

Regulatory T cells in inflammatory and infectious diseases: new horizons for old friends

Friday, March 05, 2010 8:45 am - 5:15 pm

Kennedy Lecture Theatre
UCL Institute of Child Health
30 Guilford St
London WC1N 1EH
United Kingdom

"This meeting will provide an update on phenotypic and functional aspects of regulatory T cells, aiming to inform, educate and entertain. Presentations will be delivered by world-class leaders in their respective fields and a lively discussion will follow each series of talks. Coupled with the pleasant ambience and modern facilities of the venue, this event promises to be one not to be missed"
Meeting Chair: *Dr Oliver Garden*, The Royal Veterinary College, London, UK

Abstract submissions are invited from PhD students and junior post docs (within three years of PhD graduation), from which several will be chosen to be presented as posters and three to be presented orally. Of the three best abstracts chosen for oral presentation, the audience will vote on the best overall talk, for which the presenter will be awarded a prize of a year's subscription to *Nature Reviews Immunology* or *Mucosal Immunology* (www.nature.com/mi) at the conclusion of the day. There will also be a runners up prize.

This meeting has CPD accreditation

- 8:45 – 9:15 Registration
- 9:15 – 9:25 Introduction by the Chair: *Dr Oliver Garden*, The Royal Veterinary College, London, UK
- 9:25 – 10:10 **The influence of the tumour environment on regulatory T cells**
Dr Jian-Guo Chai, Imperial College London, UK
Regulatory T cells function to dampen pathogenic immune responses and were originally identified by their role in preventing autoimmunity. Tumour antigens are also self-antigens and tumour specific immunity can be compromised by the activity of regulatory T cells. To investigate the influence of the tumour environment on regulatory T cells, we have developed models in which tumour specific or non-specific regulatory T cells are transferred into tumour bearing hosts. Using this approach, we have examined regulatory T cell expansion, the role of tumour derived TGF β , stability of Foxp3 expression, development of inducible regulatory T cells and the impact of vaccination.
- 10:10 – 10:55 **Harnessing regulatory T cells for therapy in rheumatoid arthritis**
Professor Michael Ehrenstein, Professor of Experimental Rheumatology, Windeyer Institute, University College London, UK
Regulatory T cells (T reg) from patients with RA have defective suppressor function. Following anti-TNF- α (infliximab) treatment, Treg from RA patients appear to regain their suppressive function, but only in those individuals who respond to therapy. However, infliximab, rather than restoring the defect in Treg, induces the differentiation of a distinct and potent population of CD62L⁻ Treg from responder T cells. The suppressive capability of these CD62L⁻ Tregs is dependent upon TGF- β and IL-10, unlike Tregs from healthy individuals which suppressed through cytokine independent mechanisms. Our recent results suggest that defects in CTLA-4 could contribute to abnormal Treg function in RA, and may represent a target for therapy to induce long lasting remission.
- 10:55 - 11:00 **Speakers' photo**
- 11:00 – 11:20 **Mid-morning break and poster viewing**
- 11:20 – 12:05 **Induction and function of antigen specific Tr1 cells in human diseases**
Dr. Silvia Gregori, San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Italy
Adaptive IL-10-producing regulatory T (Tr1) cells develop in the periphery upon chronic antigen-stimulation in the presence of IL-10 secreted by tolerogenic APC. Tr1 cells are required for tolerance induction to self- and non-self antigens. Indeed high percentages of allo-specific Tr1 cells, with regulatory functions, are present in peripheral blood of patients who developed persistent chimerism after allogeneic hematopoietic stem cell transplantation. We recently identified a population of human tolerogenic DC, called DC-10, present *in vivo* and inducible *in vitro*, that are powerful inducers of antigen-specific Tr1 cells. DC-10 can be used to generate *ex vivo* Tr1 cells for cell therapy to modulate pathological immune-responses to different antigens.

