Q&A from "*Fundamentals of the Immune System*"

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Q1. *Can CD8+ T cells can be activated without CD4+ T cells, or independently can be activated in response to virus infected cell?*

A1. CD8+ T cells can be primed (activated) by activated dendritic cells alone, but CD4+ T cells are required for generating a memory CD8+ T cell response. [This is similar to B cells being activated by antigen in the absence of CD4+ T cells, but again, good memory responses require CD4+ T cells.]

Q2. *Can B cells recognize anything else besides protein antigens (e.g., DNA, RNA, lipids)?*

A2. Yes. Among B cells in one's B-cell repertoire there will be clones whose B-cell receptor (BcR) binds strongly to / recognizes many different types of antigen, including nucleic acids, lipids, carbohydrates, and drug-like small molecules (haptens). In fact, it was shown a long time ago that newly invented small molecules which had never existed before could be bound by BcRs/antibodies, attesting to the vast diversity of the BcR repertoire. [Note that lymphocytes (including B cells) that recognize self antigens are typically deleted or made non-reactive (anergic), so they are not included in this category.]

However, in order to generate a good memory response, CD4+ T cells that recognize protein, must also be involved. That was shown by "hapten-carrier" studies in which animals (*e.g.*, mice or rabbits) are immunized with a "conjugate" comprising a small-molecule hapten (*e.g.*, dinitrophenyl; DNP) covalently attached to an immunogenic "carrier" protein (*e.g.*, egg albumin, AKA ovalbumin). The animal's B cells will recognize and produce antibodies against both the hapten and the protein, whereas its T cells will respond to the carrier protein. The immunized animal will elicit a strong the anti-DNP antibody response with a protein carrier, but not if the DNP is instead covalently linked to a non-protein carrier (like dextran). The non-protein conjugate will elicit an antibody response, but it will not be strong, or lasting, nor will it elicit a "germinal center" reaction that produces class switching, affinity maturation and significant somatic hypermutation. CD4+ T cells are needed for that!

Q3. *What is the difference in lymphocyte development in the bone marrow and thymus if any (we collect bone marrow and not thymus in mice)?*

A3. B cells develop from hematopoietic stem cells in the bone marrow (primary lymphoid tissue) up to the point of displaying a BcR and thus being an "immature B cell". They then travel to the spleen (and probably other sites as well) to undergo positive and negative selection as "transitional B cells", finally leaving to circulate to the secondary lymphoid tissues (lymph nodes, peyer's patches of the gut, including the spleen, etc.) in search of antigen.

In contrast, T cells also develop from the very same hematopoietic stem cells in the bone marrow, however before once committed to the T cell lineage, and before they begin rearranging their V, D and J genes that will lead to a full T cell receptor (TcR), they migrate to the thymic cortex, where TcR generation takes place, and where the T cell will commit to being either a CD4+ or CD8+ T cells during positive selection, and where self-reactive T cells are

deleted during negative selection in the thymic medulla. Naïve CD4 and CD8 T cells leave the thymus to circulate to the secondary lymphoid tissues in search of antigen. That is a very brief summary of the difference in development between the "classic" B- and T-cell subsets.

Q4. *How do APCs choose which fragments to present among the pool of randomly digested protein antigens?*

The class-I major histocompatibility complex (MHC) molecules of almost all cells have certain pockets to which "anchor residues" in a given peptide will bind. This allows the class-I MHC molecule to fold up and travel to the cell surface to present those binding peptides. Binding is tight enough to allow long-lived display; should the peptide disengage and diffuse away, the MHC molecule will be recycled. The peptides displayed by class-I MHC molecules tend to come from cytoplasmic sources where they are broken down by proteasomes; they are pumped into the rough ER where they meet and bind to nascent MHC molecules. Surface display of peptide-MHC complexes allows $CD8^+$ T cells that have been primed on an APC in the secondary lymphoid tissues, to attack and kill any cell that is displaying the peptide it was primed against, albeit presentation must also be occur on the same MHC molecule that was involved in priming. That is because TcRs recognize peptide antigens "in the context" of MHC molecules.

