Meeting Harriet & Muhammad 24/04/23

* bootstrapping, reduce search space by restriction to likely protein cleavage sites, MHC binding, common epitopes, HLA reading, test pipeline on validated associations eg.
* sydenham's chroea, guillian barre, coeliac disease before testing on psychosis ?namdr proteins

IMAT (Immunological Mechanisms of Antipsychotic Treatment) Meeting 27/04/23

* Neuropathology manifestations (cf. humoral vs. cell-mediated):
  + Autoimmunity towards specific antigens recruits innate cells eg macrophages glial cells, eg RA T1DM, long-lasting due to clonal selection (memory) vs.
  + Non-immune pathology (non-clonotypic .: no memory) – alarmins, DAMPs, eg GBS
    - Infections (?mimitope) can play a precipitating role in both
    - Eg EBV & MS
* Lipids can also be presented to TCRs by CD1?? Not just peptides. Also polysaccharides.
* Janeway, Shlomchik showed role of B-cells in T1DM: NOD mouse expressing mIgs that are expressed on cell membrane but not secreted to investigate APC role as Ig secretion not thought to be pathogenic. Findings: insulitis increased in severity and diabetic incidence increased <https://diabetesjournals.org/diabetes/article/53/10/2581/11451/Investigation-of-the-Role-of-B-Cells-in-Type-1>
* AIRE-deficient patients harbour unique high-affinity disease-ameliorating autoantibodies (eg anti-TNFalpha) <https://www.cell.com/fulltext/S0092-86741630792-9>
  + AIRE role in thymic maturation
  + Why are these people alive. Anti type 1 interferon ab’s ??protective
  + Also: Exploring the association between brain-derived neurotrophic factor (BDNF) levels and longitudinal psychopathological and cognitive changes in Sardinian psychotic patients <https://doi.org/10.1192/j.eurpsy.2022.414>
* **Therefore !hypothesis: anti-IL32 protective in high-MS-index pts confers protective role**
* Desmoglein antibodies germinated by antecedent infections produces blistering disease following ab somatic hypermutation (switch from binding pathogenic epitope to self-epitope) <https://pubmed.ncbi.nlm.nih.gov/22996451/>
* Michel Nussenzweig (Rockefeller) study (think it’s this paper) B-cell memory compartment has variable antigenic affinity? <https://www.cell.com/cell/fulltext/S0092-8674(20)31304-0> & regulation of T follicular helper cell selection <https://pubmed.ncbi.nlm.nih.gov/33536617/>
* Selection pressure for pathogenic antigens to mimic self-antigens eg SARS-CoV2 spike protein
* F. Cucca: SLE & MS BAFF <https://www.nejm.org/doi/full/10.1056/nejmoa1610528>
* Amisulpride paper <https://insight.jci.org/articles/view/165024/pdf>
  + TNF drives fibroblasts which make cytokines
* Dectin-1 signaling on colonic γδ T cells promotes psychosocial stress responses <https://www.nature.com/articles/s41590-023-01447-8>
* IL17 (immune-CNS crosstalk) Meningeal γδ T cell–derived IL-17 controls synaptic plasticity and short-term memory <https://www.science.org/doi/10.1126/sciimmunol.aay5199>: IL17 promotes glial BDNF production (general idea: complement, ILs play different role in CNS)
* VDJ recombination, somatic hypermutation (FDCs in B-cell LN region select positively BLs with high-affinity Abs and negatively BLs with low-affinity Abs (by inducing apoptosis))