- 12:05- 12:20 **Cell Processing tools for preclinical and clinical studies in immunotherapy and regenerative medicine**
Dr Catherine Barjot, **CellGenix**, Germany
 CellGenix is a German biopharmaceutical company that develops, produces and markets research and GMP grade cell processing tools for cell therapy. CellGenix was founded in 1994 as a spin-off of the University Medical Center of Freiburg. Initially focused on immunotherapy, CellGenix has developed serum free culture media and cytokines for culture and expansion of human T cells, NK, Dendritic Cells and Hematopoietic Stem Cells. The CellGenix serum free media and a lot of the CellGenix cytokines such as IL-15 and IL-7 are available as GMP grade products.
- 12:20 – 12:30 **Characterisation of umbilical cord blood derived regulatory T cells**
Sima Hirani NHS Blood and Transplant, Colindale, UK
- 12:30 – 12:40 **Evidence for natural regulatory T cells in the horse and their decline in aged individuals**
Melissa Robbin, **The Royal Veterinary College**, London, UK
 Tregs have been described in numerous species such as humans, mice, cats, dogs and pigs. We have recently identified appropriate reagents to identify Tregs in horses using CD4 and FOXP3 as markers. We observed an increase in FOXP3 expression after stimulation, and a decrease in expression in aged individuals within CD4+ lymphocytes. We further attempted to phenotypically characterise these Tregs by analysing cytokine secretion after stimulation. Further research aims to demonstrate suppressive function of these equine Tregs using suitable antibodies that detect equine CD25, which will aid in highlighting species-specific differences that exist within Tregs.
- 12:40 – 12:50 ***Helicobacter pylori*-induced protection from allergy is associated with peripheral blood regulatory T cells**
Dr Emily Staples, **Nottingham Digestive Diseases Centre**, Nottingham, UK.
- 12:50 -14: 00 **Lunch and Networking**
- 14: 00 - 15:00 **Keynote address: Regulatory T cell control of pathogenic and effector T cells - implications for cancer and autoimmunity**
Professor Kingston Mills, Professor of Experimental Immunology and Head School of Biochemistry and Immunology, Trinity College Dublin
 IL-17-producing CD4⁺ T cells (Th17 cells) play a pathogenic role in organ specific autoimmune diseases and function with Th1 cells to mediate protective immunity to pathogens. The differentiation of Th17 cells from naïve T cells is promoted by IL-6, IL-21, IL-1 β and IL-23. Furthermore, IL-1 α or IL-1 β in synergy with IL-23 can promote IL-17 secretion from memory T cells and $\gamma\delta$ T cells. Regulatory T (Treg) cells can suppress pathogenic T cells directed against self-antigens and thereby prevent autoimmunity, but also limit collateral damage during immune responses to infection. We are examining the balance between Th17 and Treg cells, and the consequences of its disruption, in mouse models of infection, hitherto considered to be dominated by Th1 or Th2 cells. This information is being used to design approaches for selective activation of Treg cells, which can inhibit Th17 cells that mediated autoimmunity, or for selective inhibition of Treg cells, to enhance effector Th1 cells and thereby develop more effective immunotherapeutics and vaccines against infection or cancer.
- 15:00-15:30: **Afternoon break and last poster viewing**
- 15: 30-16:15 **Effector function and regulation in bacterial sepsis**
Professor Daniel Altmann, Professor of Immunology/Deputy Head of Department in the Department of Infectious Diseases and Immunity, Imperial College London, UK
 Group A streptococcal (GAS) infections can manifest as a range of presentations from sore throat to scarlet fever, rheumatic fever, septic or toxic shock and necrotizing fasciitis. Many GAS genomes harbour genes encoding superantigens, accounting for some aspects of the immune/inflammatory phenotypes associated with infection. For this reason there has been considerable focus, both in humans and mouse models, on analysing the balance between microbial and immunological contributions to pathogenesis. Gram-positive sepsis is a highly pertinent setting for questions of immunopathology versus host defense, constituting a significant clinical problem with respect to high mortality and a paucity of effective immune-therapeutics: mortality in septic shock is in the 40-70% range. As one might expect for a pathogen capable of inducing shock through an excessive inflammatory response, the acute response to infection encompasses increases in both effector T cell and Treg populations. Transcription factors associated with Tregs as well as Th1, Th2 and Th17 profiles increase strongly with the first hours after infection. Particularly important in this initial response seems to be the IL-17 response derived predominantly from gamma delta T cells.