Similarly, class-II MHC molecules are recognized by $CD4^+$ T cells, and they are mostly expressed on classic *antigen-presenting cells* (APCs). Similar to class-I MHC molecules, peptides whose "anchor residues" bind to class-II MHC molecules, stabilize them, allowing them to cycle to and be displayed as complexes on the APC surface (along with peptide-class I MHC molecule complexes). The proteins from which class II peptides originate tend to come from the cell surface or from outside the cell. They are taken up in endosomes which fuse with lysosomes, that break down the peptides and allow them to bind to class II MHC molecules.

Conventional dendritic cells (DCs) are also good at "cross presentation" of peptides; that means that a portion of the proteins taken from the cell surface or extracellular space can be broken down in the cytoplasm (instead of in endosome/lysosomes), thus entering the class-I pathway. This allows extracellular pathogens (like viruses) to be recognized by both $CD4^+$ and $CD8^+$ T cells during priming.

Q5. *Please comment on the potential differences, if any, in germinal center reactions in SLO like tonsils versus mucosal lymphoid tissues.*

A5. I'm not sure what you mean by "SLO-like" given that SLO stands for secondary lymphoid organ. The tonsils are a part of the mucosal lymphoid system, it is one component of the mucosal lymphoid tissue that forms "Waldeyer's ring" of lymphoid tissue encompassing the nasopharynx. These tissues are loosely organized and can be packed with subepithelial follicles and germinal centers whose overlying epithelium includes specialized M cells that actively transport antigen to the underlying follicle/germinal center; T cells tend to be in the adjacent extrafollicular tissue whereas B cells are located in the follicles. Germinal centers are actively generating T-cell driven antibody responses.

Q6. *I can never rationalize how antibodies with conformational epitopes can be generated from short linear MHC peptides.*

A6. I agree with you! Typically, peptides are not good at eliciting antibodies that recognize conformational epitopes on folded proteins. However, as explained above, peptides from an immunogenic protein that bind to class-II MHC peptides are required to generate CD4$^+$ T-cell driven antibody responses. Just think of these peptides as being in the "carrier" portion of the antigen. However, there is some work showing that class-II MHC-binding peptides can be a part of the actual epitope a B-cell recognizes through its BcR (and later by the antibody it produces). But even in those cases, some of the protein antigen must processed to generate the peptide that will bind to class-II MHC molecule, while another portion of that same protein antigen must remain intact for the BcR to recognize the folded conformational epitope. NOTE: there is almost never a 1-to-1 correspondence between a "peptide epitope" recognized by a TcR and the folded conformational epitope recognized by the corresponding BcR. That is because conformational epitopes are typically a lot larger (in surface area) than a peptide; the peptide only covers a fraction of the total epitope recognized by the BcR.

Q7. *How does complement recognize pathogens promiscuously versus specifically via antibody?*

By complement, I think you mean "the complement system". There are several ways in which the complement system "recognizes" pathogens. First, like any innate immune recognition, some complement components (like the ficolins) recognize "pathogen-associated molecular patterns" (PAMPs). In the case of the "lectin" pathway, multivalent complement components recognize a class of molecules, like oligosaccharides or sugars, such as mannose on a pathogen's surface (such as a bacterial cell-wall carbohydrate). Due to multivalent binding, the component changes its structure and in doing so, activates the complement cascade, leading to generation and covalent binding to the pathogen's surface by the C3b complement fragment. Bound C3b can initiate the "alternative loop" of the complement cascade, which then greatly amplifies the response. So PAMP-recognition is one way of complement activation. Another mode of complement activation is more complicated. While our cells produce several different cell surface molecules that can rapidly inhibit the complement cascade, pathogens do not have these inhibitors on their surface. It turns out that a complement-activating form of C3 that can covalently bind to surfaces and initiate the complement cascade is constantly being produced spontaneously by hydrolysis. When hydrolyzed C3 binds to our cells, it is inactivated pretty quickly, but when it binds to a pathogen, it can remain long enough to spark the complement cascade. So that's another mechanism of activating complement that does not involve specific recognition. Finally, as you suggest, pathogen-binding IgM or adjacently bound IgG molecules can also initiate the complement cascade, and these occur because of specific recognition by antibody.

