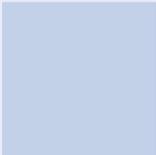


Program



3rd CELLAID Symposium



**Cell therapies for
autoimmune diseases**



20 - 22 Feb 2007
Florence, Italy



Sponsors

Generous support for the 3rd Symposium is provided by



Welcome

Dear friends and colleagues,

Welcome to the 3rd Cellaid Symposium at this inspiring location in the centre of Florence.



We are now joining in Florence for our third CELLAID Symposium. It will be the last event within the EU funding contract for the CELLAID project and the first meeting as the CELLAID working party of the EULAR standing committee for investigative rheumatology (ESCIR). Our previous meetings in Berlin 2005 and Brussels 2006 have been really productive leading to direct exchange of data, development of new concepts. We established a multidisciplinary network of scientists and clinicians with the perspective to perform pan-European studies and trials and the integration of cell based therapies for autoimmune diseases into the 7th Framework Programme of the European commission.

During this Symposium and the meeting of the ESCIR working party, we will discuss science, possible collaborations with the European Bone marrow transplant group (EBMT), the translation of research concepts into clinical practise and relevant regulatory affairs as well as dissemination and continuation of the CELLAID activities.

We aim to maintain the network as open communication platform for pan-European experts to promote the development of this promising area of research.

Looking forward to stimulating discussions!

Andreas Radbruch

Time Schedule

Tuesday, February 20th

8:00 pm Arrival, registration, media transfer & tuscan dinner

Wednesday, February 21th

08.30 - 09.00 am

CELLAID welcome

09.00 - 10.00 am

CELLAID integration

10.30 - 12.15 am

Mechanisms in autoimmunity

12.15 - 1.30

Lunch

01.30 - 03.30 pm

Cell targeting in vivo

03.30 - 04.00

Coffee

04.00 - 05.30 pm

Reset of tolerance

05.30 - 06.30 pm

Meeting of the working group for cell therapies
of the ESCIR

07.00 pm

Dinner

Thursday, February 22nd

08.45 - 09.30 am

Keynote lecture

09.30 - 10.30 am

Abstract session - late breaking news

10.30 - 11.00

Coffee

11.00 - 12.30 am/pm

Regulatory affairs

12.30 pm

Lunch

Transfer to EWRR, Sheraton

General Information

Program committee

Renate Arnold, Germany
Andrew Cope, UK
Falk Hiepe, Germany
Rikard Holmdahl, Sweden
Christian Jorgensen, France
Lars Klareskog, Sweden
Roland Martin, Germany
Wlodzimierz Maslinski, Poland
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Sergio Romagnani, Italy
Stefan Rose-John, Germany
Riccardo Saccardi, Italy
Reinhold Schmidt, Germany
Andreas Thiel, Germany
Rene van Lier, Netherlands

Organising committee

Renate Arnold, Germany
Falk Hiepe, Germany
Marco Matucci Cerinic, Italy
Andreas Radbruch, Germany
Riccardo Saccardi, Italy
Andreas Thiel, Germany

Time line

Feb 20th arrival and Florence dinner
Feb 21st full day conference
Feb 22nd half day conference &
transfer to EWRR

EWRR 2007

The 27th European workshop for Rheumatology research will take place in Florence, 22 - 24 February 2007.

www.ewrr2007.org

Location

Grand Hotel Baglioni, Firenze

This prestigious Florence hotel stands in the heart of the historic center, a few minutes from the Exhibition Center, Convention Center and main museums, and three hundred meters from Santa Maria Novella railway station.

www.hotelbaglioni.it

Program

Tuesday, February 20 th	
8:00 pm	Arrival, registration, media transfer & dinner

Program

Program

Wednesday, February 21 st		
8. ³⁰ - 9. ⁰⁰ am	CELLAID welcome	
	CELLAID: Welcome & perspectives	Andreas Radbruch
	Introduction and Integration of the EWRR	Marco Matucci Cerinic
	EBMT ADWP activity and integration at the European level	Riccardo Saccardi
9. ⁰⁰ - 10. ⁰⁰ am	CELLAID integration , Andreas Thiel, Falk Hiepe	
	CELLAID: European competence and concepts in cellular therapies for autoimmune diseases	Falk Hiepe
	EBMT mission	Dietger Niederwieser
	Cell therapies for autoimmune diseases: seeking guidance from animal models	George Kollias
10. ⁰⁰ -10. ³⁰	Coffee	
10. ³⁰ -12. ¹⁵ am	Mechanisms in autoimmunity , Rene van Lier	
	FOXP3 ⁺ CD4 ⁺ regulatory T-cells in patients with rheumatic disease	Vivianne Malmström
	Monitoring quantitative changes in the T-cell repertoire	Niek de Vries
	Thymic and Peripheral CD4 ⁺ CD25 ⁺ FoxP3 ⁺ Regulatory T Cells	Arne Akbar
	Demonstration of circulating allergen-specific CD4 ⁺ CD25 ⁺ Foxp3 ⁺ T regulatory cells in both nonatopic and atopic individuals	Francesco Annunziato
	Arthritis provoked by autoimmune responses against G6PI in normal mice	Thomas Kamradt
	Defining the inflammatory relevance of IL-6 trans-signalling in arthritis.	Simon A. Jones
	The human autoantigen hnRNP-A2 (RA33) is a major stimulator of autoimmunity in rats with pristane-induced arthritis	Günter Steiner
12. ¹⁵ - 1. ³⁰	Lunch	

Wednesday, February 21 st		
1. ³⁰ - 3. ³⁰ pm	Cell targeting in vivo , Alan Tyndall, Andrew Cope	
	B-cell Therapy	Paul Emery
	Immune regulation and tolerance in chronic arthritis - cellular therapy in JIA	Wietse Kuis
	MSCs and cellular therapy	Francesco Dazzi
	Anti-CD4 targeting	Jan-Matthias Braun
	Targeting CD28 with stimulatory and blocking antibodies	Thomas Hünig
	NK-cell mediated shaping of adaptive immunity	Alessandro Moretta
	Engineering mesenchymal stem cells for targeting, committed differentiation to cartilage and inflammation control	Yuti Chernajovsky
	MSCs for the treatment of experimental neurological diseases	Antonio Uccelli
3. ³⁰ - 4. ⁰⁰	Coffee	
4. ⁰⁰ - 5. ³⁰ pm	Reset of tolerance , Paolo Muraro, Pierre Miossec	
	Antigen-specific, cell-based tolerization strategies in autoimmune diseases specifically in MS	Roland Martin
	Stem cell transplantation: Special features in autoimmune diseases	Renate Arnold
	ASTIS: Autologous stem cell Transplantation International Scleroderma Trial	Alan Tyndall
	Tolerance induction - preventive or therapeutic?	Antonio Coutinho
	Imprinted Suppressors	Alf Hamann
	Chondrogenic differentiation of mesenchymal stem cells: a new insight through Genostem program	Christian Jorgensen
5. ³⁰ - 6. ³⁰ pm	Constitution of the working group for cell therapies of the ESCIR Election of the chair person; membership, mission and perspective of the CELLAID initiative	
7. ⁰⁰ pm	Dinner	

Thursday, February 22nd

8. ⁴⁵ - 9. ³⁰ am	Keynote lecture , Joachim Kalden	
	Generating the immune system in vitro	Fritz Melchers
9. ³⁰ - 10. ³⁰ am	Abstract session - late breaking news , Iain McInnes, Sergio Romagnani	
	Establish tolerance in MS with peptide-pulsed, fixed antigen presenting cells	Andreas Lutterotti
	Role of T-cell Costimulation in Sexual Dimorphism of Autoimmune Diseases	Rosa María Licón Luna
	Extracorporeal photochemotherapy: early ex-vivo experience on healthy controls and a multiple sclerosis patient to investigate a new potential cell therapy	Roberta Rigolio
	Multipotent mesenchymal stromal cells - a novel treatment for steroid induced avascular osteonecrosis in children	Ingo Müller
	CD49b ⁺ regulatory T cells are able to cure arthritis	P. Louis Plence
10. ³⁰ - 11. ⁰⁰	Coffee	
11. ⁰⁰ - 12. ³⁰	Regulatory affairs & 7th Framework program , Reinhold Schmidt	
	Regulatory aspects of advanced therapy products in the European Union	Klaus Cichutek
	Regulatory affairs for cell therapies in Europe	Ineke Slaper-Cortenbach
	FP7 - Perspectives for health and biotechnology related research	Jürgen Sautter
	Concluding remarks	Andreas Radbruch
12. ³⁰ pm	Lunch	
	Transfer to EWRR, Sheraton	

Abstracts

Wednesday, February 21st9.⁰⁰ - 10.⁰⁰ am

CELLAID integration, Steffen Gay, Wlozimierz Maslinski

Falk Hiepe**CELLAID: European competence and concepts in cellular therapies for autoimmune diseases**

Falk Hiepe, Jutta Steinkötter, Tobias Alexander, Andreas Thiel, Andreas Radbruch
Charite Universitätsmedizin Berlin and Deutsches Rheuma-Forschungszentrum Berlin

Keywords: autologous stem cell transplantation, candidate cellular targets, refractory autoimmune disease, European network



CELLAID, a specific support action of the 6th framework program of the European Commission, aims to initiate a pan-European research network for curative cell therapies in autoimmune diseases and define research demands with relevance for the upcoming 7th framework program. Three Symposia are the main activities of the project and they serve as multidisciplinary communication platforms for basic and translational scientists, clinicians, industry, regulatory experts and members of the European commission.