16: 15-17:00

Barriers and challenges in regulatory T cell therapy for solid organ transplantation

Professor Giovanna Lombardi, Division of Medicine, King's College London, UK

Naturally arising CD4⁺CD25⁺ regulatory T cells play a pivotal role in the prevention of autoimmunity. Strategies are under development to establish ways of expanding Tregs in vitro for clinical use to achieve tolerance in autoimmunity and transplantation. In the last few years, we have built a platform of data in murine models which support the hypothesis that Tregs are very efficient in inducing allograft survival. However, we and others have shown recently that human Tregs under inflammatory conditions are prone to conversion to proinflammatory Th17 cells, which have been shown to be key effector cells in autoimmune responses and in graft rejection.

In this study we have investigated the mechanisms of human Treg to Th17 conversion. We have shown that the major cytokine involved in the conversion of human Tregs to Th17 in vitro is IL-1 β and that this effect is mediated by a shift from functional activation of STAT5 to STAT3. This conversion was critically dependent on STAT3 as Tregs from human patients with dominant-negative mutations in STAT3 did not produce IL-17 in response to IL-1 β . Despite the role of IL-6 in activating STAT3, this cytokine did not convert Tregs to Th17. Analysis of SOCS proteins demonstrate that IL-1 but not IL-6 could promote a permissive environment for SOCS protein. Our data provide mechanistic insight into the actions of IL-1 β induction of Treg to Th17 conversion. Different strategies are now under investigation to identify molecular difference between Tregs pre- and post-conversion, to prevent their subversion.

The success of these strategies will influence the design of the first trial in which Tregs will be used in organ transplantation, where inflammation is present by avoiding the risk of conversion to IL-17 producing cells.

17:00-17:15

Chairman's summing up

This meeting was organised by Euroscicon (<http://www.euroscicon.com/>), a team of dedicated professionals working for the continuous improvement of technical knowledge-transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry.

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About the Chair

Dr Oliver Garden graduated from the Royal Veterinary College (RVC) in 1993, having completed an intercalated BSc at King's College London. Subsequent clinical and scientific training followed, with both a rotating internship in small animal medicine and surgery and a PhD in gastroenterology and immunology at the RVC; a Research Training Fellowship in molecular biology at the University of South Carolina Medical School; a Residency in Small Animal Internal Medicine at Cornell University; and a Wellcome Trust Advanced Fellowship in cellular immunology at the Hammersmith Campus of Imperial College London (ICL). From 2004, Oliver has held a tenured appointment at the RVC and an honorary appointment at ICL. His research focuses on regulatory T cells and autoimmune disease.

About the Speakers

Professor Daniel Altmann is Head of the Human Disease Immunogenetics Group and Professor of Immunology/Deputy Head of Department in the Department of Infectious Diseases and Immunity, Imperial College London.

He worked for a number of years in the area of CD4 T cells and HLA class II in disease models. He moved to Imperial College in 2001 following several years as a tenured scientist with the Medical Research Council Clinical Research Centre. Prior to that he worked at Imperial Cancer Research Fund, the Weizmann Institute of Science and University of Bristol.

Professor Michael Ehrenstein leads a research group investigating the immunoregulation of autoimmune rheumatic disease and is particularly interested in how novel therapies modulate the autoimmune response in the context of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). The use of novel biologic therapies represents an important tool to understand the aberrant immune responses found in patients with autoimmunity. In this context, his research group has been studying the phenotype, functional and molecular characteristics of regulatory T cells in patients with RA and SLE before and after therapy. He has also been focusing on the pathogenic and regulatory properties of B cells in patients with SLE as well as utilizing animal models to investigate the tolerogenic nature of secreted IgM.

Melissa Robbin studied at the University of Glasgow, where she completed a BSc Honours degree in Immunology. She is currently in her second year of her PhD at the Royal Veterinary College researching the effects of equine trophoblast cells on T regulatory cells.

Professor Kingston Mills is The Professor of Experimental Immunology and Head of The School of Biochemistry and Immunology, Trinity College Dublin. He trained at University College London and NIMR Mill Hill, before joining the Scientific Staff of NIBSC Herts, UK. He returned to an Academic post at National University of Ireland, Maynooth in 1993, where he was Director of The Institute of Immunology. He was appointed to a Personal Chair at Trinity College Dublin in 2001. He heads a research team of over 20 scientists focusing on immune regulation and T cells in infection, cancer and autoimmunity.

