1 – NSI abstract ID

Title

Combined inhibition of complement (C3/C5) and the Toll-like receptors co-factor CD14 in inflammation induced by Candida spp.

Authors

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Absract

Candida albicans is one of the foremost fungi causing opportunistic infections in immunocompromised patients. Complement and the Toll-like receptors (TLRs) are two main components of our innate immune system, reacting upstream by being initial recognizers of pathogens. The complement system is often evaded by Candida albicans. It has been shown before that double inhibition of complement, directly targeting C3 or C5, and of CD14, a key co-receptor for TLR4, TLR2, and others, in Gram-negative Escherichia coli and Neisseria meningitides, as well as several Gram-positive Staphylococci species, significantly decreases the inflammatory response in the whole blood. Now, we for the first time describe the effect of this double inhibition in fungi, using an ex vivo human whole-blood model of Candida albicans induced inflammation. Complement activation products (terminal C5b-9 complement complex [TCC] and C3 activation [C3bc]), granulocyte (myeloperoxidase) and platelet activation (β-thromboglobulin), cytokines (tumor necrosis factor [TNF], IL-1β, IL6, and IL-8), and leukocyte activation (CD11b) were quantified, by enzyme-linked immunosorbent assays, multiplex technology, flow cytometry, and iLite® Cell-Based Solutions. In Candida albicans-incubated whole blood, preliminary results of the double inhibition of both C5 and CD14 showed a decrease of most inflammatory markers. As seen before for bacteria, when compared to the single inhibition, the double inhibition strategy presented a synergistic effect, with CD14 inhibition potentiating the blockade of C5. In conclusion, despite the limitations of our ex vivo model, the double inhibition of complement and CD14 shows potential as a future therapeutic approach for immunocompromised patients suffering Candida albicans infections and sepsis with adverse innate immunity-induced inflammatory responses.

2 – NSI abstract ID

Title

Development of anti-CD37 T cell Antigen Coupler (TAC)

Authors

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Abstract

Chimeric antigen receptor (CAR) targeting CD19 have shown impressive results in patient with B-ALL, however, the treatment of B-cell non-Hodgkin lymphoma (B-NHL) was less effective. We and others have previously developed a CAR construct against an alternative target, CD37. This molecule is a surface protein present on B-NHL, which revealed to be more stably expressed than CD19 in patient samples. Our seminal report was based on transient expression of the CD37 CAR molecule as mRNA, but we and others observed that CD37 CAR when constitutively expressed showed some fratricide activity affecting CAR T cell manufacturing. We here show that this effect is not affecting the capacity of the CAR T cell to control tumour development in vivo, however, it might have a negative impact for clinical production. T cell antigen coupler (TAC) has been recently developed and shown to reduce unspecific activity; thus, we designed an anti-CD37TAC and compared it to our CD37 CAR. We followed the expansion and expression of primary T cells transduced with both constructs, assessed the T cell cytotoxicity and measured the release of cytokines in a coculture assay. Moreover, we used transduced Jurkat NFAT cells to evaluate the construct's functionality. We observed a positive effect of the CD37 TAC design for the T cell production while maintaining similar functionalities than the CD37CAR, providing an original alternative to improve therapeutic antigen receptor-based therapies.

3 – NSI abstract ID

Title

A new candidate CAR for B-cell malignancies

Authors

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Abstract

Chimeric antigen receptor (CAR) T cell therapy has had considerable success in the treatment of B cell malignancies. Targeting of CD19 has brought great success to the treatment of acute lymphoblastic leukemia (ALL), however relapse remains an issue in many cases. Such relapse can result from downregulation or loss of CD19 from the malignant cell population. In certain cases, this may also be achieved by expression of alternate isoforms of target antigens. Consequently, there remains a requirement to target alternative B-cell antigens, and to diversify the spectrum of epitopes within the same antigen; whether for use in isolation, or in combination with existing options. CD22 has been identified as a substitute target in cases of for CD19-negative relapse. One anti-CD22 antibody, clone m971, targets a membrane-proximal epitope of CD22, and has been widely validated and used in the clinic. Here we have compared m971-CAR with a CAR derived from IS7, an antibody that targets an epitope with a more distal position. We first showed that the IS7-CAR has superior affinity and avidity, and is active and specific against CD22 positive targets, including primary patient samples. Side-by-side comparisons indicated that while IS7-CAR killed less rapidly than m971-CAR in vitro, it remains efficient in controlling lymphoma xenograft models in vivo. Thus, IS7-CAR presents a potential alternative candidate for treatment of refractory B-cell malignancies.

4 – NSI abstract ID

Title

Statistical analysis of synthetic AIRR-datasets to guide the development and benchmarking of AIRR-based machine learning

Authors

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Abstract

Machine-learning on adaptive immune receptor repertoire (AIRR) data has shown great potential to recover hidden immune patterns (signals), which is crucial for AIRR-based diagnostics and therapeutics design. Experimental AIRR data, labeled with immune events such as immune state or antigen specificity, does not generally represent ground truth data since complete knowledge on immune-event-related as well as unrelated (potential confounding factors) (sub)sequences is unavailable. The lack of large-scale ground truth data renders the development and benchmarking of robust, explainable, and interpretable machine learning approaches unfeasible. To address the lack of large-scale ground truth data, simulation frameworks have been recently developed. However, it remains unclear to what extent a simulation approach and the complexity of hidden immune signal impacts a dataset both in terms of its nativeness as well as it classifyability. To address this knowledge gap, we developed the LIgO software suite that enables the modular ("lego"-like) assembly of immune-event-labeled synthetic AIRR-datasets for the development and benchmarking of AIRR-based machine learning. Specifically, LIgO contains different methods for simulating immune events and immune signals, and we provide a data-driven discussion of the advantages and disadvantages of each simulation approach for user guidance. In three case studies that simulate scenarios that are as of yet not available experimentally, we explore prediction performance and signal recovery using baseline machine learning methods on repertoire-scale paired chain data, repertoire-scale antigen-annotated data, and different sequence-based data leakage scenarios. The LIgO software is integrated into the immuneML ecosystem and outputs AIRR-compliant data for ready use in any AIRR-compliant machine learning software.

5 – NSI abstract ID

Title

Genomic and proteomic antibody repertoire analysis at single-cell and single-molecule resolution

Authors

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Abstract

The diversity of the antibody repertoire is crucial to broad pathogen recognition. Therefore, investigating the relationship between the genomic (BCR) and phenotypic (serum antibody) diversity of antibodies is crucial for understanding human adaptive immunity. However, despite advances in high-throughput BCR sequencing, a joint characterization of antibody complexity at the genomic single-cell and proteomic single-molecule level remains elusive due to the fact that mass spectrometry (MS) analysis of the serum antibody repertoires has remained underdeveloped. Specifically, it remains unclear to what extent the blood B-cell receptor repertoire differs from the serum antibody repertoire or, even more fundamentally, what the number of unique antibody clonotypes in the blood is. To address these questions, we isolated blood-borne B cells from a healthy donor and sequenced the BCR repertoire at bulk and single-cell level. Simultaneously, serum antibodies of all major isotypes were isolated, digested, and sequenced with tandem MS, ensuring that the antibody repertoire is covered comprehensively. Systems immunology analysis showed high concordance between bulk- and single-cell sequencing. MS was able to identify CDRH/L3 peptides linked to specific clonotypes in the sequencing libraries, demonstrating how to reconstruct antibody clonotypes from proteomics data at single-molecule resolution. In addition, performance benchmarking of antibody MS revealed that relative CDR3-peptide concentration differences can be measured with high sensitivity. In conclusion, we developed a platform that connects bulk sequencing, single-cell sequencing, and mass spectrometry enabling the quantification of the serum antibody repertoire at unprecedented precision thereby removing a blank spot in our understanding of antibody adaptive immunity.

