented by *Wietse Kuis*, has revealed that the more benign, self-remitting form of the disease is associated with more Tregs than those with a more aggressive, erosive disease. It turns out that T cells from these same children respond to endogenous stress proteins such as hsp60 by producing IL-10, whilst T cells from children with the more severe form of the disease do not. Interestingly, hsp60 stimulation *in vitro* drives the generation of CD4⁺CD25^{bright}Foxp3⁺ Tregs. There now exists very real opportunities to exploit these immunomodulatory properties in man, especially if it is possible to develop pan-DR binding hsp60 peptides that retain the same regulatory effects *in vivo* in patients carrying different HLA-DRB1 genotypes. *Thomas Hünig* summarised the impressive modulatory effects of anti-CD28 monoclonal antibodies in murine models of type I diabetes, multiple sclerosis (MS) and allograft tolerance, emphasising that an important biological effect lies in the capacity to promote Treg subsets, including those expressing CD103, a marker associated with intrinsic migratory competence. This may arise in part through amplification of the production of IL-2, a key factor that supports and maintains Tregs *in vivo*, at least in the mouse. The cytokine storm observed in the TGN1412 study of superagonist anti-CD28 mAb in man highlights, among other things, how mouse and man may differ. These and other studies highlight the value of humanised mouse models, as elegantly illustrated by *Jan-Matthias Braun* whose studies exploited human CD4, human MHC class II transgenic mice to study *in vivo* the immunomodulatory properties of anti-hCD4 monoclonal antibodies.

Francesco Dazzi reviewed data concerning the anti-proliferative and immunosuppressive properties of MSCs. *In vitro* experiments support the concept that MSC arrest proliferating T and B cells in the cell cycle at G₀ - G₁ phase, associated with down regulation of cyclin D2 and up regulation of P27kip1. Although several putative soluble factors have been proposed, including TGFβ, IL10, hepatocyte growth factor, PGE₂ (Prostaglandin E₂) and indoleamine-2,3-dioxygenase (IDO), the effect is best seen when cell-cell contact is also present and appears to require a "licensing" factor produced by the proliferating cells. Animal models of

autoimmune diseases that have responded to MSC include baboon skin transplant, experimental autoimmune encephalitis (EAE) and acute xenogeneic graft versus host disease (GvHD). The latter model from Dazzi's group was only effective, if the MSC were given at the time of transplantation and weekly thereafter, a phenomenon observed also in other models. However, early experiences in humans with acute severe GvHD are encouraging with around 50 patients reported and with reduction in the expected mortality of this mostly fatal condition. Acute toxicity appears to be minimal. This has led to two prospective controlled randomised clinical trials under the auspices of the EBMT in both treatment and prevention of GvHD.

The session '**Reset of tolerance**' encompassed presentations from Roland Martin (Institute of Neuroimmunology and Clinical MS Research, Hamburg, Germany), Renate Arnold (Dept. of Haematology, University Medicine Charité, Berlin, Germany), Alan Tyndall (Dept. of Rheumatology, Felix-Plater Spital, Grenoble, Switzerland), Alf Hamann (Experimental Rheumatology, University Medicine Charité, Berlin, Germany) and Christian Jorgensen (University Montpellier, France) providing insight into the current understanding of mechanisms involved in the re-induction of self-tolerance and summarized the results of autologous stem cell transplantation in systemic lupus erythematosus (SLE) and systemic sclerosis (SSc).

Renate Arnold discussed the Berlin experience of autologous stem cell transplantation in SLE. Clinical data presented from seven patients are promising showing clinical and serological long-term remission in all patients for up to 8 years despite discontinuation of immunosuppressive agents. Relapse of disease was observed in one patient, emphasising the need for better understanding of cellular mechanisms underlying the transplant procedure. Overall, immune ablation in combination with autologous stem cell transplantation has provided the proof of principle that cure is possible even in severe forms of diseases.

Alan Tyndall gave an update on the use of autologous stem cell transplantation for severe SSc. This study is based on the ASTIS trial (autologous stem cell transplantation international scleroderma trial), a prospective, controlled, randomized trial to compare safety and efficacy of high dose immunosuppressive therapy conditioning (HDIT) and autologous hematopoietic stem cell transplantation (HSCT) with monthly intravenous cyclophosphamide injection in SSc patients at risk of major organ failure or early mortality. 87 patients have been enrolled in 20 European centers per January 2007. Forty patients were randomized to the transplant arm, 47 to the control arm. No unexpected toxicities have yet been observed in either arm with a median follow-up of 23 months (range 1-54). One fatality in the transplant arm was categorised as probably treatment-related. The lower than expected transplant related mortalities (2.6% for HSCT) and unexpected toxicities with 80 patients randomized underscore the feasibility of the ongoing ASTIS-trial.

Alf Hamann discussed the work of his team on imprinted suppressors. With the ambition to find novel markers for reliable Treg identification, modification of histones and methylation of DNA was studied in the Foxp3 gene. Here, a highly conserved non-coding sequence was identified proximal to exon-1, which is completely demethylated in natural Tregs both in mice and humans, and completely methylated in naïve or effector T cells. Interestingly, demethylation is not complete in murine Tregs induced by *in vitro* activation in the presence of TGF β . Furthermore, human T cells that transiently upregulate Foxp3 simply upon activation in the absence of TGF β and develop into effector cells rather than into Tregs show almost no signs of demethylation in the Foxp3 gene. Thus, the methylation status of the Foxp3 gene might provide a better criterion for "true" and stable Tregs than the expression of Foxp3 itself.

