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EURO-THYMAIDE FP6 INTEGRATED PROJECT LHSB-CT-2003-503410

Final Publishable Executive Summary

Introduction

The Euro-Thymaide FP6 Integrated Project was launched in Brussels on 1 January 2004 and gathered 20 academic laboratories with the highest expertise in the exploration of thymus physiology, together with 5 biotechnology SMEs. The final objective of the project was the design of innovative approaches for diagnosis and treatment of autoimmune diseases based on new knowledge of thymus-dependent self-tolerance. Type 1 diabetes (T1D) had been selected as the prototype autoimmune disease tackled by Euro-Thymaide.

Before reporting about the achievements completed during this 5-year long Integrated Project, it is important to briefly summarize the state of knowledge at its initiation. This new knowledge had been essentially acquired from the previous studies performed by the academic members of the Euro-Thymaide Consortium.

❖ Through a variety of cell populations and molecules, the immune system protects the host from a world of infectious and cancer agents. During evolution, the first thymus appears in jawless fishes as a novel lymphoid organ playing a unique role in homeostasis of the immune system. In addition to differentiation and generation of thymus-dependent (T) lymphocytes (thymopoiesis), the thymus is responsible for the establishment of immunological self-tolerance, *i.e.* the inability of the immune system to aggress the host that it protects. The thymus ensures this essential function in two ways: 1) deletion (negative selection) of self-reactive T lymphocytes emerging during the intrathymic random recombination of gene segments encoding the variable parts of the T-cell receptor for antigen (TCR), and 2) selection of CD4+CD25+Foxp3+ natural regulatory T cells (nT_R) that are able to inhibit in periphery self-reactive T cells having escaped thymic negative selection.

❖ The discovery of the intrathymic transcription of many genes encoding neuroendocrine-related and other peripheral tissue-specific antigens had radically changed the common scientific view about the establishment of immunological tolerance to peripheral tissues. Before that discovery, the immunologists thought that peripheral antigens were sequestered from developing T cells in the thymus, and that tolerance to peripheral tissues/cells was primarily due to peripheral tolerogenic mechanisms. Consequently, '*back to central tolerance*' had become a leitmotiv in the field of immunology to understand the pathogenesis of autoimmune diseases that result from a failure of immunological tolerance to host tissue components.

❖ The AutoImmune REgulator gene/protein (AIRE) had been recently identified as the gene whose mutations are responsible for the APECED or autoimmune polyglandular syndrome 1 (APS-1), a rare monogenic disorder characterized by several endocrine deficiencies in the newborn. *Aire* invalidation in the mouse had also been shown to promote peripheral autoimmune processes in correlation with a decrease in intrathymic transcription of several genes coding for tissue-specific antigens.

❖ Finally, some preliminary experiments were arguing for a thymus dysfunction as a crucial factor in the development of organ-specific autoimmune diseases, including T1D.

Euro-Thymaide Consortium

Work Package (WP)	Partner (P)
WP1 Intrathymic self-antigen expression: analysis and translation into clinical studies	P1 Vincent GEENEN, University of Liege Center of Immunology, Belgium **(*)** P2 Bruno KYEWSKI, German Cancer Research Center, Heidelberg, Germany **(**)** P3 Georg HOLLÄNDER, University Children'Hospital of Basel, Switzerland P4 Ludger KLEIN, Ludwig-Maximilians University, München, Germany P5 Philppe NAQUET, Centre d'Immunologie Luminy, University of Marseille II, France P6 Ricardo PUJOL-BORRELL, Autonomous University of Barcelona, Spain P7 Pärt PETERSON, University of Tartu, Estonia **(**)** P8 Alexander MARX, University of Heidelberg, Germany P9 Hamish SCOTT, Institute of Medical and Veterinary Science, Adelaide, Australia P21 Mina VALTAVAARA, Fit Biotech, Tampere, Finland P23 Nicolai SCHWABE, ProImmune, Oxford, United Kingdom P25 Alain BOSSELOIR, Zentech, Liege-Sart Tilman, Belgium
WP2 Genetic control of thymic epithelial cell differentiation	P19 Thomas BOEHM, Max Planck Institute of Immunobiology, Freiburg, Germany **(**)** P20 Eric JENKINSON, University of Birmingham, United Kingdom P3 Georg HOLLÄNDER, University Children's Hospital of Basel, Switzerland P24 Lutz ZEITLMANN, Ingenium Pharmaceuticals, Martinsried, Germany
WP3 Role of thymic infection by coxsackievirus B4 in T1D development	P10 Didier HOBER, Centre Hospitalier Regional Universitaire, Lille, France **(**)** P1 Vincent GEENEN, University of Liege Center of Immunology, Belgium P11 Rafick-Pierre SEKALY, University of Montreal, Canada P25 Alain BOSSELOIR, Zentech, Liege-Sart Tilman, Belgium
WP4 Involvement of Cbl-b pathway in self-tolerance, autoimmunity and allergy	P12 Josef PENNINGER, Institute of Molecular Biotechnology, Vienna, Austria **(**)** P13 Isabella SCREPANTI, University La Sapienza, Roma, Italy P22 Hans LOIBNER, Apeiron Biologics, Vienna, Austria
WP5 Characterisation of Treg and design of T_R-based therapies against autoimmunity and allergy	P14 Fiona POWRIE, University of Oxford, United Kingdom **(**)** P15 Catherine HAWRYLOWICZ, King's College of London, United Kingdom P16 Dolores JARAQUEMADA, Autonomous University of Barcelona, Spain P17 Antonius ROLINK, University of Basel, Switzerland P18 Joost VAN MEERWIJK, INSERM Unit 563, Toulouse, France P4 Ludger KLEIN, Ludwig-Maximilians University, München, Germany P13 Isabella SCREPANTI, University La Sapienza, Roma, Italy
Dissemination and communication	P26 Jacques VISEUR, EuroTop, Brussels, Belgium
Financial management and coordination	David MUNTZ, Waterloo, Belgium