6 – NSI abstract ID

Title

Preclinical development of an unsuspected CAR candidate for acute myeloid leukemia

Authors

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Abstract

Acute myeloid leukemia (AML) is an aggressive and heterogeneous disease of the hematopoietic system. Due to these features, AML has been challenging to treat using targeted therapy such as chimeric antigen receptor (CAR)-redirected T cells. Indeed, almost all AML CAR targets exploited to date shared expression between pathologic blasts and normal hematopoietic stem cells (HSCs) which may induce long-term adverse toxicity. Therefore, there is still a clear unmet need to define suitable AML targets. We and others recently observed that the leukocyte-specific tetraspanin protein CD37, which is predominantly found on mature B cells, is also expressed in AML samples. By interrogation of gene expression databases, we first showed that CD37 is overexpressed in AML compared to normal HSCs. We confirmed this overexpression at the protein level using AML cell lines and patient biopsies. Further characterization of patient bone marrows showed that progenitor cells-containing leukemic stem cells also express CD37. We therefore tested our anti-B cell lymphoma CAR targeting CD37 and compared it to the AML gold-standard CD33CAR, in a series of preclinical experiments. We showed that CD37CAR T cells can specifically kill AML cells, secrete inflammatory cytokines and control tumor development in several xenograft animal models. We confirmed our observations of CD37CAR efficacy against AML primary cells and provide evidence that CD37CAR is safer than CD33CAR in terms of toxicity against healthy bone marrow. From our experiments, we conclude that a CAR targeting CD37 is an attractive and safer solution to treat a broad range of AML patients.

7 – NSI abstract ID

Title

Redundancy, sensitivity, and predictability of developability parameters in natural, patent-submitted, and clinical-stage antibodies

Authors

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Abstract

One of the antibody design challenges is developability. It signifies the feasibility of antibody candidates to successfully progress from discovery to development via evaluation of selected and very specific physicochemical properties. Suboptimal developability can lead to failure in late-stage clinical trials. The ability to predict and design properties in line with clinical application requirements helps boost the success rate of therapeutic antibody development. In contrast to therapeutic antibody discovery lasting decades, the natural immune system can design antibodies with physiological developability parameters within days. However, the natural landscape of developability parameters and its alignment with the therapeutic one has not yet been described. Delineating the rules of natural antibody design can provide insights into therapeutic antibody discovery. Thus, we profile natural antibody repertoires against therapeutic antibodies to establish redundancy, sensitivity, and predictability of antibody developability in native and human-derived antibodies. We exploit a dataset of 2 million antibody sequences and their predicted structures to build a developability landscape of 42 sequence and 48 structural computationally determined real-world relevant developability parameters. We observe the greater inter-independence of structure developability parameters compared to the sequence-based ones. We show that sensitivity of developability parameters is dependent on antibody region and structure-based developability parameters are more sensitive to change than sequence-based developability parameters. We find that sequence-based developability parameters are more predictable than structure-based parameters. Therefore, exploiting the vast amount of available antibody high-throughput data can facilitate the derivation of the underlying rules of developability profiles with which to guide antibody therapeutic discovery.

8 – NSI abstract ID

Title

Structural modeling and molecular docking reveal the impact of sequence variation in CDR-H3 on binding, contact preference, and global fold of antibodies

Authors

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Abstract

The incredible diversity of antibody repertoires has been elucidated over the last decade using high-throughput sequencing. However, corresponding experimental large-scale structural insight is currently lacking. This lack has been somewhat made up for by the emergence of (deep learning-based) computational structural biology tools that can approximate antibody structure with high accuracy (think AlphaFold and co.). However, it remains unclear whether structural modeling in combination with information driven molecular docking captures the binding behavior and contact preferences of antibody variants. Here modeled ~500 000 variants of the antibody trastuzmab and created a large dataset of in silico complexes via information driven docking. We found that sequence variations in the CDR-H3 impact the global fold and contact preferences of the antibody.

9 – NSI abstract ID

Title

Topic modeling on AIRR-seq data enables identification and generation of disease-associated sequences without individual sequence labels

Authors

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Abstract

Adaptive immune receptor repertoire (AIRR) data are complex and carry disease and infection relevant information in the form of sequence-based immune signals. For an AIRR containing an immune signal, the fraction of AIR sequences bearing this signal may be very low. One of the major unresolved challenges in AIR diagnostics and therapeutics discovery, is to predictively isolate the immune signal from an AIRR and then to use this signal to engineer novel sequences. This is a machine learning (ML) problem that bridges repertoire and sequence level classification. Existing ML methods rely on engineered features or on a predefined knowledge about repertoire generation. To address this problem, We developed AIRRTM, an end-to-end generative model based on an encoder-decoder architecture and topic modeling (TM). AIRRTM reaches stable performance in identification and generation of disease specific sequences using repertoire labels but not sequence labels.

10 – NSI abstract ID

Title
CellFit: T cells fit to fight cancer

Authors
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Abstract
The use of T lymphocytes in adoptive cell therapy (ACT) shows great promise for treatment of cancers otherwise incurable. However, one of the largest challenges faced in cell-based cancer therapy is to provide an efficient and scalable production. The use of "living drugs" leads to the development of promising therapy but requires precise logistics at all stages of cell life: development, manufacturing, transport and finally the infusion to the patient. Presently, only five T-cell products have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for clinical use. Whilst the main focus of development has been on finding tumour targets and improving the genetic modifications of the immune cells in ACT, less effort has been made to efficiently produce the optimal subset of immune cells, which ideally is more homogenous and stem-cell like, for treatment. Indeed, it is now clear that the quality, efficacy, and longevity of T-cell immunity depend on the differentiation of naïve T cells (TN) into phenotypically distinct subsets with specific roles in protective immunity. These include memory stem-like (TSCM), central memory (TCM), effector memory (TEM), and highly differentiated effector (TE) T cells. Less differentiated cells like TCM and TSCM were shown to respond more persistently against cancer cells 1,2 . Now, the present ex vivo T-cell manufacturing is very different from physiological T-cell expansions occurring in vivo when T cells encounter antigen and does not generate the T-cell subsets that provide long-term therapeutic efficacy in solid cancers. To realise broader clinical applications for efficacious ACT and enhance the current therapeutic approach, the immunotherapy field has a great need to further improve therapeutic T-cell manufacturing methods. With the CellFit project, we aim to define optimal growth conditions for improved manufacturing of adapted therapeutic T cells required for solid tumour treatment. The T-cell culture methods will be translated from manual to robotic set-ups where all pipetting, cell culturing and assay read-outs will be performed in 96-well plate formats with robots coupled to incubators, spectrophotometer and high-content confocal microscope. Moreover, we will test a panel of phenotypic markers using state-of-the-art technologies such as mass cytometry (CyTOF) and cellular metabolism (Seahorse analysis) to be able to compare different culture conditions and characterise the heterogeneity of the T-cell population. Then, data acquired by these high-throughput systems will be analysed with a custom pipeline especially designed for this project and including various modern computational cytometry techniques. We will present the pipeline of the project to establish an optimal T cell product and a high throughput screening platform for cellular therapies.