Christian Jorgensen from Montpellier gave the final talk on cell based therapies for the regeneration of connective tissue: a new insight through the Genostem program. Mesenchymal stem cells (MSCs) are considered as suitable sources for cell based therapies in cartilage engineering. The identification of a factor specific for the chondrogenic lineage

represents the major issue of this study. Bone marrow-derived human MSC were induced to differentiate towards chondrocytes using the micro pellet culture technique in presence of chondrogenic medium. Using Affymetrix microarrays, 1354 genes were differentially regulated during chondrogenesis and 705 genes were up-regulated in MSC-derived chondrocytes. The transcription factor FoxO1A was increased by a 13-fold factor. Over-expression of the wtFoxO1A or active FoxO1A genes induced up-regulation of aggrecan, collagen II and down-regulation of collagen I. In parallel, the engineered cells did not reveal higher osteogenic or adipogenic potential. In vivo, cartilage staining positive for aggrecan and collagen II were detected in the areas of cell injection confirming their potential to differentiate into chondrocytes. Thus FoxO1A is one essential transcription factor involved in the early steps of chondrogenesis.

The **final abstract session** concerned diverse cellular approaches to immune modulation and tissue repair with presentations from *Andreas Lutterotti* (Institute of Neuroimmunology and Clinical MS Research, Hamburg, Germany), *L.M. Charbonnier* (Hopital Saint Eloi, Montpellier, France), Rosa Maria Licon Luna (Charité, University Hospital Berlin, Germany), Roberta Rigolio (Department of Neuroscience, University Milan Bicocca, Milan Italy) and Ingo Müller (University Children's Hospital, Tübingen, Germany).

Andreas Lutterotti (Institute of Neuroimmunology and Clinical MS Research, Hamburg, Germany) described the rationale for the therapeutic use of autologous peripheral blood mononuclear cell populations (PBMCs) coupled with a variety of peptides associated with MS pathogenesis against which demonstrable responses can be detected in patient subsets. The critical technology comprises fixation of PBMCs pulsed with peptide using the cross linker, ECDI. This approach is effective in animal models of MS (EAE) leading to disease amelioration and can be shown to tolerize targeted PB subsets against a variety of stimuli *in vitro*. An open label single centre cross over trial design will administer MS-relevant peptide pulsed and fixed PB cells to recipients with magnetic resonance imaging proven active MS. This exploratory study will provide proof of concept of the potential for this cell based immune therapeutic approach.

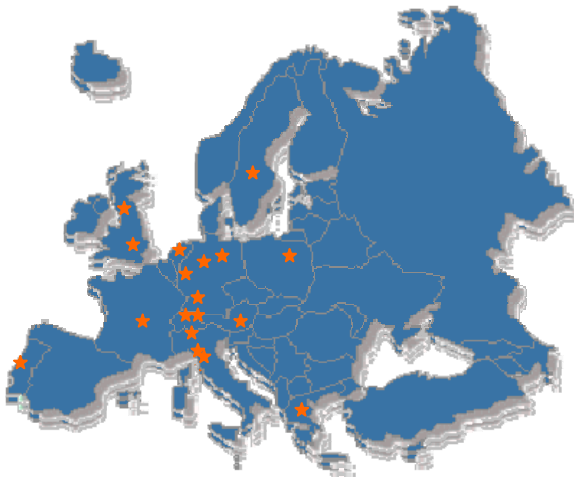
L.M. Charbonnier (Hopital Saint Eloi, Montpellier, France) reported a pre-clinical study in which a murine arthritis model was employed to investigate the mechanism of immune modulation by adoptive transfer of immature dendritic cells (iDC). Previously, iDC injection was shown to modulate the subsequent development of collagen-induced arthritis (CIA; a model of rheumatoid arthritis). In this study, the approach was shown not to be effective when iDC were transferred after the onset of disease. However, early given iDC induced the generation of a regulatory T cell population (CD4⁺, CD49b⁺) that secreted the anti-inflammatory cytokine IL-10. Transfer of these cells into established CIA suppressed the disease, suggesting that such cell populations could be generated and used in a therapeutic manner. Again, although elegant studies had been performed showing efficacy, it is likely that more mechanistic data will be required before the feasibility of this approach can be applied in the clinic.

Paul Cichutek (PEI, Langen, Germany) and *Ineke Slaper-Cortenbach* (UMC, Utrecht, Netherlands) presented the regulatory affairs situation and debated on the question of the medicinal production of cell based therapies. Any European research project should envisage these regulations and consult and collaborate with the Paul Ehrlich Institute (PEI), the European Medicines Agency (EMA) and the Joint accreditation committee of the International Society for Cellular Therapy (ISCT) and EBMT (JACIE) before planning the development and translation of any new therapeutic intervention.

Conclusions and perspectives:

There is a clear need for networking between European research institutions and clinics. This will enhance standardisation of not only clinical interventions and quality assessment but also of regulatory requirements, which will help patients across Europe to receive the benefits of this. Cooperation with European scientific societies and regulatory bodies (EULAR, EBMT, EMEA etc.) will help to overcome bottlenecks and accelerate the progress of translation.

The 4th Symposium is planned to take place in November 2008 in Berlin and is currently being organised by the coordinator and the Scientific advisory board. Close collaboration is envisaged between CELLAID and the organization of the EWRR, European Workshop for Rheumatology research and the EBMT, European Bone Marrow Transplant Group.



CELLAID member states

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