B cell Modeling of Cell Communication and Signal Transduction Pathways: How can students visualize cellular discussions?

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Abstract

How are B-lymphocytes activated and triggered to express antibodies? To increase students' comprehension of cell signaling pathways, we need multiple avenues of study to visualize immune cell communication in generating antibody response against invading pathogens. Unit alignment gives students a model system of study in B-cell communication as they research and write about cell communication pathways and cellular regulation. Students will build relevant concept maps on B-cell marker identification and cellular communications and simulate an immunological assay with flow cytometry data analysis collected through the summer research experience to draw conclusions about the cell marker recognition and internal protein expression in B-cell lymphomas. They will illustrate the pathways for expression of immunoglobulin by completing an SDS-PAGE electrophoresis protocol to separate protein chains and inquire into the make-up of IgG and protein expression in cell lysates used to study potential cancer chemotherapeutic compounds. Students will further discuss expression of cell markers and protein cascade before completing the summative assessment research paper to connect and expand their current model to either the role of apoptosis or cellular marker recognition of infectious diseases such as EBV, HIV, HBV, HCV, etc. for targeted treatments, current findings and further research questions.

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I. Overview:
Students will cover concepts of cell communication while using the immunological system as a model. Activities and laboratory experiences will be completed to address the goals of visualizing cellular discussions as in immunological cell lineages. The material best fits within a cellular biology larger unit of study or as a subset of biotechnology application.

II. Science Backgrounds
Content knowledge can be covered through extra reading as attached, which students will complete throughout the unit.

III. Student Outcomes
Students will complete activities, lectures, and discussions on general cellular communication and signal transduction pathways as well as specific model receptors and pathways in immunological cellular processes.

IV. Learning Objectives & Curriculum Standards:
AP Biology Enduring Understanding
3.D: Cells communicate by generating, transmitting, and receiving chemical signals. Students will learn mathematical skills that apply to the fields of immunology and biotechnology while completing SDS-PAGE and analyzing data collected from flow cytometry and Western-blot.
3.D.1: Cell communication processes share common features that reflect a shared evolutionary history.
3.D.2: Cells communicate with each other through direct contact with other cells or from a distance via chemical signaling.
3.D.3: Signal transduction pathways link signal reception with cellular response.
3.D.4: Changes in signal transduction pathways can alter cellular response.

4.B.2: Interactions between cells affect the fitness of the organism.
4.C.1: Variation in molecular units provides cells with a wider range of functions.

Science Practices 1-7:
Students can use representations and models to communicate scientific phenomena and solve scientific problems; can use mathematics appropriately; can engage in scientific questioning to extend thinking or guide investigations; can plan and implement data collection strategies appropriate to a particular scientific question; can perform data analysis and evaluation of evidence; can work with scientific explanations and theories; can connect and relate knowledge across various scales, concepts, and representations in and across domains.

KY Core Academic Standards & Expectations:
2.3 Students identify and analyze systems and the ways their components work together or affect each other.
2.1 Students understand scientific ways of thinking and working and use those methods to solve real-life problems.

KY Enduring Understandings:
Students will understand that:
*the many body cells in an individual can be very different from one another even though they are all descended from a single cell and thus have essentially identical genetic instructions. Different parts of the instructions are used in different types of cells.
*identify a variety of specialized cell types and describe how these differentiated cells contribute to the function of an individual organism as a whole
*describe and classify a variety of chemical reactions required for cell functions *describe the processes by which cells maintain their internal environments within acceptable limits

Skills & Concepts:
Students will:
*identify and investigate areas of current research/innovation in biological science. Make inferences/predictions of the effects of this research on society and/or the environment and support or defend these predictions with scientific data
* describe how science and technology interact. Research and investigate the impact of technology on society and how technological advances have driven scientific research

http://education.ky.gov/curriculum/docs/Documents/POS%20with%20CCS%20for%20public%20review.pdf p.509
http://education.ky.gov/curriculum/sci/Pages/Curriculum-Documents-for-Science.aspx

V. Time Requirements
Time allotments in lesson are for 90 minutes blocks meeting every other day.

American Association of Immunology
Heidi Anderson: Lesson Plan for Cell Signaling and Immune System

Unit Alignment

Day1: Students will review prior knowledge and build connections to cell communication.

