7.342 A Double-Edged Sword: Cellular Immunity in Health and Disease

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Fall 2018. Wednesdays, 11 am - 1 pm. (Class day and time are flexible.) Room 68-150.

Course Description

Immune cells are a diverse group of cells that function as foot soldiers to protect our bodies from both self-derived threats and exogenous pathogens, while keeping peace with normal cells and non-harmful or beneficial commensal microbiota. Immune cells are equipped with a variety of powerful and adaptable mechanisms to detect and subsequently resolve a wide spectrum of insults, a capacity that is essential for maintaining homeostasis under normal physiological conditions. However, the same mechanisms can backfire upon immune evasion of invading pathogens or under physiological stress and instead result in severe disorders, such as immunodeficiency, chronic infection and inflammation, autoimmune diseases, allergy, degenerative diseases, and cancer. Basic and translational research studies of immune cells have led to novel strategies to treat some of these disorders. In this course, we will discuss the connections between normal physiology (defense against infections, immune surveillance, and homeostasis) and disease (immune deficiency, chronic inflammation, and autoimmunity) by examining primary research papers that range from the classic to the most recent. We will discuss the developmental relationship between innate and adaptive immune cells as well as the functions and malfunctions of both types of immune cells. Our topics will include both basic biological principles (such as inflammatory and non-inflammatory cell death and immune cell signaling) and clinical applications (such as immune checkpoint blockade and chimeric antigen receptor-T or CAR-T cells). This course will familiarize students with basic immunological regulatory mechanisms and examples of strategies that apply knowledge of this fundamental biology to improve human health. More generally, students will learn how to identify relevant primary research literature, critically evaluate experimental data, and reach their own conclusions based on primary data. We will have the opportunity to learn how fundamental knowledge can be translated into a therapeutic treatment by visiting Merck, one of the largest pharmaceutical companies in the world.

Course Format

The class will meet weekly for two hours at a time convenient for all participants. Each week two primary research papers will be made available for students to read critically and thoroughly prior to class. During class, the students will critique the papers, focusing on two aspects: 1) overall evaluation of the results and impact of the papers; 2) detailed analysis of experimental design, methods utilized, controls, and key data points that allow authors to reach their conclusions. At the end of each session, two papers for the upcoming week will be previewed.

Course Expectations/Objectives/Goals

The course will introduce students to the critical reading of the primary scientific literature in the field of cellular immunity. By understanding the background of the papers and critiquing the methods, data, and conclusions in the papers, students will learn to:

1. Extract the overarching questions and hypothesis and understand how the background motivated the authors to work on the questions and form the underlying hypotheses.
2. Identify assumptions and critique the logic of the arguments in the papers, assess strength and weakness of the arguments, and design follow-up studies.
3. Explain experimental approaches commonly used to probe questions in immunology.
4. Identify key experiments and data (including design, controls, and statistical analyses).

5. Consider alternative approaches and compare the advantages and disadvantages of different approaches.

6. Learn how to rigorously draw conclusions from data.

Assignments

For students to practice communicating skills, there will be a midterm written assignment (1-2 pages) and a final oral presentation (around 15 slides).

Written Assignment (due Week 8, October 24th, 2018):

Students will write a critical evaluation of a primary research paper (not included as assigned weekly readings) that relates to the course contents. The student should briefly describe the paper's main questions and hypotheses and critically discuss the key experimental data, controls, and interpretations. Selected papers should be sent to the instructor at least one week in advance of the due date for approval.

Written assignments should be within 1-2 single-spaced letter pages with margins at least 0.5 inches. Figure legend font size can be smaller providing still legible.

Oral Presentation: Oral presentations will be scheduled for the last class session (**Week 15, December 12th**). Students will prepare a slide deck for a 12-minute talk with a 3-minute question-and-answer presentation discussing a chosen primary research paper. Students should present a primary research paper that is different from the paper for the written assignment and is not included in the assigned weekly readings (upon approval by course instructor one week before oral presentation).

The presentation slide deck should consist of 10-15 slides with the follow components: title slide (1 slide), background slides (1-2 slides), hypothesis for paper presentation/one aim for proposals (1 slide), results for paper focused on the key experiments and the key control(s) (3-8 slides), and conclusions or possible future experiments for proposals (1 slide).

Grading & Absence Policy

This half course (6 credits) is graded pass/fail. As the course is discussion-based, attendance at every class is mandatory and participation is a requirement for a passing grade. Should an emergency occur, please contact the instructor as soon as possible for a make-up assignment.