Q8. *How are self-reactive lymphocytes identified and eliminated when exposed to a limited variety of self-antigens in the bone-marrow. How does the body know they're self-reactive?* (Slide 7)

A8. Self-reactive T cells are generated in the thymus along with other T cells. Their TcRs bind to self peptides bound to class I or class II MHC molecules. They are "negatively selected" (and told to die) by binding tightly to those complexes in the thymic corticomedullary junction and medula. Similarly, T cells that do not recognize class I or class II MHC molecules at all also die there. That leaves T cells whose TcRs bind weakly to self peptides bound to MHC molecules; they are positively selected and their binding signals the cell to survive. Tolerance is the ability to accept self. Tolerance mechanisms that occur in the primary lymphoid tissues are referred to

as "central tolerance". There are also several tolerance mechanisms that operate outside the primary lymphoid tissues called "peripheral tolerance". In contrast to central T-cell tolerance, central B-cell tolerance occurs in the bone marrow and both positive and negative selection also acts on B cells in the bone marrow and on transitional B cells in the spleen.

Q9. *Do CD8 T cells need CD28 co-stimulation for cytotoxicity activation?*

A9. Yes. CD8 T cells become primed on classical DCs in the secondary lymphoid tissues. The DCs must be activated, and they must express CD28 on the T cell surface. However, once a CD8$^+$ T cells has been primed against a given peptide-class-I MHC complex, it does not need co-stimulation to recognize and kill a cell displaying that complex. For instance, that allows virally infected cells to be killed only because they display the peptide-MHC complex that the T cells was primed on.

Q10. *Is the process of differentiating from naive lymphocyte precursor pool to mature lymphocytes only occurring during development/childhood or does it occur throughout life?*

A10. The process of lymphocyte development occurs throughout life, though it is greatly reduced for T cells. That is because the primary lymphoid tissue for T cell generation, the thymus, "involutes" with its tissue being replaced by adipose tissue over time. The "T-cell compartment" of the body changes with time from being dominated by "naïve" T cells in young individuals to being dominated by "central and effector memory" T cells in older individuals. Notably, the naïve T cell population also sustains itself by replication, so it does not disappear altogether even with thymic involution. A similar shift occurs for B cells, but is not as dramatic.

Q11. *What is the value in knowing the germline BCR/TCR sequence in addition to the BCR/TCR sequence of the clone?*

A11. There are two answers for this. First, and foremost, B cells, especially those involved in CD4$^+$ T-cell driven, germinal center reactions, will undergo somatic mutation of their expressed, V(D)J recombined gene regions. One needs to know the germline gene sequence in order to determine, by comparison, the somatic mutations that an expressed gene has undergone. For chronic infections, like HIV-1, the clonal lineages of mutated B cells can be very complex and extensive. Of course, recombined TcR V(D)J genes do not undergo somatic mutation so they pretty much reflect the germline sequences (except at V-D, D-J and V-J joining sites).

The second reason for knowing the germline V, D and J genes of an individual is so that one can determine gene segments that may be important for health and/or disease. It turns out very few genetic markers, single-nucleotide polymorphisms (SNPs) have been determined for the immunoglobulin and TcR loci in the genome thus these regions are excluded from genome-wide association studies (GWASs). Thus, it is almost impossible to determine whether the absence or presence of a given germline gene might have an effect on one's health (*e.g.*, one's susceptibility to disease). So, knowing the set of germline genes for an individual may eventually lead to predictions regarding disease susceptibility. Further, in future, a thorough understanding of the entire chromosomal region of an immunoglobulin or TcR locus, may help one predict the features of the naive BcR or TcR repertoire.