Dr Jian-Guo Chai graduated from medical school in 1986 (Shandong, China), then completing an MSc course (1989; Beijing, China) and a PhD degree (1995; Tokushima, Japan). He started his first post-doc within the Department of Immunology, Royal Postgraduate Medical School (1995-2000), and undertook a second within the Transplantation Biology Group, Clinical Sciences Centre, MRC (2000-2003), before becoming a Principal Investigator (2003-2006) and a Senior Cancer Research Fellow funded by Cancer Research UK (2006-2012). Dr Chai is currently head of the Cancer Immunotherapy Group within the Section of Immunobiology, Department of Medicine, Imperial College London, and his main research interest is tumour immunology, especially T cell subsets in response to tumours and T cell gene therapy for treating cancer in pre-clinical mouse models.

Dr Catherine Barjot obtained a PhD in Biology in France. She started working in gene therapy as a research fellow at the University of Michigan in the United States. After returning to France, Catherine Barjot worked for a biotech focused on cell therapy where she contributed to setting-up a Dendritic Cells-based clinical trial. During this time Catherine Barjot became familiar with CellGenix where she purchased cytokines for this clinical trial. In 2005, Catherine Barjot started working for CellGenix. Drawing on her experience with CellGenix products, Catherine Barjot became the area sales manager for ex-vivo therapeutics for western Europe.

Professor Giovanna Lombardi obtained her degrees (BSc and PhD) in Rome working on the regulation of human T cell responses to *Candida albicans*. In 1987 she moved to London. During the first few years she elucidated the molecular basis of allorecognition of MHC molecules. In more recent years she has focused on the understanding of the mechanisms involved in peripheral tolerance. Recently, she was involved in the discovery of CD4⁺CD25⁺ Tregs in the human. Two years ago she moved to KCL from Imperial College and is now studying the phenotype and function of Tregs and the use of alloantigen-specific Tregs for immunotherapy in transplantation. Recently she has also developed an interest in the differentiation, function and manipulation of human dendritic cells.

This meeting has been organised in collaboration with the



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EVIDENCE FOR NATURAL REGULATORY T CELLS IN THE HORSE AND THEIR DECLINE IN AGED INDIVIDUALS

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T regulatory lymphocytes (Tregs) play an important role in transplantation, pregnancy, infectious diseases and autoimmunity. Tregs constitutively express cell surface markers CD4 and CD25 and the intracellular transcription factor FOXP3 as described in numerous species: humans, mice, pigs, dogs and cats. We have recently identified appropriate reagents to characterise Tregs in the horse. The aim of this study was to determine the phenotype of Tregs in the peripheral blood of healthy horses.

Peripheral blood mononuclear cells (PBMC) were isolated from healthy horses of mixed ages, sexes and breeds (n=18). Cells were either analysed immediately, or cultured in the presence or absence of 2.5 µg/ml pokeweed mitogen for 24, 48 or 72 hours. Cell surface labelling was performed using antibodies directed against equine CD4, CD8 and LFA-1. Intracellular labelling was performed using antibodies directed against human FOXP3 (PCH101), bovine interferon gamma (IFNG) and equine interleukin 10 (IL10). Labelled cells were analysed by flow cytometry following lymphocyte gating.

A mean of 2.2 % of CD4+ cells (n=18) and 0.5 % of CD8+ (n=14) expressed FOXP3. All FOXP3 positive cells also expressed LFA-1. Within the CD4 population, there was no statistical difference in FOXP3 expression between males and females, and horses and ponies. FOXP3 expression was significantly decreased in horses aged 15 years and above when compared with those aged 6 and under. FOXP3 expression was induced in CD4 cells upon cell activation (n=4). A peak in FOXP3 expression was observed at 72 hours, that represented a 4-fold increase when compared with non-stimulated cells. To further investigate the phenotype of the cells with induced FOXP3 expression, we performed double intracellular staining for FOXP3 combined with IFNG or IL-10. A small number of the FOXP3+ cells also expressed IFNG or IL-10, with the majority of FOXP3+ cells IFNG and IL-10 negative.

These data provide evidence that nTreg exist in the horse, comprising 2.2% of CD4 cells. The number of FOXP3+ CD4+ cells decreased in aged horses, which is in contrast to studies in humans and mice that have shown an observed increase in FOXP3+ expressing CD4+ lymphocytes in aged individuals. Equine FOXP3 expression was induced in CD4+ lymphocytes *in vitro*, similar to that observed in studies in human cells. Finally, equine FOXP3+ lymphocytes that did not express either IL-10 or IFNG may encompass nTregs, with those expressing IL-10 reflecting iTregs, which are reported to secrete cytokines to exert their suppressive function. FOXP3+IFN γ + lymphocytes may reflect activated cells.