11 – NSI abstract ID

Title

Characterizing the infiltrating immune microenvironment in bone tissue engineered constructs

Authors

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Abstract

Bone tissue engineering aims to treat clinically challenging bone defects by combining biomaterial scaffolds and bone marrow mesenchymal stem cells (BMSC) as bone tissue-engineered (BTE) constructs. These constructs encounter the immune system after implantation, and this response plays a critical role in the process of bone regeneration following damage. The aim of this study was to optimize a method to isolate single cells from in vivo implanted BTE constructs and characterize the immune cell populations responding to these constructs. Bi-calcium phosphate scaffold granules (BCP) alone or seeded with BMSC were implanted subcutaneously in mice or calvaria defects in rats. The scaffolds were harvested after two weeks, and a single-cell suspension was obtained through rounds of optimized enzymatic digestions. Isolated cells were analyzed using a multiparametric flow cytometry panel for a broad landscaping of infiltrating immune cell populations. Scaffold-associated immune cells from calvarial samples presented a higher number of T and B cells in comparison with other immune cell types, while macrophages and neutrophils were below 20% of total CD45+ cells. The composition of infiltrating cells was comparable in BCP alone and BCP+BMSC, except for a slightly higher proportion of B cells in BCP+BMSC. To our knowledge, this is the first report of immunophenotyping for single cells isolated from BTE constructs. In conclusion, we successfully optimized a protocol for isolating immune cells from BTE constructs that can be used for multiple downstream analyses to study the function of specific immune cells involved in the success of bone regeneration therapy.

12 – NSI abstract ID

Title

Immunovirotherapy with Semliki Forest virus encoding interferon-γ eradicates and induces protection against breast cancer in mice

Authors

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Abstract

We have previously shown that a viral vector based on Semliki Forest virus (SFV) can express functional interferon-gamma (IFN-γ) that activates macrophages towards a tumoricidal phenotype in combination with the Toll-like receptor (TLR)2/1 agonist Pam3CSK4 in vitro. Here we tested the efficacy of and investigated the mechanism behind immunovirotherapy with SFV vector encoding IFN-γ (SFV-Ifng) in combination with Pam3CSK4 in the E0771 mouse model for breast cancer. Orthotopic mammary adenocarcinoma tumors were treated by intratumoral injections. Combined immunotherapy with SFV-Ifng and Pam3CSK4 was highly effective at completely eradicating established tumors in 83-100% of treated animals, as was SFV-Ifng alone eliminating up to 83% of established tumors showing its efficacy as a single agent. Therapeutic efficacy was dependent on presence of encoded IFN-γ since lower treatment efficacy was observed in mice treated with SFV control vector lacking IFN-γ in combination with Pam3CSK4 or when used as a single treatment. Therapeutic efficacy of SFV-Ifng and Pam3CSK4 combination was completely abrogated by depletion of CD8 T cells. Survivor mice were protected against orthotopic re-challenge with E0771 tumor cells at the contralateral mammary fat pad long-term after initial tumor elimination or against re-challenge at a non-orthotopic site with a double amount of E0771 tumor cells. Our study reveals that local immunotherapy with SFV-Ifng in combination with TLR agonist is a highly effective treatment to eradicate murine breast tumors that is mediated by CD8 T cells and provides systemic long-term immune protection against cancer recurrence.

13 – NSI abstract ID

Title

Multimodal human thymic profiling reveals trajectories and cellular milieu for T agonist selection

Authors

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Abstract

Developing T cells in the thymus, termed thymocytes, recombine their T cell receptor (TCR) genes to express a highly diverse TCR repertoire. To ensure that only functional thymocytes without self-reactive capabilities are allowed to complete maturation, they are assessed for TCR avidity towards self-peptide-MHC complexes presented by thymic antigen-presenting cells. While thymocytes expressing self-reactive TCRs should be induced to undergo apoptosis, a process termed negative selection, divergence into tolerogenic, agonist selected lineages represent an alternative fate. Understanding factors influencing the development and differentiation of agonist selected T cell populations is needed in order to benefit from their immunoregulatory effects in clinical use. As thymocyte development, selection, and lineage choices are dependent on spatial context and cell-to-cell interactions, we have performed Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-seq), single cell TCR and BCR sequencing, and spatial transcriptomics on the young paediatric human thymus. Thymocytes expressing markers of strong TCR signalling diverged from the conventional T cell developmental trajectory prior to CD4+ or CD8+ lineage commitment, while markers of different agonist selected T cell populations (CD8$\alpha\alpha$(I), CD8$\alpha\alpha$(II), T(agonist), Treg(diff), and Treg) exhibited variable timing of induction. Expression profiles of chemokines and co-stimulatory molecules indicated that dendritic cells, B cells, and stromal cells contribute to agonist

14 – NSI abstract ID

Title
Specific adaptive immune response following vaccination

Authors
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Abstract
The purpose of vaccination is to stimulate the formation of pathogen-specific memory responses able to efficiently respond to a future exposure. The ability to form specific responses is due to the expression of highly diversified receptors on T and B cells. Following receptor recognition of antigen, an immunological cascade may be activated to result in development of protective immunity. However, the formation of an efficient immune response following vaccination differs among individuals in the population. Environmental factors, previous exposures, and genetics background may influence immune cell activation, and also which epitopes responses will be directed at. As an example, human leukocyte antigen (HLA) molecules have an inherent difference with respect to which peptides they can encompass. Could this translate into differences in T cell repertoires that again influences the formed antibody responses? Our strategy is to evaluate the T and B-cell responses formed in healthy volunteers as a response to vaccination in the context of the individual genetic background. A better understanding of the dynamics of these interactions at the individual level will enable development of improved strategies for next generation vaccine development.

15 – NSI abstract ID

Title

The infectious salmon anaemia virus 4-O-sialyl-acetyl-esterase extensively prunes target cell surfaces in infected hosts

Authors J

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Abstract

Many sialic acid-binding viruses express a receptor-destroying enzyme (RDE) that removes the virus-targeted epitope and releases viral attachment to the host cell surface. Despite growing appreciation of the role of the RDE in the infectious cycle, little is known of its direct effects on the host. We show that infection with infectious salmon anaemia virus (ISAV), an Orthomyxovirus that attaches to 4-O-acetylated sialic acids on epithelial, endothelial, and erythrocyte surfaces in Atlantic salmon, causes wide-spread loss of vascular and red blood cell surface 4-O-sialyl-acetylation as the infection progresses. The effect is specific and was absent upon infection with another virus of similar tropism. In erythrocytes, the loss of viral receptor was associated with increased availability of surface sialic acids to lectins, suggesting a potential to influence cellular signalling and/or cell-to-cell interactions. By exposing erythrocytes to ISAV ex vivo, we found that antibodies inhibiting viral attachment also prevented the virus-induced cell surface pruning. The viral haemagglutinin esterase was sufficient to induce the effect, which was obliterated by alanine mutation of its catalytic serine, Ser32, thus identifying a key role for the viral esterase. To our knowledge, this is the first characterisation of the extent to which a viral esterase can modulate cellular surfaces in infected individuals. Our findings raise the questions if the observed loss of 4-O-sialyl-acetylation affects immune functions in infected fish by modulating interactions with sialic acid immunoglobulin like lectins or in other ways contributes to the disturbance of vascular function and anaemia typically observed in infected fish.

