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SCIENTIFIC WORKSHOP 2016

INFLAMMATION & IMMUNITY IN HEALTH AND DISEASE

CeMM, Vienna, May 12th - 13th 2016

EU-LIFE scientific workshop

Inflammation & Immunity in Health and Disease

CeMM, Vienna, 12-13th May 2015

INTRODUCTION

Goal

This third EU-LIFE scientific workshop aims to bring together basic scientists and clinicians/translational researchers from EU-LIFE institutes (and collaborators) to explore and discuss inflammation and immunity in health and disease. We aim to foster a discussion on how to strengthen the interaction of basic and translational scientists with clinicians to translate research findings to diagnostic and therapeutic applications. The workshop will also be an opportunity to forge new scientific collaborations within EU-LIFE and found the basis for future joint activities and projects.

Format

Each EU-LIFE institute has invited a "**tandem of speakers**", often one being a basic researcher and the second a clinical/translational researcher.

The constellation of these "tandems" may vary:

a) one or both speakers may work at the EU-LIFE center or partnering institute/university/hospital/company, and ideally they are engaged in a cooperative project;

b) the basic researcher and the clinical/translational scientist do not have an ongoing cooperation but their work is relevant to each other.

Each tandem of speakers will have 30-minutes to present their work jointly and highlight opportunities and challenges of their collaboration. The presentation will be followed by a 10-minute discussion. Within the slot, enough time should be left for discussion.

The meeting will also include the opportunity to present their work for junior researchers. Each EU-LIFE institute will invite up to four PhDs students or postdocs to submit an abstract. To ensure maximum visibility of these presentations, we will select 12 of these abstracts for short presentations at the meeting (4 min speed talk + 1 min discussion). Fpresent their projects as poster (joint posters are also possible). Based on the submitted abstracts, 6 poster will be selected for a "Flash poster presentation" to give a maximum 3-minute (2 slide) highlight of their poster. Abstract deadline: March 15, 2016

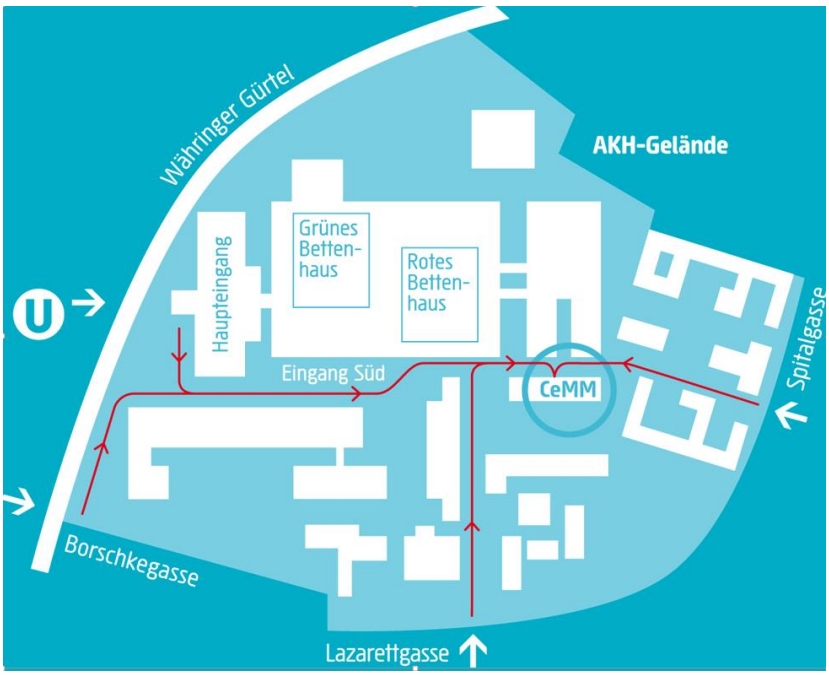
Organizers and hosts:

Andreas Bergthaler and Stefan Kubicek with the EU-LIFE Translational Working group.

GENERAL INFORMATION

GETTING TO CeMM

CeMM is located on the campus on the campus of the general hospital of Vienna (AKH)



Address:
CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences
Lazarettgasse 14, AKH BT 25.3
1090 Vienna, Austria

Transfer Vienna Airport (VIE) to City:
CAT - City Airport Train http://www.cityairporttrain.com/, one way ticket: € 11
Taxi - Airportdriver http://www.airportdriver.at/en/airport-transfer, online one way pre-booking: € 33; It takes about 35-40 min. to get from the Vienna Airport to CeMM.

For public transportation in Vienna, information on journeys and prices can be found on the following website: http://www.wienerlinien.at/eportal3/; costs for a single use ticket: € 2,20 and for a 24-hours ticket: € 7,60
More information about Vienna, including sightseeing recommendations: https://www.wien.info/en

MEETING LOCATION

The meeting will be held in CeMM's 8th floor lecture hall.
Presentations of this scientific meeting will be made available as live-stream to all EU LIFE institutes.

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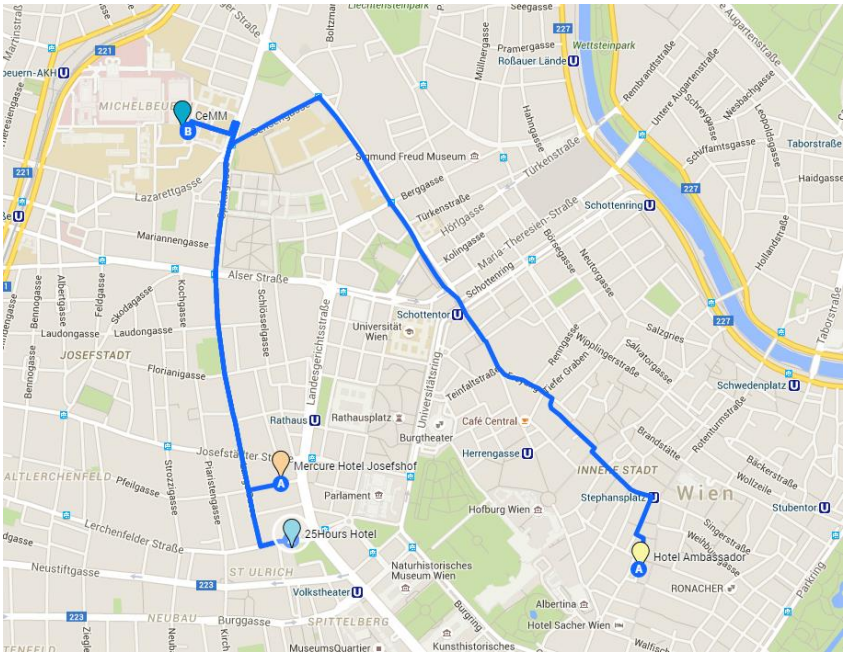
HOTELS