From the previous symposia in Berlin (2005) and Brussels (2006) the following facts clearly emerged: European science is innovative and competitive in this seminal field, though, networking and research integration, translation into the new concepts and into clinics is still lacking. We also want to state that basic knowledge is as crucial as translational efforts. Therefore, a pan-European network is required to integrate the multidisciplinary expertise and establish cohorts of patients for performing clinical trials.

Based on the scientific contributions during the first Symposia we have started to identify the most advanced concepts for innovative therapies worthwhile for translation into the clinic. Among others, candidate cellular targets that have been identified so far include proliferating activated cells, B-lymphocytes, autoreactive plasma cell memory, autoreactive T cell memory, regulatory T lymphocytes and synovial fibroblasts.

Complete immunoablation in combination with autologous stem cell transplantation which is performed in patients with refractory autoimmune diseases such as systemic lupus erythematosus, multiple sclerosis etc. has provided the proof of principle that cure is possible. Here, the elegant way for the future is to specifically eliminate or affect cells which are relevant in the pathogenesis instead of complete immunoablation.

Dietger Niederwieser
EBMT mission

George Kollias
Cell therapies for autoimmune diseases: seeking guidance from animal models

Alexander Fleming, Vari / Athens, Greece

Biomedical Sciences Research Center

The MUGEN Network of Excellence was launched by the European Commission in early 2005. Funded by the 6th Framework Programme, MUGEN links 24 leading immunology research and industrial organizations across Europe. The core activity of the MUGEN scientists is research into diseases of the immune system using integrated functional genomics in mutant mouse models and aided by technology platforms such as targeted or random genome mutagenesis, gene expression profiling and bioinformatics. MUGEN has recently launched a public searchable database of mutant mouse strains that serve as models of disease or immunological processes, as well as tools for genome modification (www.mugen-noe.org).

In my presentation, I will be discussing further specific activities of MUGEN that may prove useful to the broader scientific community investigating immunological diseases. I will also be discussing our recent results in animal models of joint and intestinal pathologies pointing to mesenchymal cells as important targets for pathogenic TNF signalling and suggesting their potential use in future cell therapies for these diseases.

10. ⁰⁰ -10. ³⁰	Coffee	
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10.30 -12.15 am

Mechanisms in autoimmunity, Rene van Lier, Rikard Holmdahl

Vivianne Malmström**FOXP3⁺CD4⁺regulatory T-cells in patients with rheumatic disease***Sukanya Raghavan, Mona Widhe, Jessica Herrath, Katrin Roth, Therese Valler-skog, Lars Klareskog, Christina Trollmo, Vivianne Malmström*

Rheumatology Unit, Dept of Medicine at Karolinska University Hospital Solna, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden

Keywords: FOXP3, regulatory T cells, arthritis, SLE, treatment

The function and possible manipulation of regulatory T cells in patients with autoimmune diseases have received much attention in recent years. We have studied patients with inflammatory rheumatic disease and the presence and function of FOXP3⁺CD25⁺CD4⁺ T cells in synovial tissue, synovial fluid and peripheral blood as well as their relationship to clinical manifestations and response to treatment.

Strikingly FOXP3⁺CD4⁺ T cells are always present in synovial fluid of patients with chronic arthritis and in higher frequency as compared to blood. These cells have a similar activated/memory phenotype as the FOXP3⁺ regulatory T cells in peripheral blood. Approximately 15% of the FOXP3⁺ T cells in synovial fluid are in active cell cycle suggesting that regulatory T cells are expanding locally in the inflamed joint.

In contrast, the T cell composition in the inflamed synovial membrane is different from both synovial fluid and blood. Here FOXP3⁺ T cells can be found within T cell infiltrates but are absent in many patients. This discrepancy between synovial fluid and tissue could have important implications in our understanding of the contribution of regulatory T cells to chronic rheumatic disease.

With regard to the effect of different treatment regimes to the presence of FOXP3⁺ T cells, we have reported an increased population of regulatory FOXP3⁺ T cells following B cell depletion by rituximab treatment in SLE patients. More recently, we have studied synovial membrane biopsies before and after local corticosteroid treatment, and can demonstrate that both aggressive and regulatory T cells are diminished following this treatment regime.

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- from the target organ of patients with rheumatoid arthritis. *Eur J Immunol.* 2003. 33:215-223
- 2)Cao D, et al. CD25brightCD4+ regulatory T cells accumulate over time in inflamed joints of patients with chronic rheumatic disease. *Arthritis Res Ther.* 2004 6:335-346
- 3)Cao D et al. FOXP3 identifies regulatory CD25brightCD4+ T cells in rheumatic joints. *Scand J Immunol* 2006 63:444-452.
- 4)Vallerskog T et al. Treatment with rituximab affects both the cellular and the humoral arm of the immune system in patients with SLE. *Clin Immunol* 2007 122:62-74

Niek de Vries

Monitoring quantitative changes in the T-cell repertoire

Hendrik P.J. Bonarius¹, Frank Baas², Esther B.M. Remmerswaal³, René A.W. van Lier³, Ineke J.M. ten Berge⁴, Paul P Tak¹, and Niek de Vries¹

¹Division of Clinical Immunology and Rheumatology, ²Department of Neurogenetics
³Department of Experimental Immunology, ⁴Division of Nephrology, Department of Internal Medicine, AMC/University of Amsterdam, Amsterdam, Netherlands

Keywords: T-cell receptor, microarray, B-cell receptor, immune response, antigen recognition

Purpose: The adaptive immune system recognizes billions of unique antigens using highly variable T-cell receptors and antibodies. Recently we designed a novel microarray based T-array technology that screens the TCR and Immunoglobulin repertoire for dominant clones and quantitative changes, yielding sequence information. Here we show its feasibility, sensitivity and validity and show its applicability in monitoring T-cell responses.

Methods & Materials: In T-array technology cDNA is amplified and subsequently hybridized to selected sets of fluorescently labelled oligonucleotides. Signals are read on 4000 spot microarrays. To test the sensitivity, specificity and quantitiveness Jurkat T-cells were diluted in random CD4 cells. To test the applicability of this technique we isolated PBMCs from a healthy HLA-A2⁺ healthy donor latently infected with CMV. Cells were stimulated in vitro with the CMV peptide NLVPMVATV, and expansion followed using FACS after tetramer staining, spectratyping and T-array analysis.

Results: T-array detected the Jurkat T cells up to a dilution of 1 in 10E6 to 1 in 10E7 CD4⁺ T-cells. This is 3 logs more sensitive than spectratyping, the current standard in repertoire analysis. Analysis was clearly quantitative and could be done in 2 days.

In the second experiment, after stimulation with CMV-peptide, the fraction of tetramer-positive cells increased from ~5% at day 0 to 60% at day 10.

Both tetramer analysis and spectratyping detected an immune response against CMV at day 6. T-array already identified the dominantly responding clone at day 0, and could show a clear increase in frequency at day 3. Conclusion: T-array has superior sensitivity, resolution and comprehensiveness compared to spectratyping. It is fast, allows quantitative monitoring of selected clones and yields nucleotide sequence information. This new technology allows identification and quantitative monitoring of expanding T- and B-cell clones specific for autoimmune disease, not feasible with other techniques.

References:

- 1) Bonarius HPJ, Baas F, Remmerswaal EBM, van Lier RAW, ten Berge IJM, Tak PP, de Vries N (2006) Monitoring the T-Cell Receptor Repertoire at Single-Clone Resolution. PLoS ONE 1(1): e55. doi:10.1371/journal.pone.0000055
- 2) Pannetier C, Cochet M, Darche S, Casrouge A, Zoller M, Kourilsky P (1993). The sizes of the CDR3 hypervariable regions of the murine T-cell receptor beta chains vary as a function of the recombined germ-line segments. Proc Natl Acad Sci U S A 90: 4319–4323.
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Arne Akbar

Thymic and Peripheral CD4⁺CD25⁺FoxP3⁺ Regulatory T Cells

Arne N. Akbar, Arthur McQuaid, Malcolm Rustin, Milica Vukmanovic-Stejic

University College London, Dept. Immunology and Molecular Pathology

The maintenance of immunity has to be balanced with appropriate immune control to prevent non-specific inflammation and immunopathology. Since thymic involution occurs, human memory T cells have to be maintained by lifelong turnover of existing populations of specific T cells. Naturally occurring regulatory CD4⁺FoxP3⁺CD25⁺ T lymphocytes can inhibit the development of autoimmunity and inflammation. However it is not known how this regulatory population is maintained during ageing in vivo. We therefore investigated the role of peripheral turnover in the maintenance of these cells in both young and old humans. Using labelling of proliferating cells with deuterated glucose in vivo, we demonstrate that regulatory CD4⁺CD45RO⁺CD25⁺T cells turnover extremely rapidly, with a doubling time of 8.3 days compared to primed/memory CD4⁺CD45RO⁺CD25⁻ (23.7 days) or naïve CD45RA⁺CD4⁺CD25⁻ (199 days) populations (1). Heteroduplex analysis demonstrates that memory and regulatory CD4⁺ T cells are closely re-

lated, suggesting that regulatory cells may originate from CD4⁺ memory T cells in the periphery throughout life. More recently it has been shown that some regulatory CD4⁺FoxP3⁺CD25⁺ T lymphocytes express CD45RA instead of CD45RO. In this talk data will also be presented indicating that the CD45RA-expressing populations are very likely to be thymically and not peripherally derived populations of regulatory cells. The representation of both populations within the total CD4⁺ regulatory T cell pool changes during ageing.

