AUTOIMMUNITY RESEARCH UNIT

BAFF is a cytokine from the TNF superfamily and essential for B lymphocyte survival without which mature B cells cannot develop[1, 2]. BAFF has 3 receptors, BAFF-R, promoting cell survival, TACI expressed on subsets of B cells important for the response to microbes and BCMA, which expression is restricted to antibody-producing cells, the plasma cells (PC). APRIL, another ligand of the TNF family also binds to BCMA and TACI [1-3]. BCMA is important for the survival of some PCs and not others, yet nobody knows which subset is supported by BCMA-mediated survival.

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Project 1: Understanding the role of BCMA in antibody production to improve vaccination
Research from Dr Robert Brink in our unit has shown that response to high affinity antigens leads to rapid and efficient PC differentiation and antibody production[4, 5]. In contrast B cell response to low affinity antigens pushes B cells to a germinal center to undergo affinity maturation via hyper-mutation of the immunoglobulin genes rather than differentiating into PC. In fact, the few PC developing in this setting do not survive and disappear very quickly.

Our hypothesis is that BCMA may play a role in the survival of PC generated in response to high affinity antigens but not low affinity antigens.

To demonstrate this we will do the following:

1- Generate mice with all B cells having a B cell receptor (BCR) specific for the antigen Hen Egg Lysozyme (HEL) which lack the BCMA receptor HEL-BCR/BCMA$^{-/-}$ mice
2- HEL-BCR/WT or HEL-BCR/BCMA$^{-/-}$ mice will be adoptively transferred in normal mice which will then be immunized with either specifically engineered high affinity or low affinity HEL antigens (HEL-2x and HEL-3x, respectively)
3- The fate of PC can be monitored by flow cytometry (FACS)
4- Look at local BAFF and APRIL expression in the bone marrow where many long-lived PC tend to reside.
5- Study BCMA$^{-/-}$ mice expressing Blimp-GFP. Blimp-1 is a molecule exclusively expressed in PC but also T cells[6] fused to a green-fluorescent protein allowing detection of PC by FACS or on tissue sections.
6- Stimulation of BCMA-/- B cells with BAFF leads to greater TLR9 and TLR7 expression. This may affect the response of these B cells to apoptotic bodies in a BAFF high environment or to T cell independent antigens.
   To check that we can reconstitute BAFF Tg mice with BCMA-/- bone marrow and immunise mice with T cell independent antigen such as NP-Ficoll.

Findings from this work will be very important. Having long-term PC generated upon vaccination is key for the efficacy of vaccines. Conversely, in autoimmune diseases having too many PC secreting autoantibodies is a problem that may be fixed by targeting BCMA.

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Project 2: Investigating the molecular bases underlying the development of autoreactive B-lymphocytes contributing to the development of Type 1 Diabetes
Type 1 diabetes (T1D) occurs when the immune system mistakenly destroys insulin producing beta cells. Autoreactive B-lymphocytes make an important pathogenic contribution to T1D by specifically capturing beta cell proteins using surface immunoglobulins and subsequently presenting them to autoreactive CD4 T cells responsible for beta cell destruction. In the NOD mouse T1D model, impaired differentiation of B-lymphocytes within the spleen promotes generation of autoreactive B-lymphocytes. To identify molecular mechanisms underlying the generation of autoreactive B-lymphocytes in NOD mice, we obtained gene expression profiles characteristic for each stage of B-lymphocyte differentiation utilizing microarrays and

compared profiles to those of counterparts from non-autoimmune prone mice. Such comparisons have led to identification of several interesting candidate genes that form the basis of this project. Gene candidates need to be confirmed at RNA and protein level using quantitative PCR and Western blotting/flow cytometry, respectively. Authenticated changes in gene products will be examined for functional consequences on B-lymphocytes using various assays that test survival, migration, activation or signalling. Finally retro-viral gene delivery systems will be used to over or under-express candidate genes in bone marrow derived haematopoietic stem cells, which will be used to prove whether gene products can alter B-lymphocyte differentiation in recipient mice.

Project 3: The role of B cells and BAFF in regulatory T cell development and functions

Regulatory T cells (Tregs) are very important cells regulating T cell function without which autoimmunity can develop. Conversely, presence of regulatory T cells in tumors can suppress T cell immune response to the tumor. BAFF Tg mice have more Tregs and this is dependent on the presence of B cells and BAFF [7].