16 – NSI abstract ID

Title

A novel target antigen for CAR therapy in metastatic osteosarcoma

Authors

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Abstract

Osteosarcoma (OS) is an aggressive malignant tumour, mainly affecting young adults. OS tends to metastasize into the lungs and patients with primary metastatic disease have a very poor prognosis. Thus, there is a clear need to identify therapeutic approaches to eliminate the metastases. An emerging path to treat solid tumours is the use of Chimeric Antigen Receptor (CAR) T cells. Indeed, for CAR T-cell therapy to be efficacious CAR T cells should recognize an epitope selectively expressed on the cell surface of OS and OS metastases, but not on healthy tissues; unfortunately no such targets have been identified yet. Herein we validated two antibodies, TP-1 and TP-3, which were generated from hybridomas in our hospital more than three decades ago and shown to be specific to OS tissues, however their target remains unknown. We designed scFv based on their sequences, linked them to a second generation CAR signaling tail and called them OSCAR-1 and OSCAR-3. We first identified their antigen as an isoform of a common and ubiquitous protein but its membrane location seemed to be a hallmark of OS development, hence a highly specific and hard-to-predict target. T cells transduced with OSCAR-1 and OSCAR-3 induced efficient and effective killing of OS cell lines, but were not reactive to hematopoietic stem cells or any healthy tissues with the exception of osteoblasts. We performed three in vivo models and observed an efficient control of tumour growth and survival. OSCARs are promising therapeutic molecules which are presently being submitted for clinical validation.

17 – NSI abstract ID

Title

Deciphering T cell intracellular signalling pathways: novel interaction partners of the SH2 domain in T cell-specific adaptor protein (TSAd)

Authors

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Abstract

Following naïve T cell activation, T cell-specific adaptor protein (TSAd) is upregulated and believed to mediate T cell receptor signalling by modulating Src family tyrosine kinase signalling. Moreover, it has been reported that TSAd-deficient mice display accelerated allograft rejection, in which TSAd mediates regulatory T cell activity. As an intracellular adaptor protein, TSAd contains several regions for protein interaction, including the Src homology 2 (SH2) domain. Prototypic binding of phosphorylated tyrosines to SH2 domains, suggest that TSAd is involved in the phosphotyrosine signalling pathway. However, the molecular details of the underlying mechanisms and the defined role of TSAd in T cell activation and biology remains elusive. Here, we perform pulldowns with exogenous TSAd in Jurkat T cells and subject it to mass spectrometry analysis. While confirming previously characterised ligands of the TSAd SH2 domain, we identify several novel interactors of the TSAd SH2 domain, including DOK2, PTPN6, PTPN11 and proteins of the COPII coat. Using immunoprecipitation and YFP-based protein fragment complementation assays, we validate and further characterise the consequence of these interactions downstream of T cell activation. Furthermore, using CRISPR-edited TSAd mutants and TSAd-deficient murine CD4+ T cells, we currently explore the affected signalling pathways that these interaction partners are implicated in. Taken together, these findings provide insight into the role of TSAd in T cell function and possible mechanisms for how TSAd acts to fine-tune T cell intracellular signalling through the TSAd SH2 domain.

18 – NSI abstract ID

Title

APC-targeted DNA vaccines against SARS-CoV-2 elicit robust immunological responses in mice

Authors

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Abstract

Background: The SARS-CoV-2 pandemic has to date resulted in over 6.5 million deaths worldwide and an even higher number of hospitalizations. Antigenic drift has reduced the efficacy of current vaccines with respect to preventing viral infection, forcing a reassessment as to which type of protection we expect after vaccination. The long-term correlate of protection against coronaviruses has yet to be established. Here, we have genetically linked Spike to different ligands for receptors expressed on antigen presenting cells. The strategy should facilitate steering of immune responses to different types and enable an evaluation of which type is more likely associated with long term protection. Methods: Mice were immunized intra muscularly with DNA vaccines encoding Spike genetically linked to a selected APC receptor ligand (either scFv against MHCII or CD11c, FliC, or the chemokines XCL1 and MIP1α). Next, we evaluated antibody formation in sera, T-cell responses, as well as antigen-specific bone marrow B-cells. Results: APC-targeting of Spike efficiently raised IgG and T-cell responses after a single vaccination, and antigen-specific B-cells were readily detected in bone marrow at day 88. Interestingly, linkage of Spike to XCL1 was found particularly efficient for induction of bone marrow B cells and long-term antibody responses. Conclusion: APC-targeting of antigen increases immunogenicity after vaccination, but further studies are needed for profiling the different types of immune responses elicited after steering of Spike towards different receptors on APC.

19 – NSI abstract ID

Title

Validation of a new imaging probe targeting CXCR4

Authors

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Abstract

Solid tumors account for more than 90% of cancers and almost 35 to 40% have a poor prognosis, there is therefore an outstanding need for new treatments. One emerging option lies in immunotherapy, which corresponds to the use of the patient immune cells to fight their cancer. However, the immune cells modified are confronted to inhibitory effects coming from the tumor microenvironment (TME). The C-X-C motif chemokine receptor 4 (CD184 or CXCR4) has become an attractive target due to its overexpression in the majority of solid tumors. Binding to its ligand, C-X-C chemokine ligand 12 (CXCL12 or SDF-1), has been described as a driver of tumor growth and metastasis. Our collaborators have previously developed a specific inhibitor, R54, which can alter cell migration. We have extended the use of R54 and are developing R54-based probe, for specific positron emission tomography (CXCR4-NAN-PET) to be used as a tracer for CXCR4-positive tumors. Since CXCR4 can also be expressed on immune cells, we have assessed the effect of R54 on immune effector cells (T- and NK-cells). Our results confirm that R54 is extremely specific against CXCR4 in immune cells: we tested its effect in a series of settings and could not find any other modifications than the ones related to CXCR4. We have further expanded our analysis to therapeutic T cells (expressing Chimeric Antigen Receptor) and did not observe interference with their therapeutic activity in vitro. Taken together we show that R54 is an extremely specific molecule, even in immune cells.

20 – NSI abstract ID

Title

Development of an Lck-SH2 domain superbinder for characterization of phosphotyrosine proteins during T cell activation

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Abstract

T cell activation via T cell receptor (TCR) requires the tyrosine kinase Lck, and is propagated through phosphorylation of tyrosine residues on various proteins. These phosphotyrosines (pTyr) may serve as docking stations for SH2 or PTB domain containing proteins, ultimately leading to altered functional activity of enzymes and adaptor proteins. While pTyr-proteins play intermediate roles in TCR signal transduction, many are still poorly characterized. This could be attributed to suboptimal enrichment with anti-pTyr antibodies and relatively low pTyr prevalence, compared to phosphoserines and phosphothreonines. An alternative to enrichment of pTyr proteins may be pTyr binding protein domains. However, the pTyr binding affinity to SH2 is comparatively low, ranging between Kd 0.1 to 10μM. Thus, there is a need for development of high affinity pTyr binding tools. Introduction of amino acid substitutions in the Fyn and Src SH2 domains has been reported to increase binding affinity 10-fold. In order to identify additional pTyr proteins dependent on Lck-signaling, we substituted the Thr159Val, Ser164Ala and Lys182Leu of the Lck SH2 domain to produce an Lck-SH2 pTyr superbinder. The mutated SH2 domain pulled down significantly more pTyr proteins from pervanadate treated Jurkat T cells, compared to wild type, as assessed by immunoblotting. Combined with mass spectrometry analysis of pull down proteins, this superbinder will aid the rapid identification of novel Lck interacting proteins. Ultimately, the results from this work may reveal potential drug targets for controlling auto-reactive T cells or promoting vaccine responsive T cells, in particular in the context of immunodeficiency.

