Next-generation infertility research

Professor Andreas Meinhardt gives an insight into his research on infertility and how his group is developing talent through German-Australian collaboration

What attracted you to the study of infertility?

I was attracted to this field as a graduate student when I received my training in cell biology while working on issues related to male fertility. I was intrigued by the complex differentiation steps leading to the production of spermatozoa, which are passively transported to the epididymis where they collect in a single duct. During the long journey through this duct, sequential biochemical steps occur that prepare them for their journey into the female tract to fertilise the egg. I still find it fascinating trying to understand these mechanisms and their impediments.

Could you briefly explain what the *in vivo* biotin tagging system is and how this aids the study of identifying macrophage migration inhibitory factor interacting proteins?

The method was not invented by us, but it is very useful for identifying interacting partners of proteins in live cells. Dr Jörg Klug from my team has adapted the method to find interacting partners for macrophage migration inhibitory factor (MIF), an important protein in many diseases. With this approach, MIF is produced in the cell with a tag – think of it as a 'hook' which pulls MIF out of the cell together with direct and indirect binding partners. As a main task we tried to understand what they do to MIF function. One (RP S19) seems to act as an endogenous inhibitor which makes it attractive as a counterbalance to increased MIF levels, as they typically are for many diseases.

Your research suggests testosterone is important in the maintenance of immunological balance in the testes. What role does testosterone play in this regard?

Testosterone, the main male sex hormone, has an anti-inflammatory capacity. This is one reason why men are thought to suffer less from autoimmune disease where an uncontrolled immune response takes place. The testosterone concentrations in the testes where it is produced are 10 times higher than in serum. Serum values drop during organ-specific and general inflammation. A junior research group in our team led by Dr Monika Fijak with Dr Magdalena Walecki as a postdoc is undertaking research to find out whether a restoration of reduced testosterone concentrations may improve the course of infection or inflammation-based fertility impairment.

Could you explain how the Giessen-Monash international research training group, a special German-Australian collaboration, came into being and where it stands? What are the key achievements of this partnership?

Our group has a longstanding relationship with Monash University in Melbourne which originated at the time of my postdoctorate there. With several groups at Monash and Giessen working in closely related fields, it was obvious that a more formalised and expanded cooperation would be beneficial to all involved. Interestingly, the German Research Foundation (DFG) offers a funding scheme named International Research Training Groups (IRTGs). In this scheme a German and an international partner both contribute funds to support projects for PhD students. These projects depend on international cooperation and aim to exchange students for longer periods to each partner's lab. Applications are highly competitive and we will be reviewed in the coming weeks. If successful in our application, PhD students at the end of their thesis should receive their award from both universities – so-called 'double badged' PhD titles.

The partnership focuses on young researchers/graduate students. Why is this such a crucial component?

First of all, it is an essential requirement of the DFG and central aim of an IRTG that young researchers, and particularly PhD students, are involved. Their research is accompanied by a special qualification programme which is complementary in nature so that they can cover topics at Monash they cannot learn here, and vice versa. In essence, our ultimate goal is to build the next generation of scientists in this field.

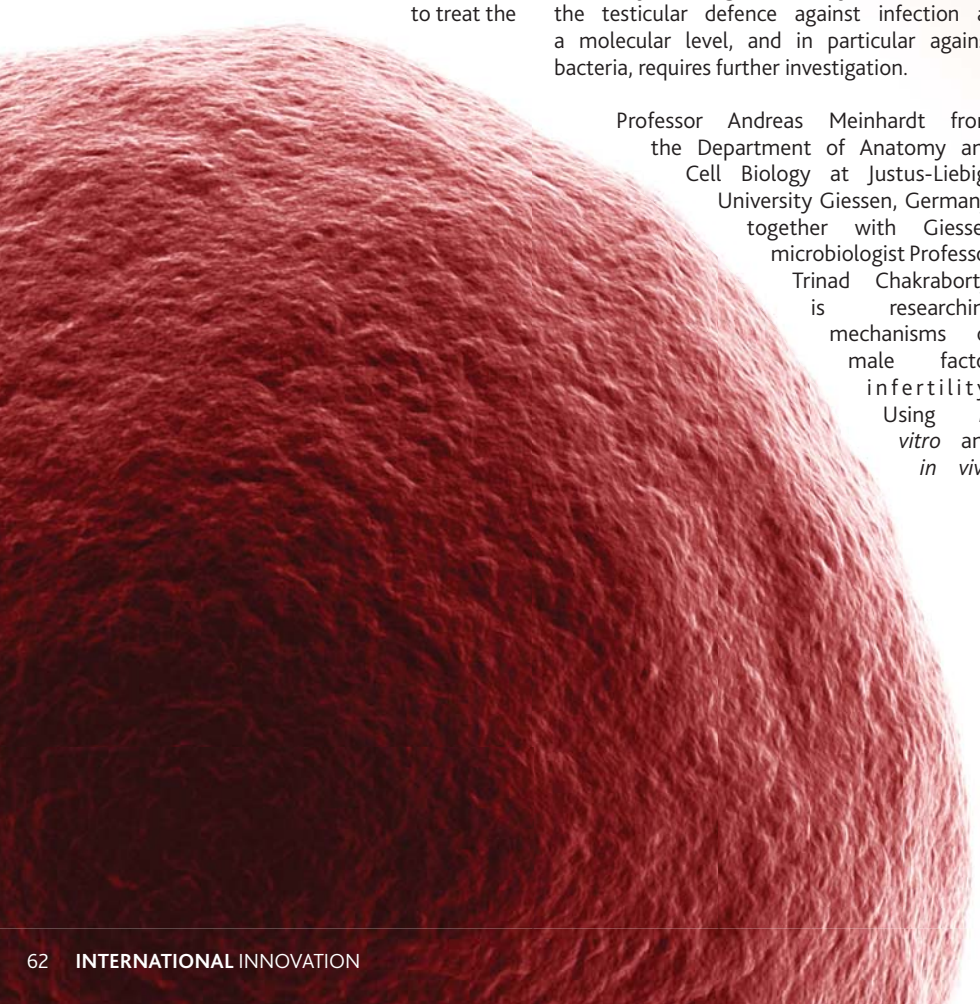
You recently received the prestigious J Christian Herr Award for Excellence in Reproductive Immunology from the American Society of Reproductive Immunology. What did you receive the award for?

