Clinically-Relevant Rapamycin Treatment Regimens Enhance CD8\(^+\) Effector Memory T Cell Function In The Skin and Allow their Infiltration into Cutaneous Squamous Cell Carcinoma

Patients receiving immunosuppressive drugs to prevent organ transplant rejection exhibit a greatly increased risk of developing cutaneous squamous cell carcinoma (SCC). However, not all immunosuppressive drugs confer the same risk. Randomised, controlled trials demonstrate that switching renal transplant recipients receiving calcineurin inhibitor-based therapies to mammalian target of rapamycin (mTOR) inhibitors results in a reduced incidence of de novo SCC formation, and can even result in the regression of pre-existing premalignant lesions. However, the contribution played by residual immune function in this setting is unclear. We examined the hypotheses that mTOR inhibitors promote the enhanced differentiation and function of CD8\(^+\) memory T cells in the skin. Here, we demonstrate that the long-term oral administration of rapamycin to achieve clinically-relevant whole blood drug target thresholds, creates a "low rapamycin dose" environment in the skin. While both rapamycin and the calcineurin inhibitor tacrolimus elongated the survival of OVA-expressing skin grafts, and inhibited short-term antigen-specific CD8\(^+\) T cell responses, rapamycin but not tacrolimus permitted the statistically significant infiltration of CD8\(^+\) effector memory T cells into UV-induced SCC lesions. Furthermore, rapamycin uniquely enhanced the number and function of CD8\(^+\) effector and central memory T cells in a model of long-term contact hypersensitivity provided that rapamycin was present during the antigen sensitization phase. Thus, our findings suggest that patients switched to mTOR inhibitor regimens likely experience enhanced CD8\(^+\) memory T cell function to new antigen-challenges in their skin, which could contribute to their lower risk of de novo SSC formation and regression of pre-existing premalignant lesions.
Genetically Induced Tumors in the Oncopig Model Invoke an Antitumor Immune Response Dominated by Cytotoxic CD8β+ T Cells and Differentiated γδ T Cells Alongside a Regulatory Response Mediated by FOXP3+ T Cells and Immunoregulatory Molecules

In recent years, immunotherapy has shown considerable promise in the management of several malignancies. However, the majority of preclinical studies have been conducted in rodents, the results of which often translate poorly to patients given the substantial differences between murine and human immunology. As the porcine immune system is far more analogous to that of humans, pigs may serve as a supplementary preclinical model for future testing of such therapies. We have generated the genetically modified Oncopig with inducible tumor formation resulting from concomitant KRASG12D and TP53R167H mutations under control of an adenoviral vector Cre-recombinase (AdCre). The objective of this study was to characterize the tumor microenvironment in this novel animal model with respect to T-cell responses in particular and to elucidate the potential use of Oncopigs for future preclinical testing of cancer immunotherapies. In this study, we observed pronounced intratumoral T-cell infiltration with a strong CD8β+ predominance alongside a representation of highly differentiated γδ T cells. The infiltrating CD8β+ T cells displayed increased expression of the cytotoxic marker perforin when compared with the peripheral T-cell pool. Similarly, there was robust granzyme B staining localizing to the tumors; affirming the presence of cytotoxic immune cells within the tumor. In parallel with this antitumor immune response, the tumors displayed enrichment in FOXP3-expressing T cells and increased gene expression of indoleamine 2,3-dioxygenase 1 (IDO1), cytotoxic T-lymphocyte-associated protein 4 (CTLA4), and programmed death-ligand 1 (PDL1). Finally, we investigated the Oncopig immune system in mediating antitumor immunity. We observed pronounced killing of autologous tumor cells, which demonstrates the propensity of the Oncopig immune system to recognize and mount a cytotoxic response against tumor cells. Together, these findings suggest innate and adaptive recognition of the induced tumors with a concomitant in vivo suppression of T-cell effector functions. Combined, the data support that the Oncopig may serve as a valuable model for future preclinical testing of immunotherapies aimed at reactivating tumor-directed cytotoxicity in vivo.