References:

1) Vukmanovic-Stejic M. et al (2006). Human Regulatory CD4⁺CD25⁺Foxp3⁺ T Cells Are Derived Through Rapid Turnover From Memory Populations in vivo. *J. Clin. Invest.* 116:2423.

Francesco Annunziato

Demonstration of circulating allergen-specific CD4⁺ CD25^{high}Foxp3⁺ T regulatory cells in both nonatopic and atopic individuals

Laura Maggi, BSc, Veronica Santarasci, MD, Francesco Liotta, MD, Francesca Frosali, BSc, Roberta Angeli, BSc, Lorenzo Cosmi, MD, Enrico Maggi, MD, Sergio Romagnani, MD, Francesco Annunziato, PhD.

Excellence Center for Research, Transfer, and High Education DENOTHE, University of Florence; Florence, Italy

Key words: Allergy, Immune suppression, T regulatory cells

Background: CD4⁺CD25⁺Foxp3⁺ regulatory T (Treg) cells play a fundamental role in the control of autoimmunity, a function associated with their capacity to recognize self antigens. Whether human CD4⁺CD25⁺Foxp3⁺ Treg cells that recognize foreign antigens, including allergens, also exist is still unclear.

Objective: To investigate the existence in humans of circulating Treg cells able to recognize exogenous antigens.

Methods: CD4⁺CD25^{high}Foxp3⁺ and CD4⁺CD25⁻Foxp3⁻ cells were purified from human peripheral blood and cultured for 15 days with autologous dendritic cells (DCs), unloaded, or loaded with Dermatophagoides pteronyssinus group 1 (Der p 1) allergen or the bacterial antigen streptokinase (SK).

Results: CD4⁺CD25^{high}Foxp3⁺ circulating T cells obtained from healthy non-atopic subjects and cultured with Der p 1-loaded DCs, but not those cultured with either unloaded or SK-loaded DCs, suppressed the proliferative response to Der p 1 of autologous Der p 1-specific T cells generated from the CD4⁺CD25⁻Foxp3⁻ subset. The antigen specificity of either Der p 1- or SK-CD4⁺CD25^{high}Foxp3⁺ T cells was confirmed even at clonal level. Finally,

under the same experimental conditions, functionally active Der p 1-specific Treg cells were obtained from the pool of circulating CD4⁺CD25^{high}Foxp3⁺ T cells of Der p 1-sensitive, atopic, individuals.

Conclusion: These data provide the first demonstration on the existence of human CD4⁺CD25^{high}Foxp3⁺ circulating Treg cells specific for exogenous antigens, including the Der p 1 allergen, and indicate that CD4⁺CD25^{high}Foxp3⁺ Treg cells specific for Der p 1 are present and functionally active in both non atopic and Der p1-sensitive, atopic, individuals.

Thomas Kamradt

Arthritis provoked by autoimmune responses against G6PI in normal mice

Oliver Frey¹, Lisa Bruns¹, Andreas Reichel¹, Lars Morawietz², David Schubert³, Robert Bockermann⁴, Rikard Holmdahl⁴, Veit Krenn², Thomas Kamradt¹

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Keywords: Arthritis, Th cells, Treg cells, B cells, FcgR

The antigens that trigger the pathogenic immune response in rheumatoid arthritis (RA) remain unknown. Until recently it was assumed that joint-specific antigens were the targets of arthritogenic T and B-lymphocytes in RA. Consequently, murine models of arthritis are induced by immunization with either joint-specific antigens such as type II collagen or microbial products such as streptococcal cell wall. In the K/BxN T-cell receptor transgenic mouse model, arthritis is caused by a systemic autoimmune response to the ubiquitously expressed glycolytic enzyme glucose-6-phosphate isomerase (G6PI). More recently it was shown that G6PI immunization induces severe symmetrical peripheral polyarthritis in genetically unaltered DBA/1 or SJL mice. T cells are indispensable for both the induction and the effector phase of G6PI-induced arthritis. Arthritis is cured by depletion of CD4 cells. In contrast, Abs and FcgR effector cells are necessary but not sufficient for G6PI-induced arthritis in genetically unaltered mice. Both the induction and effector phase of arthritis induced by a systemic autoimmune response can be dissected and preventive and therapeutic strategies evaluated in this model.

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2. Bockermann R, Schubert D, Kamradt T, Holmdahl R. Induction of a B cell dependent chronic arthritis with glucose 6 phosphate isomerase. *Arthritis Research & Therapy* 2005;7:R1316-24.

Simon A. Jones**Defining the inflammatory relevance of IL-6 trans-signalling in arthritis**

Anwen S. Williams¹, Peter J. Richards¹, Mari A. Nowell¹, Sara Carty¹, Jürgen Scheller², Nicholas Topley¹, Stefan Rose-John² and Simon A. Jones¹

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Keywords: IL-6, sIL-6R, gp130, sgp130, arthritis

Interleukin (IL)-6 responses are transmitted via gp130, which serves as the universal signal-transducing receptor subunit for all IL-6-related cytokines. Although this classically occurs through IL-6 engagement of a membrane-bound receptor (IL-6R), it is evident that a soluble form of IL-6R affords IL-6 with a second mechanism of gp130 activation. This alternative mode of cell activation is termed IL-6 trans-signalling. Using a monoarthritic model of antigen-induced arthritis (AIA) we have shown that IL-6 trans-signalling promotes synovial hyperplasia, STAT3 activation and mononuclear leukocyte recruitment. Indeed, by adopting a recurrent model of AIA studies suggest that repeated inflammatory activation leads to a distortion in the IL-6 control of these processes, which affects joint destruction and retention of the inflammatory infiltrate within the inflamed synovium. Documentation of these activities has been aided by our demonstration that two soluble forms of gp130 (sgp130 and gp130-RAPS) selectively counteract IL-6 trans-signaling in vivo. We therefore reasoned that sgp130 might represent a promising therapeutic modality for the treatment of rheumatoid arthritis. To validate this approach, we have now used collagen-induced arthritis (CIA) as a systemic model of inflammatory arthritis. CIA was induced in wild type mice and the efficacy and potency of an sgp130Fc fusion protein examined in both early and late intervention regimes. On induction of CIA, sgp130Fc was administered (2-50 mg/mouse, i.p.) every second day over a 14-day period. This approach resulted in a dose-dependent improvement

in clinical score with 50 mg/mouse significantly reducing synovial hyperplasia, inflammatory infiltrate/exudate, joint erosion, and systemic serum amyloid-A levels. Similar benefit was also recorded when sgp130Fc was administered (i.p.) daily following the onset of established disease (>80% arthritis incidence). Collectively, these results endorse a role for IL-6 trans-signaling in arthritis progression, and advocates the selective targeting sIL-6R-mediated responses as a therapeutic strategy.

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- 1) P.J. Richards, et al., *Arthritis & Rheumatism* 54: 1662 (2006)
- 2) S.A. Jones J. *Immunol.* 175 (9): 3463 (2005)
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Günter Steiner

The human autoantigen hnRNP-A2 (RA33) is a major stimulator of autoimmunity in rats with pristane-induced arthritis

Markus H Hoffmann, Dept. of Rheumatology, Medical Univ. of Vienna, Austria; Jonatan Tuncel, Section for Medical Inflammation Research, Univ. of Lund, Sweden; Karl Skriner, Dept. of Rheumatology, Charité Univ. Hospital, Berlin, Germany; Makiyeh Tohidast-Akrad, L. Boltzmann Institute for Rheumatology; Vienna, Austria; Birgit Türk, Dept. of Rheumatology, Medical Univ. of Vienna; Guy Serre, Laboratory of Epidermis Differentiation and Rheumatoid Autoimmunity, CNRS-Toulouse III University, Toulouse, France; Georg Schett, Dept. of Rheumatology, Medical Univ. of Vienna; Josef S Smolen, Dept. of Rheumatology, Medical Univ. of Vienna; Rikard Holmdahl, Section for Medical Inflammation Research, Univ. of Lund, Sweden; Günter Steiner, Dept. of Rheumatology, Medical Univ. of Vienna, Austria

Department of Rheumatology, Internal Medicine III, Medical University of Vienna

Keywords Rheumatoid Arthritis, Autoantigens, Autoantibodies, T cells, Animal models