Review prior knowledge/vocabulary and concepts:
Biochemistry, macromolecules, glycoprotein &integral membrane proteins, phosphorylation, mitosis & cyclin/CdK, cell proliferation, gene expression acetylation/methylation, transcription/translation, mutations, phagocytosis

Pre-Test over cell communication & immune system

Overview & discussion: Cell Communication & Signal Transduction Pathways

Day2: Students will read text on the immune system as a model of cell communication and discuss mechanisms by which these cells and chemical signals are used in innate and adaptive immunity.

Reading Activity: Bondada, S., Chelvarajan, R. & M. Gururajan, 2005. "B Lymphocytes" Encyclopedia of Life Science, John Wiley & Sons, Ltd.

Introduction: WBC classification with Innate vs. Adaptive Immunity, Cellular vs. Humoral Immunity Compare/Contrast

Lecture: Innate vs. Adaptive (Cellular vs. Humoral) Immunity

Activity A: Concept Mapping Cellular Interactions, with cytokines and cell receptor identifications

New Terminology:

Cells	*Markers/Receptors*	*Protein Cascade*	Cytokines/ Chemokines	Immunoglobulins
Leukocytes	CD19	BID	IL-1	IgM
Neutrophils	CD4+	BCL XL	IL-2	IgG
Eosinophils	CD8+	BCL-2	IL-4	IgE
Basophils	CD3	TCL	IL-5	IgA
Macrophages	BCR	PAR-4	TNF-β	IgD
Mast cells	TCR	BKT	γ–IFN	
Dendritic cells	MHC I	cIAP2		
T cells (Th1, Th2, CTL or Tc, Treg)	MHCII			Opsonization
B cells (Plasma, Memory)				
NK cells				
Lymphocytes				

Day 3: Students will read and discuss MHC and the means by which our immune system tells self from non-self as well as complete lab activities to learn how to collect evidence and analyze data for immunological studies.

Reading: Gregory, E. 2005 "An Introduction to the Major Histocompatibility Complex" Marieb, E. & K. Hoehn, 2007. *Human Anatomy & Physiology:"Too clean for our own good?"* p.824

Writing: Students will form a concept map on MHC classification and diagram relationships between cellular and marker vocabulary from article. Students will make connections between cell lineages and the role each cell plays and how scientists can identify differences through cell markers. Students will then use the concept map to write a short summary of the article and the implications of MHC.

Lab Activity B: Cell Counting and Density Calculations Worksheet
1. Ward's Catalog WBC Counts as a Diagnostic Tool Lab Kit 36W0049
2. Assay Trials Calculations Worksheet for determining solution concentrations in trials

Lab Activity C part 1: Drug Treatment Assay Flow Cytometry Data Analysis for presence of T cell, B cell and Macrophage Activity

Activity B: Concept Map additions: Immune System Overview Concept Map: *Supplement:* **"**Response to Antigen Invasion" as found in Marieb, E.N. & K. Hoehn. 2007. *Human Anatomy & Physiology Study Guide*, 7th ed. Pearson Publishing p.591.

*Disease Research Activity: Students will research specific disease impacting different immunological cell lines: Students research one for homework and share in class the main pattern of infection and present findings in a short 2-3 minute informal presentation:
Potential Pathogen/Host Cell interaction Diseases: HIV, EBV, HBV, HCV, Toxic Shock…
Potential Autoimmune Diseases: Lupus, Arthritis, Grave's, Thrombocytopenia, Asthma…

Day4: Students will model cellular interactions and communication within immune system.

Activity: Play-doh modeling of cells and interactions from readings -Make stop action clay-mation or cartoon videos of the cellular and cytokine cascades after normal antigen presentation to immune cells-

Discussion: Cell Signal Cascades in B cell pro- & anti-apoptosis proteins: BID, BCL-xl, BCL-2. Pro- & anti-apoptotic signal pathways are important in the mitochondrial membrane for cellular regulation or energy regulation in cells and change then during apoptosis so measuring levels can determine the impact of chemotherapy treatments on cell populations.