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KRAS(G12D) and TP53(R167H) Cooperate to Induce Pancreatic Ductal Adenocarcinoma in Sus scrofa Pigs

Although survival has improved in recent years, the prognosis of patients with advanced pancreatic ductal adenocarcinoma (PDAC) remains poor. Despite substantial differences in anatomy, physiology, genetics, and metabolism, the overwhelming majority of preclinical testing relies on transgenic mice. Hence, while mice have allowed for tremendous advances in cancer biology, they have been a poor predictor of drug performance/toxicity in the clinic. Given the greater similarity of sus scrofa pigs to humans, we engineered transgenic sus scrofa expressing a LSL-KRAS(G12D)-TP53(R167H) cassette. By applying Adeno-Cre to pancreatic duct cells in vitro, cells self-immortalized and established tumors in immunocompromised mice. When Adeno-Cre was administered to the main pancreatic duct in vivo, pigs developed extensive PDAC at the injection site hallmarkcd by excessive proliferation and desmoplastic stroma. This serves as the first large animal model of pancreatic carcinogenesis, and may allow for insight into new avenues of translational research not before possible in rodents.

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CD4<sup>+</sup>CD8<sup>+</sup> double-positive T cells in skin-draining lymph nodes respond to inflammatory signals from the skin

CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP), mature, peripheral T cells are readily detectable in a variety of species and tissues. Despite a common association with autoimmune and malignant skin disorders, however, little is understood about their role or function. Herein, we show that DP T cells are readily detectable in the blood, spleen, and peripheral lymph nodes of naïve C57BL/6 mice. DP T cells were also present in Jα<sup>-</sup> and CD1d<sup>-</sup> mice, indicating that these cells are not NK-T cells. After skin administration of CASAC adjuvant, but not Quil A adjuvant, both total DP T cells and skin-infiltrating DP T cells increased in number. We explored the possibility that DP T cells could represent aggregates between CD4<sup>+</sup> and CD8<sup>+</sup> single-positive T cells and found strong evidence that a large proportion of apparent DP T cells were indeed aggregates. However, the existence of true CD4<sup>+</sup>CD8<sup>+</sup> DP T cells was confirmed by Amnis Image-Stream (Millipore Sigma, Billerica, MA, USA) imaging. Multiple rounds of FACS sorting separated true DP cells from aggregates and indicated that conventional analyses may lead to ~10-fold overestimation of DP T cell numbers. The high degree of aggregate
contamination and overestimation of DP abundance using conventional analysis techniques may explain discrepancies reported in the literature for DP T cell origin, phenotype, and function.

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BFI (2015): BFI-level 1
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BFI (2008): BFI-level 1
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Scopus rating (2007): SJR 2.443 SNIP 1.093
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Low antigen dose formulated in CAF09 adjuvant Favours a cytotoxic T-cell response following intraperitoneal immunization in Göttigen minipigs

The relationship between the antigen dose and the quality of an immune response generated upon immunization is poorly understood. However, findings show that the immune system is indeed influenced by the antigen dose; hence underlining the importance of correctly determining which dose to use in order to generate a certain type of immune response. To investigate this area further, we used Göttigen minipigs as an animal model especially due to the similar body size and high degree of immunome similarity between humans and pigs. In this study, we show that both a humoral and a cell-mediated immune (CMI) response can be generated following intraperitoneal immunization with tetanus toxoid (TT) formulated in the CAF09 liposomal adjuvant. Importantly, a low antigen dose induced more TT-specific polyfunctional T cells, whereas antigen-specific IgG production was observed upon high-dose immunization. Independent of antigen dose, intraperitoneal administration of antigen increased the amount of TT-specific cytotoxic CD8β+ T cells within the cytokine-producing T-cell pool when compared to the non-cytokine producing T-cell compartment. Taken together, these results demonstrate that a full protein formulated in the CAF09 adjuvant and administered to pigs via the intraperitoneal route effectively generates a cytotoxic T-cell response. Moreover, we confirm the inverse relationship between the antigen dose and the induction of polyfunctional T cells in a large animal model. These findings can have implications for the design of upcoming vaccine trials aiming at establishing a cytotoxic T-cell response.
The Oncopig Cancer Model: An Innovative Large Animal Translational Oncology Platform