Q12a. *Can we say that MHC I and TCR recognize the same peptide but bind to different epitope?*
A12a. One can say that MHC and TcR can BIND to different sites on the same peptide. The peptide's epitope comprises the side that is bound by the TcR's paratope. The anchor residues comprise the side chains that allow binding to the MHC molecule. This is true for both class I and II MHC molecules

Q12b. *Will their binding affinity differ significantly?*
A12b. Yes. Binding to the MHC molecule has to be very tight (with a long off-rate) so as to keep the peptide-MHC molecule complex intact. Binding to the peptide (in the context of the MHC molecule!!) is much weaker to allow on-off signaling.

Q12c. *So, the idea of TCR-T can also apply to MHC-T?*
A12c. I'm not sure what you mean by that. The two binding mechanisms are similar in being driven by non-covalent interactions (van der Waal's interactions, H-bonds, salt bridges, polar bonds and hydrophobic interactions), but otherwise, they are very different.

Q13. *How does the self-antigen binding work? Is there expression of every single self-epitope expressed somewhere?*

A13. The binding mechanism to self antigen by an antibody or by a TcR or MHC molecule is similar in being driven by non-covalent interactions, as described above.

With regard to expression of all self epitopes in a single location or compartment, the closest mechanism that fulfills this occurs in the thymic medulla, where the transcription factor, the autoimmune regulator (AIRE), mediates the random expression of most if not all proteins encoded in the genome. This allows many, many proteins to be processed and tested for binding on one's MHC molecules, thus allowing for negative selection to more thoroughly operate on developing T cells, and remove self-binding T cells from the final naïve T cell repertoire.

Q14. *How is self antigen expressed in the bone marrow to check for BCR self reactivity?*

Negative selection of self reactive B cells only begins in the bone marrow. I do not know of a system similar to AIRE that produces many types of protein for negative selection to act on in the bone marrow. However, be aware that B cells are still immature (and thus unable to respond to antigen) when they move to the spleen, where they are in contact with many more antigens (remember, the spleen is filtering the blood and capturing antigens in the process). So, the various stages of transitional B cell and their differentiation into "follicular" vs. "extrafollicular" (*e.g.*, marginal zone) B cells occur in the spleen (and very likely in gut-associated lymphoid tissues (GALT) as well!).

Q15. *What is cross presentation? And what is the basic difference between class I and class II MHC molecules?*

A15. Please see the answer to Q4 above.

Q16. *Assuming you have an unknown antibody and wanted to find its target protein, can CDR sequences be used to predict epitope sequences?*

A16. Better progress has been made for TcRs than for antibodies on this score. That is because the CDR-3 loops on the alpha and beta chains directly contact the core of the peptides exposed face, and the dimensions and structure of TcR paratopes are much less variable than those of antibody paratopes. Predictive algorithms, like Jacob Glanville's GLIPH, have had success in predicting TcRs in a T-cell repertoire that bind to the same antigen. Not exactly the same thing you're asking… but close!

Q17. *How common is it to have an antibody and TCR with the same CDR sequences?*

A17. Extremely unlikely given that CDRs 1 and 2 are encoded in germline V gene segments, and CDR3 of the BcR heavy chain and the TcR beta or delta chain is also partially encoded by a germline D gene segment. Throw imprecise joining and N-region addition into the mix and the chances of similarity are further reduced.

Q18. *Does the total number of B-cells and T-cells increase after all this proliferation throughout our lives? If not, why?*

There seems to be an imaginary, but very real, "size" to the B- and T-cell compartments, that restricts the number of cells to a given number. The total number of cells does not increase over one's lifetime, though the balance between naïve and memory cells does shift (see A10 above).

I like to imagine these compartments as containing a limited set of possible points where B and T cells can receive "survival signals"; cells that do not receive enough survival signals will eventually die off. In this way, competition among lymphocytes for a set number of survival signals restricts the number of cells in a B- or T-cell compartment to an optimal and maximal number.

My thanks to you all for participating in this webinar and for your excellent questions!! Feel free to contact me at jkscott@sfu.ca anytime if you have more questions or clarifications.