We have reserved several rooms including breakfast at the following hotels in Vienna.
By mentioning “EU-LIFE”, the meeting participants will get a better price. Booking deadline is March 15, 2016 for all three hotels (to guarantee the special deals).



Mercure Hotel Josefshof http://josefshof.com/en/hotel
Josefsgasse 4-6, 1080 Vienna, T: +43-1-40419, reservierung@josefshof.com
Single Room: € 102,10 / Double Room (can also be booked for single use): € 122,20
4 star hotel near Vienna City Hall/Rathaus, walking distance to CeMM: 1,5 km
Check-in after 2.00 pm, Check-out until 12.00

25Hours Hotel http://www.25hours-hotels.com/en/museumsquartier/home/home.html
Lerchenfelder Strasse 1-3, 1070 Vienna, T: +43-1-52151-820, Events.Wien@25hours-hotels.com
Single Room: € 184,00 / Double Room (can also be booked for single use): € 199,00
4 star hotel near Museumsquartier, walking distance to CeMM: 1,8 km
Check-in after 3.00 pm, Check-out until 12.00

Hotel Ambassador http://www.ambassador.at/default-en.html
Kärtner Strasse 22, 1010 Wien, T: +43-1-96161-0, sales@ambassador.at
Double Room Vienna Classic (also for single use): € 230,00
5 star hotel near St. Stephen’s Cathedral/Stephansdom, walking distance to CeMM: 2,5 km
Check-in after 2.00 pm, Check-out until 12.00

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Agenda

Thursday, May 12th 2016

13.30	**Welcome**
13.30-14.00	Welcome, aims, feedback from strategy meeting
14.00	**Session 1: Autoimmunity**
14.00-14.40	Janna Saarela (FIMM) *Filtering through population bottlenecks: variants causing primary immunodeficiency and autoinflammation in a population isolate* Mikko Seppänen (FIMM) *Clinical exome sequencing challenges and strengths*
14.40-15.20	Kim Bak Jensen (BRIC) Ole Haagen (BRIC) *Inflammatory bowel disease - from clinical manifestations to molecular mechanisms of tissue repair*
15.20-15.30	Speed talks selected from abstracts
15.30-16.00	**Coffee Break**
16.00	**Session 2: T-cells in cancer immunology**
16.00-16.40	Olivier Lantz (Institut Curie) *Characterization of the immune response towards uveal melanoma in different clinical setting* Emanuela Romano (Institut Curie) *Fc-mediated depletion of Tregs via ipilimumab-dependent ADCC in advanced melanoma patients*
16.40-17.20	Thomas Blankenstein (MDC) Il-Kang Na (Experimental and Clinical Research Center of MDC and Charité) *Adoptive T cell therapy of cancer*
17.20-17.30	Speed talks selected from abstracts
17.30-18.10	Pia Kvistborg (NKI) *What T cells see on human cancer* Seth Coffelt (NKI) *Immune cell dominoes: falling in line to promote breast cancer metastasis*
18.10-18.50	Rahul Roychoudhuri (Babraham Institute) *Making all the wrong decisions: Transcription factors in tumour immunosuppression* Marc Veldhoen (Babraham Institute) *Environmental influences on T cell immunity*
18.50-19.00	Speed talks selected from abstracts
19:30	**Conference dinner including poster session – NHM?**

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Friday, May 13th 2016

9.00	**Session 3: Innate pathways in cancer immunology**
9.00-9.40	Bill Keyes (CRG) *The senescence associated secretory phenotype instructs stem cell function and regulation* Clemens Schmitt (MDC/Charité) *The dangerous liaison of therapy-induced senescence, senescence-associated cancer stemness and treatment failure*
9.40-10.20	Jo Van Ginderachter (VIB) *Functionally distinct macrophages and dendritic cell populations in the tumor microenvironment* Max Mazonne (VIB) *Hypoxic TAMs in cancer progression*
10:20-10:30	Speed talks selected from abstracts
10.30-11.00	**Coffee Break**
11.00-11.40	Luca Mazzarella (IEO) *Combined metabolic and epigenetic modulation in the treatment of Acute Myeloid Leukemia* Anna Giulia Sanarico (IEO) *Metabolic alterations in FLT3-ITD acute myeloid Leukemia*
11.40-11.50	Speed talks selected from abstracts
11.50	**Session 4: Infection**
11.50-12.30	Michael Trauner (Medical University of Vienna, Austria) Andreas Bergthaler (CeMM)
12.30-13.40	Speed talks selected from abstracts
12.40-13.30	**Lunch**
13.30-14.10	Miguel Soares (Instituto Gulbenkian de Ciência) Luis Ferreira Moita (Instituto Gulbenkian de Ciência)
14.10-14.50	Pavel Plevka (CEITEC) *Structure and genome release of Myoviridae phage with double layered baseplate* Mary O'Connell (CEITEC) *ADAR1 is crucial for discriminating between 'self' and 'non-self' cellular RNAs*
14:50	Wrap-up
15:00	**End of meeting**

ABSTRACTS

SESSION 1: AUTOIMMUNITY

Filtering through population bottlenecks: variants causing primary immunodeficiency and autoinflammation in a population isolate

Janna Saarela
Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland

The population history of Finland, characterized by a restricted number of founders, isolation and several population bottlenecks, has caused enrichment of certain rare disease causing variants and losses of others, as part of a phenomenon called the Finnish Disease Heritage. Accordingly, rare founder mutations cause the majority of observed Finnish cases in these mostly autosomal recessive disorders that consequently are more frequent in Finland than elsewhere. Here I describe two examples of gene defects causing PID in Finland.