Despite an improved understanding of cancer molecular biology, immune landscapes, and advancements in cytotoxic, biologic, and immunologic anti-cancer therapeutics, cancer remains a leading cause of death worldwide. More than 8.2 million deaths were attributed to cancer in 2012, and it is anticipated that cancer incidence will continue to rise, with 19.3 million cases expected by 2025. The development and investigation of new diagnostic modalities and innovative therapeutic tools is critical for reducing the global cancer burden. Toward this end, transitional animal models serve a crucial role in bridging the gap between fundamental diagnostic and therapeutic discoveries and human clinical trials. Such animal models offer insights into all aspects of the basic science-clinical translational cancer research continuum (screening, detection, oncogenesis, tumor biology, immunogenicity, therapeutics, and outcomes). To date, however, cancer research progress has been markedly hampered by lack of a genotypically, anatomically, and physiologically relevant large animal model. Without progressive cancer models, discoveries are hindered and cures are improbable. Herein, we describe a transgenic porcine model—the Oncopig Cancer Model (OCM)—as a next-generation large animal platform for the study of hematologic and solid tumor oncology. With mutations in key tumor suppressor and oncogenes, $TP53_{R167H}$ and $KRAS_{G12D}$, the OCM recapitulates transcriptional hallmarks of human disease while also exhibiting clinically relevant histologic and genotypic tumor phenotypes. Moreover, as obesity rates increase across the global population, cancer patients commonly present clinically with multiple comorbid conditions. Due to the effects of these comorbidities on patient management, therapeutic strategies, and clinical outcomes, an ideal animal model should develop cancer on the background of representative comorbid conditions (tumor macro- and microenvironments). As observed in clinical practice, liver cirrhosis frequently precedes development of primary liver cancer or hepatocellular carcinoma. The OCM has the capacity to develop tumors in combination with such relevant comorbidities. Furthermore, studies on the tumor microenvironment demonstrate similarities between OCM and human cancer genomic landscapes. This review highlights the potential of this and other large animal platforms as transitional models to bridge the gap between basic research and clinical practice.

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The Pig as a Large Animal Model for Studying Anti-Tumor Immune Responses

The immune system plays a crucial role in cancer development and progression. Cancer immunoediting encompasses three phases: elimination, equilibrium, and escape; together, describing the complex interplay between tumor and immune cells. Specifically, the immune system both protects against cancer but also generates a selective pressure, which may lead to selection of tumor cell variants with reduced immunogenicity; thereby, increasing the risk of tumor escape. Cancer immunotherapy includes treatment strategies aimed at activating anti-tumor immune responses or inhibiting suppressive and tumor-favorable immune mechanisms. One of the promising arms of cancer immunotherapy is peptide-based therapeutic vaccines; yet, no such vaccine has been approved for use in human oncology. For many years, mouse models have provided invaluable understanding of complex immunological pathways; however, the majority of preclinical results are lost in translation from mice to humans. In particular, the success rate when translating therapeutic cancer vaccines has been extremely low; thus leaving room for improvement.

The overall aim of this Ph.D. project was to investigate the potential for the pig as a large animal model for cancer immunology research and preclinical testing of cancer immunotherapies. We hypothesized that a physiologically relevant model with high degree of homology with humans can provide a crucial link between murine studies and human patients. This may increase the success rate when translating preclinical findings in the future.

As T cells are important mediators of anti-tumor immune responses, we first developed an immunization protocol allowing the induction of a cytotoxic T lymphocyte (CTL) response and evaluation of the effect of vaccine antigen dose. Göttingen minipigs received intraperitoneal (i.p.) injections with tetanus toxoid, an exogenous model antigen, formulated in CAF09 adjuvant. We demonstrate induction of a polyfunctional CTL response upon low antigen dose immunization, while a CAF09-formulated high antigen dose generates antigen-specific IgG antibodies.