Severe erosive arthritis can be induced in genetically susceptible DA rats by a single intradermal injection of the mineral oil pristane. Pristane-induced arthritis (PIA) closely mimics rheumatoid arthritis (RA) showing a chronic disease course, symmetrical involvement of peripheral joints and the presence of rheumatoid factor (RF). Although it is well established that PIA is driven by autoreactive T cells, it has not been possible to link the immune response to joint antigens or other endogenous components. We

therefore analyzed B and T cell responses in rats with PIA to autoantigens potentially involved in the pathogenesis of RA, including IgG, citrullinated proteins, stress proteins, glucose-6-phosphate isomerase and heterogeneous nuclear ribonucleoprotein (hnRNP)-A2 (RA33). In these studies autoantibodies to hnRNP-A2 were detectable by ELISA and Western blotting in sera of pristane-primed rats already one week before disease onset, reaching maximum levels during the initial acute phase. In addition, a transient increase in IgG-RF was observed during acute phase, while autoantibodies to other antigens were generally not observed. CD4+ lymph node cells isolated 10 days after pristane injection produced interferon-gamma but not interleukin-4 in response to stimulation with hnRNP-A2, whereas none of the other candidate antigens elicited cytokine secretion. Surprisingly, hnRNP-A2 also stimulated non-primed lymph node cells to produce inflammatory cytokines such as TNF and IL-6. Cytokine production was not seen in MyD88 deficient cells suggesting involvement of Toll-like receptors. Finally, hnRNP-A2 was highly overexpressed in joints of rats with PIA as revealed by immunohistochemistry and Western blotting. The presence of autoantibodies and autoreactive Th1-like cells directed to hnRNP-A2 shortly after pristane injection and before disease onset suggests this autoantigen to be among the primary inducers of autoimmunity in PIA. Therefore, hnRNP-A2 might play a pivotal role in the pathogenesis of PIA and possibly also human RA.

References

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12. ¹⁵ - 1. ³⁰	Lunch
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1.³⁰ - 3.³⁰ pm

Cell targeting in vivo, Alan Tyndall, Andrew Cope

Paul Emery
B-cell Therapy

Academic Section of Musculoskeletal Disease, Leeds Institute of Molecular Medicine, University of Leeds, UK

Keywords: B-cells, rheumatoid arthritis, b-lymphocyte depletion

B-cells have long been known to be critical components of immune response contributing to the pathogenesis of rheumatoid arthritis. The availability of B-cell directed therapies, initial pilot studies were followed by randomised controlled studies that have proven the benefit of lymphocyte depletion. Interestingly completion depletion has not been associated with major infectious problems, and repeat dosage has proved feasible. Levels of immunoglobulin remain stable initially but IgM falls below normal levels after pre-infusions in approximately 25% of cases.

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Wietse Kuis**Immune regulation and tolerance in chronic arthritis - cellular therapy
in JIA**

Department of Pediatric Immunology and Rheumatology, UMC Wilhelmina
Children's Hospital, Utrecht, Netherlands

We investigated immune regulatory mechanisms in children with chronic arthritis. Therefore we investigated 2 onset types of Juvenile Idiopathic Arthritis i.e. the oligoarticular and the polyarticular onset type. The oligoarticular onset type is an unique example of a rather benign and selflimiting and sometimes even self-remitting disease, while the polyarticular onset form is mostly a serious disease with risk of erosive changes in the joints in due time.

Immune regulatory mechanisms differ in both diseases. We found that PBMC of oligoarticular JIA patients respond to auto-antigens like HSP60 with the production of cytokines like IL10, while patients with a polyarticular course do not recognize HSP60. This indicates that self HSP60 (auto reactive) T cells in JIA are related to a good prognosis and have a regulatory phenotype. This makes (epitopes of) HSP ideal candidates for immune therapy. Accordingly we selected pan DR-binding epitopes that could be recognized by patients with JIA. These epitopes are suitable candidates for immune therapy.

What is the nature of these immune regulatory cells? Are they related to well characterized immune regulatory cells like for example CD4+CD25+ T reg cells? Therefore we incubated CD4+CD25- T cells with HSP 60 and looked for the induction of CD4+CD25+ T reg cells. We found a significant production of CD4+CD25+ T reg cells with especially in CD25+ bright cells the expression of FoxP3 cells. Accordingly we showed that these HSP induced T reg cells can suppress T cell responses. If a deficiency of T reg's plays a role in the pathogenesis of chronic arthritis, the question is if we can restore the T reg compartment.

Therefore we determined the course of T reg cells before and after autologous stemcell's in children with therapy resistant arthritis.

In more than 60% of these patients a remission could be induced. Interestingly before transplantation T reg cells were decreased, while there was a catch up after transplantation. So, autologous stemcell transplantation can restore immune regulatory mechanisms in these patients. The next step is treatment of (severe) arthritis patients with pan DR-binding HSP60 epitopes. The first clinical results with DNA J (other HSP60 epitope) will be shown.

In summary these results show that immunotherapy in these patients is an option for treatment.

Francesco Dazzi
Mesenchymal stem cells and cell therapy*F Dazzi, V Tisato, L Weng, N Horwood*

Kennedy Institute of Rheumatology and Department of Haematology - Imperial College, London, United Kingdom.

Cell therapy, pioneered for the treatment of malignancies in the form of bone marrow transplantation, has subsequently been tested and successfully employed in autoimmune diseases (AD). Autologous haemopoietic stem cell transplantation (HSCT) has become a curative option for conditions with very poor prognosis in which targeted therapies have little or no effect. Although HSCT remains a non specific approach, the knowledge gained in this field has led to the identification of new avenues. In fact, it has become evident that the therapeutic efficacy of HSCT cannot merely be the consequence to a high dose immunosuppression but rather the result of a resetting of the abnormal immune regulation underlying autoimmune conditions. We will report the results of a new study whereby we show that the preparatory regimen for HSCT is associated with a proportional expansion of regulatory T (Treg) cells the specificity of which can be skewed by the presence of an antigen. The induction of tolerance to the putative antigen is strictly dependent on the presence of Treg cells but not its maintenance.

The identification of professional and non professional immunosuppressive cells and their biological properties is generating a huge interest for their clinical exploitation. More recently, mesenchymal stem cells of bone marrow origin have been shown not only to be able to differentiate into multiple tissues but also to exert a potent anti-proliferative effect which results in the inhibition of immune responses and prolonged survival of haemopoietic stem cells. Mesenchymal stem cells (MSC) are progenitor cells of stromal origin which can differentiate into multiple lineages. MSC also possess immunosuppressive properties by selectively halting immune cells at the G0-G1 phase. For these reasons MSC have been used to manipulate graft-versus-host disease (GvHD). We tested the therapeutic potentials of human MSC to prevent and/or treat GvHD in a xenogeneic model. When MSC were given with human PBMC at weekly intervals, there was a marked decrease in human T cell engraftment and none of the mice developed GvHD. If MSC were administered when GvHD had already developed, T cell expansion and the course of the disease were comparable to controls. MSC engrafted only in the BM if injected alone but were detectable also in the lungs, liver and peritoneal washing in the mice in which MSC were administered with PBMC. These findings are consistent with the notion that the immunosuppressive

effect of MSC resembles split energy, thus supporting the use of MSC as a prophylactic rather than a therapeutic approach. All these potential resources clearly need to be investigated further but support a great deal of enthusiasm for cell therapy of AD.

Jan-Matthias Braun Anti-CD4 targeting

Jan Matthias Braun, Marc Brulport, Jan Hengstler, Bianka Bussmann, Peter Ahnert, Michael Heinrich, Franziska Kießling, Sonya Faber, Frank Emmrich

Universität Leipzig, Translational Centre for Regenerative Medicine (TRM–Leipzig), Fraunhofer Institute for Cell Therapies and Immunology (IZI), and Regenerative Medicine Network Leipzig-Halle (RegMedNet.org), Germany

Keywords: immunological tolerance, cell therapy, non-depleting anti-human CD4 monoclonal antibody

Introduction: The goal of anti-CD4 therapy is a reduction in the rate of tissue rejection, the induction or the reset of immunological tolerance in autoimmune disease or in cell or tissue transplantation, while maintaining a medium and long-term competent cellular and humoral immune system effective in combating infectious agents, tumours, and toxins. A pre-clinical cell transplantation model was used to show successful induction of tolerance.

Materials and Methods: Cultured human SV40LT immortalised hepatocytes were transplanted into immunocompetent C57BL6 mice with a murine CD4 knock-out geno- and phenotype, while expressing physiologically human CD4 molecules on T-helper cells and human HLA-DR molecules on antigen presenting cells. Mice were treated with two single doses of antibody.

Results: An increased number of human hepatocytes that had successfully engrafted into the mouse liver could be observed in mice treated with anti-CD4 therapy, while cells transplanted into PBS treated animals showed no engraftment.

Conclusions: These findings provide evidence that the anti-human CD4 monoclonal antibodies in liver cell transplantation are promising candidates for an effective antigen-specific induction of immunotolerance against transplant tissues or cells. This model is proposed for use in pre-clinical and immunotoxicity testing of cells, native or engineered tissues, or biohybrid and biomaterial constructs. Particularly stem and precursor cell therapy, or islet cell transplantation may benefit clinically from induction therapy.

Summary: Clinically applicable anti-human CD4 antibody therapy (mAb

Max16H5) was used in an experimental transgenic animal model. Human liver cells were transplanted into fully immunocompetent mice receiving mAb, but no immunosuppressive agents, thereby inducing antigen specific tolerance.