First, exome sequencing of a multi-case Finnish family with a Hyper IgM type 2 phenotype identified a rare, homozygous, variant (c.416T>C, p.Met139Thr) in the AICDA gene, found to be significantly enriched in the Finnish population compared with other populations of European origin (38.56-fold, $p < 0.001$). Further screening of all currently known Finnish patients with HIGM2 showed them to be homozygous for the same variant. Furthermore, all carriers of the p.Met139Thr in Finnish population cohorts originated from the eastern and northeastern parts of Finland, shared more of their genome identity by descent (IBD) than Finns in general ($p<0.001$), and carried a 207.5kb ancestral haplotype containing the variant. Based on these finding we suggest HIGM2 as a novel Finnish Disease Heritance disorder.

The second example describes identification of a novel PID gene by NGS screening of three unrelated Finnish kindreds with immunodeficiency and autoinflammation. In all 13 affected individuals, we identified heterozygous variants in NFKB1, encoding for the NF-κB family members p50/p105. Patients harboring a p.I553M variant presented with antibody deficiency, infection susceptibility, and multi-organ autoimmunity. In addition to hypogammaglobulinemia, patients with a p.H67R change suffered from autoinflammatory episodes including aphthae, gut disease, febrile attacks and small vessel vasculitis characteristic of Behcet's disease. The main symptom in patients with a p.R157X stop-gain variant was a severe hyperinflammatory response to routine surgery.

Functional analyses indicated that both missense variants led to a reduction in p50/p105 function, whereas the p.R157X variant likely leads to rapid elimination of the truncated transcript. The p.H67R variant reduced nuclear entry of p50, which in luciferase-reporter assays led to a decrease in transcriptional activity. The p.I553M mutation, in turn, reduced phosphorylation of the NF-κB inhibitor p105 decreasing its stability during TNF induction. Both missense mutations also led to altered protein-protein interactions in affinity purification mass spectrometry. These findings suggest that a subset of autoinflammatory diseases may be caused by rare, monogenic variants and highlight the importance of careful homeostasis between components of the NF-κB pathway.

Clinical exome sequencing challenges and strengths

Mikko Seppänen
Rare Disease Center, Helsinki University Hospital (HUH), Helsinki, Finland

From the known protein-protein interactions it has been predicted that, instead of the known approximately 350 primary immunodeficiency (PID) and bone marrow failure (BMF) genes, there are over 3100 potential genes that could cause PID. For many of these, both gain and loss of function seem plausible, and the data on protein-protein interactions is still far from comprehensive. When diagnosing PIDs in isolated populations like the genetic Finns, these predicted numbers seems fully likely. Finns have relatively little “background noise” when filtering for potential novel PID candidate genes. Both the clinical phenotypes and the known or suspected gene candidates after whole exome and/or genome sequencing differ strongly from those found from better studied populations. This has led to a situation where a substantial number of patients harbor likely novel diseases or phenotypes caused by known or novel candidate genes. The rate limiting steps are of course effective and reliable variant filtering as well as gathering enough functional data to prove that the candidate gene in fact causes the disease in patients or even large kindreds. Similarly as in other genetic isolates, such as the populations of Middle Eastern ancestry, studying PIDs in the Finns has already led to identification of novel PID causing genes. However, creation of a comprehensive map of genes involved in rare disorders, such as PIDs and bone marrow failure, will require European wide and even a global collaboration.

Inflammatory bowel disease - from clinical manifestations to molecular mechanisms of tissue repair

Ole Haagen Nielsen[1] and Kim B. Jensen[2]
1. Department of Gastroenterology, Medical Section, Herlev Hospital, University of Copenhagen, DK-2730 Herlev, Denmark
2. BRIC - Biotech Research and Innovation Centre, University of Copenhagen, Ole Maaloes Vej 5, DK-2200 Copenhagen N, Denmark

Inflammatory bowel disease is an umbrella term of a number of distinct disorders characterised by uncontrolled inflammation in the gastrointestinal tract of which ulcerative colitis and Crohn's disease are the most prevalent entities. Ulcerative colitis is confined to the colon, whereas Crohn's disease may affect any part of the GI tract, and the prevalence of both diseases is increasing world-wide possibly due to yet unknown factors in the environment. A compromised barrier function of the epithelium in the GI tract causes additional problems due to the direct exposure to the commensal microbiota. Currently, patients are treated with glucocorticoids, immunomodulators (i.e. thiopurines and methotrexate) as well as biologics (i.e. TNF-α inhibitors and α4β7 anti-integrins) and oral administered small molecules and antisense therapeutics are expected in the near future to control the intestinal inflammation and allow the GI tract to restore a normal barrier function. We will discuss how understanding clinical manifestations of inflammatory bowel disease as well as the process of normal epithelial tissue repair will provide new insights into disease development and help us to identify novel therapeutic strategies for a more tailored approach to target the disease in the future.

SESSION 2: T-CELLS IN CANCER IMMUNOLOGY

Characterization of the immune response towards uveal melanoma in different clinical setting

Olivier Lantz on behalf of the Institut Curie Uveal melanoma translational research group
Clinical Immunology laboratory, Institut Curie, Paris, France.

Once metastatic the prognosis of uveal melanoma (UM) is very poor. In the absence of active conventional or targeted therapy, immunotherapy would be very attractive. However, only anecdotic responses to anti-check point blockers have been reported. UMs do not harbor somatic mutations that would be the source of neoantigens. Yet, immune infiltrates are found in both primary tumors and metastatic lesions. In addition, increased proportion of oligoclonal effector CD4 and CD8 T cells are found in the blood of metastatic patients suggesting the existence of an anti-tumor response (1).