Secondly, we investigated the effect of antigen dose, when immunizing Göttingen minipigs against Indoleamine 2,3-dioxygenase (IDO); an endogenous target relevant for cancer immunotherapeutic purposes. By repeated i.p. administration of CAF09-adjuvanted IDO-derived peptides, we show a vaccine-induced break in the peripheral tolerance towards IDO and the establishment of an antigen-specific cell-mediated immune (CMI) response. When comparing the different CAF09-formulated antigen doses, we demonstrate the induction of a CMI-dominant response upon exposure to a low endogenous peptide dose. In contrast, a mixed CMI and humoral immune response could be shown following repeated high peptide dose immunization. Together, our data underline the importance of correctly determining the first-in-human vaccine antigen dose, which may be more accurately predicted in a large animal like the pig.

Finally, we performed a T-cell focused immunological characterization of the novel transgenic Oncopig model. Following injection with an adenoviral vector Cre-recombinase (AdCre), these animals develop sarcomas at the injection site resulting from expression of two mutant transgenes: KRASG12D and TP53R167H. We demonstrate pronounced T-cell infiltration to the tumor site with a specific enrichment in both regulatory and cytotoxic subsets when compared to peripheral blood. Thus, Oncopig subcutaneous tumors can be classified as hot in accordance with the Immunoscore classification.

In an in vitro setup, we show immune-mediated specific lysis of autologous tumor cells, underlining the capacity of the Oncopig immune system to mount a cytotoxic anti-tumor response. Using the results from RNA-seq analysis, we propose a potential mechanism for in vivo inhibition of anti-tumor cytotoxicity based on elevated expression of the immunosuppressive genes IDO1, CTLA4, and PDL1 within Oncopig leiomyosarcomas. As a high rate of spontaneous regression of subcutaneous tumors occurs over time, we speculate that the anti-tumor immune responses become dominant at the later stages post AdCre injection; eventually leading to tumor elimination. Combined, our data support that the Oncopig provides a crucial platform for studying anti-tumor immune responses in a large in vivo system, although the model currently only allows preclinical testing of therapeutics against the early stages of cancer.

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Altering the balance between immune activation versus regulation in the skin to promote CD8+ T-cell activity within epithelial cancers

The Human Papilloma Virus (HPV) 16 is a high-risk HPV known to be a causative agent in numerous cancers including cervical cancer. While prophylactic vaccines exist to combat the spread of HPV16, successful therapeutic vaccines to combat established HPV16-associated disease remain elusive. The expression, in a mouse model ("E7"), of the HPV16 E7 gene in keratinocytes under the control of the K14 promoter, leads to a local immune suppressive environment, as evidenced by the lack of graft rejection when E7 skin grafts are placed on WT recipient mice. Furthermore, well healed (>30 days) E7 skin grafts are not rejected when mice are immunised with E7 peptide in combination with Quil A- or CASAC-based adjuvants. This is despite a substantial increase in E7 peptide/H-2Db pentamer staining in the blood, and marked killing of E7-peptide expressing TC-1 cells when injected i.v., confirming that CD8 T-cells respond to vaccination and differentiate into CTL capable of killing E7-expressing target cells. We hypothesised that the removal of regulatory T-cells (T-reg) might lead to E7 graft rejection in immunised mice. The co-administration of an anti-CD4-depeting antibody at the time of immunisation led to rejection of ~50% of grafts. To confirm a role for T-reg, E7-grafted T-reg-deficient Rag1-/- mice received purified donor CD8 T-cells from E7-vaccinated WT mice. FACS staining of Rag1-/- lymph nodes 30 days post CD8+ T-cell transfer confirmed the absence of classical CD4+FoxP3+ Treg, however the E7 grafts did not reject. As in the WT mice however, rejection could be induced through the coadministration of an anti-CD4 antibody. The data suggest that the removal of a CD4+, non T-reg cell, leads to CD8+ T-cell activity in the skin as evidenced by E7 skin graft destruction.