Immunology Notes

* iNKT (invariant NKT) cells are a subset of lymphocytes that bridge the gap between innate and adaptive immunity. They have TCRs on their surface for glycolipid antigen recognition. They also have natural killer (NK) cell receptors (? generic PRRs). Play a regulatory role in the development of autoimmune diseases, and deficiency/dysfunction exacerbates autoimmune diseases. NK cells play a role in adaptive immune responses by way of antibody-dependent cellular cytotoxicity or ADCC where they bind to and kill cells to which antibody molecules have bound.
  + .: B-cell aetiological role?? IgM half-life 6 days, IgG half life 3 weeks
* Lymphocytes in the innate immune system – Gamma:delta T-lympocytes aka intraepithelial T-lymphocytes/IELs may have PRRs & are cytotoxic. Do they require co-presentation (eg MHC + CD)?. **B-1 lymphocytes secrete IgM against PAMPs/MAMPs.**
  + Do regions of the NAMDR subunit resemble MAMPs
* Epitopes: peptides (5-15AA) or polysaccharides (3-4 sugar residues)
* Some cases in TCR presentation where CD1/MHC not necessary
* Because they (IgD) recognize molecular shapes that occur as a result of the 3-dimensional folding of an antigen, B-cell receptors can bind directly to epitopes on peptide, protein, polysaccharide, nucleic acid, and lipid antigens. .: tertiary structure does matter??
  + Both IgM and IgG or lead to opsonisation – undergo conformational change upon binding antigen that allows complement protein C1q to associate with the Fc region of the antibody. C1q association eventually leads to the recruitment of complement C4b and C3b, both of which are recognized by complement receptor 1, 3, and 4 (CR1, CR3, CR4), which are present on most phagocytes.
* And even for TCR – if epitope is eg in hydrophobic protein domain core, epitope will not be immunogenic.
* BL present exogenous epitopes to CD4+ TL which produce cytokines to allow BL to differentiate and mature to become plasma cell. Hence most proteins are T-dependent antigens.
* 5 primary differentiated CD4+ types: TH1, TH2, TH17, Treg, TFH
* T-independent (TI) antigens (normally carbohydrates or lipids, includes foreign polysaccharides & unmethylated CpG DNA) can activate BLs without effector CD4+ lymphocytes – two types. TI-1 are PAMPs, bind to BL PRRs (not BCRs). TI-2 activate BLs by simultaneously crosslinking a number of B-cell receptors.
* Autoreactive B-cells are induced in the bone marrow to either undergo apoptosis or receptor editing if immature (gene rearrangement)
* APCs do not discriminate between self- and non-self antigens during MHC-II presentation. Hence TLs can uniquely discriminate between self- and non-self pMHCs eg cells infected with HIV have only 8–46 HIV-specific pMHCs, compared with 100,000 total pMHCs, per cell. Does this implicate a defective self-reactive TH1/2 cell as a key mediator of autoimmunity? Additionally, proteasome cleavage is only part of the MHC-I pathway, NOT MHC-II (which utilises proteolysis). These molecules respectively bind 8-10mers and 13-25mers. pMHCs will have a lifetime based on peptide-MHC affinity. The kinetic proofreading model links this to the likelihood of successive initially reversible tyrosine phosphorylations in the TCR complex (Immunoreceptor tyrosine-based activation motif, ITAM). ITAMs are phosphorylated by the Src kinase Lck, which is anchored to the plasma membrane by CD4 (specific to helper T cells & regulatory T cells) or CD8. Lck competes with phosphatase CD45 which removes phosphates from ITAMs. High-affinity pMHC binding disrupts this Lck/phosphatase balance. <https://en.wikipedia.org/wiki/T-cell_receptor>
* MHC classes I-III <https://en.wikipedia.org/wiki/Major_histocompatibility_complex>
  + In MHC class I, any nucleated cell normally presents cytosolic peptides, mostly self peptides derived from protein turnover and defective ribosomal products. During viral infection, intracellular microorganism infection, or cancerous transformation, such proteins degraded in the proteosome are as well loaded onto MHC class I molecules and displayed on the cell surface. T lymphocytes can detect a peptide displayed at 0.1%-1% of the MHC molecules.
  + In MHC class II, phagocytes such as macrophages and immature dendritic cells take up entities by phagocytosis into phagosomes—though B cells exhibit the more general endocytosis into endosomes—which fuse with lysosomes whose acidic enzymes cleave the uptaken protein into many different peptides. Via physicochemical dynamics in molecular interaction with the particular MHC class II variants borne by the host, encoded in the host's genome, a particular peptide exhibits immunodominance and loads onto MHC class II molecules. These are trafficked to and externalized on the cell surface.
  + binding of peptides between the alpha-helix walls, upon a beta-sheet base. Class I primarily makes contact with backbone residues at the Carboxy and amino terminal regions, while Class II primarily makes contacts along the length of the residue backbone. The precise location of binding residues is determined by the MHC allele
  + MHC only sensitive to linear epitopes (cf conformational epitopes eg BCR)
  + A key challenge in identifying T cell epitopes is that their recognition varies substantially between individuals. One factor driving this variability is that the genes encoding for MHC molecules (called HLA in humans) are the most polymorphic in the human genome. Different MHC molecules have distinct binding specificities, which results in them presenting different MHC ligands to T cells. As a result, different individuals in the human population will present different epitopes.