Lab Activity C part 2: How does compound 1919x affect pro- and anti-apoptotic protein cascades? Using Collected Data for Analysis of Western-Blots

Day5: Students will complete an SDS-PAGE lab while analyzing data for protein expression.

Discussion & Diagramming Activity: Immunoglobulin classification, expression from B-cells and protein make-up with five subclasses of antibodies:
Immunoglobulin A (IgA), high concentrations in the mucous membranes lining the respiratory passages and gastrointestinal tract, saliva and tears.
Immunoglobulin G (IgG), the most abundant antibody protects against bacterial and viral infections.
Immunoglobulin M (IgM), found in the blood and lymph fluid, first made to fight a new infection.
Immunoglobulin E (IgE), associated with parasitic diseases and with allergic reactions, found in the lungs, skin, and mucous membranes.
Immunoglobulin D (IgD), exists in the blood in small amounts and is the least understood antibody.
http://kidshealth.org/parent/system/medical/test_immunoglobulins.html
http://pathmicro.med.sc.edu/mayer/igstruct2000.htm

Pre-lab Analysis of Western-blot films

Lab Activity D: SDS-PAGE Electrophoresis Protocols for IgG Separation and Molecular Weight measures

Day6: **Final Summative Assessment**:

Essential Question: How do cells communicate by generating, transmitting, and receiving chemical signals? What is the role of apoptosis in immune system? How are pathogens able to attack immune cells?

Task: After researching primary and secondary literature on cell communication, immune response, and apoptosis in immunity, students will write an essay/research paper that explains the role of programmed cell death (apoptosis) in living organisms, pathogen host cell recognition for diseases of immune cells, or a specific autoimmune disorder of their choosing. What conclusions or implications can you draw from literature search? Cite at least 5 sources, pointing out key elements from each source and addressing the credibility and origin of sources. Identify gaps and unanswered questions in the research.

Supplemental Resources for Unit:

Calculations for Assay Trials on B-cell Populations
Lab Supplement Activity B

Directions: Complete the following calculations for a drug trial using the equation $C_1V_1 = C_2V_2$

1. The cell concentration we start with is 6.3×10^6 cells/mL from our cell counts taken using a hematocytometer. We wish to have a final concentration of cells equal to 0.5×10^6 cells/mL in 1000μL. What volume of the original cell concentration will we need to add to media to make a final volume of 1000μL?

C_1=
V_1=
C_2=
V_2=

2. In 1919x Trial #1, we will dilute a 5mM concentration of treatment drug 1919x stock solution to a 0.1mM concentration of 100μL. What is the volume of old stock that we use to dilute to a new concentration of 0.10mM drug 1919x concentration with final volume of 100μL?

C_1=
V_1=
C_2=
V_2=

3. In 1919x trial #1, we need to further dilute the New 0.1mM drug stock to a 0.3μM concentration with a total volume of 1000uL. What volume of the new stock should we use to make a 0.3μM solution?

C_1=
V_1=
C_2=
V_2=

4. The control treatment for 1919x trial #1 will include LPS (lipopolysaccharide) in an original concentration of 5000μg/mL diluted to 5μg/mL concentration in 1000μL. Df(dilution fold)=5

C_1=
V_1=
C_2=
V_2=

KEY
Calculations for Assay Trials on B-cell Populations
Lab Supplement Activity B

Directions: Complete the following calculations for a drug trial using the equation $C_1V_1 = C_2V_2$

1. The cell concentration we start with is 6.3 $x10^6$ cells/mL from our cell counts taken using a hematocytometer. We wish to have a final concentration of cells equal to 0.5$x10^6$ cells/mL in 1000µL. What volume of the original cell concentration will we need to add to media to make a final volume of 1000µL?

C_1= 6.3$x10^6$ cells/mL
V_1= ? solution V_1= 80µL
C_2=0.5$x10^6$ cells/mL
V_2= 1000 µL

2. In 1919x Trial #1, we will dilute a 5mM concentration of treatment drug 1919x stock solution to a 0.1mM concentration of 100µL. What is the volume of old stock that we use to dilute to a new concentration of 0.10mM drug 1919x concentration with final volume of 100µL?