Herein, characterized the immune response observed in the blood of patients either at the time of primary treatment or when operated for liver metastasis. We observed the presence of effector CD4 T cells at the time of diagnosis in a fraction (≈20%) of the 85 patients we studied. The correlation with genomic anomalies is ongoing. At the time of liver metastasectomy, effector CD4 T cells were observed in the blood of about half of the metastatic patients. We also characterized the immune infiltrated in the liver lesions of 15 patients using both immunochemistry and cytometry. In comparison with adjacent "healthy" liver, we observed an increased proportion of Tregs and CD8 T cells with a concomitant decrease in MAIT and NKT cell numbers. By immunochemistry, T cells were found in both the periphery and inside the tumors.

Altogether, these results suggest that contrary to what is usually assumed uveal melanoma tumor cells are seen by the immune system. The antigen specificity of the effector T cells observed in UM patients will be characterized in further work and may allow the development of therapeutic vaccine strategy. The probable presence of tumor reactive T cells in UM patients also justifies the use of therapeutic schemes including anti-checkpoint blockers.

Peguillet et al. Cancer. Res. 2014. 74(8):2204-16

Fc-mediated depletion of Tregs via ipilimumab-dependent ADCC in advanced melanoma patients

Emanuela Romano
Institut Curie, Paris, France

Enhancing immune responses with immune-modulatory monoclonal antibodies (mAbs) directed to inhibitory immune-receptors is a promising modality in cancer therapy. Clinical efficacy has been demonstrated with antibodies blocking inhibitory immune checkpoints such as CTLA-4 or PD-1/PD-L1. Treatment with ipilimumab, a fully human CTLA-4 specific mAb, showed durable clinical efficacy and improved overall survival in metastatic melanoma; its mechanism/s of action, however, are only partially understood. Recent studies in melanoma mouse models revealed that the anti-tumour activity of CTLA-4 blockade is mediated by FcgRIV-expressing macrophages in the tumour microenvironment (TME) via in-trans depletion of tumour-infiltrating Tregs. We speculated that a similar mechanism might operate in melanoma patients responding to ipilimumab. To investigate this hypothesis, we interrogated peripheral blood mononuclear cells (PBMCs) and matched melanoma metastases from 15 patients responding (R) and 14 non-responding (NR) to ipilimumab. Our findings show, for the first time, that ipilimumab leads to the depletion in vitro of regulatory T cells (Tregs) via an antibody-dependent-cellular-cytotoxicity (ADCC) mechanism, selectively mediated by FcgRIIIA (CD16)-expressing, non-classical monocytes. In contrast, classical monocytes, lacking the FcgRIIIA expression, are unable to deplete Tregs in an ADCC assay. Interestingly, patients responding to ipilimumab displayed significantly higher baseline peripheral frequencies of non-classical monocytes than non-responder patients. Evaluation of matched melanoma metastases from pre- and post-ipilimumab time-points by IHC revealed that, in the TME, responders had the highest CD68+/CD163+ macrophage ratios at baseline, and showed decreased infiltration of Tregs after treatment. Notably, baseline Treg infiltration was comparable between the two groups. Our results provide novel mechanistic insight into the clinical activity of ipilimumab, highlighting the contribution of the tumour stroma into the final outcome of antibody-based immunomodulatory therapies and suggest non-classical monocytes as a potential biomarker of response.

Adoptive T cell therapy

Thomas Blankenstein
Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Strasse 10, 13092 Berlin, Germany
Charité Centrum für Tumormedizin, Institut für Immunologie, Campus Berlin-Buch, Lindenberger Weg 80, 13125 Berlin

Adoptively transferred T cells have been shown to reject large established tumors. In such models, T cells recognize the tumor antigen as foreign. The task is to generate human T cell receptors (TCR) that recognize human tumor-associated (self) antigens as foreign and use these TCRs for gene therapy. We use mice with a humanized TCR repertoire to isolate therapeutic TCRs.

Adoptive T cell therapy of cancer

Il-Kang Na
Department of Hematology, Oncology and Tumor Immunology, Charité University Medicine CVK, Berlin, Germany
Experimental and Clinical Research Center (ECRC), Berlin, Germany

Adoptively transferred T cells require successful infiltration into the tumor tissue and activation on site for efficient tumor eradication. Using a dual-luciferase transgenic mouse, which enables us to concurrently and longitudinally visualize migration and activation of T cells in vivo, we compare activation and effector function of T cell subpopulations when targeting a tumor-specific or a tumor-associated antigen and test combinatorial drug and adoptive T cell therapies to prevent tumor relapse.

What T cells see on human cancer

Pia Kvistborg
The Netherlands Cancer Institute

Human tumors contain large numbers of mutations, of which many hundreds can be present within expressed genes. As the resulting altered protein sequences are foreign to the immune system, immune recognition of such 'neo-antigens' is likely to be of significant importance to the activity of clinically used immunotherapeutics such as anti-CTLA-4 and anti-PD-1 in melanoma. However, the vast majority of the mutations in human cancers are unique to individual patients and, because of this, broadly applicable approaches to link the consequences of DNA damage in human cancer to tumor-specific T cell activity have long been lacking.

Using in-house developed technologies for monitoring of T cell activity, we have recently demonstrated the feasibility of cancer exome-driven analysis of both tumor-specific $CD8^+$ T cell reactivity and $CD4^+$ T cell reactivity in human melanoma.

The data obtained demonstrate that T cell recognition of the consequences of DNA damage is a common feature in human melanoma. Furthermore, based on the distribution of mutation loads in other major human cancer types, we propose that also in many other human tumors, the repertoire of mutant antigens provided by DNA damage (the ‘neo-antigen space’) will suffice to allow T cell recognition.

Collectively, these data indicate that mutational load may form a biomarker in cancer immunotherapy, and that the development of ‘personalized immunotherapies’ that exploit cancer genome information to target patient-specific mutant antigens should be explored.

