Module 3 Assignment

585.751.81 Immunoengineering

1. Explain in your own words these four mechanisms of tolerance: (30 points)
2. Central Tolerance

Central tolerance happens for T cells in the thymus and B cells in the bone marrow. Central tolerance consists in the selection mechanisms to keep these cells that recognize self-antigens (positive selection) but not too strongly (negative selection). Cells which fail positive selection, undergo apoptosis and cells which fail negative selection may also go into apoptosis, anergy (functionally inactive), or mature into Treg cells. Receptor editing is a central tolerance process that attempts to change the specificity of the immature B cell receptor to the self-antigens, involving rearrangements of the immunoglobin (Ig) genes. If receptor editing fails, the B cells die, if successful, the cells may survive and undergo positive selection.

1. Antigen Segregation

Antigen segregation refers to the distribution and compartmentalization of antigens within tissues such as the eye, or brain or privileged site modulating local or global immune response. Factors influencing antigen segregation include expression of Fas by tissues, which can induce apoptosis of immune cells, or the fact that antigen do not pass through conventional lymphatics.

1. Peripheral Anergy

When self-reactive T cells escape the central tolerance, peripheral tolerance will make these cells non-responsive to antigen presentation. Anergy can occur when T cells receive signal 1 on APC without the appropriate co-stimulatory signal 2.

1. Regulatory T cells

T regulatory cells (Treg) can come from the thymus and have strong binding within their TCR but instead of being eliminated by negative selection they become Treg. They can also be induced from T cells.

Treg participate to the peripheral tolerance, including:

* They can secrete IL10 and TGF-beta cytokines which render inactive immune cells, including auto reactive T cells.
* Treg can express inhibitory receptors such as LAG3 or CTLA4 inhibiting the function of APCs, thereby suppressing immune responses.
* They can secrete granzymes leading to apoptosis of the effector T cells, contributing to immune suppression.
* In addition, they can decrease cytotoxic activity of CD8+ cells and they can skew the response Th1 or Th2 cells decreasing proliferation.

1. From the following examples of autoimmune diseases, select one and research further:

As you research, answer briefly (3-5 bullet points for each): (40 points)

1. Psoriasis
2. **Rheumatoid arthritis**
3. Chron’s disease
4. Graves’ disease
5. Multiple sclerosis
6. Systemic lupus erythematosus

As you research, answer briefly (3-5 bullet points for each):

1. What immune cells are involved in this pathology?

* B cells secrete pro-inflammatory cytokines, rheumatoid factors (RFs), anti-citrullinated protein antibodies (ACPA). Autoreactive B cells can also act as antigen presenting cell (APC) in stimulating T cell maturation and differentiation into memory CD4+ T cells.
* Autoreactive CD4+ T cells contribute to synovial tissue proliferation, and cartilage destruction.
* CD8+ T cell can be activated promoting inflammation by targeting and killing infected or damaged cells, contributing to tissue damage in RA.
* B cells express RANKL molecules promoting osteoclasts (OCs) differentiation which causes bone resorption.
* B cells also activate macrophages and fibroblasts and transform them into tissue-destructive cells. Macrophages in inflamed joint regulates the secretion of pro-inflammatory cytokines and damaging enzymes associated with inflammatory responses [1], [2].

1. What signaling molecules are involved (e.g. cytokines, chemokines, MHC, etc.)?

* In early onset of RA, cytokines such as IL-6 and tumor necrosis alpha (TNF-alpha) have been reported to promote RA. These cytokines stimulate the activation of both chondrocytes and osteoclasts that degrade the matrix of articular cartilage leading to bone resorption. Other cytokines such as IL-13, IL-14, and IL-15 secreted from T cells and stromal cells participate in the inflammatory response and contribute to the chronic inflammation.
* Activation of Wnt pathway can cause synovial proliferation, bone damage and destroy joint.
* Altered expression of certain signaling lymphocytic activation molecules (SLAMF) receptors have been associated with RA and their specific role in RA pathogenesis is being investigated[1], [2].

1. What is thought to be a trigger of the disease?

Several potential triggers or risk factors have been identified, including:

* Genetics: the HLA-DRB1 has been thought to explain 30-50% of the genetic risk of RA
* Environment factors: also play a role like exposure to pollutants, or smoking.
* Autoimmune response: auto-reactive B cells are normally eliminated by the central and peripheral B-cell tolerance checkpoints. In RA patients, both checkpoints are usually defective leading to accumulation of mature B cells and Treg cells. The underlying cause can be a mutation in PTPN22 gene disrupting the B-cell receptor (BCR) pathway impairing the central tolerance checkpoint [1], [2].

1. Which of the mechanisms of tolerance does this break?

The mechanisms underlying B cell proliferations break the central and peripheral tolerances:

* Central tolerance: immature B cells in the bone marrow undergo central tolerance mechanisms to eliminate autoreactive B cells (negative selection). However, if autoreactive B cells escape elimination, they can become mature self-reactive B cells.
* Peripheral tolerance: If peripheral tolerance mechanisms are deficient, self-reactive B cells become activated and proliferate and promote autoimmunity inflammation.