C_1= 5mM
V_1= ? solution V_1=2µL
C_2= 0.1mM
V_2=100µL

3. In 1919x trial #1, we need to further dilute the New 0.1mM drug stock to a 0.3µM concentration with a total volume of 1000uL. What volume of the new stock should we use to make a 0.3µM solution?

C_1= 0.1mM =100µM
V_1= ? solution V_1=3µL
C_2= 0.3µM
V_2=1000µL

4. The control treatment for 1919x trial #1 will include LPS (lipo-polysaccharide) in an original concentration of 5000µg/mL diluted to 5µg/mL concentration in 1000µL. Df(dilution fold)=5

C_1= 5000µg/mL
V_1= ? solution V_1= 1µL
C_2= 5µg/mL
V_2=1000µL

Lab Activity C Part 1. Analysis of Flow Cytometry Data

Background: Flow cytometry is a laser-based technology used in cell counting and biomarker detection. Cultures of spleen cells are grown *in vitro* and then grown in a control or treatment group to determine the response of cells lines to treatment and finally exposed to antibodies that bond to set antigens on immunological cell lines with florescent markers for detection by lasers in the flow cytometer.

*Students read further details and schematic from
http://www.unsolvedmysteries.oregonstate.edu/flow_06

Objectives: Students learn how to read flow cytometry data from experimental treatments *in vitro* culture assays of spleen cells:

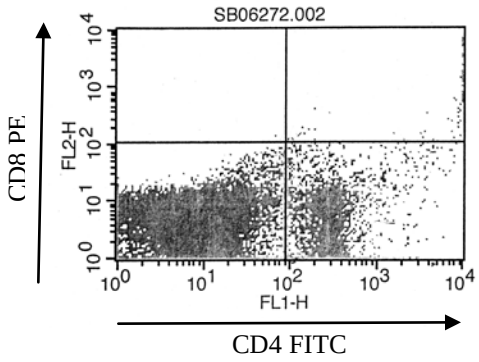
Experimental Design:

1) In lanes B2, C2, D2, E2, of cell culture trays, we will use PE CD19, FITC Class II, and BiotinPECY5 IgM antibodies to fluoresce cells for identification of B-cells.
Positive results in *Fig.2*

2) In Lanes B3, C3, D3, E3, of cell culture trays, we will use FITC CD4, PE CD8, BiotinPECY5 F4/80 antibodies to fluoresce cells for identification of T-cells.
Positive results in *Fig.1&3*

A. The data profiles are read as follows for media only controls:

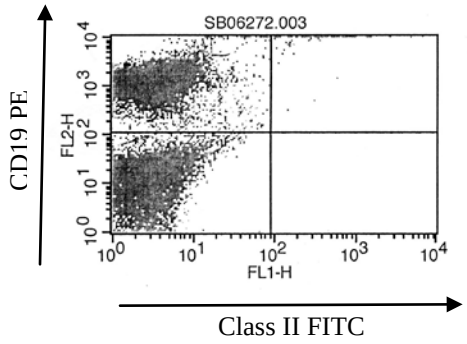
Standards: CD4 Th cells, CD19 B cells, Cytotoxic T Lymphocytes, Immunoglobulin expression



File: SB06272.002 Acquisition Date: 27-Jun-12
Gate: G1 Gated Events: 29791
Total Events: 59199 X Parameter: FL1-H (Log)
Y Parameter: FL2-H (Log) Quad Location: 91, 108

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean
UL	2	0.01	0.00	80.01	79.50	125.71
UR	73	0.25	0.12	7697.71	5144.77	2035.72
LL	24929	83.68	42.11	12.57	9.04	5.18
LR	4787	16.07	8.09	381.71	302.25	7.69

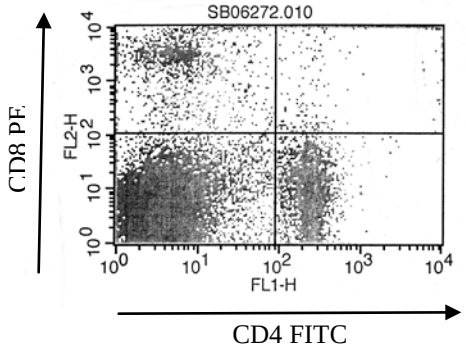
Fig. 1 positive test CD4 T-cells, lower-right quadrant