We wish to understand the role of B cells in generating Tregs in the presence or absence of BAFF. Several cytokines can drive Tregs in vitro such as TGFb. We will use in vitro assays to incubating various B cell subsets plus naïve T cells and cytokines to study Treg formation. We will use B cells mutant for various BAFF receptors to understand which receptor mediates T reg formation. Using a transwell system we will test whether contact between naïve T cells and B cells is requird for Treg formation. We can also adoptively transfer B cell subsets that generate more Tregs in BAFF Tg mice lacking B cells to see whether these B cells can enhance Treg formation. Finally, autantibody to IL-2 may form complexes with IL-2 and induce the generation of Tregs. ELISA will be done to test whether BAFF Tg mice make autoantibodies to IL-2 and if serum transfer of Ig-sufficient or Ig-depleted serum from BAFF Tg mice into a BAFF Tg mouse lacking B cells has an effect on Treg formation.

Project 4: The role of colon B2 B cells and peritoneal B1 B cells in driving autoimmunity in BAFF Tg mice

A new population of B cells has been identified in the colon and this population [8], unlike B1 B cells requires BAFF for their development. We have never looked at these cells in BAFF Tg mice and we would like to see whether they play a role in autoimmunity.

These B cells have a specific phenotype and can be identified by FACS using specific markers. The presence of these cells will be checked in the colon and secondary organs and inflamed tissues of BAFF Tg mice. Gut B2 B cells, B1a and B1b peritoneal B cells will be sorted from WT and BAFF Tg mice and gene-profiled using affymetrix microarray. Gene specific for colon B2 B cells and B1 B cells will be identified and compared to our gene array data of other B cell subsets already available. B1 or colon B2 B cells will be adoptively transferred in BAFF Tg mice lacking B cells to see which subsets drives autoantibody production and nephritis. Markers specific for the B cell subset involved in disease will be targeted (raising antibodies).

Project 5: The role of regulatory T cells in protecting BAFF Tg mice against experimental autoimmune encephalomyelitis (EAE)

We have discovered that BAFF Tg mice are protected in a model of multiple sclerosis (EAE) in mice. This is quite surprising as blocking BAFF in the same model in normal mice protects against EAE. We thinks that BAFF has two functions normal BAFF levels increase the capacity of B cells to present to T cells and activate them. Then blocking BAFF would prevent T cell activation. But excess BAFF increases B cell auto-reactivity and the numbers of regulatory T cells which are potent inhibitors of EAE. We need to understand better these 2 possibilities to find news strategies to treat multiple sclerosis. In vitro assays addressing the antigen-presentation activity of B cells with BAFF will be done, using WT mice and mice lacking BAFF or each of the receptors. Finally, the role of excess BAFF in generating regulatory T cells will be investigated using various BAFF receptor-deficient mice.

References

1. Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. Nat Rev Immunol 2002;2:465-75.
2. Mackay F, Schneider P, Rennert P, Browning JL. BAFF and APRIL: a tutorial on B cell survival. Ann Rev Immunol 2003;21:231-64.
3. Dillon SR, Gross JA, Ansell SM, Novak AJ. An APRIL to remember: novel TNF ligands as therapeuthic targets. Nature Reviews Drug Discovery 2006;In Press.
4. Phan TG, Amesbury M, Gardam S, et al. Immunoglobulin (Ig) class switch recombination and production of IgG autoantibodies by anergic self-reactive B cells. J Exp Med 2003;197:845-60.
5. Phan TG, Paus D, Chan TD, et al. High affinity germinal center B cells are actively selected into the plasma cell compartment. J Exp Med 2006;203(11):2419-24.

6. Martins G, Calame K. Regulation and functions of Blimp-1 in T and B lymphocytes. Annu Rev Immunol 2008;26:133-69.
7. Groom JR, Fletcher CA, Walters SN, et al. BAFF and MyD88 signals promote a lupuslike disease independent of T cells. J Exp Med 2007;204(8):1959-71.
8. Shimomura Y, Ogawa A, Kawada M, et al. A unique B2 B cell subset in the intestine. J Exp Med 2008;205(6):1343-55.