1. Can the disease be resolved?

* According to the NIAMS web site, there are therapeutics to improve the symptoms but no cure and no ‘preventive’ measures for RA: there are therapies available to improve the symptoms of RA including disease-modifying anti-rheumatic drugs (DMARDs) or nonsteroidal anti-inflammatory drugs (NSAIDs) for pain relief.
* There is active research to discover new drugs which can improve the outcome. Phase I trial for the promising Rheumavax has been completed but since then there has not been any new development.
* In theory, therapies targeting pro-inflammatory cytokines, such as denosumab (targeting RANKL) or developing antigen-specific T cell could potentially lead to a breakthrough [1], [2].

**Suggested Resources/reading:**

* Janeway Immunobiology or other immunology textbook
* <https://www.nature.com/articles/nature05663>
* <https://www.nature.com/articles/nrdp201682>
* <https://www.cdc.gov/arthritis/basics/rheumatoid-arthritis.html>
* <https://nccih.nih.gov/health/RA/getthefacts.htm>
* <https://www.niams.nih.gov/health-topics/rheumatoid-arthritis>
* <https://www.sciencedirect.com/science/article/abs/pii/S1568997214000251#f0010>
* <https://www.nature.com/articles/nrdp201639>
* <https://ghr.nlm.nih.gov/condition/multiple-sclerosis>
* <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4622286/>
* Any published literature: Search key terms in Google Scholar (make sure to cite all sources used)

1. Review the study comparing the Hutterite vs. Amish populations in the citation below. How does the study probe at the hygiene hypothesis of growing levels of allergy and autoimmunity? In your response please answer: (40 points)
2. What differentiates the two populations?

The main differences of these two populations include:

* Farming practices: the Hutterite live on large, very industrialized, communal farms whereas the Amish live on a single-family dairy farm and use horses for transportation.
* Peripheral-blood leukocytes has increased proportion in Amish children of neutrophils, decreased proportions of eosinophils and similar proportions of monocytes compared with samples from Hutterite children.
* Neutrophils from Amish children expressed lower levels of chemokine receptor CXCR4, and adhesion molecules CD11b and CD11c than in neutrophils from Hutterite children.
* Monocytes from Amish children, compared to the ones in Hutterite children, exhibited lower levels of human leukocyte antigen DR (HLA-DR), and higher expression levels of ILT3.
* Median levels of 23 cytokines were lower in the Amish children than in the Hutterite children.
* 1,449 genes were up-regulated in the peripheral-blood leukocytes of Amish children, notably TNFAIP3 and IRF7, as compared with 1,360 genes up-regulated in the cells of Hutterite children with notably the gene TRIM8.

1. How did these differences contribute to cellular and molecular changes of the immune system?

The Amish exposed to an environment rich in microbes, higher levels of allergen and endotoxin, required more activation of their innate immune system. In response to microbes, 18 genes of the tumor necrosis factor (TNF) and interferon regulatory network factor 7 (IRF7), which regulates type I interferon transcription and is critical for the innate airway responses against pathogens, were overexpressed in Amish leukocytes.

1. How did they test this in a model experimental system?

To test the effect of the dust on asthma and the activation of the innate immune system, they administered Hutterite dust extracts to a mouse model allergic asthma for 4 to 5 weeks, and then compare their airway reaction to the ones of only induced asthma mouse treated with ovalbumin. Inhalation of Amish dust was enough to inhibit ovalbumin-induce airway hyperresponsiveness. By comparison, they repeat a similar experiment with MyD88-deficient mice switching to Hutterite dust extracts. They measured levels of eosinophils, neutrophils and cytokine responses, T reg and found similar results observed in humans.

1. What interventions can you think of that could be implemented?

Similar studies could be conducted among populations with comparable farming and livelihood conditions but exposed to different microbial environments, such as tribes in Africa or South America. By comparing the findings of these studies with this study, the goal will be to draw similar conclusions. Additionally, implementing longitudinal studies, would allow to track immune profiles and asthma symptoms over time, providing a better understanding of the relationships between farming practices, environmental exposure and immune responses.

Stein, Michelle M., et al. (2016). [Innate immunity and asthma risk in Amish and Hutterite farm children](https://www.nejm.org/doi/full/10.1056/NEJMoa1508749). *New England Journal of Medicine, 375*(5), 411-421.

[1] H.-Y. Yap, S. Z.-Y. Tee, M. M.-T. Wong, S.-K. Chow, S.-C. Peh, and S.-Y. Teow, “Pathogenic Role of Immune Cells in Rheumatoid Arthritis: Implications in Clinical Treatment and Biomarker Development,” *Cells*, vol. 7, no. 10, p. 161, 2018, doi: 10.3390/cells7100161

[2] S. Jang, E.-J. Kwon, and J. J. Lee, “Rheumatoid Arthritis: Pathogenic Roles of Diverse Immune Cells,” *Int. J. Mol. Sci.*, vol. 23, no. 2, p. 905, 2022, doi: 10.3390/ijms23020905