Antigen-Encoding Bone Marrow Terminates Islet-Directed Memory CD8+ T-Cell Responses to Alleviate Islet Transplant Rejection

Islet-specific memory T cells arise early in type 1 diabetes (T1D), persist for long periods, perpetuate disease, and are rapidly reactivated by islet transplantation. As memory T cells are poorly controlled by "conventional" therapies, memory T cell–mediated attack is a substantial challenge in islet transplantation, and this will extend to application of personalized approaches using stem cell–derived replacement β-cells. New approaches are required to limit memory autoimmune attack of transplanted islets or replacement β-cells. Here, we show that transfer of bone marrow encoding cognate antigen directed to dendritic cells, under mild, immune-preserving conditions, inactivates established memory CD8+ T-cell populations and generates a long-lived, antigen-specific tolerogenic environment. Consequently, CD8+ memory T cell–mediated targeting of islet-expressed antigens is prevented and islet graft rejection alleviated. The immunological mechanisms of protection are mediated through deletion and induction of unresponsiveness in targeted memory T-cell populations. The data demonstrate that hematopoietic stem cell–mediated gene therapy effectively terminates antigen-specific memory T-cell responses, and this can alleviate destruction of antigen-expressing islets. This addresses a key challenge facing islet transplantation and, importantly, the clinical application of personalized β-cell replacement therapies using patient-derived stem cells.
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CD4⁺ CD8⁺ double-positive T-cells regulate CD8⁺ single-positive T cell function in the skin

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Novel regulators of CD8+ T-cell functions in the skin

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The pig as a large preclinical model for therapeutic human anti-cancer vaccine development

Development of therapeutic cancer vaccines has largely been based on rodent models and the majority failed to establish therapeutic responses in clinical trials. We therefore used pigs as a large animal model for human cancer vaccine development due to the large similarity between the porcine and human immunome. We administered peptides derived from porcine IDO, a cancer antigen important in human disease, formulated in Th1-inducing adjuvants to outbred pigs. By in silico prediction 136 candidate IDO-derived peptides were identified and peptide-SLA class I complex stability measurements revealed 89 stable (t½ ≥ 0.5 hour) complexes with expressed SLA alleles. By IFN-γ ELISpot we showed that it was possible to break the peripheral tolerance and induce a cell-mediated response to an endogenous antigen.

Mounting a proper Th1 response is highly dependent on peptide dose; we therefore designed a dose titration study with 15 Gottingen minipigs receiving intraperitoneal injections of either 1 µg, 10 µg or 100 µg of 30-31mer peptides covering the majority of IDO-derived potential cytotoxic T lymphocyte (CTL) epitopes. Peptides were formulated in CAF09, an adjuvant comprised of cationic DDA liposomes decorated with poly (I:C) and MMG as immune modulators. Interestingly, the 1 µg group was the only one showing responses to all immunization peptides following seven injections as determined by IFN-γ ELISpot. These data show that a reduction in dose can result in a highly specific Th1-biased response. To test the CTL functionality we designed an in vivo cytotoxicity assay, where purified autologous PBMCs fluorescently labelled and pulsed with IDO-derived target peptides were administered intravenously into each donor and killing capacity was measured by flow cytometry. All animals receiving 10 µg peptide immunizations showed specific killing of peptide-pulsed
target cells one week post i.v. transfer with certain animals reaching close to 60% specific killing capacity in vivo.
The pig as a large preclinical model for therapeutic human anti-cancer vaccine development

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Tracking the elusive cytotoxic T cell response in pigs

Quantitative and qualitative assessment of antigen-specific cytotoxic T cell (CTL) responses in pigs is not a straightforward process. Through the years we have developed a series of reagents, tools and protocols to characterize peptide-specific CTL responses in pigs.

The most common recombinant SLA heavy chains were produced and peptide binding motifs were determined by assays measuring the affinity and stability of the peptide-SLA complex (pSLA) interaction. These results have been used to train neural networks to predict the binding of any pSLA (http://www.cbs.dtu.dk/services/). Recombinant SLA molecules complexed with verified binding peptides can be assembled to SLA multimers for staining of peptide-specific CTLs, and measured by flow cytometry, as we have shown with FMDV and influenza. This, however, requires SLA-matched pigs for which we have developed two methods: a sequence-based, high-resolution SLA genotyping method by standard PCR for specific detection of eight in-house SLA molecules; and a next-generation sequencing method for parallel detection of up to 50 samples of barcoded cDNA PCR products spanning exon 2 and 3. The latter for a wider characterization of expressed alleles in candidate pigs.