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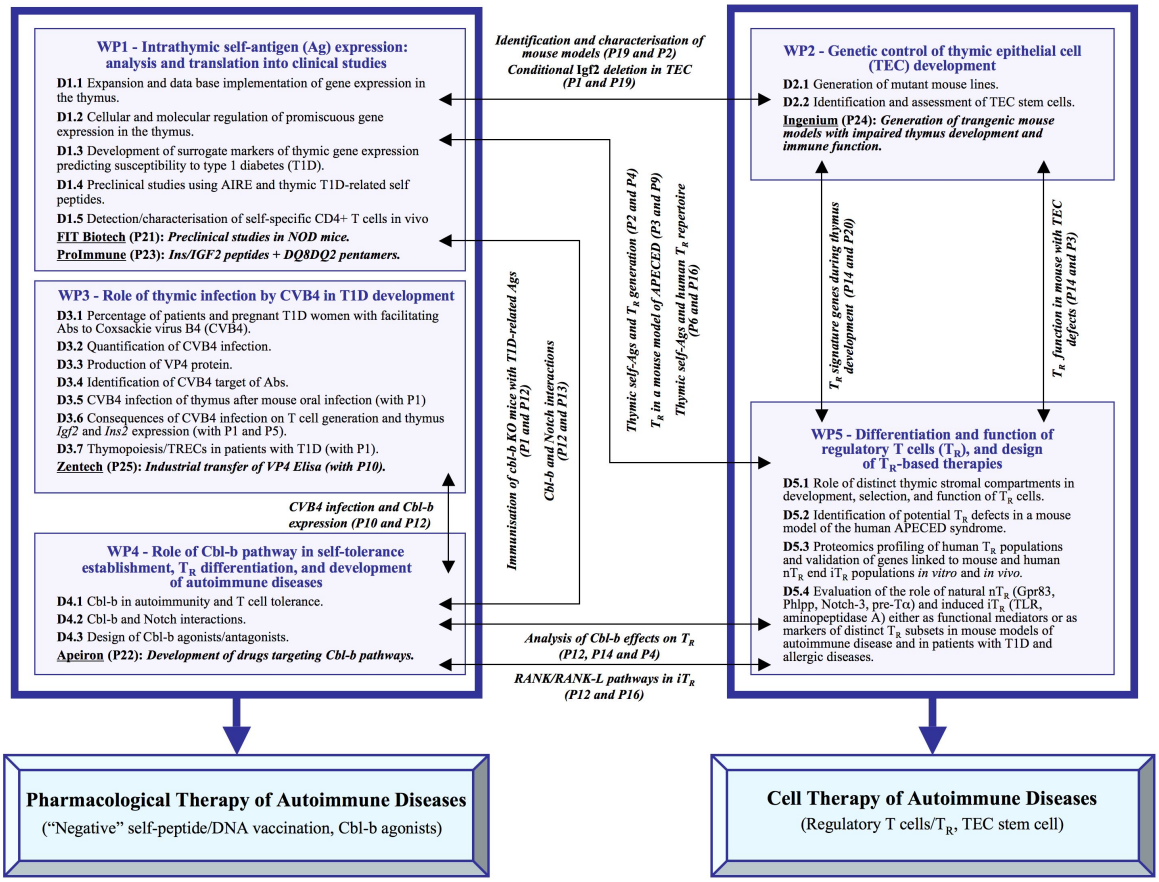
Flow-diagram of the Euro-Thymaide FP6 Integrated Project