The award is given annually to contributions in this area. Most of reproductive immunology revolves around implantation of the embryo and the placenta, with past awards going to researchers in this field, and this time round the committee obviously considered the male aspects of fertility. It should be mentioned that although this award was technically received by one person, it was many years of teamwork which led to our contributions and success, so I see it as a real acknowledgement of our team's efforts.

Tackling male infertility

Professor Andreas Meinhardt's research into the mechanism of impairment of testicular function by immune evasive activity of uropathogenic *Escherichia coli* looks to combat infections and falling fertility rates in men

APPROXIMATELY ONE IN SEVEN couples worldwide are affected by infertility and while the cause of the problem remains controversial, in nearly half of cases it can be attributed to the male. The incidence of male factor infertility in the general population is approximately 7 per cent, making it more prevalent than Types 1 and 2 diabetes combined, and this proportion is expected to rise. Roughly a third of infertile men exhibit idiopathic infertility – that is, infertility whose origins are unclear – and approximately half are unable to father children without some form of assisted reproductive technology, the most successful method of which is intracytoplasmic sperm injection, which carries an approximate 25 per cent success rate per cycle. This outcome is significant considering the financial and psychological costs to the 75 per cent of couples for whom the treatment fails. Above all, the need to treat the female partner for what is essentially a male problem remains a major shortcoming of current therapies.



GERM CELL LOSS

According to the World Health Organization, some 13-15 per cent of all male infertility cases can be attributed to bacterial infection or inflammation of the genital tract, manifesting as urethritis, prostatitis, epididymitis or epididymo-orchitis; with the latter seeing over 600,000 cases annually in the US alone. Acute testicular inflammation is symptomatic, whereas sub-acute or chronic disease is mostly asymptomatic, and even successful interventionist therapies can fail to prevent a silent inflammatory reaction in the testes which leads to functional and structural damage of the seminiferous tubules, ultimately resulting in infertility. Understanding the testicular defence against infection at a molecular level, and in particular against bacteria, requires further investigation.

Professor Andreas Meinhardt from the Department of Anatomy and Cell Biology at Justus-Liebig-University Giessen, Germany, together with Giessen microbiologist Professor Trinad Chakraborty, is researching mechanisms of male factor infertility. Using *in vitro* and *in vivo* models, his work focuses on the molecular mechanism of impaired spermatogenesis caused by uropathogenic *Escherichia coli* (UPEC) infection of the testes and epididymis, and the genes responsible for the pathogenicity of UPEC on testicular cells (led by Dr Sudhanshu Bhushan) and sperm in epididymis (led by Dr Tali Lang). UPEC is the most common of pathogens to cause acute and chronic bacterial genitourinary tract infections in men, yet despite its ability to adversely affect and impair spermatogenesis, the testes' defence against this infection is still poorly understood: "In cooperation with local, national and international partners, our research aims to unravel the pathomechanisms which lead to male infertility as a consequence of an infection or autoimmune process," he outlines. "The aim of our studies is to define the essential targets involved in bacterial infection in the testes."