21 – NSI abstract ID

Title

Humanization of murine Chimeric Antigen Receptors (CARs) for immunotherapy of Osteosarcoma

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Abstract

CAR T cells have shown remarkable efficiency against liquid malignancy. However, such efficient responses were not observed for treatment of solid tumors. Aside from challenges linked to tumor microenvironment, proper targeting of the tumor is critical. We have generated OSCAR-1 and OSCAR-3, chimeric antigen receptors (CARs) targeting osteosarcoma (OS) from two mouse antibodies, TP-1 and TP-3, isolated in our hospital, and proven highly specific for OS-derived lung metastases. Metastatic OS still represents a medical unmet need, and immunotherapy is an attractive option. OSCARs recognize a unique target in around 90% of primary samples, and OSCAR T cells demonstrated efficacy and specificity. Thus OSCARs are promising for cellular therapy of OS patients. However, the murine TP-antibodies raise concerns regarding immunogenicity of OSCAR T cells. Antibody sequences from murine origin can be humanized to dampen immunogenicity. We outsourced humanization of our single chain fragment variables (scFv's) to two companies and obtained sixteen humanized sequences. These sequences were cloned as second generation CARs and compared to original OSCARs. They demonstrated varying levels of staining with an anti-mouse fragment antigen-binding (mFab) antibody. We assessed their reactivity with a reporter system and observed substantial variations for some humanized CARs compared to murine CARs. The sequences obtained from one of the companies were not reactive as CARs and did not bind their target when produced as soluble antibodies. The reactive sequences showed specific efficiency as CAR T cells in cytotoxic activity and functional assays against relevant cell lines. Consequently, we selected similar candidates for further characterization.

22 – NSI abstract ID

Title

Unveiling Addison's disease by adrenal imaging mass cytometry

Authors

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Abstract

Autoimmune Addison's disease (AAD) is characterized by destruction of the hormone producing cells in the adrenal cortex, leading to impaired production of cortisol and aldosterone. AAD occurs isolated or in autoimmune polyendocrine syndromes and is associated with risk loci implicating both arms of the immune system. However, adrenal biopsies are not collected for ethical reasons, representing a gap in knowledge about the immune system involvement in the affected tissue. To bridge this gap, we have utilized autopsy material from individuals with and without AAD. By utilizing imaging mass cytometry (IMC), which allows spatial studies of dozens of individual protein markers, we have developed an antibody panel to scrutinize immune-adrenal ongoings. Analyses of non-AAD human adrenal glands support earlier reports describing an abundance of macrophages, but also provide novelty in showing putative tissue resident CD8+ T-cells throughout the adrenal cortex. We also identify unique target cells with elevated expression of known as well as putative novel autoantigens. Conversely, adrenal IMC analysis of AAD recapitulates seminal findings with an abundance of CD4+ and CD8+ T-cell within the cortex infiltrate. However, we also reveal novelty by identifying macrophages, mesenchymal cells and innate lymphocytes within the infiltrating lymphocytes. This identification of novel cellular suspects in AAD aids in elucidating the pathogenesis and progression of the disease and may ultimately point towards strategies to dampen the autoimmune adrenal process.

23 – NSI abstract ID

Title
Vaccine efficacy during obesity

Authors
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Abstract
Vaccines are less effective in obese individuals as compared to those with a recommended body fat content. Neither increasing the dosage of antigen nor the inclusion of adjuvants in vaccines seem to significantly increase protection during obesity. With the prevalence of obesity steadily increasing, finding new ways to induce protective immunity in these individuals is imperative. Our project aims to examine how the chronic low-grade inflammation associated with obesity influences antibody functionality, and to develop vaccine strategies able to improve vaccine efficacy. To enable detailed investigations into the immunological characteristics of obesity, our starting point was mouse studies. We have established an obesity mouse model using a high-fat diet to increase weight. We have demonstrated significant differences in weight and obesity markers such as interleukin 6, leptin, and glucose between the obese and non-obese groups. A comparison of the immunological profiles following vaccination with Pandemrix and selected subunit vaccines also demonstrated significant variations in antibody induction, particularly a reduction of IgG2a during obesity. We have also observed reduced protection against a lethal influenza challenge in obese as compared to healthy weight mice. Our preliminary data suggests that qualitative differences in antibody functionality may explain the reduced protection of obese individuals following vaccination.

24 – NSI abstract ID

Title

CombiCAR design rescues low affinity CAR and provides enhanced selectivity

Authors

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Abstract

Chimeric antigen receptor (CAR) T cells have led to remarkable improvements in therapeutic outcome for the treatment of B-cell malignancies. CD19 targeting CARs were the first approved CAR T cells for treatment of leukemia and lymphoma. Despite substantial rates of durable remission, a subset of patients relapsed mainly as a consequence of antigen loss. It is thus highly crucial to identify and characterize alternative targets. However, the quest for novel cancer markers has shown limitation due to their distribution in healthy tissues. Secondary modifications on exposed proteins are emerging as target of choice for some cancers: aberrant glycosylation leading to exposure of unusual carbohydrate residues are excellent antigens. Among those are N-glycans capped with α2,6-linked sialic acid (α2,6NeuAc) overexpressed in lymphoma. Three decades ago, we isolated an antibody specific for -B cell lymphoma, HH2, which turned out to be specific towards α2,6NeuAc-containing N-glycans. We identified the antibody coding sequence, designed an scFv molecule, and cloned it as a second generation CAR. HH2CAR showed cytotoxic activity against B cell lymphoma cell lines, however, the construct failed to induce detectable cytokine release. Consequently, CAR T cells could not control tumor progression in vivo. We undertook a screen of different CAR designs in order to boost the T cell response and found that combining HH2 with CD19 could improve T cell reactivity. In addition, this combinatorial CAR strategy rekindles the low efficiency HH2 targeting CAR while preventing potential off-tumor on-target toxicity induced by CD19 targeting.

25 – NSI abstract ID

Title
Screening for small-molecule immunomodulators

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Abstract
Ways to therapeutically target the immune system are highly sought after. Here, we present the discovery of small-molecule inhibitors of previously undrugged enzymes implicated in DNA repair but with emerging roles in epigentics and immunogenic gene regulation. We also demonstrate SINTEFs pipeline of in vitro immune assays for the assessment of safety and efficacy of therapies that modulate the immune system.

26 – NSI abstract ID

Title

A proteome-based score as measure of gluten induced mucosal damage in celiac disease

Authors

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Abstract

Histological assessment of gluten-induced mucosal damage is the gold standard for diagnosis of celiac disease (CeD) and is used as end point in clinical drug trials. However, measurement of histological changes requires good biopsy orientation and skilled observers, and has poor interobserver reproducibility. More robust and unbiased methods are therefore needed. We have shown that proteome analysis of formalin-fixed paraffin-embedded (FFPE) biopsy sections comprehensively capture disease processes in CeD intestine, and even reveal disease processes prior to evident changes in histology. To enable use in clinical trial settings we have set up a simple protocol for MS-based proteome analysis of single, glass-mounted FFPE biopsy sections. Here, we use a single sample rank-based gene set enrichment approach based on proteins that significantly change in the intestine of CeD patients in presence or absence of gluten. The applied rank-based proteome scoring of our training datasets show agreement with Marsh classification and good correlation with Vh:Cd ratio. The rank-based score is robust for samples with > 800 proteins. Our pipeline show very low variation between serial sections from the same biopsy block, and can be used on both unstained and HE-stained cover-glass mounted sections, facilitating retrospective analysis. A proteome-based score from single glass mounted FFPE sections will be a great supplement as endpoint in clinical trials to monitor disease activity and evaluate drug efficacy.

