JAMES RENNIE BEQUEST

REPORT ON EXPEDITION/PROJECT/CONFERENCE

Expedition/Project/Conference Title: Keystone Symposia; Lymphocyte activation and Signalling

Travel Dates: 2nd February 2008 – 8th February 2008…….

Location: Snowbird Resort, Utah, USA .....

Group Member(s): Joanne Konkel .....

Aims: Presentation of my data in the form of a poster, form links with other laboratories .....

OUTCOME (not less than 300 words):-

Keystone symposia are excellent conferences, and are regarded as such by the scientific community. This conference proved to be no exception, as a host of world renowned scientists came together to discuss the mechanisms of T and B cell activation and function. I will discuss my personal conference highlights, what I learnt and the scientific links I managed to develop.

On the first day Steve Reiner (University of Pennsylvania) gave a really interesting talk on T cell fates. He discussed how a naïve T cell can be activated and induced to proliferate, and how that one cell can differentiate and acquire two different cell fates (for example effector T cell and memory T cell). He demonstrated how a prolonged T cell; APC contact prior to T cell proliferation can lead to asymmetric cell division. This would give to two distinct daughter cells, one of which inherited the synaptic proteins, the other inherits the more distal components of the cell. Performing adoptive transfer experiments of distal and proximal daughters, he saw that both could proliferate to initial stimulation. However if the cells were re-challenged after 2 months, the proximal daughters could no longer respond but the distal daughter cells could. In this very interesting talk he went onto show that asymmetric cell division doesn't lock a T cell into a definite cell fate but does influence the likelihood of a T cell developing a certain way.

Later that day Anjana Rao (Harvard Medical School) spoke on Treg cells and Th17 cells. This was a particularly interesting talk for me as a number of people within my lab work on the generation and role of Th17 cells in EAE. Prof. Rao discussed a selective inhibitor of Th17 differentiation called MS17. She showed that this inhibitor needs to be added to a differentiation culture within the first 24hours to have any effect of Th17 differentiation. MS17 works by preventing the late stage phosphorylation and activation of STAT3, the important intracellular mediator of Th17 differentiation. She also discussed calcium signalling in Tregs, an area I am particularly interested in. She showed that Tregs are dependent on store-operated calcium signalling, mice in which only CD4+ T cells were defective in intracellular calcium release, failed to generate any natural Tregs.

Dan Littman (New York University School of Medicine) continued on the Th17 theme. He focused on the roles of RORγt and TGFβ in immune system homeostasis. He showed that RORγt and foxp3 physically interact, and that foxp3 restrains Th17 differentiation through this interaction with RORγt. He showed in vivo data of a small population of CD4+ T cells which express both foxp3 and RORγt, and that these calls did not express IL-17.

The following day Lawrence Samelson (NIH) spoke on signalling at the T cell receptor (TCR). His talk focused on LAT, a transmembrane adaptor protein. Using a mutant LAT which could no longer cause calcium flux within the T cell he showed the cells were still able to a activate ERK. Further experiments highlight a second adaptor protein Bam32, which could substitute for LAT.

Other excellent talks were given by Pamela Schwartzberg (NIH) and Paula Oliver (University of Pennsylvania). Pamela Schwartzbergs' presentation focused on X-linked proliferative disease, which is caused by mutations in SAP. She presented data showing that SAP deficient T cells fail to provide help to B

cells, as they fail to form long-lived contacts with them. Paula Oliver gave a short talk on Nedd 4, a ubquitin ligase, which is thought to be involved in tolerance and anergy. She demonstrated that following T cell activation Nedd 4 levels are high and function to keep cbl-b levels low (cbl-b is an inhibitor of T cell responses). Following a tolerogenic signal Nedd 4 levels are low, which results in high cbl-b levels and therefore reduced T cell responses.

On Wednesday afternoon there was a workshop which specifically focuses on costimulation. This was specifically relevant to me as I work on costimulatory molecules and their involvement in T cell tolerance. This was an intimate session held during the winter sports break where speakers gave short talks and lots of discussions were had. Topics included mTOR, how glucose metabolism must change in order to generate a T cell response, ICOS, the involvement of fyn in B cell tolerance, and CD40. Later that day Micheal Karin (University of California) also spoke on CD40, discussing the complexes downstream of it. He demonstrated that a complex of TRAF2;MEKK1;MKK4/7;IKKγ;Ubc13 is formed in response to CD40 engagement. He termed this the 'signalsome', which is released into the cytosol to initiate signalling (a similar signalsome has been discovered following TLR stimulation).