These studies provide the first characterization of Tregs in the horse and are essential to future studies that will address the role of Tregs in equine diseases, for example responses to endotoxin, for which the horse is particularly sensitive. Current studies are focusing on demonstrating suppressive function of equine Tregs via the identification of suitable antibodies that detect equine CD25. These studies also highlight both species differences and similarities in phenotypic characteristics of Tregs. Further understanding of the equine immune system will aid in comparative mammalian studies, shedding light on both the evolution, and behaviour, of Tregs.

CHARACTERISATION OF UMBILICAL CORD BLOOD DERIVED REGULATORY T CELLS

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Introduction Umbilical cord blood (UCB) is recognised to be a valuable alternative to bone marrow (BM) as a source of haematopoietic stem cells (HSC). The occurrence of Graft-vs-Host Disease (GvHD) after UCB transplantation has been reported to be less severe in comparison to BM transplants. In addition to the naive state of immune cells, the action of immuno-suppressive cells such as regulatory T cells (Treg) may contribute to the positive aspects observed in UCB transplants. This study investigated the phenotypic and functional characteristics of UCB Treg and their potential for expansion in culture. If expanded Treg retain their regulatory function they may prove to be a useful clinical source in immunotherapy.

Methods & Results Six-colour flow cytometry was used to determine markers of Treg definition (CD4, CD25 and CD127), function (FOXP3, CD152 and CD39), and naive status (CD45RA/RO, CD95 and CD31). Preliminary results show that both UCB and Adult peripheral blood (AB) contain a population of putative Treg, defined as CD4+FOXP3+CD25+CD127^{low} cells, percentages of these cells were equal in UCB and AB. UCB Treg purification using magnetic beads yields a population which is highly pure (85%) for CD4+CD25+FOXP3+CD127^{low} and displays suppressive abilities. UCB contains higher percentages of naive (>50% CD45RA+ and 90% CD31+) Treg, in comparison to AB Treg (>90% CD45RO+ and CD31-). The expression of IL-2, IFN- γ , or IL-17 has not been detected in these isolated populations. However, UCB Treg cells have increased levels of IL-10 production in comparison to AB Treg. Expansion of UCB Treg, using IL-2 (300U/ml) and anti-CD2/CD3/CD28 beads, has yielded a 325-500 fold expansion over a 3 week period. UCB Treg display lower expansion rates than those reported for AB under the same conditions. Expanded UCB Treg displayed suppressive abilities, which were more potent than before expansion. UCB Treg did not produce IL-2 or IL-17 after expansion, which implies there is no detectable conversion of Treg into inflammatory cells during expansion.

Conclusion Cells from UCB display different properties to those of AB. The characterisation of UCB Treg may clarify the cellular interactions in clinical settings in which UCB is currently used and highlight potential future uses.

HELICOBACTER PYLORI-INDUCED PROTECTION FROM ALLERGY IS ASSOCIATED WITH PERIPHERAL BLOOD REGULATORY T-CELLS.

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Introduction

The Gram-negative bacterium *Helicobacter pylori* (*Hp*) is the major cause of peptic ulceration and gastric cancer, but disease only occurs in around 10-15% of cases. *Hp* induces a strong gastric Treg response [1-4], which downregulates the mucosal inflammation, facilitating persistence of the infection. Epidemiological studies show that *Hp* infection is inversely associated with atopy, asthma and allergy [5-7]. We hypothesized that a systemic Treg response to *Hp* is linked to protection against allergy, and aimed to characterize this Treg response and examine correlations with serum IgE levels. We also used an animal model to determine if *Hp* infection could indeed influence allergy.

Methods

Samples were donated by 49 *Hp*⁺ and 58 *Hp*⁻ consenting adult patients undergoing upper GI endoscopy. *Hp* status was determined by biopsy urease test, *Hp* culture and histopathology. Blood samples were collected, plasma separated, and peripheral blood mononuclear cells (PBMCs) purified. PBMCs were cultured with *Hp* lysate antigen, or PMA/ionomycin (positive control) or with medium alone (negative control), then stained for Treg markers and analysed by flow cytometry [1]. IgE concentrations in the plasma samples were determined by ImmunoCAP.