* Autoimmunity
  + T cells need three signals to become fully activated. Signal 1 is provided by the T-cell receptor when recognising a specific antigen on a MHC molecule. Signal 2 comes from co-stimulatory receptors such as CD28, presented on the surface of other immune cells. It is expressed only when an infection was detected by the innate immune system, it is a "Danger indicating signal". This two-signal system makes sure that T cells only respond to harmful pathogens and not to self-antigens. An additional third signal is provided by cytokines, which regulate the differentiation of T cells into different subsets of effector T cells.
  + Defective tolerance
    - Central tolerance: presentation of self-antigens initiated by mTECs but reinforced by thymic dendritic cells after expression of AIRE and engulfment of mTECs during maturing T-lymphocyte negative selection
    - Peripheral tolerance (failsafe): tissue-specific self-antigen presentation within LN by dendritic cells (here role of cross-presentation ie *extracellular* antigen uptake for TL presentation, as many viruses able to interfere with antigen processes eg herpesviridae). Induction of anergy, apoptosis or T-regulatory state for high-self-affinity CTLs.
  + On surfaces of helper T cells are CD4 receptors, as well as TCRs. When a naive helper T cell's CD4 molecule docks to an APC's MHC class II molecule, its TCR can meet and bind the epitope coupled within the MHC class II. This event primes the naive T cell. According to the local milieu, that is, the balance of cytokines secreted by APCs in the microenvironment, the naive helper T cell (Th0) polarizes into either a memory Th cell or an effector Th cell of phenotype either type 1 (Th1), type 2 (Th2), type 17 (Th17), or regulatory/suppressor (Treg), as so far identified, the Th cell's terminal differentiation. MHC class II thus mediates immunization to—or, if APCs polarize Th0 cells principally to Treg cells, immune tolerance of—an antigen. The polarization during primary exposure to an antigen is key in determining a number of chronic diseases, such as inflammatory bowel diseases and asthma, by skewing the immune response that memory Th cells coordinate when their memory recall is triggered upon secondary exposure to similar antigens. B cells express MHC class II to present antigens to Th0, but when their B cell receptors bind matching epitopes, interactions which are not mediated by MHC, these activated B cells secrete soluble immunoglobulins: antibody molecules mediating humoral immunity. These are T-independent antigens, and produce antibodies with relatively poor affinities, and generally only IgM, with some differentiation into long-lived memory B-cells but predominantly short-lived cells.
  + Endpoint: autoreactive CTLs or secretory BLs
    - The threshold for activation of CD8+ cells is very high, and the process can occur via two pathways: thymus-independent (by infected APCs) or thymus-dependent (by CD4+ T cells). In the thymus-independent pathway, because the APC is infected, it is highly activated and expresses a large number of co-receptors for coactivation. If APCs are not infected, CD4 cells need to be involved: either to activate the APC by co-stimulation (more common) or to directly activate the Tc cell by secreting IL-2.
    - B-cells: Once a BCR binds a TD antigen, the antigen is taken up into the B cell through receptor-mediated endocytosis, degraded, and presented to T cells as peptide pieces in complex with MHC-II molecules on the cell membrane.[19] T helper (TH) cells, typically follicular T helper (TFH) cells recognize and bind these MHC-II-peptide complexes through their T cell receptor (TCR).[20] Following TCR-MHC-II-peptide binding, T cells express the surface protein CD40L as well as cytokines such as IL-4 and IL-21.[20] CD40L serves as a necessary co-stimulatory factor for B cell activation by binding the B cell surface receptor CD40, which promotes B cell proliferation, immunoglobulin class switching, and somatic hypermutation as well as sustains T cell growth and differentiation.[1] T cell-derived cytokines bound by B cell cytokine receptors also promote B cell proliferation, immunoglobulin class switching, and somatic hypermutation as well as guide differentiation.[20] After B cells receive these signals, they are considered activated.[20] Once activated, B cells participate in a two-step differentiation process that yields both short-lived plasmablasts for immediate protection and long-lived plasma cells and memory B cells for persistent protection.[16] The first step, known as the extrafollicular response, occurs outside lymphoid follicles but still in the SLO.[16] During this step activated B cells proliferate, may undergo immunoglobulin class switching, and differentiate into plasmablasts that produce early, weak antibodies mostly of class IgM.[21] The second step consists of activated B cells entering a lymphoid follicle and forming a germinal center (GC), which is a specialized microenvironment where B cells undergo extensive proliferation, immunoglobulin class switching, and affinity maturation directed by somatic hypermutation.[22] These processes are facilitated by TFH cells within the GC and generate both high-affinity memory B cells and long-lived plasma cells.[16] Resultant plasma cells secrete large amounts of antibody and either stay within the SLO or, more preferentially, migrate to bone marrow.[22]