IMMUNE PRIVILEGE

Understanding how immune cells such as testicular macrophages respond to bacterial pathogens, as well as how such pathogens manipulate our defence pathways, is therefore crucial. Comparing various testicular cell types, Dr Bhushan from Meinhardt's team has discovered that Sertoli cells – the 'mother' or 'nurse' cells of the testes located in the seminiferous tubules, which nourish the developing sperm throughout spermatogenesis – react to UPEC infection by activating the Toll-like receptor 4 pathway (TLR4). These pathways play a vital role in pathogen recognition and the activation of innate immunity against microbial infection. Interestingly, activation of the TLR4 pathway is initiated but not completed, thus the cells sense the bacteria, but are unable to adequately react and combat the pathogens as cytokine production is suppressed.

It is assumed that undiagnosed previous infections or autoimmune processes might be responsible for infertility in many cases, and Meinhardt's research aims to elucidate the effects of this 'immune privilege' – a condition thought to arise from the need to prevent immune responses against antigens of meiotic

and haploid germ cells which first appear in the testes at puberty. Paradoxically, in later life the balance can tip in the other direction, with infection and inflammation causing severe damage to the testes. "The testicular immune environment is quite unique, being tolerant to molecules that first appear in puberty and long after the establishment of self-tolerance around birth," Meinhardt explains. "If the testes were less immune-tolerant, men would experience constant inflammation, with sterility as a consequence."

SYNERGY

The UPEC project is one of two group-funded projects promoting excellence in science in a programme of the German state of Hesse called LOEWE-MIBIE which deals exclusively with infection and inflammation in the male tract, bringing together clinicians, microbiologists, veterinarians and others with exciting opportunities that improve understanding, diagnosis and treatment.

Primarily being a locally well-linked project at Justus-Liebig-University Giessen, the work is now being extended to Monash University in Melbourne, Australia. "For our long-term research plans, we are currently trying to build a group funding scheme with Monash University in Melbourne, which, if successful, could tie us together for many years," reveals Meinhardt. His research also takes him all over the world to universities in Australia, Finland and the USA, among others: "Progress is much quicker if several partners pull at the same string," he reflects.

Despite the high incidence of male reproductive disorders and many groups working in the field, only a relative few larger, internationally-competitive centres are devoted to addressing this topic in concerted action. These include Münster, Rome, Florence, Copenhagen, Malmö, Edinburgh and two or three centres in the USA, as well as Giessen and Melbourne. The kind of complementarity and synergy required to solve the obvious medical need is possible only by the cooperation of large centres such as that seen through the Giessen-Monash link.

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"For both the Australian and German teams, the combining of resources in graduate education is an ideal means of advancing the professional expertise of young researchers through the exchange of ideas, methods and international experience", explains Professor Kate Loveland, the Monash coordinator of the programme. Each student shall benefit from a minimum of 12 months spent between the German and Australian laboratories, and as both institutions rank among the world's largest and most productive research centres specialising in male reproduction, their alliance can only further strengthen existing scientific ties and improve the quality of their output. The resultant network could form an ideal basis for future collaborative grant applications across Australia and the European Union.

IMPROVED DIAGNOSIS

Meinhardt believes that the future will take his work in several directions, with one important avenue being the development of a test for improving diagnoses of clinically-silent, painless impairment of spermatogenesis: "To this end we are cooperating with andrologists and urologists in Giessen, Münster and Bonn on the clinical aspects, and our transfer agency, Transmit, which helped us to find finance and an industry partner to develop a test fulfilling all necessary industry standards, is proving a great help".

A final multi-centre effort will be required to demonstrate the suitability of the test as the team attempts to understand the precise constitution of the uniquely immune-tolerant environment in the testes, and why it is so prone to error both in infectious and autoimmune diseases.

INTELLIGENCE

MECHANISM OF IMPAIRMENT OF TESTICULAR FUNCTION BY IMMUNE EVASIVE MECHANISM OF UROPATHOGENIC *ESCHERICHIA COLI*

OBJECTIVES

The research attempts in cooperation with local, national and international partners to unravel the pathomechanims that are leading to infertility in men as a consequence of infection or autoimmune processes.

KEY COLLABORATORS

Germany:

Professor Chakraborty; **Dr Tchatalbachev**; **Dr Hossain**, Microbiology, Giessen • **Professor Weidner**, Urology and Andrology, Giessen • **Professor Engel**, Göttingen • **Dr Mallidis**, Münster

Rest of the world:

Professor Bucala, Yale University, New Haven, USA • **Professors Loveland**; **Professor Hedger**, Monash University, Melbourne, Australia

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PROFESSOR ANDREAS MEINHARDT

obtained his PhD in 1994 from Philipps-University of Marburg. After a postdoctoral stint at Monash University in Melbourne, Australia, he returned to Marburg where he lectured up to 2001. Meinhardt has since been Professor of Anatomy and Cell Biology at Justus-Liebig-University Giessen, where his research interest in male reproductive processes continues to this day. He recently received the J Christian Herr Award for Excellence in Reproductive Immunology from the American Society of Reproductive Immunology.

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN