ARTEMIS – Final Report

Our immune system has the central role to protect us from infectious agents, but at the same time has to avoid reacting with our own tissues. The ARTEMIS project has investigated the balance between autoimmune reaction and tolerance, with a special focus on the molecular discrimination of self (host-derived nucleic acids and mitochondrial components) and non-self (bacterial and viral components).

Impact

The project was initiated by two publications; one senior-authored by the Researcher at the Return Host (Brinkmann et al., 2013) and one co-authored by the Researcher at the Outgoing Host (Chatterjee et al., 2013). The former demonstrated a central role of a branch of our immune defense called the complement system in directing homeostatic clearance of mitochondria, while the latter demonstrated a crucial role of the complement system in preventing emergence of self-reactive B cells.

At the Outgoing Host, the Researcher undertook an ambitious and ground-breaking research effort, which resulted in first author publications in Cell (Degn, van der Poel, Firl, Ayoglu, et al., 2017c), Clinical and Experimental Immunology (Degn, Alicot, & Carroll, 2017a), and Oncotarget (Degn, van der Poel, & Carroll, 2017b), and collectively have made a leap in our understanding of autoreactive germinal centers and the handling and transport of autoantigens. The Researcher won a poster prize at the Annual Scientific Retreat of the Program in Cellular and Molecular Medicine (2016) and gave and invited talk at the Keystone Symposium on B Cells at the Interface of Innate and Adaptive Immunity in Stockholm (2016).

At the Return Host, the Researcher has undertaken dissemination activities to contribute a knowledge transfer, including a seminar with the title "Visualizing clonal evolution in autoreactive germinal centers by intravital microscopy" in the two-photon user group at Aarhus University. Furthermore, the Researcher now teaches on the PhD course entitled "Advanced In-vivo Optical imaging techniques" with the lecture "Clonal evolution in germinal centres studied by 2-photon in-vivo microscopy". The Researcher has additionally fulfilled teaching duties including lectures, seminars, and lab courses for medical students and Master's students in molecular medicine at the Return Host.

The Researcher has given several talks disseminating scientific findings of the project, including at the annual meeting of the Danish Immunological Society in Odense, Denmark (May 24th, 2017), in a Departmental meeting for senior postdocs and junior group leaders ("Spangroups", November 29th, 2016) and in the Master's Program at the University of Patras Medical School, Greece (October 6th, 2017), as well as an internal lab workshop (Moesgaard Museum, Aarhus, May 3rd 2017), local journal club at the Department of Biomedicine, Aarhus University (October 2nd 2017), and in a departmental presentation meeting with the new Dean at the Faculty of Health, Aarhus University (May 9th 2017).

Broader dissemination and outreach activities have included numerous features in the Danish press and online scientific news platforms (for example Videnskab.dk, DR Viden,

Dagenspharma.dk, Dagensmedicin.dk, BT.dk, Scleroseforeningen), as well as at the Outgoing host (Vector at Children's Hospital Boston, Scicasts, EurekAlert! at AAAS, Brightsurf, Dotemirates, MedicalXpress, Science Codex, and ScienceNewsline), and a Snapchat story at Boston Children's Hospital entitled "Dr Michael Carroll's lab", which was directly prompted by the media coverage surrounding one of the abovementioned publications.

Finally, upon conclusion of the Return Phase and the ARTEMIS project, the Researcher has transitioned to the independent researcher stage, on August 1st 2017 assuming a tenure-track Assistant Professor position and status as Group Leader at the Department of Biomedicine, Aarhus University. The Researcher was awarded a sizeable Starting Grant, amounting to 10 million DKK for a period of 5 years, from an independent Danish research foundation.

The project has directly resulted in 3 publications and 1 manuscript in submission, as well as 4 associated publications.

Scientific Summary

The ARTEMIS project revolved around the balance between autoimmune reaction and tolerance, with a special focus on the molecular discrimination of self (host-derived nucleic acids and mitochondrial components) and non-self (bacterial and viral components).

Mitochondria, the powerhouses of our cells, are remnants of a eubacterial endosymbiont. Because mitochondria retain many hallmarks of their bacterial origin, they have recently been implicated in the etiology of the sterile systemic inflammatory response syndrome (SIRS), a frequent cause of death in intensive care units (Zhang et al., 2010). However, we found that mitochondria are immunologically inert under normal homeostatic conditions, as they are cleared in a complement-dependent manner in the absence of overt inflammation (**Associated Publication 1**) (Brinkmann et al., 2013). Taken together, these contrasting observations exemplify the fundamental question of *how the immune system distinguishes self from non-self.* The central role of complement in this context was investigated (**Associated Publication 2 & 3**) (Chatterjee et al., 2013; Degn et al., 2014) and reviewed (**Associated Publication 4**) (Bajic, Degn, Thiel, & Andersen, 2015).