Major scientific achievements

WP1 - The phenomenon of promiscuous gene expression (pGE) in the thymus has been established as a cornerstone of immunological self-tolerance. The scope of pGE has been more precisely delineated in medullary thymic epithelial cells (TEC) of mice, rats and humans. Genomic mapping of this gene pool showed significant genomic clustering and a high content of tissue-restricted antigens. These studies revealed high species conservation in size, composition and organization at the gene pool. Comparative analysis of pGE expression patterns at the population level and in single medullary TEC and peripheral tissues showed that the same genes are differentially regulated at both sites.

Our understanding of the role of AIRE in pGE has been refined with respect to its cellular expression pattern, its target gene pool, its influence on T-cell deletion, antigen presentation, T-cell migration, and its molecular action.

Studies on the role of pGE and central tolerance in 3 autoimmune diseases (T1D, myasthenia gravis and Graves' thyroiditis) uncovered common pathophysiological mechanisms, which led to a completely new paradigm. Genetic polymorphisms in the regulatory regions of target self-antigens indirectly influence the thymic transcription of these antigens by AIRE. These variations in the level of self-antigen display set threshold for self-tolerance and thus co-determine susceptibility to disease.

Certain thymomas (TEC malignancies) can now be viewed as disturbed microenvironments that still sustain T-cell development but fall short of proper tolerance induction due to defects in AIRE expression, pGE and delivery of tolerogenic signals.

Based on the finding that *Igf2* expression is required for full tolerance to insulin, the patent *'Thymus-based tolerogenic vaccine against T1D' WO2004019965* has been extended and delivered in Australia. New knowledge about the neuroendocrine control of thymus physiology is also the basis of a spin-off project supported by the Walloon Region of Belgium (*ThymUp* First Spin-off project).

WP2 - Previous studies in the group have shown that the forkhead transcription factor N1 (Foxn1) is responsible for initial TEC differentiation. The major impact contributed by this work package has been to identify Foxn1-dependent bipotent TEC progenitors capable of regenerating both cortical TEC and medullary TEC lineages in the thymus. The expression of RANK by thymic epithelium was shown to be essential for the generation of AIRE+ medullary TEC and induction of central self-tolerance. In parallel with this, the identification of key molecular regulators in the development of TEC provides the basis for future studies manipulating these cells in order to restore thymic function in either autoimmune disease conditions or in different kinds of thymus-dependent immune deficiencies (including age-related immunosenescence).

WP3 - Thymus infection with coxsackievirus B4 (CVB4) was shown to be associated with significant troubles in T-cell differentiation that could play a role in the development of the diabetogenic autoimmune process. Quantification of thymopoiesis in T1D patients has been initiated. The industrial transfer of the detection of 'facilitating' anti-CVB4 antibodies is under current progress with Zentech (P25). Vaccination against CVB4 could be another way of T1D prevention, particularly in countries with prevalence of CVB4 infections.

WP4 - The partners have further identified the Casitas B-cell lymphoma-b protein (Cbl-b) as a major gatekeeper that regulates CD28-mediated co-stimulation of T-cell response, controls immunotolerance as well as the response of effector T cells to T_R-mediated suppression, and controls spontaneous anti-tumour activity by endogenous T cells. *Cbl-b* inactivation in mice has revealed an essential role of this molecule in tolerance induction. Cbl-b is the first ubiquitin ligase ever found that has a direct effect on the immune system leading to an explosion of studies on the role of ubiquitination pathway in the control of the immune response. The results are under current translation for the development of novel drugs to activate the anti-tumour immune response (in collaboration with Apeiron Biologicals that is now a partner of Oncotyrol, a new research centre for personalized cancer medicine).