Immune cell dominoes: falling in line to promote breast cancer metastasis

Seth Coffelt (NKI) & Karin E. de Visser
The Netherlands Cancer Institute

Metastasis remains the primary cause of death for breast cancer patients. This is due in large part to the lack of knowledge about the mechanisms underlying cancer spread. Metastatic disease is still largely unexplored, poorly understood and incurable. Emerging evidence indicates that immune cells are critical regulators of the different steps of the metastatic cascade. Recently, we showed that mammary tumors in *K14-cre;Cdh1*$^{F/F}$*;Trp53*$^{F/F}$ mice elicit a systemic inflammatory cascade to dampen anti-tumor T cells and maximize metastasis formation. This cascade is initiated by tumor microenvironment-derived IL1β that activates IL17-producing γδ T cells, leading to G-CSF-dependent neutrophil expansion and repolarization. In turn, these neutrophils suppress $CD8^+$ T cells, allowing disseminated cancer cells to go unnoticed. Current efforts are underway to determine how genetic makeup of different mammary tumors regulates the γδ T cell – IL17 – neutrophil axis. Together, these data provide further insight into how immune cells participate in metastasis and uncover several putative targets for metastatic breast cancer.

Making all the wrong decisions: Transcription factors in tumour immunosuppression

Rahul Roychoudhuri
Lymphocyte Signalling and Development Laboratory, The Babraham Institute, Babraham Research Campus, Cambridge, CB22 3AT, UK.

Through functional diversification, cells of the innate and adaptive immune system either drive or constrain immune reactions within tumours. Thus, while the immune system has a powerful ability to recognize and kill cancer cells, this function is often suppressed, preventing clearance of disease. The transcription factor (TF) BACH2 regulates the differentiation and function of multiple innate and adaptive immune lineages, but its role in controlling tumor immunity is not known. We have found that BACH2 is required to establish immunosuppression within tumors. Growth of tumours was markedly impaired in Bach2-deficient mice and coincided with intratumoural activation of both innate and adaptive immunity. However, augmented tumor clearance was dependent upon adaptive immunity. Analysis of tumor-infiltrating lymphocytes in Bach2-deficient mice revealed high frequencies of CD4+ and CD8+ effector cells expressing the inflammatory cytokine interferon (IFN)-γ. T cell activation correlatedwith reduction in the frequency of intratumoral CD4+ Foxp3+regulatory T (Treg) cells. Mechanistically, BACH2 promoted tumor immunosuppression through Treg-dependent inhibition of intratumoural CD8+ T cells.

These findings demonstrate that BACH2 is a key component of the molecular program of tumor immunosuppression and identify a new target for development of therapies aimed at reversing immunosuppression in cancer.

Environmental influences on T cell immunity

Marc Veldhoen
Lymphocyte Signalling and Development Laboratory, The Babraham Institute, Babraham Research Campus, Cambridge, CB22 3AT, UK.

Epithelial barrier sites, such as the gastrointestinal tract and skin, show a remarkable compartmentalisation with respect to T lymphocytes and their function. The top most layer, the intraepithelial compartment and epidermis contain a mix of innate-like T cells capable of making interferon gamma (IFNγ) whilst the deeper tissue layers, the lamina propria and dermis, are enriched for interleukin (IL)-17 producing T cells. We have previously shown that particular environmental factors, such as those derived from the diet, are able to directly affect Th17 as well as intraepithelial lymphocytes (IELs) via the arylhydrocarbon receptor.

For some time we have been probing the requirements for Th17 cell differentiation. Although IL-6 and IL6R/Stat3 signalling is a requirement for Th17 cell polarisation and although TGFβ and TGFβR are reportedly required, no known downstream TGFβR signalling pathway, such as the SMADs, are essential of Th17 development. We hypothesised if alternative pathways are required for Th17 cell development. We will present data on an alternative pathway with unexpected results.

Recent observations showed a strong correlation between tumours and Th17 cell numbers. This observation in combination with the sites where Th17 cells are enriched made us hypothesise if alterations in metabolic availability in the microenvironment could influence Th17 cell differentiation. We will present new data on how metabolic disturbances specifically influence Th17 cell differentiation, with implications for chronic inflammatory disorders and cancer.

SESSION 3: INNATE PATHWAYS IN CANCER IMMUNOLOGY

The senescence associated secretory phenotype instructs stem cell function and regeneration

Bill Keyes
Centre for Genomic Regulation (CRG), Barcelona

Senescence is a form of cell cycle arrest that prevents the proliferation of damaged cells through the activation of tumor suppressor networks. As a result, senescence is described as a tumor suppressive mechanism. However, even while arrested, senescent cells can interact extensively with their microenvironment through the secretion of a variety of proteins termed the senescence-associated secretory phenotype (SASP). This secretome is largely composed of growth factors, cytokines/chemokines and extracellular-matrix remodeling proteins secreted by the arrested cells, and can function to reinforce the arrest, or recruit immune cells to clear the senescent population. Conversely however, in certain conditions, the SASP can favor tissue growth and repair, such as during wound healing and as we recently demonstrated, during embryonic development. In some cases, the SASP can even promote tumor growth. These functions suggest a broader more complex physiological role for senescence and the SASP than currently understood.

Here we describe that the induction of oncogene-induced senescence (OIS) in primary mouse keratinocytes leads to a dramatic increase in stem cell gene expression. Interestingly, this signature can also be induced in normal cells upon exposure to the SASP, suggesting that secreted factors by the senescent cells induce stem cell gene expression. In order to examine stem cell functionality in these cells, we performed full-thickness skin grafting with senescent or SASP-treated cells. Interestingly, cells transiently exposed to the SASP acquired full-stem cell capacity, and could regenerate skin and hair follicles in the recipient host. However, transplantation of fully senescent cells induced the formation of papilloma, a pre-malignant skin lesion that is maintained by aberrantly proliferating stem cells. Together, our data suggests that the SASP is in fact a regenerative mechanism that instructs stemness, but that if left uncurtailed, leads to tumor formation through aberrant regenerative mechanisms.