The in vivo generation of CTL responses to antigens following peptide immunizations is thought to require cross-presentation in appropriate dendritic cells (DC). In mice this was linked to targeting of CD103+DCs recruited after intraperitoneal immunizations. We have therefore developed a protocol for intraperitoneal delivery of peptides formulated in poly(I:C)/MMG-decorated liposomes (CAF09) to investigate the influence of peptide dose on the generation of CTL vs. antibody responses. Finally, the induced CTL killing was assessed by an in vivo cytotoxicity assay, where purified autologous PBMCs, fluorescently labeled and pulsed with target peptides, were reinjected into the donor. The in vivo killing of peptide-pulsed cells was measured by flow cytometry relative to non-pulsed PBMCs at different time points after cell transfer.

General information
Does the nature of residual immune function explain the differential risk of non-melanoma skin cancer development in immunosuppressed organ transplant recipients?

Patients receiving immunosuppression to prevent organ transplant rejection are at a greatly increased risk of developing nonmelanoma skin cancer. In recent years a correlation has been identified between the class of immunosuppressant that these patients receive and their subsequent cancer risk; in particular, patients switched from calcineurin inhibitors to mammalian target of rapamycin (mTOR) inhibitors not only displayed a dramatic reduction in new tumor formation but also in some cases a regression of their existing lesions. Studies of cancer models in mice and cell lines in the laboratory have attributed these discrepancies in cancer risk to the ability of immunosuppressants such as mTOR inhibitors to elicit direct anticancer effects, including suppressing angiogenesis and increasing autophagy-mediated DNA repair. Recent evidence from the immunological literature however, suggests a significant alternative contribution of mTOR inhibitors; namely the promotion of memory T-cell function. Recent advances in understanding memory T-cell establishment and the demonstration of their critical role in long-term immunity make it timely to review the available evidence as to whether the improved nonmelanoma skin cancer outcome shown by patients switched to mTOR inhibitor treatment regimens may be associated with the retainment of memory T-cell function.
Elucidating the T-cell reactivity against porcine IDO and RhoC to establish the pig as an animal model for vaccine development against human cancer

Immune therapy of cancer has recently experienced a great breakthrough with prolonged overall survival in patients with metastatic disease following the use of checkpoint inhibitors and T cell therapy with ex vivo expanded CD8+ cytotoxic T cells (CTLs). In the further development of immune therapies against cancer, vaccine formulations tailored to mount in vivo CTL responses towards co-delivered cancer antigens will be an important hallmark. Recognition of antigen-derived peptides presented in the context of major histocompatibility complex (MHC) class I molecules on cancer cells is a requirement for activation of CTLs. Previously, the development of therapeutic anti-cancer vaccines have largely been based on rodent models, in particular mice; however the majority of these fail to establish a therapeutic response once put into clinical trials. Pigs have the potential to serve as a model superior to rodents as they are more closely related to humans in terms of immunology and physiology. Here, we introduce pigs as a supplementary large animal model for human cancer vaccine development via the use of our unique technology for swine leukocyte antigen (SLA) production. IDO and RhoC, two tumor antigens previously identified as important players in human cancer development and progression, were used as vaccine targets. Using peptide-MHC-I binding predictors we identified IDO-derived and RhoC-derived candidate peptides potentially binding to five different broadly distributed SLA molecules. We measured the peptide-SLA complex stability of these and found a total of 89 stable (t½ ≥ 0.5 hours) peptide-MHC complexes with SLA-
Establishing the pig as a large animal model for vaccine development against human cancer