Thomas Hünig

Targeting CD28 with stimulatory and blocking anti-bodies

Institute for Virology and Immunology, University of Würzburg, Germany

CD28 is the most important costimulatory receptor for both effector and regulatory T-cells. We have developed two types of mouse-anti-mouse mAb. Conventional mAb E18 binds to the natural ligand binding site, costimulates in vitro and blocks CD28 in vivo. The „superagonistic“ mAb D665 binds to the C“D loop of CD28, and induces T-cell proliferation without the need for TCR engagement. In vivo, D665 preferentially activates regulatory T-cells and, as previously shown in the rat system, is highly effective in preventing or treating autoimmunity. While the cytokine storm observed in a human trial with CD28 superagonists presently excludes this approach in human disease, we show that polyclonal and transient Treg activation as a general principle is very efficacious, and may even be superior to the use of Treg cells with known specificity for an autoantigen in some situations.

Blocking the activation of effector T-cells with conventional, blocking CD28 specific mAb is an interesting alternative to the currently used blockade of CD28 ligands by CTLA-4-Ig, because it leaves the CTLA-4 pathway intact. A caveat in CD28 blockade may be a gradual loss of regulatory T-cells due to the high dependence of this subset on CD28 stimulation in cis and in trans (for IL-2 production). We show that at least in the mouse model, this is not a major concern because Treg reduction is only partial and is rapidly reversed after cessation of mAb treatment.

Alessandro Moretta

NK-cell mediated shaping of adaptive immunity

Dept. of Experimental Medicine, University of Genova, Italy

Keywords: NK cells, Innate Immunity, dendritic cells

Natural Killer (NK) - cells are capable of discriminating between normal and virus-infected cells or cells undergoing tumor transformation. The selective

elimination of abnormal target cells, based on classical NK-mediated cytotoxicity, is the result of the combined function of activating receptors including NCRs and NKG2D(1) and inhibitory receptors such as Killer Ig-like receptors (KIRs) and CD94/NKG2A on NK cells as well as of the expression of their specific ligands on target cells. However a number of recent studies have highlighted the novel concept that the actual role of natural killer (NK) cells is not only confined to the destruction of virus-infected cells or tumors. Indeed NK cells, by interacting with myeloid DCs during the early phases of inflammation, appear to play a crucial role in shaping both innate immune reactions (within inflamed peripheral tissues) and adaptive immune responses (in secondary lymphoid compartments) (2). Interestingly, this novel function assigned to NK cells is essentially mediated through the aggression of normal immature myeloid DCs. Only DCs undergoing optimal maturation become refractory to NK cell killing and will obtain the permission to prime Th1 cells after migration to lymph nodes. In addition interactions between NK cells and other cells of the innate immune system may occur during the early phases of acute inflammation, secondary to infection. Along this line recent studies were focused on the crosstalk between NK cells and plasmacytoid dendritic cells (PDC) (3), mast cells, basophils, eosinophils and neutrophils. Thus a complicated network of interactions can take place after the recruitment of these different cells to inflammatory sites in response to tissue damage resulting from invasion by pathogens (or tumor cells) (4).

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Yuti Chernajovsky

Engineering mesenchymal stem cells for targeting, committed differentiation to cartilage and inflammation control

Marinela Mendez, Pertuz, Gordon Daly, Chris Hughes, David Gould, Bjarne Faurholm, Ahuva Nissim, Andrea Hoffmann, Gerhard Gross* and Yuti Chernajovsky*

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Keywords: Cell engineering, molecular targeting, anti-inflammatory therapy, transcriptional regulation, gene therapy

In order to target MSC to the joint extracellular matrix, we have expressed under tight tetracycline regulation from a lentiviral vector, a chimeric receptor with an extracellular domain of a collagen type II-specific monoclonal scFv (1-3) linked to the transmembrane and signalling domain of the FGFR3 or BMP receptors. Both the tetracycline trans-activator rTA-2SM2 and the repressor tetR-KRAB are expressed from a second constitutive lentiviral vector driven by the SFFV promoter and linked via an IRES. This regulated expression is needed to mimick the embryological control of receptor expression.

Expression of these receptors is dose dependent on Doxycycline and signalling cascades are activated when collagen type II is in the extracellular space. In monolayer cultures, the BMP receptor chimera activates the SMAD signalling pathway and the FGFR3 chimera activates the ERK phosphorylation cascade. Expression of these receptors in micromass cultures leads to chondrocyte differentiation without external addition of collagen type II or other growth/differentiation factors as the cells endogenous production of collagen type II is sufficient for receptor activation.

We have also engineered these cells to express recently developed latent cytokines that became active only at sites of high MMP activity (4).

Antonio Uccelli

MSCs for the treatment of experimental neurological diseases

Uccelli A, Gerdoni E, Casazza S, Frassoni F, Mancardi GL. Department of Neurosciences Ophthalmology and Genetics - University of Genoa

Neuroimmunology Unit, Department of Neurosciences, Ophthalmology and Genetics, University of Genoa

Keywords: EAE, autoimmunity, stem cells, T cells

Mesenchymal stem cells (MSC), a subset of adult stem cells derived from the bone marrow stroma, have generated much enthusiasm as possible cell source for tissue repair including the nervous system. Recent studies have

shown that MSC can also modulate T and B cell responses thus providing a rationale for their use as therapy for experimental autoimmune disease such as experimental autoimmune encephalomyelitis (EAE). In this study we show that i.v. injected MSC can ameliorate both relapsing-remitting (PLP-induced) and chronic progressive (MOG-induced) EAE before and after disease onset. In vivo T cell response and antibodies production against the immunizing antigens and mitogens was significantly decreased in MSC treated mice. The adoptive transfer of encephalitogenic PLP- sensitized, preconditioned with MSC, induced a very mild disease compared to controls thus suggesting the induction of peripheral tolerance. Upon i.v injection, Green Fluorescent Protein labeled MSC were detected inside the lymph nodes early upon injection and inside the inflamed CNS at later stage. MSC injection resulted in a decreased number of inflammatory infiltrates, reduced demyelination, axonal loss and, in contrast, sparing of neurons, astrocytes and oligodendrocytes. We observed no evidence of GFP labeled transdifferentiation into neural cells. Overall, we propose that MSC may block the autoimmune attack against the CNS and protect neural cells from death and therefore may be effective for the treatment of autoimmune diseases where tissue degeneration is associated with inflammation.

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3.³⁰ - 4.⁰⁰

Coffee

4.⁰⁰ - 5.³⁰ pm**Reset of tolerance**, Paolo Muraro, Pierre Miossec**Roland Martin****Antigen-Specific, Cell-Based Tolerization Strategies in Autoimmune Diseases Specifically in MS***Roland Martin, MD (inims), Andreas Lutterotti, MD (inims)*

Institute for Neuroimmunology and Clinical MS Research (inims*), Center for Molecular Neurobiology Hamburg (ZMNH), University Medical Center Eppendorf (* inims is supported by the Gemeinnützige Hertie Stiftung)

Keywords: antigen-specific therapies, multiple sclerosis, clinical trials

Antigen-specific tolerization to autoantigens remains a long-term goal for clinical and basic immunologists. In the last decades, many strategies towards this aim for human autoimmune diseases such as multiple sclerosis (MS) have been considered based on promising data from in vitro data or animal experiments. A number of them have been explored in early clinical trials. However, none of these finally advanced to approval by the regulatory agencies. The main reasons for failure included lack of efficacy in humans and/or unexpected and intolerable side effects. Despite the fact that previous attempts towards antigen-specific immunomodulation have often been disappointing, there is renewed interest in therapies that aim at re-establishing tolerance to autoantigens at the level of either T cell- or antibody-mediated immune responses or both. Such antigen-specific immunotherapies in theory offer to correct pathological immune reactivity against autoantigens in a highly specific and effective manner, and also to achieve this goal with relatively little side effects. The most interesting approaches that are currently being pursued and use cell-based strategies for antigen-specific immunotherapies will be presented.