Prerequisites

No previous immunology courses are required. Basic knowledge of molecular biology, cell biology and biochemistry will be helpful. Students are recommended to have taken at least one of the following courses: 7.03 (Genetics), 7.06 (Cell Biology), or 7.28 (Molecular Biology). Prerequisites may be waived with permission by the instructor.

Class Field Trip

To complement the papers we read in the course, we will take a field trip to the pharmaceutical company Merck on topics related to cellular immunity (such as immune checkpoint therapies). A lab tour and a career panel discussion will be included in the trip tentatively scheduled for the week of December 3, 2018.

Syllabus

Week 1. Class introduction

- Introductions: instructor and students
- Overview of the syllabus and expectations

- Overview of primary research papers and how to find them (demo of literature search on PubMed).
- Introduction to cellular immunology
    - Functions and cellular components of the immune system.
    - Development of immune cells during embryogenesis and postnatal development.
    - Overview of some commonly used methods in immunology: flow cytometry, immunofluorescence, immunoblotting, genetic approaches to generate engineered cells or animals, transplantation assays to reconstitute immune system (mostly in recipient mice).
- Introduction of papers to be discussed in week 2.

Week 2: Development of immune cells

In mammals, immune cells include numerous specialized hematopoietic blood cells that are differentiated from hematopoietic stem cells (HSCs) located mostly in the bone marrow of adult animals. HSCs were one of the first well-characterized classes of somatic stem cells: they can self-renew in the bone marrow, and through a process of multi-step differentiation, generate all types of hematopoietic blood cells. In addition to the immune cells circulating in blood, a different group of cells collectively referred to as resident myeloid cells is thought to arise through a different developmental history. These cells are formed from precursors in the yolk sac during early embryogenesis and then migrate to developing organs to maintain the homeostasis of resident myeloid cells independently of HSCs. This week we will read one classic paper that contributed to our fundamental knowledge about lymphoid progenitors that give rise to lymphoid cells during hematopoiesis and a more recent paper focusing on a completely different development paradigm/model of resident myeloid cells.

(2A) Kondo, M., Weissman, I.L., and Akashi, K. (1997). Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell *91*, 661-672.
(2B) Schulz, C., Gomez Perdiguero, E., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., Prinz, M., Wu, B., Jacobsen, S.E., Pollard, J.W., Frampton, J., Liu, K.J., and Geissmann, F. (2012). A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science *336*, 86-90.

Week 3. DNA rearrangements in adaptive immune cells

The innate immune system consists of the cells and mechanisms that provide the first line of defense from infection in a non-specific manner. However, if a pathogen overwhelms innate immunity, an adaptive immune response is initiated in a pathogen-specific manner. B cells and T cells mediate the adaptive immune response thorough antigen-specific antibodies and pathogen-specific effector T cells (various types of T cells that exert different functions in adaptive immune response against particular pathogens), respectively. The capability of B cells and T cells to specifically recognize enormously diverse antigens relies on V(D)J recombination (recombination of immunoglobulin (Igs) and T cell receptor (TCRs) variable (V), diversity (D), and joining (J) segments), a fundamental process that occurs during the early developmental stages of T and B cell maturation. This week we will read a classic paper identifying the *Recombination Activating Gene 1* (*Rag1*) genes that encodes a critical endonuclease instrumental to V(D)J recombination. Additionally, we will read a recent study examining the chromosomal mechanism controlling this locus-specific recombination frequency. Higher frequency of recombination is achieved through CTCF-binding elements (DNA sequences that are bound by the transcription factor CCCTC-binding factor (CTCF), which acts as a regulator of chromatin architecture). CTCF-binding elements slow the chromatin-scanning movements of the RAG complex (composed of both Rag1

and Rag2), thereby improving the recombination frequency for recombination sites close to CTCT-binding elements.

(3A) Schatz, D.G., Oettinger, M.A., and Baltimore, D. (1989). The V(D)J recombination activating gene, RAG-1. Cell *59*, 1035-1048.
(3B) Jain, S., Ba, Z., Zhang, Y., Dai, H.Q., and Alt, F.W. (2018). CTCF-binding elements mediate accessibility of RAG substrates during chromatin scanning. Cell *174*, 102-116 e114.