Immunotherapy has increased overall survival of metastatic cancer patients, and cancer antigens are promising vaccine targets. To fulfill the promise, appropriate tailoring of the vaccine formulations to mount in vivo cytotoxic T cell (CTL) responses toward co-delivered cancer antigens is essential. Previous development of therapeutic cancer vaccines has largely been based on studies in mice, and the majority of these candidate vaccines failed to induce therapeutic responses in the subsequent human clinical trials. Given that antigen dose and vaccine volume in pigs are translatable to humans and the porcine immunome is closer related to the human counterpart, we here introduce pigs as a supplementary large animal model for human cancer vaccine development. IDO and RhoC, both important in human cancer development and progression, were used as vaccine targets and 12 pigs were immunized with overlapping 20 mer peptides spanning the entire porcine IDO and RhoC sequences formulated in CTL-inducing adjuvants: CAF09, CASAC, Montanide ISA 51 VG, or PBS. Taking advantage of recombinant swine MHC class I molecules (SLAs), the peptide-SLA complex stability was measured for 198 IDO- or RhoC-derived 9-11mer peptides predicted to bind to SLA-1*04:01, −1*07:02, −2*04:01, −2*05:02, and/or −3*04:01. This identified 89 stable (t½ ≥ 0.5 h) peptide-SLA complexes. By IFN-γ release in PBMC cultures we monitored the vaccine-induced peptide-specific CTL responses, and found responses to both IDO- and RhoC-derived peptides across all groups with no adjuvant being superior. These findings support the further use of pigs as a large animal model for vaccine development against human cancer.
The pig as a model for therapeutic human anti-cancer vaccine development, elucidating the T-cell reactivity against IDO and RhoC

Immunotherapy against cancer has shown increased overall survival of metastatic cancer patients and is a promising new vaccine target. For this to succeed, appropriate tailoring of vaccine formulations to mount in vivo cytotoxic T cell (CTL) responses towards co-delivered cancer antigens is important. Previous development of therapeutic cancer vaccines has largely been based on studies in mice and the majority of these candidate vaccines failed to establish therapeutic responses in subsequent human clinical trials. Since the porcine immunome is more closely related to the human counterpart, we here introduce pigs as a superior large animal model for human cancer vaccine development via the use of our unique technology for swine leukocyte antigen (SLA) production. IDO and RhoC, both known to be important in human cancer development and progression, were used as vaccine targets. Pigs were immunized with overlapping 20-mer peptides spanning the entire porcine IDO and RhoC sequences formulated in a panel of CTL-inducing adjuvants. 198 candidate IDO- and RhoC-derived 9-11mer peptides potentially binding to SLA- *1*04:01, -*1*07:02, -*2*04:01, -*2*05:02 and/or -*3*04:01 were identified in silico, and peptide-SLA complex stability measurements revealed 89 stable (t½ ≥ 0.5 hour) complexes. Vaccine-induced peptide-specific CTL responses were monitored using IFN-γ release as a read out. We found responses to IDO- and RhoC-derived peptides across all groups; surprisingly non-stably binding peptides also induced responses. None of the adjuvants was found to be superior as they were all capable of generating CTL responses to IDO and RhoC hence supporting the further use of pigs as a large animal model for vaccine development against human cancer.

Uncovering new pathways of CD8 T-cell regulation in the skin

Uncovering new pathways of CD8 T-cell regulation in the skin

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CD4+/CD8+ double-positive T cells: more than just a developmental stage?

CD4+/CD8+ DP thymocytes are a well-described T cell developmental stage within the thymus. However, once differentiated, the CD4+ lineage or the CD8+ lineage is generally considered to be fixed. Nevertheless, mature CD4+/CD8+ DP T cells have been described in the blood and peripheral lymphoid tissues of numerous species, as well as in numerous disease settings, including cancer. The expression of CD4 and CD8 is regulated by a very strict transcriptional program involving the transcription factors Runx3 and ThPOK. Initially thought to be mutually exclusive within CD4+ and CD8+ T cells, CD4+/CD8+ T cell populations, outside of the thymus, have recently been described to express concurrently ThPOK and Runx3. Considerable heterogeneity exists within the CD4+/CD8+ DP T cell pool, and the function of CD4+/CD8+ T cell populations remains controversial, with conflicting reports describing cytotoxic or suppressive roles for these cells. In this review, we describe how transcriptional regulation, lineage of origin, heterogeneity of CD4 and CD8 expression, age, species, and specific disease settings influence the functionality of this rarely studied T cell population.