The final day was begun by Alexander Rudensky (University of Washington). He talked about the importance of Tregs in immune responses, focusing on viral infections. In mice, using a conditional knockout of Tregs and a genital HSV infection, he showed that Tregs are vital for anti-viral responses. Removal of Tregs during and post infection led to decreased mouse survival and increased viral titres. He went onto demonstrate that Tregs are needed to facilitate the recruitment of immune cells to the site of infection. Fiona Powrie (University of Oxford) also focused on Tregs, talking on the balance between Treg and T effector cells in the intestine. She showed how the CD103+ DC present in the gut, which generate Tregs, ensure that the environment is more regulatory than inflammatory. She also discussed the role of IL-23 in Crohns Disease. Using her transfer model of colitis she presented data showing that in the absence of Tregs, IL-23 is not required for the development of disease. An interesting short talk was given by Jeff Rathmell (Duke University). He talked about the role of glucose metabolism in T cell responses. He had created a T cell transgenic mouse in which the T cells expressed elevated levels of Glut1, the primary glucose transporter on T cells. He showed that these T cells gave increased responses when stimulated. Transgenic mice also developed an autoimmune-like phenotype, in that there was an accumulation of Ig in the kidneys of the mice. Yoshiyuki Minegishi (Tokoyo Medical and Dental University) talked about Hyper IgE syndrome (HIES). Exploring the genetics of HIES patients he identified that a high proportion of patients had defects in STAT3. Stimulating patient T cells with anti-CD3 and anti-CD28 he showed that patient T cells make equal amount of IFNγ to controls but much less IL-17. A symptom of HIES is the 'cold abscess', this is an extracellular bacterial infection which produces no signs of infection, such as fever. He suggested that the cold abscess is a result of reduced inflammatory responses due to reduced IL-17 production and insufficient neutrophil recruitment. The final talk was given by Edward Wakeland (University of Texas Southwestern Medical Centre) who explored the mechanisms which mediate loss of B cell tolerance. Looking at the NZM2410 lupus prone mouse he showed how mutations in Ly108 were responsible for the breach in tolerance. Ly108 is a SLAM family member expressed highly in thymocytes, of which there are two isoforms. His discussion focused on data which showed that preferential expression of the Ly108-1 isoform reduces deletion of double positive thymocytes. This reduced deletion was due to cells expressing altered levels of Ly108-1 having an altered activation threshold and hence escaping negative selection. This resulted in more mature T cells emigrating from the thymus, and this enhanced the peripheral T cell repertoire and hence likelihood of autoimmunity.

The poster sessions were held in the evening, following the days talks. These, were fairly small affairs, with about 60 posters in each session, but this allowed good discussions of the presented data. They also went on until late and so it was possible to get round to see all the posters I was interested in. During the first session I spoke to F Flores-Borja (UCL). His data showed that Tregs from Rheumatoid arthritis (RA) patients expressed reduced levels of CTLA-4 compared to controls, and that the CTLA-4 expressed was recruited much more slowly to the immunological synapse. This has implications for the balance of co-stimulatory signals for the T cell at activation. My poster session was on the second day. I received a considerable amount on attention from a variety of people including pharma company reps, journal editors and pure scientists. A number of people at the conference were presenting data on PD-1 and they all came to see my poster. I also had interest from Scott Stuart-Tharp, a member of the John O-Shea group, who was

specifically interested in the models I used to ask my specific scientific questions. The editor of JEM also spent a considerable amount of time talking with me, as much of the data I presented is somewhat controversial. I also spoke to Sara Morgan from Oxford who works on human PD-1. She has a superagonist antibody against PD-1, we discussed the possibility of using this antibody in my T cell transfer system and in EAE to see if it would promote tolerance.
During the final poster session I spoke to S. You (Universite Rene Descartes). Her poster looked at the role of GITR/GITRL in diabetes development in the NOD mouse. She had performed similar experiments to me, using a blocking antibody in a disease setting, to explore the role of GITR/GITRL in the NOD mouse. Discussion with her gave me some ideas about some in vitro assays I can do. I also spoke with M. Srinivasan, who looks at the signalling events within anergic CD8+ T cells. As I have been trying to look at this in tolerant CD4+ T cells I found her poster very interesting, and she gave me some good ideas about how to look at my cells. She also suggested a few pointers on how to look at calcium signalling within the CD4+ T cells I have. I also spoke with F. Schnell from Brain Evavolds lab. I discussed with him the phenotype of these uniquely tolerant CD4+ T cells. He suggested a number of possible targets I could look at in these cells, specifically he suggested I look at SHP1 a phosphotase which binds the IL-2 receptor.

Overall this was a very interesting conference. As well as giving me a number of good ideas for future directions of my projects, it also gave me the chance to talk with people about the current techniques I employ, and I was able to get good feed back onto how to best improve these. I also made many useful contacts which I hope will help me with my future career.