Mitochondria are centrally involved in initiation and execution of programmed cell death. This process may both release endogenous danger-associated molecular patterns (DAMPs) and pathogen-associated molecular pattern (PAMP) mimetics, as well as produce reactive oxygen species (ROS), which in turn can generate neoepitopes. Autoantibodies to mitochondrial components, e.g. cytochrome C, have been observed in patients with lupus and related autoimmune diseases (Mamula, Jemmerson, & Hardin, 1990). Such autoantibodies are thought to arise in response to oxidative damage caused by free radical generation in cells at sites including the mitochondria, endoplasmic reticulum and nuclear membranes (Casciola-Rosen, Anhalt, & Rosen, 1994). Accordingly, we investigated the release, transport and uptake of such apoptotic cell-derived antigenic material using intravital two-photon imaging (**Project Publication 1**) (Degn, Alicot, & Carroll, 2017a). Labeled apoptotic cells were generated *in situ* in a fluorescent reporter strain (BFP-SSB) by UVB irradiation. We were able to visualize these components *in situ* but they were efficiently cleared, and did not drain in intact form to skin-draining lymph nodes. An embedded OTII epitope in the BFP-SSB reporter was used to

specifically address T cell responses in this scenario. Adoptive transfer of transgenic OTII T cells demonstrated efficient surveillance of such neo-autoantigen in skin-draining lymph nodes, indicating drainage of degraded antigen. Yet following transient activation, responding T cell populations contracted, *demonstrating a regulatory response*.

An exciting observation springing from these initial investigations was that a restricted autoreactive B cell repertoire is sufficient to drive autoreactive germinal center reactions, recruiting endogenous proto-autoreactive B cells. This process may be a fundamental one underlying the concepts of epitope spreading and repertoire conversion. Autoantibodies are common in autoimmune conditions, and class-switched affinity-matured antibodies to nucleic acid antigens are a hallmark of systemic lupus erythematosus (SLE) (Rahman & Isenberg, 2008). Anti-double stranded-DNA antibody is more specific for SLE (>75%) compared with healthy controls (~0.5%) and typically comes up a few years before SLE is diagnosed (Arbuckle et al., 2003). SLE patients examined closer to or after onset of disease demonstrate drift in their serological reactivities towards a variety of nuclear, nucleolar, and protein-DNA complexes which may reflect functional epitope spreading and which may propagate disease states. Epitope spreading is driven in part by chronic immune responses and functionally refers to diversification of VJ segment utilization by germinal center (GC) B cells which leads to new reactivities. Presence and levels of titers representing these reactivities are used to guide clinical management without mechanistic understanding of either their role in disease or their widely varying penetrance and magnitude (Arbuckle et al., 2003; Cornaby et al., 2015; Vanderlugt & Miller, 2002). GCs are the primary sites of clonal expansion, class-switching and affinity maturation of B cells, directing the production of high-affinity antibodies. Yet the dynamics of self-reactive B cell engagement and persistence in chronic autoreactive GCs remained poorly understood. We developed a new model system to evaluate factors influencing break of tolerance and development of autoimmune disease, which we termed the ARTEMIS model, to signify "AutoReactive B cell driven T-dependent Epitope Migration toward Immunity to Self.

We found that a self-reactive transgenic B cell clone (derived from the 564 model, (Berland et al., 2006)) is sufficient to break tolerance and initiate autoreactive GCs composed predominantly of wild type-derived B cells (**Project Publication 2**) (Degn, van der Poel, Firl, Ayoglu, et al., 2017c). Autoreactive GCs depended on T help, were self-sustained and long-lived. Employing a novel Aicda-Cre^{ERT2} Confetti reporter system (Tas et al., 2016), we found that they evolved towards pauciclonality at a decreased rate compared with GCs elicited by foreign antigen. Using photoactivatable (PA)-GFP reporters (Victora et al., 2010), we examined single GCs. Clonally they converged on stereotypic autoreactive sequence elements, which were mirrored serologically by functional epitope spreading. These studies were extended in a recently developed intravital imaging setup (Meijer et al., 2017). Using this chronic window implantate, we could follow the evolution of several parallel germinal centers within inguinal lymph nodes of individual mice longitudinally for up to 3 weeks (**Project Publication 3**) (Firl et al., manuscript submitted to *Nature Communications*).

Our findings have fundamental implications for the understanding of important pathophysiological processes such as epitope spreading and repertoire conversion, and point to future points of treatment intervention in autoimmune diseases (**Project Publication 4**) (Degn, van der Poel, & Carroll, 2017b).