Renate Arnold**Stem cell transplantation: Special features in autoimmune diseases**

Charité University Medicine Berlin

Alan Tyndall**Autologous stem cell transplantation for severe systemic Sclerosis:
Update on the ASTIS Trial**

J. M. Van Laar¹, . D. Farge², . C. Bocelli-Tyndall³, . J. K. Sont⁴, . R. Saccardi⁵, . I. Miniati⁶, . M. Matucci-Cerinic⁶, . K. Machold⁷, . S. Arf⁸, . D. Adoue⁹, . F. Sarrot-Reynauld¹⁰, . J. Constans¹¹, . P. Roblot¹², . I. Queré¹³, . J. M. Durand¹⁴, . J. Sibilía¹⁵, . A. Himself¹⁶, . J. Kaltwasser¹⁶, . H. Peter¹⁷, . I. Koetter¹⁸, . S. Kleinert¹⁹, . H. Tony¹⁹, . A. Fasso²⁰, . A. E. Voskuyl²¹, . M. Vonk²², . F. H. Van den Hoogen²², . P. Villiger²³, . S. Weiner²⁴, . S. Dass²⁵, . P. Emery²⁵, . A. Gratwohl²⁶, . A. Tyndall²⁷

¹Rheumatology, Leiden University Medical Center, Leiden, Netherlands, ²Internal Medicine, Hopital St Louis, Paris, France, ³Rheumatology, Felix-Platter Spital, Basel, Switzerland, ⁴Medical Decision Making, Leiden University Medical Center, Leiden, Netherlands, ⁵Hematology, ⁶Rheumatology, Univ. of Florence, Florence, Italy, ⁷Rheumatology, Allgemeines Krankenhaus, Vienna, Austria, ⁸Internal Medicine, CHU La Meynard, Fort de France, ⁹Internal Medicine, Hop Purpan, Toulouse, ¹⁰Internal Medicine, CHU de Grenoble, Grenoble, ¹¹Internal Medicine, Hop St André, Bordeaux, ¹²Internal Medicine, Hop St Bernard, Poitiers, ¹³Internal Medicine, CHU de Montpellier, Montpellier, ¹⁴Rheumatology, Hop de la Conception, Marseille, ¹⁵Rheumatology, Hop de Haute pierre, Strassbourg, France, ¹⁶Rheumatology, Klin JW Goethe-Uni, Frankfurt, ¹⁷Rheumatology, Albert-Ludwigs Uni, Freiburg, ¹⁸Rheumatology, Med Uni Tübingen, Tübingen, ¹⁹Rheumatology, Med Uni Würzburg, Würzburg, Germany, ²⁰Hematology, Papanikolaou Hosp, Thessaloniki, Greece, ²¹Rheumatology, VU-MC, Amsterdam, ²²Rheumatology, UMCN, Nijmegen, Netherlands, ²³Rheumatology, Insel Spital, Bern, Switzerland, ²⁴Internal Medicine, Med Uni, Herne-Bochum, Germany, ²⁵Rheumatology, Leeds General Infirmary, Leeds, United Kingdom, ²⁶Hematology, Kantonspital, Basel, ²⁷Rheumatology, Felix-Platter Spital, Grenoble, Switzerland

Background: High dose immunosuppressive therapy (HDIT) and hematopoietic stem cell transplantation (HSCT) is a potential treatment for patients with severe systemic sclerosis (SSc). Previous studies showed responses in two thirds of patients durable up to 3 yrs after HSCT in one third(1). This strategy is being investigated through the ASTIS-trial (autologous stem cell transplantation international scleroderma trial), a prospective, controlled, randomized trial to compare safety and efficacy of HDIT + HSCT versus monthly i.v. cyclophosphamide in SSc patients at risk of major organ failure or early mortality.

Objectives: To evaluate whether HDIT + HSCT is superior to conventional treatment in terms of safety and efficacy in SSc patients, and to assess potential predictive factors of response.

Methods: SSc patients with early active diffuse disease with or without major organ involvement are eligible. SSc patients randomized to the transplant arm undergo mobilization with cyclophosphamide 2x2 g/m², conditioning with cyclophosphamide 200 mg/kg, rbATG 7.5 mg/kg, followed by reinfusion of CD34+ selected autologous HSCT. Those randomized to the control arm are treated with 12x monthly i.v. bolus cyclophosphamide 750 mg/m². The primary endpoint is event-free survival, defined as survival until death or development of major organ failure during 2 years follow-up.

Results: 87 SSc patients have been enrolled in 20 European centers per Jan 2007 : mean age 43 yrs, mean disease duration 1,8 yr, mean VC 81%, mean DLCO 59%. 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.

Conclusion: The lower than expected TRM (2.6% for HSCT) and unexpected toxicities with 80 SSc patients randomized underscores the feasibility of the ongoing ASTIS-trial.

References: van Laar JM, Farge D, Tyndall A, on behalf of the EBMT/EULAR Scleroderma Study Group. The ASTIS-trial, hope on the horizon. *Ann Rheum Dis* 2005;64:1515.

Antonio Coutinho

Tolerance induction - preventive or therapeutic?

António Coutinho, Santiago Zelenay, Francisca Fontes & Jocelyne Demengeot

Instituto Gulbenkian de Ciencia, Oeiras, Portugal

Keywords: Tolerance; regulation; lymphocytes

This question could amount to ask whether the mechanisms that ensure natural tissue-specific tolerance (e.g. Treg) are as effective in naïve vs primed individuals. Previous experiments have shown that haplotype-specific Treg selected on thymic epithelium, which efficiently suppress rejection of primary skin grafts, are unable to regulate rejection of secondary grafts. Yet, recent evidence in several experimental autoimmune disease models, some of which will be presented, indicates that elevated numbers or activity of Treg may halt disease progression or even reverse symptoms and pathology. Perhaps the question, after all, amounts to list the differences between deviations of physiological autoreactivity that result in a chronic autoimmune process, vs a conventional immune response to non-self. In any event, preventive measures require that pathogenic autoimmune states

are identified prior to eclosion of clinical manifestations and/or establishment of pathology. In turn, this commands more efforts in the development of predictive tests in this area.

Alf Hamann Imprinted Suppressors

Alf Hamann, Jochen Huehn, Stefan Floess, Jennifer Freyer, Katrin Baumann, Udo Baron), Sven Olek*)*

Experimental Rheumatology, Charité University Medicine Berlin, *) Epiontis GmbH, Berlin, Germany

Keywords: regulatory T cells, Foxp3, epigenetics, TGFb

The detection of Foxp3 in regulatory T cells (Tregs) promised to solve the problem of their identification. However, the issue seems not to be as easy. Natural Tregs are generated in the thymus as a permanent cell lineage, but Foxp3+ Tregs can also be induced under appropriate conditions from conventional, naive T cells. Yet, how stable are these induced Tregs? Do induced as well as natural Tregs remain stable upon activation and expansion in vitro, as highly desirable for cellular therapies? The reliability of Foxp3 as marker for Tregs was challenged when it was observed that human T cells might transiently express Foxp3 upon activation without acquisition of a suppressive phenotype.

We wondered whether Tregs display properties of stable lineages where expression patterns are imprinted by epigenetic mechanisms. An emerging paradigm assigns chromatin remodeling a key role in the permanent fixation of cellular differentiation events. It involves modification of histones and methylation of DNA which change, in a heritable way, the accessibility of genes crucial for function.

Our analysis of the foxp3 gene provided compelling evidence that this mechanism is indeed operative in Tregs 1). We identified a highly conserved non-coding sequence proximal to exon -1, which is completely demethylated in natural Tregs, both in mice and man, and completely methylated in naive T cells or effector T cells, suggesting that epigenetic regulation is involved in the differentiation into a stable lineage. Interestingly, demethylation is not complete in murine Tregs induced by activation in vitro in the presence of TGFbeta; in fact, Foxp3 expression in such induced Tregs is rather unstable. Furthermore, human T cells that transiently upregulate Foxp3 simply upon activation, in the absence of TGFbeta, and develop into effector cells rather

than into Tregs, were found to show almost no signs of demethylation in the *foxp3* gene.

Together, these findings suggest that an analysis of the methylation status of the conserved region in the *foxp3* gene might provide a better criterion for „true“ and stable Tregs than the mere expression of *Foxp3* and might become an important analytical tool in the quality control of Tregs destined for cellular therapies.

References:

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Christian Jorgensen

Chondrogenic differentiation of mesenchymal stem cells: a new insight through Genostem program

Djouad F.^{1,2}, Bony C.^{1,2}, Louis-Plence P.^{1,2}, Apparailly F.^{1,2}, Noël D.^{1,2}, Jorgensen C.^{1,2,3}

¹Inserm U475, Montpellier, France, ²Université Montpellier, ¹UFR de Médecine, Montpellier, France, ³CHU Montpellier, Hôpital Lapeyronie, Unité Clinique d'Immuno-Rheumatologie, Montpellier, France

Mesenchymal stem cells (MSC) are considered suitable sources for cell-based therapies in cartilage engineering. However, their potential to regenerate a fully functional and mature tissue relies on the presence of a differentiation factor. The identification of such a factor specific for the chondrogenic lineage has still to be performed and represents the major issue of this study.

Methods: Bone marrow-derived human MSC were induced to differentiate towards chondrocytes using the micropellet culture technique in presence of chondrogenic medium containing hBMP-2 for 21 days. Total RNA were hybridized on DNA microarrays (Affymetrix U133A). Quantitative RT-PCR was performed on RNA extracted at various time points during chondrogenesis. The C3H10T1/2 murine MSC line was stably transfected with a plasmid expressing the wild type (Wt) or a constitutively active form of *Foxo1A* (Δ) and cultured in chondro-, osteo-, adipogenic conditions or injected intra-articularly. Expression of specific markers was assessed by RT-PCR.