File: SB06272.003 Acquisition Date: 27-Jun-12
Gate: G1 Gated Events: 29831
Total Events: 55459 X Parameter: FL1-H (Log)
Y Parameter: FL2-H (Log) Quad Location: 91, 108

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean
UL	17805	59.69	32.10	3.97	2.92	1251.07
UR	100	0.34	0.18	522.49	327.08	9042.09
LL	11926	39.98	21.50	3.35	2.65	15.74
LR	0	0.00	0.00	***	***	***

Fig. 2 positive test CD19 B cells, upper-left quadrant



File: SB06272.010 Acquisition Date: 27-Jun-12
Gate: G1 Gated Events: 29543
Total Events: 63225 X Parameter: FL1-H (Log)
Y Parameter: FL2-H (Log) Quad Location: 91, 108

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean
UL	1699	5.75	2.69	13.89	7.31	3721.34
UR	1407	4.76	2.23	2814.71	1315.19	9010.94
LL	22251	75.32	35.19	6.15	4.06	7.02
LR	4186	14.17	6.62	266.53	248.20	12.09

Fig.3 positive test CD8+ upper-left quadrant, CD4+ T cells lower-right quadrant

Protein Separation using SDS-PAGE Lab Protocol: Activity D

Objective: Students will learn the steps of protein electrophoresis and be able to analyze the products by protein molecular weights and expression.

Pre-lab Activity: Analysis of Western-blots from treatments from activity B. link1, link2, link3, link 4

Background: Students have completed simple gel electrophoresis of DNA extraction prior to completing a protein analysis using SDS-PAGE to now study immunological and muscle proteins by molecular weight.

Materials:

1. Ward's Scientific Catalog
Mini-protean TGX precast gels
SDS Solution 10%
Tris-Gly SDS Running Buffer
Comparative Proteomics Kit I: Protein Profiler
Protein Fixative Stain
Colored PAGE Marker

2. Carolina Biological Catalog
Serum Antibody Set

or

Fisher Scientific Catalog
IgG Goat Antihuman 0855127

3. Microcentrifuge Tubes
Micropipettes & Tips 10-100ul
Floating Microtube rack
Hot water bath

4. Sample buffer: from Bondada Lab

with 2MEA
2 ml 0.5M Tris HCL (ph6.8)
2 ml 100% glycerol
0.4g SDS
2mg Bromophenol Blue
0.25 ml 2MEA
0.75 DH2O

without 2MEA
2 ml 0.5M Tris HCL (ph6.8)
2 ml 100% glycerol
0.4g SDS
2mg Bromophenol Blue
0.75 DH2O

Procedures:

1. Load Precast SDS-PAGE gels and 1X running buffer into apparatus (wear PPE, gloves for safety while handling gels and buffer)
2. In a microcentrifuge tube, place 10ul IgG + sample buffer without 2MEA and in second microcentrifuge tube place 10 ul IgG + sample buffer with 2MEA. Vortex vigorously.

3. Heat tubes 10 minutes in boiling water and vortex again.
4. Load wells with Colored PAGE Marker and then 2 samples of IgG preparations. Load remaining wells with samples from Protein Profiler Kit for comparative proteomics.
5. Run SDS PAGE gels for ~1 hour at 120V (35 amps)
6. Fix and Stain gels and then visualize with light table.

Bibliography

Bondada, S., Chelvarajan, R. & M. Gururajan, 2005. "B Lymphocytes" *Encyclopedia of Life Science* , John Wiley & Sons, Ltd.

Gregory, E. 2005. "An Introduction to the Major Histocompatibility Complex"
Marieb, E. & K. Hoehn, 2007. *Human Anatomy & Physiology:"Too clean for our own good?"* p.824.

Marieb, E.N. & K. Hoehn. 2007. *Human Anatomy & Physiology Study Guide,* 7th ed. Pearson Publishing p.591.

Ward's Catalog: WBC Counts as a Diagnostic Tool Lab Kit 36W0049