The dangerous liaison of therapy-induced senescence, senescence-associated cancer stemness and treatment failure

Clemens A. Schmitt and colleagues
Charité – University Medical Center, Medical Department of Hematology, Oncology and Tumor Immunology, and Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

Cellular senescence is a stress-responsive cell-cycle arrest program that terminates further expansion of (pre-)malignant cells. Interestingly, many key signaling components of the senescence machinery also operate as critical regulators of stem cell functions (collectively termed 'stemness'), among them the p53 axis and the transcriptionally repressive lysine 9-trimethylated histone H3 (H3K9me3) mark. We report here our experimental approach to address two pivotal questions: (I) May chemotherapy-induced senescence evoke stem cell-related functionalities in malignant cells of murine and human origin? (II) Is cellular senescence a potentially reversible program, thereby allowing cells to execute their stem-like capacity in a division-coupled manner?

Gene expression and functional analyses comparing chemotherapy-induced senescent vs. non-senescent Bcl2-overexpessing (and, thus, apoptosis-blocked) Eμ-myc transgenic B-cell lymphomas unveiled massive upregulation of an adult tissue stem cell signature, activated Wnt signaling, and de novo expression of distinct stem cell markers in senescence. We previously demonstrated that the H3K9-targeting histone methyltransferase Suv39h1, like p53, operates in a senescence-essential fashion in these murine B-cell lymphomas. Utilizing conditional Suv39h1- and p53-based, genetically switchable 'matched pair' models of senescence to mimic spontaneous escape ('previously senescent', PS), we found PS cells to re-enter the cell-cycle with strongly enhanced and Wnt-dependent clonogenic growth when compared to their equally chemotherapy-exposed but never senescent (NS) counterparts. In vivo, these PS lymphoma cells presented with a much higher tumor initiation potential, which was neutralized upon pharmacological or genetic Wnt inhibition. Strikingly, temporary enforcement of senescence in a p53-regulatable leukemia model reprogrammed non-stem bulk PS leukemia cells into self-renewing leukemia-initiating stem cells, while equally chemotherapy-exposed NS bulk cells did not acquire stemness potential. Our data, further supported by consistent findings in various human cancer cell lines and primary tumor samples, uncover senescence-associated stemness as an unexpected, cell-autonomous feature that exerts its detrimental potential upon escape from the cell-cycle block. These findings indicate a hitherto unappreciated role of senescence in stress-evoked tissue damage but also raise concerns about the long-term benefit of senescence-inducing cancer therapies, and provide new mechanistic insights into the plasticity of the "cancer stem cell" condition. In turn, we present synthetic lethal targeting of senescence-associated stemness as a conceptually novel, outcome-improving anticancer treatment strategy.

Functionally distinct macrophage and dendritic cell populations in the tumor microenvironment.

Jo A. Van Ginderachter
Myeloid Cell Immunology Lab, VIB; Cellular and Molecular Immunology Lab, Vrije Universiteit Brussel, Brussels, Belgium

Tumor-associated macrophages (TAM) are exposed to multiple microenvironmental cues in tumors, which collaborate to endow these cells with protumoral activities. Given the inherent plasticity of macrophages, we hypothesized that different macrophage types might exist in distinct tumor regions. We showed that the myeloid infiltrate in tumors is complex and encompasses three ontogenically distinct tumor-associated dendritic cell (TADC) populations and at least two major TAM subsets, designated as MHC-IIlow MMRhi and MHC-IIhi MMRlow TAM, both of which were derived from tumor-infiltrating Ly6Chi monocytes. MHC-IIlow TAM express higher levels of prototypical M2 markers and reside in more hypoxic regions, as shown by in vivo molecular imaging using MMR-specific nanobodies. Employing different in vivo strategies, our results show that hypoxia is not a major driver of TAM subset differentiation and polarization per se, but rather specifically fine-tunes the phenotype of M2-like MHC-IIlow TAM. We now identified M-CSFR signaling as an important regulator of monocyte and macrophage differentiation in tumors, which seems especially important for regulating the MHC-IIlow TAM population. Conversely, GM-CSFR signaling rather fine-tunes the phenotype of MHC-IIhi TAM. Ongoing work in our lab is further exploring the distinct functions and effector molecules of these TAM subsets and strategies to target them in vivo.

The identification of functionally distinct tumor-associated DC (TADC) subpopulations could prove essential for the understanding of basic TADC biology and for envisaging targeted immunotherapies. We demonstrated that multiple mouse tumors as well as human tumors harbor ontogenically discrete TADC subsets. Monocyte-derived TADC are prominent in tumor antigen processing, but lack strong T-cell stimulatory capacity due to NO-mediated immunosuppression. Pre-cDC-derived TADC have lymph node migratory potential, whereby cDC1 efficiently activate CD8+ T cells and cDC2 induce Th17 cells. Mice vaccinated with cDC2 displayed a reduced tumor growth accompanied by a reprogramming of pro-tumoral TAM and a reduction of MDSC, while cDC1 vaccination strongly induces anti-tumor CTL. Our data might prove important for therapeutic interventions targeted at specific TADC subsets or their precursors.

Influence of Macrophage Metabolism on Vessel Shape: Implications for Cancer

Max Mazzone
VIB

Cancer typically has an inflammatory nature. Of all immune cells invading the tumor, the role of macrophages remains controversial because of their functionally distinct differentiation states. Classically activated (M1-like) macrophages and alternatively activated (M2-like) macrophages represent the two extremities of a continuum, in which the former are proinflammatory and tumoricidal, whereas the latter display pro-angiogenic and protumoral activities. This heterogeneity reflects the plasticity and versatility of these cells in response to microenvironmental signals. The tumor environment is constituted of cancer cells and stromal cells that coexist under different oxygen tensions and the influence of soluble factors. A plethora of (hypoxia-induced) cytokines and chemokines can define the macrophage phenotype and their positioning within the tumor. We have elucidated an elegant mechanism by which macrophages are recruited and retained in avascular hypoxic niches of the tumor, where they exert their immunosuppressive and pro-angiogenic activity. Hypoxia by itself indeed strongly affects macrophage function, as the cells are forced to adapt and alter their metabolism, which is accompanied by a drastic change in their transcriptome and proteome. We have found that the resultant metabolic changes drive macrophages towards a pro-angiogenic phenotype in an attempt to restore oxygenation. Soluble factors derived from the tumor exacerbate this macrophage shift by further enforcing this metabolic adaptation. As a consequence, tumor blood vessels are abnormal and dysfunctional, thus perpetuating hypoxia, which in turn fosters metastasis. By using genetic tools in mice, we show that inhibiting macrophage recruitment in hypoxic niches, or altering their metabolic and energetic state can break this feed-forward loop, resulting in normalized blood vessels and thus, strongly reducing metastatic dissemination.