Week 4. T cells: Th2 and cytotoxic T cells

T cells are critical mediators of the adaptive immune response. This week we focus on two classes of T cells: CD4+ helper T cells (Th) and CD8+ cytotoxic T cells. We will read a classic paper about a master regulator that is necessary and sufficient to specify a particular set of helper T cells (Th cells), Th2 cells that induce humoral immunity. Additionally, we will examine how the immune checkpoint pathway (regulations of immune activation to prevent autoimmune disorders) in cytotoxic T cells contributes to immune evasion (strategies used by pathogens and cancer cells to evade immune responses) of HIV-infected cells.

(4A) Zheng, W., and Flavell, R.A. (1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell *89*, 587-596.
(4B) Trautmann, L., Janbazian, L., Chomont, N., Said, E.A., Gimmig, S., Bessette, B., Boulassel, M.R., Delwart, E., Sepulveda, H., Balderas, R.S., Routy, J.P., Haddad, E.K., and Sekaly, R.P. (2006). Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. Nature Medicine *12*, 1198-1202.

Week 5. Cell death and immune processes

Programmed cell death is critical for homeostasis and immunity. This week we read a fundamental paper in the field of the cell death that describe how *C. elegans* developmental genetics identified critical players in the programmed cell death (apoptosis) pathway that subsequently proved to also be used when immune cells eliminate virus-infected cells. The second paper describes pyroptosis, a relatively new mode of cell death that is generally inflammatory, in contrast to the generally non-inflammatory process of apoptotic cell death.

(5A) Yuan, J., Shaham, S., Ledoux, S., Ellis, H.M., and Horvitz, H.R. (1993). The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell *75*, 641-652.
(5B) Kayagaki, N., Stowe, I.B., Lee, B.L., O'Rourke, K., Anderson, K., Warming, S., Cuellar, T., Haley, B., Roose-Girma, M., Phung, Q.T., Liu, P.S., Lill, J.R., Li, H., Wu, J., Kummerfeld, S., Zhang, J., Lee, W.P., Snipas, S.J., Salvesen, G.S., Morris, L.X., Fitzgerald, L., Zhang, Y., Bertram, E.M., Goodnow, C.C., and Dixit, V.M. (2015). Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. Nature *526*, 666-671.

Week 6. Lymphocytes in disease

The mechanisms immune cells employ to protect against pathogens can backfire and instead contribute to disease progression. We examine two examples this week. First, recombination of DNA is critical for the diversity of immunoglobulins and TCRs. However, this process of recombining DNA can contribute to leukemia as discovered in Erikson *et al.*: the *Igh* (*Immunoglobulin heavy chain*) locus experiences a high frequency of double-stranded DNA breaks and error-prone non-homologous end-joining repair, and this process can lead to translocation (chromosome abnormality caused by rearrangement of parts between nonhomologous chromosomes) of the *c-Myc* locus to *Igh* locus. *Cis*-regulatory elements of the *Igh* locus then will drive high expression of *c-Myc* and leukemogenesis. Doitsh *et al*. described

how pyroptotic cell death, a process thought to function in antimicrobial defense, contributes to cell death of HIV-infected CD4 T cells, thereby facilitating AIDS pathogenesis.

(6A) Erikson, J., ar-Rushdi, A., Drwinga, H.L., Nowell, P.C., and Croce, C.M. (1983). Transcriptional activation of the translocated c-myc oncogene in burkitt lymphoma. Proceedings of the National Academy of Sciences of the United States of America *80*, 820-824.
(6B) Doitsh, G., Galloway, N.L., Geng, X., Yang, Z., Monroe, K.M., Zepeda, O., Hunt, P.W., Hatano, H., Sowinski, S., Munoz-Arias, I., and Greene, W.C. (2014). Cell death by pyroptosis drives CD4 T cell depletion in HIV-1 infection. Nature *505*, 509-514.

Week 7. Th17 cells

T helper (Th) cells are critical components of the immune system, and different Th cell types respond to different stimuli. Th1 produces INF-gamma and mediates the response to intracellular infection by killing infected cells. Th2 produces the cytokines IL4, IL5, and IL13, which are thought to mediate a "weep-and-sweep" response to remove extracellular pathogens by regulating smooth muscles cells and mucus-secreting cells. IL17-producing Th17 cells constitute a proinflammatory group of T helper cells that mediate inflammation and are implicated in autoimmune disease and other chronic inflammatory conditions. This week we read a paper describing RORγt as a master regulator of Th17 cells. The second paper examines how Th17 cytokines accelerate initiation and progression of one of the most deadly of human malignancies, pancreatic ductal adenocarcinoma (PDAC).