27 – NSI abstract ID

Title

Miniaturization of bioprocess development for immunotherapies

Authors

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Abstract

The rapid increase in novel immunotherapies necessitate alternative approaches to process development, to increase capacity and provide opportunities for flexible and personalized production of a large variety of products. Miniaturized devices can be used for faster bioprocess development for both protein and cell products, with high-throughput screening of cultivation parameters, reduced volumes and efficient process read-outs. Different miniaturized formats have been developed, such as shaken vessels (e.g. microtiter plates, mainly for microbial processes) and stirred tank microreactors (e.g. ambr15, tailored to mammalian cell cultures). Here, we asked whether high-throughput systems mainly employed for microbial processes could be adapted to achieve scalable production of immunotherapeutic antibodies and T-cells. Furthermore, we asked how process parameters traditionally measured manually (e.g. cell culture density) could be replaced by high-throughput-compatible analyses and automatic read-outs. Cell cultivation and antibody production were optimized in microtiter plates and a microreactor system (Biolector). We demonstrated that the platform could be used for efficient screening of culture media, nutrient addition, feeding regimes and process parameters (e. g. temperature, pH). The processes were compared with larger scale industry- and clinic-relevant systems (stirred bioreactors and culture flasks) in relation to cell proliferation, nutrient consumption, metabolites and product yield, and image-based high-throughput methods were developed for quantification of cell culture density. The established platform is generic and readily translatable for process optimization of a wide range of cells and therapeutic proteins expressed in mammalian cell hosts.

28 – NSI abstract ID

Title

Isolation of a cytolytic subpopulation of extracellular vesicles derived from NK cells containing NKG7 and cytolytic proteins

Authors

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Abstract

NK cells can broadly target and kill malignant cells via release of cytolytic proteins. NK cells also release extracellular vesicles (EVs) that contain cytolytic proteins, previously shown to induce apoptosis of a variety of cancer cells in vitro and in vivo. The EVs released by NK cells are likely very heterogeneous, as vesicles can be released from the plasma membrane or from different intracellular compartments. In this study, we undertook a fractionation scheme to enrich for cytolytic NK-EVs. NK-EVs were harvested from culture medium from the human NK-92 cell line or primary human NK cells grown in serum-free conditions. By combining ultracentrifugation with downstream density-gradient ultracentrifugation or size-exclusion chromatography, distinct EV populations were identified. Density-gradient ultracentrifugation led to separation of three subpopulations of EVs. The different EV isolates were characterized by label-free quantitative mass spectrometry and western blotting, and we found that one subpopulation was primarily enriched for plasma membrane proteins and tetraspanins CD37, CD82, and CD151, and likely represents microvesicles. The other major subpopulation was enriched in intracellularly derived markers with high expression of the endosomal tetraspanin CD63 and markers for intracellular organelles. The intracellularly derived EVs were highly enriched in cytolytic proteins, and possessed high apoptotic activity against HCT-116 colon cancer spheroids. To further enrich for cytolytic EVs, immunoaffinity pulldowns led to the isolation of a subset of EVs containing the cytolytic granule marker NKG7 and the majority of vesicular granzyme B content. We therefore propose that EVs containing cytolytic proteins may primarily be released via cytolytic granules.

29 – NSI abstract ID

Title

The T-cell receptor repertoire in human peripheral blood encodes T1D status

Authors

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Abstract

Although Islet autoantibodies (AAb) are predictive of type 1 diabetes (T1D) risk and are clinically used in the diagnosis of disease, AAb only serve as a marker of autoantigen presentation by B cells and are not involved in disease pathogenesis. On the other hand, T cells are hypothesized to be directly pathogenic and responsible for the destruction of β-cells, highlighting the importance of developing a T-cell biomarker for T1D. So far, there exist no clinically feasible T-cell biomarkers of T1D. We have sequenced 2286 TCRβ repertoires containing 1112, 720, 68 and 386 repertoires for T1D, first degree relatives, second degree relatives and healthy controls, respectively. The standard repertoire analysis (such as diversity profile, Morisita Horn similarity index etc.) were not able to classify the T1D repertoires from the healthy ones. Therefore, we implemented a Deep learning model entitled "DeepRC" to (i) classify the T1D and healthy repertoires and (ii) identify the biomarker associated with T1D. The DeepRC method showed classification accuracy of ~80% on the test dataset. The T1D associated biomarkers identified by DeepRC were further analyzed for their association with high risk class II HLA and autoantibody presence, showing positive association. We have also compared the performance of DeepRC with other state of the art in silico protocols to identify biomarkers. The study shows that deep learning-based models are significantly better than the current methods. Moreover, we have identified a biomarker associated with T1D status, which has been validated robustly on blind test dataset.

30 – NSI abstract ID

Title

Human Gut organoids: mini-tissues in culture to study intestinal physiology

Authors

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Abstract

The gastrointestinal tract is an organ that takes in food, digests it, and absorbs food-derived nutrients. However, the gut also protects against insults after exposure to numerous pathogens. Intestinal epithelial cells generate multiple barriers to protect the intestinal mucosa from commensal microbes or invading pathogenic microorganisms. Because of the cellular and molecular complexity of the intestine, elucidating the intrinsic mechanisms underlying the regulation of epithelial homeostasis is difficult. Thus, the current three-dimensional gut organoid culture has revolutionized fundamental and biomedical science research. Organoid are mini-guts and show high regeneration potential from normal and diseased primary tissues and mimicking the physiological features of their tissue of origin. We have developed healthy duodenal organoids from five patients undergoing Whipple surgery at Rikshospitalet. Duodenal organoids could be culture for up to six months after splitting every 10- 12 days. We observed that proliferation and differentiation could be controlled by high (50 % Wnt3a and 20 % R-Spondin 1) and low (5 % Wnt3a and 5 % R-Spondin 1) concentrations of growth factors. We observed an increase in MUC2+ Goblet cell number by decreasing the concentration of Wnt3a & R-Spondin 1 and in the absence of Nicotinamide and SB202190- a p38 MAPK inhibitor. Bulk RNA sequencing after treatment of duodenal organoids with cytokines will reveal various aspects of immune cell- epithelial crosstalk. In summary, methods for maintenance and expansion of human duodenal organoids are established. We will elucidate novel aspects of crosstalk between the intestinal epithelium and immune cells during homeostasis and disease conditions.

31 – NSI abstract ID

Title

Diversity of intratumoral regulatory T cells in B-cell non-Hodgkin lymphoma

Authors

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Abstract

Tumor-infiltrating regulatory T cells (Tregs) contribute to an immunosuppressive tumor microenvironment. Despite extensive studies, the prognostic impact of tumor-infiltrating Tregs in B cell non-Hodgkin lymphomas (B-NHL) remains unclear. Emerging studies suggest substantial heterogeneity in the phenotype and suppressive capacities of Tregs, emphasizing the importance of understanding Treg diversity and the need for additional markers to identify highly suppressive Tregs. Here, we combined single-cell RNA sequencing with high-dimensional mass cytometry to decipher the heterogeneity of intratumoral Tregs in diffuse large B-cell lymphoma and follicular lymphoma and in non-malignant tonsillar tissue. We identified three distinct transcriptional states of Tregs; resting, activated and LAG3+FOXP3- Tregs. Activated Tregs were enriched in B-NHL tumors, co-expressed several checkpoint receptors and had stronger immunosuppressive activity compared with resting Tregs. We used a computational approach to develop a unique gene signature matrix which was used to enumerate each Treg subset. This facilitated estimation of their frequencies in cohorts with bulk gene expression data. In two independent follicular lymphoma cohorts, activated Tregs was the major subset, and high abundance was associated with adverse outcome. This study demonstrates that Tregs infiltrating NHL tumors are transcriptionally and functionally diverse and include highly immunosuppressive activated Tregs co-expressing several checkpoint receptors, which distinguish them from peripheral blood Tregs. Our data suggest that a deeper understanding of Treg heterogeneity in B-NHL could open new paths for rational drug design, facilitating selective targeting to improve anti-tumor immunity.