Meeting Harriet, Katharina & Muhammad 02/05/23

* Viral proteome eg coxsackie B4 > CTL cleavage prediction > MHC II HLA alleles high affinity binding predictions > BLAST to compare vs self-antigens of interest > n peptides
* Then use other positive controls eg <https://doi.org/10.1016/j.jaut.2018.10.012>
  + Coxsackie B4/HLA DR3, DR4/GAD \*PXVKEK\* (T1DM)
  + EBV/?HLA alleles/??antigen (MS)
  + Gliadin/HLA DQ2/??intestinal antigen (coeliac disease)
  + Swine flu vaccine/?HLA allele/hypocretin aurexin neurons (narcolepsy)
  + GABHS/?HLA/? antigen (Rheumatic heart disease)
* Reviews on molecular mimicry
* Neuropeptides as a possible target (endocrine disruption in scz?)
* RIPKE 2014 GWAS scz
* Databases constraints/methods

Meeting w James Campbell, Bioinformatics 04/05/23

* Jupyter notebooks long-term
* Virtual env for python (Venv?)
* Conda allows shell scripting on CAMP (CAMP runs on linux anyway)

Meeting w Harriet, 10/05/2023

* Has somebody worked on a similar epitope mimicry pipeline before?
* Endoproteosome processing to restrict search space (speak to Le, Hannah soon)
  + Le suggested speaking to Oliver Schultz downstairs
    - suggested Mishto lab or someone outside Crick
      * Emailed Leipe
* HLA/MHC I molecular mimicry – does it happen? Is it allele-dependent?
  + Ultimate question is – do we need to worry about the intricacies of antigen processing or can we do BLAST immediately and worry about search space restriction later on?
  + Also look at other Mimitope reviews or epitope mimicry pipelines
* Read Katharina email