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Combined metabolic and epigenetic modulation in the treatment of Acute Myeloid Leukemia

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Introduction. There is increasing interest in the therapeutic modulation of metabolic pathways in cancer. Tumor cells preferentially use aerobic glycolysis to meet their energetic demands, but can switch to alternative substrate usage when specific nutrients are limiting. The molecular basis of metabolic adaptation is poorly understood. Recently, the histone demethylase LSD1 (Lysine-Specific Demethylase 1) has been implicated in the control of oxidative phosphorylation (OXPHOS) in adipocytes through its interaction with NRF1 (Nuclear Respiratory Factor 1), a master regulator of metabolic gene transcription (1). We hypothesized that LSD1 could regulate metabolic adaptability and be a therapeutic target upon metabolic modulation through Caloric Restriction (CR) in Acute Myeloid Leukaemia (AML) and specifically in APL (Acute Promyelocytic Leukaemia), which we showed to be sensitive to body fatness in the clinic (2). Methods. APLs were generated in mice expressing the PML-RARa fusion under the control of the Cathepsin G promoter (3). Primary leukemias were transplanted into recipients subjected to 30% CR or Standard Diet (SD). We scored the effect of CR alone or in combination with the LSD1 inhibitor IEO368 (4) on mouse survival, Leukemia Initiating Cell (LIC) frequency and epigenomic, transcriptomic and metabolic parameters. Results. Compared to SD controls, CR-fed recipients experienced an initial dramatic decrease in the total leukemic burden accompanied by cell cycle slowdown ("adaptation phase"); this was followed by a delayed disease progression that brought animals to death ("terminal phase") (median survival 91 vs 51 days, p=0.038). Limiting-dilution transplantation of CR-conditioned leukemias revealed increased frequency of LICs (estimated frequency 1/3064 cells in SD vs 1/947 in CR, p=0.003) and increased aggressiveness (median survival reduced to 49 vs 70.5 days with 5000 cells injected, p<0.0001). Thus, CR limits the expansion of leukemic cells but enriches for cells with increased ability to regrow. RNAseq of leukemic cells purified during the terminal phase (but not earlier) showed that a dramatic transcriptional reprogramming in CR, characterized by upregulation of genes controlling OXPHOS, Krebs' cycle and nucleotide and protein biosynthesis, and downregulation of insulin signaling and glucose transporters. In particular, superenhancer-associated genes were uniformly downregulated in CR, suggesting enhancer "decommissioning", a process dependent on LSD1 in embryonic stem cells. Strikingly, co-treatment of leukemic mice with CR and our LSD1 inhibitor IEO368 (4) resulted in macroscopic and microscopic eradication of disease. LSD1 inhibition alone was also effective but did not produce bona fide disease eradication. Importantly, some of the features of the CR-LSD1 interaction could be modeled by combining LSD1 and an IGF1/Insulin inhibitor. In vivo, this combination was synergistic and led to durable responses (median survival 121 vs 50 days in untreated controls, p=0.0143, vs 65.5 and 78.5 days with Insulin/IGF1 Inhibitor and IEO368 respectively). Conclusion: the combination of LSD1 inhibition and insulin/IGF1 signaling reduction by pharmacological or dietary intervention appears as an extremely effective therapeutic strategy and deserves further investigation. Preclinical studies are ongoing to verify its applicability to other models of cancer.

Metabolic alterations in FLT3-ITD acute myeloid Leukemia

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Mutations in the tyrosine kinase FLT3, usually by insertion of Internal Tandem Duplications (ITD) in the region encoding the autoinhibitory juxtamembrane domain, are associated with poor prognosis in Acute Myeloid Leukemia (AML). Attempts to target FLT3 by direct kinase domain inhibitors have provided small albeit significant survival advantages in clinical trials. However, mutations involving drug-binding sites rapidly appear and confer resistance. Therefore, a better understanding of FLT3 biology is needed to develop suitable treatments.
We previously showed that in a subtype of AML, Acute Promyelocytic Leukemia (APL), increased body mass is associated with worse outcome. Intriguingly, we found that overweight or obese patients had a 4-fold higher incidence of FLT3 mutations. Promotion of FLT3ITD-associated leukemia by obesity was confirmed in transgenic FLT3ITD mice, in which long term high fat diet (HFD) resulted in significantly accelerated leukemic mortality. These findings suggested that the obese “milieu” might provide a selective advantage for FLT3-mutated clones, prompting us to investigate specific metabolic requirements induced by FLT3ITD. Analysis of syngeneic cell lines overexpressing WT or ITD FLT3 showed that FLT3ITD generates significant ER stress that cannot be alleviated through reduction in protein biosynthesis rate due to persistently elevated mTOR signaling. Multiple adaptive pathways including SREBP1/2-dependent lipid biosynthesis are modulated at the transcriptional level and required for FLT3ITD cell survival, providing a possible mechanistic link with obesity. The net result of this metabolic rewiring is an unbalanced redox microenvironment that consumes reduced glutathione; depletion of glutathione or ablation of glutathione-replenishing enzymes induced rapid cell death, revealing a potential new avenue for FLT3ITD targeting in leukemia.