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Comparative Immune Phenotypic Analysis of Cutaneous Squamous Cell Carcinoma and Intraepidermal Carcinoma in Immune-Competent Individuals: Proportional Representation of CD8$^+$ T-Cells but Not FoxP3$^+$ Regulatory T-Cells Is Associated with Disease Stage.

Squamous Cell Carcinoma (SCC) is a type of non-melanoma skin cancer prevalent in immune-suppressed transplant recipients and older individuals with a history of chronic sun-exposure. SCC itself is believed to be a late-stage manifestation that can develop from premalignant lesions including Intraepidermal Carcinoma (IEC). Notably, while SCC regression is rare, IEC typically regresses in response to immune modifying topical treatments, however the underlying immunological reasons for these differential responses remain unclear. This study aimed to define whether IEC and SCC are associated with distinct immune profiles. We investigated the immune cell infiltrate of photo-damaged skin, IEC, and SCC tissue using 10-colour flow cytometry following fresh lesion digest. We found that IEC lesions contain higher percentages of CD3$^+$ T-cells than photo-damaged skin, however, the abundance of CD3 CD56$^+$ Natural Killer (NK) cells, CD11c$^+$HLA-DR$^+$ conventional Dendritic Cells (cDC), BDCA-2$^+$HLA-DR$^+$ plasmacytoid DC (pDC), FoxP3$^+$ Regulatory T-cells (T-reg), Vα24$^+$Vβ11$^+$ invariant NKT-cells, and γδ T-cells did not alter with disease stage. Within the total T-cell population, high percentages of CD4$^+$ T-cells were associated with SCC, yet CD8$^+$ T-cells were less abundant in SCC compared with IEC. Our study demonstrates that while IEC lesions contain a higher proportion of T-cells than SCC lesions in general, SCC lesions specifically display a lower abundance of CD8$^+$ T-cells than IEC. We propose that differences in CD8$^+$ T-cell abundance contribute critically to the different capacity of SCC and IEC to regress in response to immune modifying topical treatments. Our study also suggests that a high ratio of CD4$^+$ T-cells to CD8$^+$ T-cells may be an immunological diagnostic indicator of late-stage SCC development in immune-competent patients.
Targeting antigen to DC permits therapeutic termination of memory CD8+ T-cell responses by HSC-mediated gene therapy under immune-preserving conditions

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Accelerating development of vaccines against cancer with pigs as a large animal model

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Accelerating development of vaccines against cancer with pigs as a large animal model

Accelerating development of vaccines against cancer with pigs as a large animal model (CANVACPIG) This project runs July 1st 2014 and until December 31st 2017. It is budgeted for 5.7 million DKK and is financed by the Danish Council for Independent Research, ID: DFF – 4005-00428. Current project participants •Professor Gregers Jungersen, DTU National Veterinary Institute •Postdoc Thomas Mørch Frøsig, DTU National Veterinary Institute •PhD student Nana Haahr Overgaard, DTU National Veterinary Institute •Master’s thesis student Rikke Selbeck Andersen, DTU National Veterinary Institute •Postdoc Maria Rathmann Sørensen, DTU National Veterinary Institute •Professor Søren Buus, University of Copenhagen •Professor Mads Hald Andersen, Center for Cancer Immune Therapy, Herlev Hospital Former project participants •Master’s thesis student Zina Al-Shatrawi, DTU National Veterinary Institute •Master's thesis student Mette Ilsøe, DTU National Veterinary Institute The project Development of vaccines against cancer requires the use of animal models before new vaccines can be used in patients. The mouse is most often used, but there are large differences in the immune system between humans and mice. The immune system of the pig on contrary resembles the human one in several ways, and we aim therefore to accelerate the development of cancer vaccines by using pigs as an animal model for the correct activation of the immune system by vaccination. The goal is to identify the optimal vaccine composition for stimulation of the immune system and the generation of killer cells specifically attacking the cancer cells. Vaccines, consisting of cancer-related molecules (peptides) and the combination with different immune-activating substances will be tried in healthy pigs. By using luminescent tissue type molecules in the laboratory we can monitor the development of killer cells after the vaccination, specifically recognizing the cell surface of the cancer cells.

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