Meeting w/ Harriet, Katharina & Muhammad 16/05/2023

* Pipeline
  + Take viral proteins from human viruses in ViralZone
  + Run them through NetChop
  + Filter to 13-25 nucleotides
  + Run through MixMHC2 and find peptides that bind (will need to do reading here regarding IC50 cutoffs, can look at positive control references e.g. PEVKEK sequence)
  + BLAST alignment against human proteome
  + Find positively- and negatively-associated HLA alleles (will need BABS help here)
    - Can also use some form of MHC-II binding model looking at eg RMSD eg
      * Epitopedia (<https://doi.org/10.1016/j.immuno.2023.100023>): human proteome (protein databank +/- alphafold) & all T- and B-cell and MHC ligands and associated source sequences from IEDB > BLAST for sequences >=5 AA > filtered to sequences with >=3 consecutive AAs with relative accessible surface area >20% > filter by epitope structure availability from EPI-3D or alphafold if not available (remaining hits reported as 1D mimics) > TM-align for RMSD for all pair structural alignments, calculate accessibility agreement > list molecular mimicry between inputs & IEDB epiropes, including top list of 3D mimics with RMSD <1 angstrom
      * EMOMIS (preprint on bioarxiv, <https://doi.org/10.1101/2022.02.05.479274>): BLAST using AA sequence > results filtered using sequence length, surface accessibility, sequence similarity in antibody-antigen interface region > check for 3d structural alignment > antibody-antigen binding using deep learning model (Gainza et al. 2020)
      * Arthritogenic alphaviridae epitopes (most similar to ours, <https://doi.org/10.1038/s41598-019-55730-6>): BLAST for human/viral common proteome > in silico analysis for T-cell epitopes (ProPred II for HLA class II allele-specific binding ability, molecular docking analysis using GalaxyPepDock, chemical binding analysis using LigPlot+) and B-cell epitopes (three different prediction algorithms: Bepipred linear epitope prediction, Emini surface accessibility scale and Kolaskar and Tongaonkar antigenicity scale > triple hits were submitted for 3d accessibility analysis using Ellipro). SKDVYANTQLVLQRPAA identified in this study as being a potential mimitope was verified as such in vivo in mice (see discussion).
      * <https://doi.org/10.1371/journal.pone.0273494>
      * <https://doi.org/10.1093/oxfimm/iqac009>
      * <https://doi.org/10.1186/s12859-019-2867-5>
      * <https://doi.org/10.3390/v14071415>
      * another thing i need to do is look through the citations for the discussion in this paper <https://www.nature.com/articles/s41579-022-00770-5> around figure 2 (the EBNA1 mimitope regions) to see how those authors came to their findings
      * this is a commercial platform but looks very much worth going through due to their consideration of discontinuous primary structures with tertiary apposition <https://www.biosynth.com/biologics/epitope-mapping>

IMAT Meeting 17/05/2023

* Grant goals
  1. Describe longitudinal peripheral immune phenotypes related to antipsychotic treatment response. In treatment-seeking individuals with psychosis (N=200), we shall use easily applicable, high-content immunophenotyping and machine learning to compare immune markers before and after antipsychotic treatment.
  2. Characterise brain-specific immune components relevant to antipsychotic treatment response. In a follow-up study in treatment-seeking individuals with psychosis (N=300) and healthy individuals (N=150), we shall combine immunophenotyping with targeted immunological assays for relating brain-specific immune markers to antipsychotic treatment response.
  3. Elucidate immune mechanisms mediating antipsychotic treatment response. In an immunological mouse model, we shall combine our translational psychosis-like behavioural assays with genetic and pharmacological immune manipulations, including adoptive cell transfers, to dissect immune causation vis-à-vis antipsychotic treatment responses.

IMAT Meeting 31/05/2023

* Nuts & bolts of aim 1: Describe longitudinal peripheral immune phenotypes related to antipsychotic treatment response. In treatment-seeking individuals with psychosis (N=200), we shall use easily applicable, high-content immunophenotyping and machine learning to compare immune markers before and after antipsychotic treatment. Not restricted to first-episode psychosis.
* Will aim 2 be run sequentially: Characterise brain-specific immune components relevant to antipsychotic treatment response. In a follow-up study in treatment-seeking individuals with psychosis (N=300) and healthy individuals (N=150), we shall combine immunophenotyping with targeted immunological assays for relating brain-specific immune markers to antipsychotic treatment response. CSF to be taken from N=50 of each group.
* Abs? T/B clones? In blood & CSF