Ten C57BL/6 mice were infected with the B128 7.13 *Hp* strain and ten received oral doses of vehicle only. One month later five infected and five uninfected mice received intra-nasal (i.n.) doses of house dust mite extract followed by a challenge i.n. dose of purified Der p 1. The remaining five control animals in each group received i.n. doses of PBS. Serum IgE levels were assayed by ELISA.

Results

1. Peripheral blood Treg response and serum IgE. The frequency of CD4⁺CD25^{hi} cells in PBMCs from *Hp*⁺ patients was 2.5-fold higher than in uninfected patients (p=0.018). Levels of CD4⁺CD25^{hi}IL-10⁺ cells were also 2.5-fold increased (p=0.007). Stimulation of PBMCs with *Hp* lysate resulted in a significant increase in CD4⁺CD25^{hi}IL-10⁺ events in *Hp*⁺ patients but no significant difference in CD4⁺CD25^{hi}IL-10⁺ cell numbers in *Hp*⁻ patients. The proportion of CD4⁺CD25^{hi} cells expressing CTLA-4 was 2-fold higher in PBMCs from *Hp*⁺ patients compared to *Hp*⁻ patients. There were no significant differences in the proportions of FOXP3⁺, GITR⁺ and CD127^{lo} CD4⁺CD25^{hi} events. Total and allergen-specific IgE concentrations were low when there was a strong IL-10⁺ Treg response. This association was only statistically significant amongst the *Hp*⁺ group.

2. *Hp* infection and Der p 1 sensitization/challenge in a mouse model of allergy. The total IgE response was reduced by 38% in *Hp*-infected/Der p 1 sensitized mice compared to uninfected/Der p 1 sensitized mice (p=0.007). There was also a small but statistically significant reduction in Der p 1 specific IgE (p=0.01).

Conclusions

Hp induces a systemic IL-10⁺CD4⁺CD25^{hi} Treg response in humans, and high numbers of these Tregs were associated with low serum IgE levels in *Hp*⁺ but not *Hp*⁻ patients. The Treg response may therefore play a role in *Hp*-mediated protection against allergy. We have shown directly, and for the first time, that *Hp* infection can suppress the development of sensitization in an animal model of allergy.

References

1. Robinson, K., et al. *Gut*, 2008. 57: p. 1375-85. 2. Rad, R., et al. *Gastroenterology*, 2006. 131(2): p.525-37. 3. Raghavan, S., et al. *Clinical & Experimental Immunology*, 2003. 132(3): p. 393-400. 4. Lundgren, A., et al. *Infection and Immunity*, 2005. 73: p. 523-31. 5. Kosunen, T.U., et al. *Clinical & Experimental Allergy*, 2002. 32(3): p. 373-8. 6. Pessi, T., et al. *International Archives of Allergy & Immunology*, 2005. 137(4): p. 282-8. 7. Chen, Y. and M.J. Blaser. *Archives of Internal Medicine*, 2007. 167(8): p. 821-7.

LOW-LEVEL *HELICOBACTER PYLORI*-INDUCED REGULATORY T-CELL RESPONSES IN THE HUMAN GASTRIC MUCOSA ARE ASSOCIATED WITH THE PRESENCE OF PEPTIC ULCER DISEASE AND PRE-MALIGNANT PATHOLOGY.

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Introduction:

H. pylori (*Hp*) is the leading cause of peptic ulcer disease (PUD) and gastric adenocarcinoma. The majority of infections result in asymptomatic gastritis, however. A polarised T-helper 1 (Th1) immune response has been shown to reduce bacterial colonisation but exacerbate mucosal inflammation, leading to PUD and cancer development¹. Regulatory T cell (Treg) responses play a role in suppressing the inflammatory response to *Hp* and promote persistence of the infection².

We hypothesise that upregulated Treg responses are associated with reduced risk of PUD and premalignant pathology in the infected human gastric mucosa. We aimed to investigate the mucosal Treg response during *Hp* infection and assess the relationship with pathology and disease status.