Results: Among the 1354 differentially regulated genes during chondrogenesis, 705 genes were up-regulated in MSC-derived chondrocytes. We first focused our attention on transcription factors and in particular, on *Foxo1A* which was shown to be increased by a 13-fold factor using real time PCR, as soon as day 2 of chondrogenesis. After the over-expression of the wt*Foxo1A*

or Δ Foxo1A genes in the C3H10T1/2 cells, we could show the up-regulation of aggrecan, collagen IIB and the down-regulation of collagen I, suggesting that Foxo1A is sufficient to induce chondrogenesis. In parallel, the engineered cells did not revealed higher osteogenic or adipogenic potential than naïve C3H10T1/2 cells when cultured in specific inducing conditions. In vivo, we could detect the formation of cartilage staining positive for aggrecan and collagen II in the areas of engineered cell injection confirming their potential to differentiate into chondrocytes. On the contrary, knock-down of Foxo1A in the CL1 bipotent cell line resulted in the decrease of differentiation as shown by diminution of collagen II and aggrecan mRNA levels.

Conclusion: Our results suggest that Foxo1A is one essential transcription factor involved in the early steps of chondrogenesis. Further studies will aim at determining the mechanism underlining the activation of chondrocyte-specific markers.

5. ³⁰ - 6. ³⁰ pm	Meeting of the working group for cell therapies of the ESCIR Election of the chair person; membership, mission and perspective of the CELLAID initiative	
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7. ⁰⁰ pm	<i>Dinner</i>	
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Wednesday, February 21st

8. ⁴⁵ - 9. ³⁰ am	Keynote lecture , Joachim Kalden
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Fritz Melchers
Generating the immune system in vitro

Max Planck Institute for Infection Biology

9. ³⁰ - 10. ³⁰ am	Abstract session - late breaking news , Iain McInnes, Sergio Romagnani
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Andreas Lutterotti**Establish Tolerance In MS With Peptide-Pulsed, Fixed Antigen Presenting Cells - A Mri-Controlled, Single Center, Baseline To Treatment Cross-Over, Phase Iia Trial In Relapsing-Remitting MS Patients**

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Keywords: multiple sclerosis, immune tolerance, peptide, ECDI

Multiple Sclerosis (MS) is the most frequent debilitating neurological disease of young adults in Europe, with half of patients needing a walking aid 10-15 years from onset of the disease. Current therapies for MS have only modest effects on clinical relapses, all need to be injected for long periods of time and are associated with considerable side effects.

We propose to conduct an open-label, single center, baseline-to-treatment cross-over phase Iia clinical trial to assess the safety, preliminary efficacy and in vivo mechanisms of action of i.v. administration of autologous peripheral blood lymphocytes (PBLs) coupled with a cocktail containing seven immunodominant myelin peptides (MBP13-32, MBP83-99, MBP111-129, MBP146-170, PLP139-154, MOG1-20 and MOG35-55) and fixed with the cross linker 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (ECDI). Only patients with active disease, as measured by MRI, who show autoreactivity against one of the myelin antigens used will be included. After a 4 months screening phase, patients will receive a single iv injection of 5x10⁸ antigen coupled ECDI fixed PBL and followed for 6 months. Monthly neurological, MRI and immunological exams will be performed to assess the safety, efficacy and in vivo mechanisms of action of this regimen. We expect that this protocol will decrease the average number of monthly contrast-enhancing MRI lesions by 50% or greater (primary outcome) and reduce the number and/or change the phenotype of myelin peptide-specific T cells from a pro-

inflammatory Th1/Th17 to an anti-inflammatory Th2-like type.

This protocol has been shown to be safe and highly effective in preventing and treating animals of different T cell mediated autoimmune diseases (Kohm et al., 2005). In vitro tolerization of human T cells by autologous antigen-coupled APC treated with ECDI is effective as shown by failure of tolerized T cells to proliferate or to produce Th1 cytokines and a decreased expression of costimulatory molecules on these cells (Vandenbark et al., 2000).

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Rosa María Licón Luna

Role of T-cell Costimulation in Sexual Dimorphism of Autoimmune Diseases

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Keywords: Cytotoxic T cell antigen-4 (CTLA-4), Experimental Autoimmune encephalomyelitis (EAE), Multiple Sclerosis (MS), costimulatory molecules, sexual dimorphism.

Autoimmune diseases, such as multiple sclerosis (MS), rheumatoid arthritis (RA), systemic lupus erythematosus, and thyroiditis affect approximately 8% of the population, 78% of whom are women (1). The high prevalence of autoimmunity in females may be a consequence of a bidirectional signaling network between hormones and the immune system (5). The chronic inflammation characteristic of autoimmune diseases is mainly initiated and maintained by autoreactive CD4+ T lymphocytes. Costimulation is required for an optimal response of T-lymphocytes: CD28 is a T-cell activator, whereas CTLA-4 (CD152) downregulates T-cell activity and both together provide a balance in the T-cell immune response (2, 3, 4).

To elucidate the role of costimulation in the quality of sex-related immune responses, we use the SJL mouse model in the development of Experimental Autoimmune Encephalomyelitis (EAE), which mimics very well pathogene-

sis and the higher incidence in females that occurs in the human MS counterparts. Using a unique sensitive staining technique, we found that activated T lymphocytes from male mice express significantly higher frequencies of CTLA-4 on their cell surface suggesting that the inhibitory molecule CTLA-4 is preferentially used by male mice to dampen inflammatory responses. Additionally, we found that immune responses of females reveal a higher frequency of IFN γ cells compared to male-derived ones, probably the most inflammatory population, leading to enhanced inflammatory responses. Our results suggest that induction of CTLA-4 expression could serve as a target for an immunomodulatory strategy to downregulate immune responses during the highly sexually dimorphic autoimmune diseases.

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Roberta Rigolio

Extracorporeal photochemotherapy: early ex-vivo experience on healthy controls and a multiple sclerosis patient to investigate a new potential cell therapy

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Keywords: Extracorporeal Photochemotherapy (ECP), proinflammatory cytokines,

multiple sclerosis

Extracorporeal photochemotherapy (ECP) is a cell based procedure effective in the treatment of different T-cell mediated diseases such as cutaneous T-cell lymphoma and Graft-versus-Host Disease. During ECP treatment the patient's blood is processed by means of a cell separator to collect leukocytes which are then added with the photoactive drug 8-methoxypsoralen (8-MOP), exposed to ultraviolet-A light (UV-A) and reinfused into the patient.

Even if the mechanisms of action of ECP remain elusive, it has been shown to own in-vivo immunomodulatory effects and to be effective also on experimental allergic encephalomyelitis (EAE) animals and in a small pilot study on multiple sclerosis (MS) patients.

It has been suggested that during ECP not only UV-A irradiation but also changes in the environmental condition may be relevant. Therefore, we developed a new peristaltic-based bench device which reliably mimics ex-vivo the complete ECP cycle using 50 ml peripheral blood sample. Peripheral blood mononuclear cells (PBMC) were then collected and treated with 8-MOP and/or UV-A under the same conditions used for the patients' therapy. Using this strategy we investigated 8-MOP and/or UV-A effect on the production of the inflammatory cytokines IFN- γ , IL-2 and TNF- α in PBMC after polyclonal stimulation.

We observed a significant decrease in activated CD4+ and CD8+ T-lymphocytes producing cytokines after UV-A irradiation and a further decrease in the presence of 8-MOP.

The decrease in cytokines production seemed to be both cytokine- and cell type-related. In fact TNF- α production was reduced to a lesser extent than IFN- γ and IL-2 ones, while CD4+ T-cells seemed to be more sensitive than CD8+ lymphocytes when IFN-gamma and IL-2 production was considered. Both T-cell population showed similar behaviour when TNF- α production was evaluated.

Following this preliminary experience, this ex-vivo protocol will be used to deeply investigate the effect of ECP on EAE rats.

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Ingo Müller**Multipotent mesenchymal stromal cells - a novel treatment for steroid induced avascular osteonecrosis in children***N. Tzaribachev¹, M. Vaegler¹, P. Reize², M. Rudert², R. Handgretinger¹, I. Müller¹*¹University Children's Hospital, Tuebingen; ²University Hospital, Dept. for Orthopaedics, Tuebingen*Keywords: multipotent mesenchymal stromal cells, avascular necrosis*

Avascular osteonecrosis (AVN) is a common adverse effect in the treatment of autoimmune diseases in children. Inflammation of the bone, predisposing thrombogenic conditions (e. g. in systemic lupus erythematoses) and the use of high dose steroids promote bone vulnerability. Necrotic lesions are most frequently located in the femur, although other bone regions may be involved. Still there are no convincing therapeutic strategies for the treatment of AVN and surgical techniques including core decompression techniques are standard measures to restore bone integrity.

Multipotent mesenchymal stromal cells (MSC) can be differentiated into osteoblast with high efficiency. They easily can be obtained from bone marrow aspirates of 10 ml in patients with autoimmune diseases. Expansion in an animal protein-free process allows a fast proliferation and generation of large amounts of MSC in typically 3 weeks.

In our department, 3 patients with severe steroid induced AVN received a MSC based therapy. After retrograde core decompression surgery and MSC application. All patients tolerated the procedure well and showed no adverse affects, in particular no infection. All three patients achieved alleviation of the acute pain symptoms. MRI scans after 7 months in the first patient suggested restorative processes of the bone integrity in the lesion areas. The time period is yet too short for a precise prognosis.

Further fields of scientific interest will be the use of scaffolds carrying MSC (e. g. collagen scaffolds) for the creation of a better bone adhesion and integrity.

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P. Louis Plence**CD49b+ regulatory T cells are able to cure arthritis**

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Keywords : Regulatory T cells, Dendritic cell, Immunotherapy, Arthritis.

Objective : Dendritic cells (DCs) are specialized antigen presenting cells with an important role in the initiation and regulation of immune responses. We previously demonstrated that immature DCs (iDCs) can prevent arthritis by inducing a regulatory population of T lymphocytes (Treg) (1). These Tregs were characterized by the expression of the CD49b molecule and by interleukin-10 (IL-10) secretion. In the present study we evaluated and compared the therapeutical potential of iDC versus Treg to cure established arthritis.

Methods : The Treg population was purified from the liver and spleen of DBA/1 mice that were repetitively injected with 5×10^5 iDCs by sorting the CD4+CD49b+ cells. These Tregs were adoptively transferred in DBA/1 mice (n=7-8/group) with established collagen-induced arthritis (day 28). In parallel iDC were repetitively injected in arthritic mice in order to evaluate their therapeutic potential and compared with therapeutical potential of T regs.

Results: We showed that the repetitive injections of iDC just after the boost in incomplete freund adjuvant did not protect the mice from severe arthritis. However a single injection of less than 100 000 T reg cells was able to decrease the severity of arthritis and protect mice until day 43. The severity score of the Treg treated group was 0.2 versus 2.4 for the control group. We are currently working on the characterization of the Treg cells in order to better define this new regulatory population.

Conclusion: These results suggest that the use of iDC vaccination in an inflammatory setting has to be carefully addressed and the in vivo maturation of such iDC has to be blocked before their injection. Such strategy has been evaluated with IL-10 or dexamethasone modulated DC. More importantly, we demonstrated in this study the high therapeutical potential of the CD49b+ regulatory T cells on established arthritis in the CIA model.

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10. ³⁰ - 11. ⁰⁰	Coffee	
11. ⁰⁰ - 12. ³⁰	Regulatory affairs & 7 th Framework program, Reinhold Schmidt	

Klaus Cichutek

Regulatory aspects of advanced therapy products in the European Union

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According to the proposal of the European Commission, advanced therapy products include gene therapy, somatic cell therapy and tissue-engineering products. General technical requirements to be presented in a licensing application can be found in Directive 2003/63/EC amending Directive 2001/83/EC. General guidance for gene therapy („Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)“ and cell therapy (guideline on human cell-based products in preparation) is or will be available. Marketing authorisation will follow the centralized procedure, co-ordinated by the European Medicines Agency (EMA) in London, according to Council Regulation (EG) No. 726/2004, for some products possibly after a transition period. Clinical trials can be initiated following a positive appraisal by an ethics committee and clinical trial authorization by the competent authority. This is now established in all member states of the European Union (EU), where more than 100 cell or gene therapy clinical trials have been registered in EudraCT since 2004. In conclusion, easily available and common regulations of clinical trials in member states pave the way to centralized licensing in all EU member states after filing a single application.

Donation, procurement and storage of cells to be used in or as medicinal products is regulated by Directive 2004/23/EC. Additional guidance may be found in Directive 2006/17/EC.

The CHMP (Committee for Human Medicinal Products) Gene Therapy Working Party (GTWP) and the CHMP Cell-based Product Working Party (CPWP) allow for informal briefing meetings with applicants on specific scientific matters of medicinal product development, where input from European regulators would be helpful. The formal scientific advice procedure provides rapid regulatory discussions with the CHMP Scientific Advice Working Party, optimal for later phases of clinical development and similar to pre-IND meeting. For orphan medicinal products an orphan drug status may be given by EMA which enormously reduces the finances needed for

European procedures including licensing and scientific advice, then called protocol assistance. Licensing under exceptional circumstances and fast track procedures allow for rapid licensing decisions. Thus, although EMEA is a network of European regulatory authorities, systems have been put in place that provide foreseeable and reliable procedures for licensing. Scientific advice at an early stage of medicinal product development is offered by EU members state competent authorities like the Paul-Ehrlich-Institut in Germany (klinpruefung@pei.de). The ICH-Gen Therapy Discussion Group extends the information exchange between regulators to an additional international level, especially to discussions among Japanese, North American and EU experts.

Ineke Slaper-Cortenbach
Regulatory affairs for cell therapies in Europe

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Since cell therapy is a field with a worldwide exchange of products, an urgent need was felt within the European Community to have a unified regulatory framework ensuring high standards of quality and safety of tissues and cells.

The European Union therefore published the European Directive (EUD) 2004/23/EC on April 7, 2004, entitled: 'Setting standards on quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of tissues and cells'.

Of importance for most cell therapy facilities is the fact that this EUD is applicable for all tissues and cells including haematopoietic peripheral blood, umbilical-cord (blood) and bone marrow stem cells, reproductive cells (eggs, sperm), foetal tissues and cells, and adult and embryonic stem cells.

After the publication of this directive, it took a great deal of time to discuss and publish the actual technical annexes, which describe the implementation of this EUD.

These technical annexes were divided into two parts.

- 1) The EUD 2006/17 published on 8 February 2006 deals with the technical requirements for the donation, procurement and testing of human tissues and cells, including the donor selection and evaluation criteria.
- 2) The EUD 2006/86 published on October 25, 2006 defines the traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.

In this presentation, the importance of these directives, the bottle-necks and

the steps taken so far by a number of organisations to solve these issues in cellular therapy in Europe, will be discussed.

Jürgen Sautter

FP7 - Perspectives for health and biotechnology related research

European Commission – Research DG

Keywords: European Union, Seventh Framework Programme, Biotechnology for Health, research funding

The multi-annual Seventh Framework Programme (2007 - 2013) of the European Union provides support and funding of research and development activities on a European level. It is also the instrument to implement the objectives and strategies of EU policies, which mainly are to improve and promote the health of the European population and to improve the competitiveness of European health care biotechnology and medical technology sectors, where SMEs and pharmaceutical industries are the main drivers. During its 7 years duration, FP7 will have a total budget of 50.5 billion Euros of which 6050 million Euros alone will be allocated to Collaborative Research under the „Health Theme“. Biomedical research will be mainly focussed under this Health Theme within two activities: „Biotechnology, generic tools and medical technologies for human health“ and „Translating research for human health“.

Research on major diseases, including autoimmune diseases, and research involving cell therapy and regenerative medicine, gene therapy or immunotherapy may find several entry points in the above-mentioned activities. Thus, among others, the first two calls will encompass the following topics: „In vivo image-guidance for cell therapy“ (e.g. immune cells or stem cells), „Development and production of new generation antibodies“, „Stem cell lines for cell-based therapies“, „Adding value to EU stem cell therapy research: scientific communication and future perspectives“, „Modelling of T-cell activation“, „Natural course and pathophysiology of rare diseases“ (... incl. immunological diseases...), „Early processes in the pathogenesis of chronic inflammatory diseases“ (... incl. chronic inflammatory diseases, such as asthma, rheumatoid arthritis and autoimmune conditions).

The emphasis will be on translational research as well as on tools and technologies. This approach should lead to new knowledge and applications in medicine and biotechnology. Research may be implemented through Collaborative Projects of various scales, Networks of Excellence and Coordination and Support Actions.

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- 1)Seventh Framework Programme: http://europa.eu.int/comm/research/future/index_en.cfm
- 2)Information on research programmes and projects: <http://www.cordis.lu>
- 3)European Research Council (ERC): http://ec.europa.eu/erc/index_en.cfm
- 4)Independent Experts for Evaluation of Proposals and monitoring and reviewing of projects:
http://cordis.europa.eu/experts/fp6_candidature.htm
- 5)Information requests : research@ec.europa.eu

Andreas Radbruch
Concluding remarks

<i>12.30 pm</i>	<i>Lunch</i>
	Transfer to EWRR, Sheraton

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Notes

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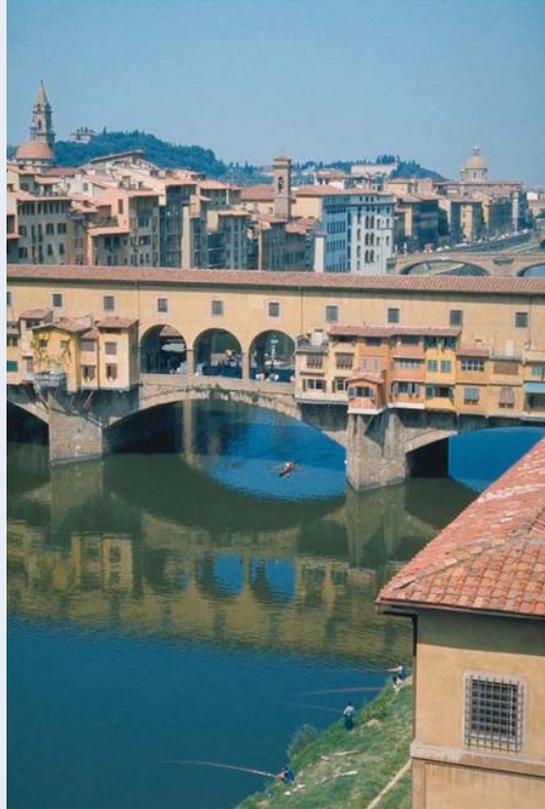
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