32 – NSI abstract ID

Title

The presence of transglutaminase 2 derived from shed enterocytes in the small intestinal lumen

Authors

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Abstract

Background: The enzyme transglutaminase 2 (TG2) is involved in the pathogenesis of the celiac disease (CeD) in two ways. First, by deamidating gluten peptides and creating highly immunogenic T-cell epitopes, and second, by being the target of highly-disease-specific autoantibodies. Celiac disease is caused by the adverse immune response to gluten, and accumulating evidence suggests that interactions between TG2-specific B cells and gluten-specific T cells are important in pathogenesis. A big remaining question is where enzymatically active TG2 encounters gluten. We recently suggested that TG2 derived from shed enterocytes could be the source of pathogenically relevant TG2 in celiac disease. Objective: In this study, we aim to detect shed enterocytes in the lumen of the small intestine of mice. As it is still controversial whether enterocytes are extruded as apoptotic or non-apoptotic cells, we will assess if we can detect live enterocytes. Method: We will perform flow cytometry and cytospin to detect enterocytes in small intestinal lavage fluid. We will use a western blot to detect TG2 protein. Mice deficient for TG2 and mice with a specific deletion of TG2 in enterocytes will serve as controls. Pathogenic conditions with increased cell shedding will be mimicked through the injection of inflammatory cytokines. Immunohistochemistry will be performed for histopathological evaluation.

33 – NSI abstract ID

Title

Characteristics of the immunoglobulin knock-in mouse 1E03

Authors

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Abstract

Celiac disease is an autoimmune enteropathy driven by an immune response to dietary gluten. It has a strong association to the Human Leukocyte Antigen (HLA) allotypes HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8 that facilitate the interaction between disease-relevant B cells and T cells, warranting a closer look at their collaboration. The disease is associated with the generation of highly specific antibodies to transglutaminase 2 (TG2) as well as to deamidated gluten peptides (DGP). DGP-specific B cells' immunodominant epitopes are often overlapping with gluten-specific T cell epitopes, making these B cells ideally selected for T cell help. We have generated an immunoglobulin knock-in (Ig KI) mouse (1E03) expressing a B-cell receptor recognizing the immunodominant DGP epitope QPEQPFP. We aim to use these mice to study the formation of anti-DGP IgA in the gut following oral gluten exposure. Characterization of the 1E03 mouse will be done using flow cytometry and in vitro T cell – B cell proliferation assays. Gut anti-DGP-IgA responses will be induced in HLA-DQ2.5 expressing mice after adoptive transfer of DGP-specific B cells and T cells. The HLA-DQ2.5 KI mice will be orally immunized with gluten and adjuvant and anti-DGP responses will be measured in blood serum and faeces, through ELISA, in the lamina propria, through ELISPOT, and in tissue sections, through immunostaining. The majority of 1E03 B cells bind DGP and specifically interact with T cells, however, spleen weights and cell numbers may differ from wildtype mice. The B cells are functional in vitro. They are currently being investigated for in vivo functionality.

34 – NSI abstract ID

Title

Immune therapy for tissue regeneration: elucidating the impact of nanodiamond particles on dendritic cells

Authors

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Abstract

Nanodiamond particles (nDP) are carbon-derived nanoparticles (ø=4-5 nm) with low chemical reactivity and unique physical properties with a promising potential in immune-mediated tissue regeneration. nDP are modified by adding or removing functional groups to stimulate immune cells to promote regeneration or to deliver bioactive molecules that recruit and/or activate immune cells. Dendritic cells are crucial for initiating adaptive immunity, foreign body reactions and they play an important role in the tissue regeneration process. The aim of this study was to investigate how nDP modifications affect dendritic cell activation and cytokine profile to design immune-instructing regeneration therapies. Human monocyte-derived dendritic cells (moDC) were exposed to different concentrations of unmodified nDP or azide-linker modified nDP (Az-nDP) for 24h. moDC exposed to LPS served as a positive and unexposed moDC as negative control. Pro- and anti-inflammatory cytokines were evaluated at gene level, CCL22 release was measured using ELISA and moDC activation was evaluated using flow cytometry. Comparable gene expression trends were shown for pro- and anti-inflammatory genes with 0.02 mg/mL Az-nDP and LPS compared to negative control. moDC exposed to LPS, 0.02 or 0.002 mg/mL Az-nDP released significantly less CCL22 than negative control. CCL22 produced by moDC exposed to 0.02 and 0.002 mg/mL unmodified nDP was lower than unexposed. Az-nDP also showed effects on activation surface markers, CD86 and MHCII. These data suggest immunostimulatory effects of Az-nDP compared to unmodified with trends co

35 – NSI abstract ID

Title

Regulatory T cells' secretome modulates osteogenic differentiation of bone marrow mesenchymal stromal cells in vitro

Authors

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Abstract

Recent reports indicate the effect of regulatory T cells (Tregs) on tissue regeneration by regulating the immune response and/or influencing the progenitor cells. However, how Tregs regulate bone regeneration and the differentiation of bone marrow-derived mesenchymal stromal cells (BMSC) is not yet known. Therefore, the aim of this study was to evaluate whether secreted factors from Tregs modulate osteogenic differentiation of human BMSC. Tregs were isolated from human peripheral blood and expanded using anti-CD3/CD28 beads, and then starved for 48h before conditioned medium (CM), which contains Tregs' secretome, was collected. Human BMSC was cultured for 21 days under routine growth or osteogenic medium (OM) supplemented with either 10% or 50% Treg-CM. BMSC morphology and proliferation were evaluated after 7 and 14 days. Further, their osteogenic potential was investigated by quantifying alkaline phosphatase (ALP) gene expression and enzymatic activity after 7 and 14 days. Extracellular matrix mineralization was assessed by Alizarin-Red S stain after 21 days. BMSC proliferation was significantly decreased with 50% Treg-CM+/-OM compared to OM alone at day 7, 14. ALP enzyme activity of BMSC was increased significantly with 10% and 50% Treg-CM+OM compared to cells cultured with only OM alone at day 7 and 14. ALP gene level was significantly upregulated with 50% Treg-CM+OM. Interestingly, profound mineralized nodules formation were observed after 21 days with 10% and 50% Treg-CM+/-OM compared to growth medium alone. In conclusion, Tregs' secretome plays a role in enhancing the osteogenic differentiation of BMSC and further investigation on activated pathways is warranted.