(7A) Ivanov, II, McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell *126*, 1121-1133.
(7B) McAllister, F., Bailey, J.M., Alsina, J., Nirschl, C.J., Sharma, R., Fan, H., Rattigan, Y., Roeser, J.C., Lankapalli, R.H., Zhang, H., Jaffee, E.M., Drake, C.G., Housseau, F., Maitra, A., Kolls, J.K., Sears, C.L., Pardoll, D.M., and Leach, S.D. (2014). Oncogenic Kras activates a hematopoietic-to-epithelial IL-17 signaling axis in preinvasive pancreatic neoplasia. Cancer Cell *25*, 621-637.

Week 8. T cells (adoptive immune transfer)

Immune checkpoints act to maintain self-tolerance (the ability of the immune system to recognize the body's own cells or products as non-harmful and not mount immune responses). Cancer cells can hijack this process to evade immune surveillance. This week we read one of the early papers testing the hypothesis that the Programmed Cell Death Protein 1 (PD1)-mediated immune checkpoint plays a role in tumor survival and might serve as a target of immune checkpoint inhibition. Then we examine how CRISPR/Cas9-mediated genome editing of T cells generated chimeric antigen T cells (or CAR-T cells, T cells engineered to recognize a particular antigen) for adopted immune transfer to treat certain blood malignancies.

(8A) Iwai, Y., Ishida, M., Tanaka, Y., Okazaki, T., Honjo, T., and Minato, N. (2002). Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proceedings of the National Academy of Sciences of the United States of America *99*, 12293-12297.
(8B) Eyquem, J., Mansilla-Soto, J., Giavridis, T., van der Stegen, S.J., Hamieh, M., Cunanan, K.M., Odak, A., Gonen, M., and Sadelain, M. (2017). Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature *543*, 113-117.

Week 9. Autoimmunity

Multiple immune cell types function in preventing autoimmune disease. Regulatory T cells or T-regs are a class of anti-inflammatory T helper cells critical for suppressing autoimmunity. This week we discuss a paper in developmental immunology that discovered that the critical transcription factor FoxP3 specifies T-reg cell fate. Loss of *FoxP3* function in mice resulted in a lethal autoimmune syndrome. The second paper showed that loss of *MyD88* (a critical signaling component in innate immune cells) protected autoimmune diabetes in pathogen-free mice but not germ-free mice (mice without commensal microbiota). This is one of the first studies that examined the contributions of the microbiota and innate immune cells in autoimmune diabetes in mice.

(9A) Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nature Immunology *4*, 330-336.
(9B) Wen, L., Ley, R.E., Volchkov, P.Y., Stranges, P.B., Avanesyan, L., Stonebraker, A.C., Hu, C., Wong, F.S., Szot, G.L., Bluestone, J.A., Gordon, J.I., and Chervonsky, A.V. (2008). Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature *455*, 1109-1113.

Week 10. Innate lymphoid cells (ILC)

Type 2 innate lymphoid cells (ILC2) function in type 2 responses ("weep-and-sweep" protective immune response against helminth parasites) that stimulate secretion of mucus and hypercontractility of smooth muscles. ILCs play important roles in responses to parasites and allergens. This week we read two papers about the role of ILCs in the intestine and in the airway. The first paper shows interactions between tuft cells, a type of gut epithelial cells, and ILC2 through IL12. In turn, ILC2 functions by secreting IL13, which orchestrates the "weep-and-sweep" response from the gut epithelia. The second paper demonstrates that in the airway pulmonary neuroendocrine cells (PNESs), a rare type of airway epithelial cells, activate ILC2s by secreting calcitonin gene-related peptide (CGRP) and gamma-aminobutyric acid (GABA). ILC2s then activate the immune response in allergies by stimulating mucus secretion from airway epithelia.

(10A) von Moltke, J., Ji, M., Liang, H.E., and Locksley, R.M. (2016). Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature *529*, 221-225.
(10B) Sui, P., Wiesner, D.L., Xu, J., Zhang, Y., Lee, J., Van Dyken, S., Lashua, A., Yu, C., Klein, B.S., Locksley, R.M., Deutsch, G., and Sun, X. (2018). Pulmonary neuroendocrine cells amplify allergic asthma responses. Science *360*.