For further information, please contact:

Søren E. Degn, PhD, Assistant Professor, Group Leader
Department of Biomedicine, Aarhus University
Bartholins Allé 6, Building 1242, 565
8000 Aarhus C, Denmark
sdegn@biomed.au.dk
Phone: +4587167274
Web: http://degnlab.au.dk

References

Arbuckle, M. R., McClain, M. T., Rubertone, M. V., Scofield, R. H., Dennis, G. J., James, J. A., & Harley, J. B. (2003). Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *The New England Journal of Medicine*, *349*(16), 1526–1533. http://doi.org/10.1056/NEJMoa021933

Bajic, G., Degn, S. E., Thiel, S., & Andersen, G. R. (2015). Complement activation, regulation, and molecular basis for complement-related diseases. *The EMBO Journal*, *34*(22), 2735–2757. http://doi.org/10.15252/embj.201591881

Berland, R., Fernandez, L., Kari, E., Han, J.-H., Lomakin, I., Akira, S., et al. (2006). Toll-like receptor 7-dependent loss of B cell tolerance in pathogenic autoantibody knockin mice. *Immunity*, *25*(3), 429–440. http://doi.org/10.1016/j.immuni.2006.07.014

Brinkmann, C. R., Jensen, L., Dagnaes-Hansen, F., Holm, I. E., Endo, Y., Fujita, T., et al. (2013). Mitochondria and the lectin pathway of complement. *The Journal of Biological Chemistry*, *288*(12), 8016–8027. http://doi.org/10.1074/jbc.M112.430249

Casciola-Rosen, L. A., Anhalt, G., & Rosen, A. (1994). Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *The Journal of Experimental Medicine*, *179*(4), 1317–1330.

Chatterjee, P., Agyemang, A. F., Alimzhanov, M. B., Degn, S., Tsiftsoglou, S. A., Alicot, E., et al. (2013). Complement C4 maintains peripheral B-cell tolerance in a myeloid cell dependent manner. *European Journal of Immunology*, *43*(9), 2441–2450. http://doi.org/10.1002/eji.201343412

Cornaby, C., Gibbons, L., Mayhew, V., Sloan, C. S., Welling, A., & Poole, B. D. (2015). B cell epitope spreading: mechanisms and contribution to autoimmune diseases. *Immunology Letters*, *163*(1), 56–68. http://doi.org/10.1016/j.imlet.2014.11.001

Degn, S. E., Alicot, E., & Carroll, M. C. (2017a). B cell tolerance to epidermal ribonuclear-associated neo-autoantigen in vivo. *Clinical & Experimental Immunology*. http://doi.org/10.1111/cei.13066

Degn, S. E., Kjaer, T. R., Kidmose, R. T., Jensen, L., Hansen, A. G., Tekin, M., et al. (2014). Complement activation by ligand-driven juxtaposition of discrete pattern recognition complexes. *Proceedings of the National Academy of Sciences*, *111*(37), 13445–13450. http://doi.org/10.1073/pnas.1406849111

Degn, S. E., van der Poel, C. E., & Carroll, M. C. (2017b). Targeting autoreactive germinal

centers to curb autoimmunity. *Oncotarget*, 1–2. http://doi.org/https://doi.org/10.18632/oncotarget.21701

Degn, S. E., van der Poel, C., Firl, D. J., Ayoglu, B., Qureshah, Al, F. A., Bajic, G., et al. (2017c). Clonal Evolution of Autoreactive Germinal Centers. *Cell*, *170*(5), 913–926. http://doi.org/http://dx.doi.org/10.1016/ j.cell.2017.07.026

Mamula, M. J., Jemmerson, R., & Hardin, J. A. (1990). The specificity of human anti-cytochrome c autoantibodies that arise in autoimmune disease. *The Journal of Immunology*, *144*(5), 1835–1840.

Meijer, E. F. J., Jeong, H.-S., Pereira, E. R., Ruggieri, T. A., Blatter, C., Vakoc, B. J., & Padera, T. P. (2017). Murine chronic lymph node window for longitudinal intravital lymph node imaging. *Nature Protocols*, *12*(8), 1513–1520. http://doi.org/10.1038/nprot.2017.045

Rahman, A., & Isenberg, D. A. (2008). Systemic Lupus Erythematosus. *New England Journal of Medicine*, *358*(9), 929–939. http://doi.org/10.1056/nejmra071297

Tas, J. M. J., Mesin, L., Pasqual, G., Targ, S., Jacobsen, J. T., Mano, Y. M., et al. (2016). Visualizing antibody affinity maturation in germinal centers. *Science (New York, NY)*, *351*(6277), 1048–1054. http://doi.org/10.1126/science.aad3439

Vanderlugt, C. L., & Miller, S. D. (2002). Epitope spreading in immune-mediated diseases: implications for immunotherapy. *Nature Reviews Immunology*, *2*(2), 85–95. http://doi.org/10.1038/nri724

Victora, G. D., Schwickert, T. A., Fooksman, D. R., Kamphorst, A. O., Meyer-Hermann, M., Dustin, M. L., & Nussenzweig, M. C. (2010). Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell*, *143*(4), 592–605. http://doi.org/10.1016/j.cell.2010.10.032

Zhang, Q., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., et al. (2010). Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*, *464*(7285), 104–107. http://doi.org/10.1038/nature08780