SESSION 4: INFECTION

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Metabolic-Inflammatory Crosstalk in Viral Infection

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The molecular mechanisms of how viral infections cause tissue damage and clinical disease are poorly understood and cannot solely be attributed to classical immune effectors. Instead, the involved inflammatory processes are increasingly being appreciated as tightly integrated with metabolic pathways. By taking an integrated approach of virology, mouse immunology, pathology and systems biology, we unveiled a novel link between the antiviral cytokine interferon and redox metabolism. Virus-induced interferon was found to regulate the expression of key antioxidant genes, leading to the intracellular accumulation of reactive oxygen species, the death of hepatocytes and liver pathology.
Ongoing research in our laboratory focuses on the molecular interface of metabolism and inflammation in chronic viral infection. We investigate the impact of the multi-cytokine milieu on systemic metabolism, tissue damage and infection-associated cachexia. This systemic perspective aims towards a better pathophysiological understanding of infectious and inflammatory diseases.

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Tissue damage control in immune mediated inflammatory diseases

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Damage control refers to actions made towards minimizing the extent of damage associated with a given emergency situation. Depending on context, damage control may refer to emergency procedures dealing with the sinking of a ship or to surgery procedures dealing with severe trauma or even to a company in *Marvel Comics*, which repairs damaged property arising from conflicts between super heroes and villains. By extension, "tissue" damage control refers to adaptive responses that minimize the extent of tissue damage and dysfunction associated with the pathogenesis of immune mediated inflammatory conditions, including in infectious diseases[1,2]. Presumably, tissue damage control is regulated by a number of evolutionarily conserved stress- and damage-responses associated with the induction of overlapping profiles of gene expression[1]. This argues for the existence of a core number of evolutionarily conserved genes regulating tissue damage control[1]. Moreover, this might explain why overlapping stress- and damage-responses confer protection against apparently unrelated forms of stress and damage, a phenomenon known as hormesis. A subgroup of these evolutionarily conserved genes regulates iron metabolism and control the participation of iron in the production of free radicals leading to oxidative stress and tissue dysfunction. In support of this notion, immune mediated inflammatory diseases are often associated with deregulated iron metabolism and oxidative stress[3,4]. Here I will discuss how the expression of stress responsive genes controlling iron metabolism exert anti-oxidant effects that confer tissue damage control in different infectious diseases[5-7].

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Sepsis: the need for tolerance not complacency

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Sepsis is a life-threatening condition that arises as a systemic inflammatory response syndrome to an infection. Its uncontrolled progression can in frequent cases lead to multiple organ failure, which is still associated with high mortality rates. Modern antibiotics made clear that the infection is only an initiating, and not always necessary, event of this syndrome as many patients with sepsis die despite effective eradication of the inciting pathogen. This observation critically contributed to a paradigm shift that focused the pathogenesis of sepsis on the host and not on the pathogen. However, therapeutic strategies based on the inhibition of proinflammatory critical mediators of sepsis or immunostimulation have so far failed to improve sepsis outcome and, therefore, this condition urgently needs transformative therapeutic ideas and strategies. We argue that the induction of disease tolerance, a defence strategy that minimises the impact of an infection on organ function without directly affecting the pathogen burden, is perhaps the missing but essential element to add to the current components of sepsis care and treatment.

Structure and genome release of Myoviridae phage with double layered baseplate

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Bacteriophages from the family Myoviridae use double-layered contractile tails to infect bacteria. Contraction of tail sheath enables tail tube to penetrate through bacterial cell wall and serve as channel for transport of phage genome into cytoplasm. However, the mechanisms controlling the tail contraction and genome release of phages with “double layered” baseplates were unknown. We used cryo-electron microscopy to show that binding of Twort-like phage phi812 to Staphylococcus aureus cell wall requires 210° rotation of receptor-binding complexes within its baseplate. The rotation disrupts interaction of the receptor-binding complexes with tail sheath and, hence, triggers its contraction. However, the tail sheath contraction of myoviridae phages is not sufficient to induce genome ejection. We show that end of phi812 dsDNA genome is bound to one protein subunit from a connector complex that also forms interface between phage head and tail. The tail sheath contraction induces conformational changes of tail neck and connector that result in disruption of the DNA binding. The genome penetrates into the neck, but is stopped at a bottleneck before the tail tube. Subsequent structural change of the tail tube induced by its interaction with S. aureus cell is required for the genome release.

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ADAR1 is crucial for discriminating between 'self' and 'non-self' cellular RNAs

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How a cell can distinguish between self and non-self nucleic acids is critical as failure to discriminate correctly can cause autoimmune disorders. Recent technological developments have revealed that the abundance and diversity of endogenous RNAs in the cell is greater than previously anticipated. Therefore the cell must a rigorous system to prevent autoimmune reactions. Our group were the first to demonstrate that the deamination of adenosine to inosine in dsRNA marks endogenous RNA as 'self'1. In humans only 0.4% of RNA editing events catalysed by ADARs result in recoding while the majority, which is over 100 million editing events occur within transcripts encoding Alus or SINEs in other organisms. Mutations in ADAR1 have been shown to cause the autoimmune disorder Aicardi Goutières syndrome (AGS) 2. Patients with AGS display heightened levels of type-I interferon (IFN) and IFN stimulated genes (ISGs). We have characterised the editing activity of these ADAR1 mutants identified in the AGS patients and find that they have reduced editing activity that is consistent with clinical observations of the disease.
Mice lacking Adar1 also have an immune phenotype with heighten levels of IFN and ISGs1. We have rescued the embryonic lethality of Adar1-/- mice to birth by generating a double homozygous mutant between Adar1 and Mavs (Mitochondrial antiviral-signaling protein); an important protein in the innate immune RLR (RIG-I like receptors) pathway. We propose that ADAR1 plays a major role in the modification and hence the discrimination of endogenous cellular dsRNAs and in the absence of ADAR1, cellular RNAs aberrantly stimulate an innate immune response which leads to autoimmune disease phenotypes.

This immune function of ADARs is evolutionary conserved and occurs in Drosophila which has a very different immune system to vertebrates, lacking both IFN and the dsRNA sensor proteins. In addition Drosophila encode the orthologue of ADAR2 not ADAR1 as it has been lost in Diptera. Thus this evolutionary conservation of ADARs role in innate immunity is highly significant.

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