WP5 - As documented by active research in WP5, T_R cells represent a non-redundant host mechanism that controls homeostasis of the immune system. Therapeutic manipulation of T_R cells may be beneficial in a number of immune-mediated diseases including autoimmune and inflammatory diseases, organ transplantation and cancer. The results generated in WP5 have identified mechanisms of T_R generation in the thymus and periphery. Both cortical TEC and medullary TEC were shown to have the capacity to

preferentially drive antigen-specific T_R development, whilst the expression of the same antigen by thymic dendritic cells resulted in T_R deletion. Self-antigen expression in AIRE+ TEC was also shown to select antigen T_R populations in the thymus. In the intestine, CD103+ dendritic cells were shown to induce Foxp3 expression through a TGF-ß and retinoic acid dependent mechanism. Analysis of signalling pathways involved in TR function revealed an important role for Notch 3 and the pre-TCRa in the ability of nTR to suppress autoimmune diabetes, suggesting a link between Notch 3 signalling and Foxp3 expression. The knowledge of these mechanisms may be of use in terms of developing antigen-specific T_R focussed on distinct tissue sites in autoimmune diseases. In addition, studies on IL-10 secreting T_R induced by glucocorticoids and vitamin D3 have identified novel markers associated with these cells that may allow monitoring of these cells in asthma as well as pathways with which to manipulate their function.

Exploitable results

Five biotechnology were involved in the project (Zentech, Apeiron Biologicals, ProImmune Ltd,, Ingenium Pharmaceuticals and FIT Biotech. Two patents and one technological application (diagnostic test) are currently under commercial development.

As an example of applied knowledge, the involvement of the company ProImmune Ltd (UK), a research intensive biomedical SME, gave them the opportunity to complete the design of defined MHC multimers to detect T cells specific for autoimmune-related antigens. This application can be used to evaluate T-celll immunity in disease and in response to therapy, enabling investigators to accelerate their research and the discovery of new biopharmaceutical drugs in areas of major unmet needs, such as autoimmunity, cancer, infectious diseases and organ transplantation.

Dissemination and communication

Much other information including the complete list of scientific publications and video presentations for the lay people may be found on the website: http://www.eurothymaide.org/common/home.asp) that will remain open for a few years. During the project, more than 175 papers have been published, with some in most highly ranked journals such as *Science, Nature, Immunity, Annual Reviews in Immunology, Nature Reviews in Immunology, Nature Immunology, Nature Medicine, Journal of Experimental Medicine, Journal of Clinical Investigation, PNAS, Journal of Immunology, European Journal of Immunology, Immunological Reviews, Journal of Virology, Blood, Arthritis and Rheumatism, Diabetologia, American Journal of Pathology, Current Opinion in Immunology, Current Opinion in Pharmacology, Trends in Immunology, PLoS One…*

Gene expression data collected from studies of various partners have been gathered and deposited in a European Database of Thymic Self Antigens (EDTSA), which is publicly accessible (http://www.eurothymaide.org/common/EDTSA.asp). The database will be updated at regular intervals as a service to the community after closure of the Euro-Thymaide project.

Other interesting links:
http://www.eurothymaide.org/common/News.asp
http://www.eurothymaide.org/media/documents/Euro-thymaide_poster.pdf
http://www.eurothymaide.org/media/documents/Euro-thymaide_brochure.pdf

General conclusions

The Euro-Thymaide Consortium fostered in many cases collaborations between leading European research groups with complementary expertise. Cooperation at different personal levels and exchange of ideas, methods, data, transgenic animals and reagents resulted in accomplishments which otherwise would not have been possible. In this view, Euro-Thymaide represents a vivid example of the added value of such a research cluster.

The central results issued from the Euro-Thymaide FP6 Integrated Project may be summarised as follows:

1. Some 470 million years ago, mainly under the control of Foxn1, the thymus epithelium appeared with the specific charge of orchestrating the setting-up of immunological tolerance. This was a necessity because of the risk of auto-toxicity linked to the increasing level of diversity resulting from recombinase-dependent adaptive immunity. TEC, and in particular medullary TEC, are characterized by a specific property, *i.e.* promiscuous gene expression, that leads to the presentation of peripheral tissue-restricted self-antigens. By a still unexplained and paradoxical mechanism, presentation of self-antigens in the thymus is responsible both for deletion of self-reactive T cells that randomly emerge during T-cell differentiation and for selection of self-antigen specific natural T_R. The transcription of most self-antigens in the thymus is controlled by AIRE.

2. A primary or secondary thymus defect in the establishment of self-tolerance is responsible for the development of autoimmune diseases such as type 1 diabetes. This defect is mainly characterised by a decrease in self-antigen expression and/or presentation in the thymus to developing T cells. This thymus-specific defect leads to an enrichment of the peripheral T-cell repertoire with self-reactive effector T cells, as well as by a decrease in the generation of self-antigen specific nature T_R.

3. Further identification of thymic self-peptides, as well as targeting of Cbl-b mediated pathways and mechanisms involved in the generation of self-antigen specific T_R should lead in the very near future to the development of novel therapeutic strategies aiming to reinstall immunological self-tolerance that is absent, already in foetal life, at the initiation of many autoimmune diseases.