36 – NSI abstract ID

Title

Engineered albumin as a scaffold for improving the pharmacokinetic properties of antibody fragments

Authors

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Abstract

The pharmacological effect of many protein-based biologics is limited by brief target exposure due to a short plasma half-life. A solution to this is a fusion of such biologics to the IgG Fc region, which will prolong their longevity by increasing their molecular size above the kidney threshold as well as allow binding to the half-life regulator FcRn. However, Fc fusions may interact with other effector molecules and induce unwanted immune responses. Importantly, FcRn also binds albumin via a non-overlapping binding site and provides it with a long plasma half-life. While the Fc region is an N-glycosylated homodimer, albumin is a non-glycosylated single polypeptide that is effector negative. This is an advantage when the aim is to block a biological target. Here, I will discuss how knowledge about the relationship between FcRn and its ligands can be explored in the design of new formats that combine elements from antibodies with that of albumin as a strategy to achieve improved pharmacokinetics of antibody fragments or mimics

37 – NSI abstract ID

Title

Biomarkers linked to severity of disease in hospitalized influenza patients

Authors

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Abstract

Influenza is one of the greatest global health threats and understanding protective and disease associated immune conditions is important for improved clinical treatment and vaccination strategies. The aim of this study was to identify biomarkers associated with disease severity in adult hospitalized influenza patients. Blood samples (plasma, RNA and PBMC) were collected from influenza patients admitted to OUS (n=156) during acute infection and after 5-11 months. In addition, healthy controls (n=87) were included. Cellular and humoral immune responses were measured using dual IFN-γ/IL-2 fluorospot, plasma CXCL13 ELISA and hemagglutination inhibition (HI) analysis. Moreover, high dimensional immune profiling was performed on a subset of patients by gene expression analysis and mass cytometry. CXCL13 was higher in severely ill compared to moderately ill patients, while there were no differences in HAI titers or cellular responses as measured by EliSpot between the groups. Compared to healthy controls, IFN-signaling genes were upregulated and genes for adaptive immune responses were downregulated during acute infection. Only CD4 gene expression differed between moderately and severely ill patients. CyTOF analysis revealed increased levels of activated monocytes (CD3-CD169+CD11b+CD11c+CD14+CD85j+), activated NK cells (CD3-CD56+CD57+CD38+TIM-3+GranzymeB +) dendritic cells (CD3-CD14-CD11c+CD11b+CD123+CD3-HLA-DR+CD85j+), plasma B cells (CD3-CD19lowCD85j+CD95+CD38+) and cytotoxic CD8+ T cells (CD3+CD8+GranzymeB+IFNg+) and reduced levels of circulating MAIT cells (CD3+TCRVa7.2+), B cells (CD3-CD19+CXCL5+) and activated CD4+ memory T cells (CD3+CD4+CD45RA-CCR7+CD137+orPD-L2+ in severely ill compared to moderately ill patients. A severity associated immune profile for influenza was identified with elevated CXCL13 levels in plasma and altered levels of defined major innate and adaptive immune cell populations.

38 – NSI abstract ID

Title

Novel combinatorial IGK-CD19 CAR efficiently and selectively targets malignant B cells in vitro

Authors

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Abstract

Chimeric antigen receptor (CAR) T cells have exhibited great potential for the treatment of malignancies. FDA-approved CD19CAR T cells are a powerful treatment for B cell leukemia and lymphoma, but kill indiscriminately both leukemic and healthy cells. This results in B cell aplasia, which increases risk of infection. In this context, the immunoglobulin (Ig) kappa light chain (IGK) of the B cell receptor, highly expressed on malignant B cells, emerges as an attractive target to improve target cell-specificity. IGKCAR T cells demonstrated high efficacy in preclinical models, however, our preliminary data showed reduced efficiency due to their reactivity to serum Ig. We further observed that serum Ig-induced stimulation led to lower expansion during manufacture and short-lasting CAR-T effect likely due to exhaustion. To overcome these issues, we have designed a new combinatorial CAR construct, composed of IGKCAR-CD3ζ and CD19CAR-4-1BB (Kz-19BB). We hypothesized that splitting the signaling domains would provide two benefits: reduction of the Ig-dependent stimulation of IGKCAR and restrict target specificity toward IGK only. Kz-19BBCAR T cells displayed high efficacy and selectivity against malignant B cells. Here, we confirm these results using retrovirally transduced Kz-19BBCAR, seeking to improve its stability and long-term efficacy. Further, to study the therapeutic potential of Kz-19BBCAR, immunodeficient mice transplanted with leukemic B cells will be treated with Kz-19BBCAR T cells, with the aim of reducing tumor burden. Taken together, our results confirm the efficiency and selectivity of Kz-19BBCAR T cells in vitro, and appear as a promising therapy for leukemia and lymphoma.

39 – NSI abstract ID

Title

Instant and prolonged changes in activated gluten-specific CD4+ T cells upon initiation of gluten-free diet in celiac patients

Authors

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Abstract

Coeliac disease (CeD) is driven by oral gluten exposure. Treatment with gluten-free diet (GFD) normalizes the small intestinal inflammation and disease-specific antibodies. However, when gluten is re-introduced, persisting, HLA-restricted, gluten-specific T cells support pathogenic memory response. Here we monitored HLA-DQ2.5-restricted, gluten-specific CD4+ T cells and other disease-specific biomarkers the first year of treatment. We followed 17 CeD patients at five time points the first year on GFD and assessed activation and frequency of gluten-specific CD4+ blood and gut T cells with HLA-DQ2.5:gluten tetramers and flow cytometry in parallel with disease-specific serology, histology and symptom scores. In six CeD patients we followed 12 markers hallmarking gluten-specific blood T cells and serology the first three weeks on GFD. We also performed intracellular staining to investigate cell proliferation and survival. We show that gluten-specific T cells lost expression of CD38, ICOS, HLA-DR and Ki-67 while CD127 and Bcl-2 increased within the first two weeks on GFD. PD-1, CD39 and OX40 expression remained upregulated even after 12 months. The gluten-specific CD4+ T-cell frequency peaked at day 14, then decreased significantly within 10 weeks and remained detectable at all time points. In contrast, CeD-specific serology and histology normalized within 6 and 12 months, respectively. In conclusion, the transient increase in the first two weeks appears to be accumulation of non-proliferating cells forming a memory pool of gluten-specific T cells. Our findings support the notion that the phenotypic dynamics of antigen-specific CD4+ T cells associate with the chronic nature of autoimmune disorders beyond CeD.

40 – NSI abstract ID

Title

High-throughput screening for small molecular compounds targeting FoxP3 in regulatory T cells

Authors

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Abstract

Suppression of antitumor immunity by regulatory T cells (Tregs) has been found to support tumor progression and is associated with poor prognosis in most cancer types. Thus, Treg-targeted therapies have become of particular interest. Despite efforts in targeting FoxP3, the key lineage-defining transcription factor for Treg differentiation and function, most clinical trials focus on Treg surface markers, leading to immune-related adverse effects.

To characterize potential FoxP3 targets, we screened a library of 1522 approved drugs by establishing a unique, phenotypic, cell-based, high-throughput flow cytometry assay. We identified three potential hits that passed initial validation tests and met selection criteria, searched for drug-like analog compounds by in silico prediction, built sub-libraries and tested the effects on Tregs. Our preliminary data show that the hits and some of the analog compounds induced significant downregulation of FoxP3 levels and loss of Treg suppressive function, with comparable or more efficient effects than the original hits. Furthermore, these analogs could reduce the expression of some Treg markers (such as PD-1, ICOS and LAG-3) associated with its suppressive function.

Although further investigation is needed, these hits can serve as potential FoxP3 targets as well as probes for studying specific Treg mechanisms of action, increasing the repertoire of antitumor immunity strategies.