Methods:

Gastric antral biopsies were collected from 38 *Hp*⁺ and 22 *Hp*⁻ patients attending the University Hospital, Nottingham. Gastric Tregs were isolated, stained for CD4, CD25, IL10 and CTLA-4, and analysed by flow cytometry. qRT-PCR analysis for *FOXP3*, *TGF β* and *IL10* was also carried out on RNA from biopsy tissues². Tissue sections were scored for inflammation and pathology using the Sydney classification system³.

Results:

A 36-fold increase in the percentage of CD4⁺CD25^{hi}IL10⁺ cells was observed in gastric tissue from *Hp*⁺ compared to *Hp*⁻ patients ($p=0.001$). The same trend was seen with CD4⁺CD25^{hi}CTLA-4⁺ cells ($p=0.002$). The *IL10* mRNA level for *Hp*⁺ samples was >1000-fold higher than in *Hp*⁻ biopsies ($p=0.002$). *FOXP3* and *TGF β* expression levels were also upregulated ($p=0.001$; $p=0.03$).

The number of Treg cells inversely correlated with inflammation scores ($p=0.02$). Lower frequencies of CD4⁺CD25^{hi}IL10⁺ cells were observed in tissue from *Hp*⁺ donors with PUD compared to those with gastritis (2.5 fold; $p=0.02$). RT-PCR confirmed lower *IL10* expression levels in PUD samples (22-fold reduction; $p=0.03$). Reduced expression of *FOXP3* was also observed (5-fold; $p=0.005$). *TGF β* expression, however, was not significantly different.

In biopsies where atrophy was present, lower mRNA expression levels of Treg-associated genes *FOXP3* (8-fold; $p=0.006$), *IL-10* (400-fold; $p=0.027$), and *TGF β* (1.6-fold; $p=0.054$) were observed compared to those where atrophy was absent. The same trend was seen for samples with and without intestinal metaplasia (IM) (*FOXP3*: 6-fold; $p=0.048$; *IL-10*: 400-fold; $p=0.09$, and *TGF β* : 6-fold; $p=0.027$). Reduced numbers of CD4⁺CD25^{hi} cells were found in the presence of IM ($p=0.02$).

Conclusions:

An elevated IL-10⁺ FOXP3⁺ Treg response was found in the infected gastric mucosa which correlated with reduced inflammation. *Hp*-induced gastric disease and premalignant pathology was associated with a significantly reduced level of Tregs. These findings indicate that peptic ulceration and gastric cancer are less likely to occur in the presence of a sufficient Treg response.

References: ¹Robinson, Argent & Atherton (2007) *Best Pract Res Clin Gastroenterol* 21:237; ²Robinson *et al.* (2008) *Gut* 57:1375; ³Zaitoun (1994) *J. Clin Pathol* 47:810

INTERFERON ALPHA TREATMENT NEGATIVELY REGULATES PRO-INFLAMMATORY IFN α PRODUCTION BY PLASMACYTOID DENDRITIC CELLS AND PROMOTES TREG FUNCTION THROUGH IL2/IL4 SECRETION IN PATIENTS WITH SIGHT-THREATENING UVEITIS

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Interferon (IFN)- α is an effective treatment of severe sight-threatening uveitis. Initial studies suggest that patients with uveitis may have a defect in plasmacytoid dendritic cells (pDCs) ability to produce IFN α . We have therefore performed longitudinal studies on uveitis patients treated with IFN α to evaluate pDC function and possible links with altered T regulatory (Treg) cell function.

Patients with non-infectious sight-threatening uveitis (NIU) who were refractory to other treatments, were recruited prior to, during and after completing a course of IFN α . Analysis of peripheral blood CD3⁺ T cells showed an increase in basal expression of IL2 and an increase in IL4 under stimulated conditions in the patient group compared to controls. Furthermore, IFN α treatment increased the CD4⁺CD25⁺ population in patients when compared to pre-treatment levels. Interestingly, this was associated with an increase in the number of circulating pDCs which was sustained after completing the treatment course. However, pDCs from patients undergoing IFN α treatment secreted very low to zero levels of IFN α in response to CpG stimulation.

Plasmacytoid DCs may have both pro-inflammatory and immunoregulatory (tolerising) roles. We believe that in patients with uveitis, the tolerising role of pDCs is impaired. This impairment is restored following treatment with exogenous IFN α which may promote pDCs towards their tolerising role. Our data suggest that this is achieved by promoting T cell IL2 secretion, leading to an increase in Treg cell numbers and restoring immune homeostasis.