Week 11. Macrophages

Macrophages are a type of innate immune cells that engulf and digest microbes and cellular debris, and regulate local and global inflammation. However, excess inflammation operates at the cost of regular tissue function and can cause damage and reduce fitness during infection. Immune tolerance (a state of unresponsiveness of the immune system to substances that generally elicit an immune response) is another coping mechanism and is hypothesized to be more advantageous than extreme inflammation. The first paper describes the impact of heme, a metabolite increased upon lysis of red blood cells (such as in severe bacterial infection), and how heme oxygenase protects endothelial cells from apoptosis by producing carbon monoxide (CO). The second paper describes how regular HSCs upregulate the macrophage "don't eat me" signal CD47 during migration/mobilization to improve HSCs survival during migration, and how leukemic stem cells mimic normal physiological function to reduce phagocytosis by macrophages, thereby promoting leukemia progression.

(11A) Brouard, S., Otterbein, L.E., Anrather, J., Tobiasch, E., Bach, F.H., Choi, A.M., and Soares, M.P. (2000). Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis. The Journal of Experimental Medicine *192*, 1015-1026.
(11B) Jaiswal, S., Jamieson, C.H., Pang, W.W., Park, C.Y., Chao, M.P., Majeti, R., Traver, D., van Rooijen, N., and Weissman, I.L. (2009). CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell *138*, 271-285.

Week 12. Trained immunity

Innate immune cells were long thought to be of no or very limited adaptation/memory. This week we read some recent papers suggesting that the innate immune response could show features of memory, a process termed trained immunity. The first paper maps epigenetic (DNA methylations or histone modifications that regulate gene expression without altering DNA sequences) mechanisms underlying trained immunity in the process of monocyte (a precursor cell type that can differentiate into a macrophage or a dendritic cell) to macrophage differentiation. The second paper explores the trained immunity of HSCs during the immune responses to tuberculosis bacteria infection after Bacille Calmette-Guérin (BCG) vaccination.

(12A) Saeed, S., Quintin, J., Kerstens, H.H., Rao, N.A., Aghajanirefah, A., Matarese, F., Cheng, S.C., Ratter, J., Berentsen, K., van der Ent, M.A., Sharifi, N., Janssen-Megens, E.M., Ter Huurne, M., Mandoli, A., van Schaik, T., Ng, A., Burden, F., Downes, K., Frontini, M., Kumar, V., Giamarellos-Bourboulis, E.J., Ouwehand, W.H., van der Meer, J.W., Joosten, L.A., Wijmenga, C., Martens, J.H., Xavier, R.J., Logie, C., Netea, M.G., and Stunnenberg, H.G. (2014). Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science *345*, 1251086.
(12B) Kaufmann, E., Sanz, J., Dunn, J.L., Khan, N., Mendonca, L.E., Pacis, A., Tzelepis, F., Pernet, E., Dumaine, A., Grenier, J.C., Mailhot-Leonard, F., Ahmed, E., Belle, J., Besla, R., Mazer, B., King, I.L., Nijnik, A., Robbins, C.S., Barreiro, L.B., and Divangahi, M. (2018). BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. Cell *172*, 176-190 e119.

Week 13. Emerging technologies in immunology

Powerful technologies have been instrumental for the field of immunology. This week we read some emerging methods in the field. The first paper shows simultaneous measurements of 34 parameters (including multiple cell-surface and interacellular signaling components) at the single-cell level by combining flow cytometry and mass spectrometry. The second paper applies single-cell RNA-sequencing (RNA-seq) analysis for the discovery of potential new types of innate immune cells.

(13A) Bendall, S.C., Simonds, E.F., Qiu, P., Amir el, A.D., Krutzik, P.O., Finck, R., Bruggner, R.V., Melamed, R., Trejo, A., Ornatsky, O.I., Balderas, R.S., Plevritis, S.K., Sachs, K., Pe'er, D., Tanner, S.D., and Nolan, G.P. (2011). Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. Science *332*, 687-696.
(13B) Villani, A.C., Satija, R., Reynolds, G., Sarkizova, S., Shekhar, K., Fletcher, J., Griesbeck, M., Butler, A., Zheng, S., Lazo, S., Jardine, L., Dixon, D., Stephenson, E., Nilsson, E., Grundberg, I., McDonald, D., Filby, A., Li, W., De Jager, P.L., Rozenblatt-Rosen, O., Lane, A.A., Haniffa, M., Regev, A., and Hacohen, N. (2017). Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science *356*.

Week 14. Field trip. See page 2 above.

Week 15. Oral presentations and evaluations

Students will give their oral presentations, complete course evaluations, and we will discuss the course (pros and cons, things learned, and things students wish would have learned).