

BIOL 3451 Cell Biology Lab
Fall 2011
Room 330 Cherry Emerson
Wednesday - Section A 12:05-2:55 pm, Section B 3:05-5:55 pm

Instructor

Dr. Samantha Parks Office: Cherry Emerson, Rm. 231
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Office Hours: By appointment

Dr. Merrill will also be visiting frequently from the Cell Biology lecture course, and will participate in the discussions and experiments.

Teaching assistants – office hours by appointment

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Required Text

A Student Handbook for Writing in Biology by Karin Knisely

Website

This course makes extensive use of T-square (tsquare.gatech.edu). You are responsible for checking the website frequently for announcements. Experimental procedures and other important information will be posted on the site. Lab reports should be submitted via the assignments tab on T-square.

Overview

You will explore fundamental aspects of cell biology and current methods used in the cell biology laboratory by developing a group research project modeled on the experience of an individual student working under a research faculty member. Our model system is the mouse macrophage cell line, RAW264.7 and we will verify findings described in the paper “**Activated α 2 macroglobulin induces matrix metalloproteinase 9 expression by low density lipoprotein receptor-related protein 1 through MAPK-ERK1/2 and NF- κ B activation in macrophage derived cell lines**” by Cáceres *et al.* [J Cell Biochem.](#) 2010 October 15; 111 (3):607-617. You will need to bring this article with you to lab and can find it using the library website.

We will be studying the response and activation of RAW264.7 cells to LPS, a bacterial endotoxin. Five experiments will be performed to examine changes in cell morphology, proliferation and cell death, and gene expression that accompany LPS-induced activation.

Course format

Each of the five experiments will be performed over a three week period. During the first week, the relevant concepts will be presented and the class will develop a protocol to be used in the experiment by adapting standard methods posted on the course T-square site. Students are expected to keep an accurate laboratory notebook and have the necessary procedures written out in full when they arrive in class the following week. During week two, you will work in groups to carry out the experiment, analyze the data, and organize your results. During week three, students will review their findings in preparation for writing their final reports. Discussion of the next experiment will also take place at this time.



Lab Safety and Personal Protection Equipment (PPE)

In accordance with the new Georgia Tech Policy on PPE and appropriate attire (<http://www.ehs.gatech.edu/chemical/ppePolicy.php>), safety glasses and lab coats must be worn in the lab. These are to be purchased by students. Safety goggles will be worn at all times. Lab coats must be worn when handling chemicals, biologicals or radiologicals. Appropriate lab coats can be purchased at VWR in EST. Long pants and shirts (not tank tops) must be worn at all times, as well as shoes that completely enclose the foot.

Academic integrity

All students should be familiar with their rights and responsibilities under the Georgia Tech Academic Honor Code and are expected to abide by its provisions. Academic dishonesty isn't a "victimless" crime; it interferes with instruction, damages the reputation of the Institute, and ultimately harms the perpetrator who fails to learn course material or the value of individual effort. Violations of the Honor Code (<http://www.honor.gatech.edu>) are taken seriously and can result in severe disciplinary action, up to and including expulsion. Prohibited conduct includes, but is not limited to: copying from another student or allowing someone to copy your work (sharing group data when completing laboratory reports is permitted, but submission of identical written work is not), using notes in any form on a quiz without the express permission of the instructor, requesting a re-grade of an assignment after altering it, submitting someone else's work as your own, or allowing your work to be submitted under another person's name.

Written reports

After the completion of an experiment, each student should prepare a journal style article for the lab report. This should include:

- Abstract: concise summary of what happened during the experiment (2-3 sentences per experiment)
- Introduction: to provide adequate background pertaining to the cell line and experiments to give any reader knowledge of why you did the experiment. This should include your hypothesis.
- Materials and Methods: concise summary of what you did including how the cells were prepared and maintained.
- Results: This section will include both figures and written results. Here you state simply what you saw.
- Discussion: This section you will analyze your results and state why you observed what you did during the experiment. (Conclusions section)
- References: you will need to be looking for *appropriate* references to support your introduction and discussion. Don't forget to cite the paper that the lab is based on!

Lab reports will be reduced by 10 points for each day they are late.

Group presentations

On November 30, each lab group will lead the class through a discussion of Experiment 5. This will include rationale/background, methods, results and conclusions. The presentations should be ~10 minutes and may use PowerPoint.

Lab Notebooks and pre-lab questions

You need a bound composition-style notebook for this course. Lab notebooks are the single most important tool in any research lab. Each experiment should begin with a hypothesis. Detailed protocols should be written in your notebook by the experiment day for that experiment. You should be using your handwritten protocols during the lab session. During the lab, you should note any changes to the protocol, clarification, etc. You should write down observations and finish up with conclusions. If the lab results were not ideal, include a sentence or two about trouble shooting – this will help with Experiment 5 and your lab reports. We will have two labs that will require more in depth planning, and we will discuss those closer to the appropriate time. Graphs or other data should be assembled and pasted into your notebook by the beginning of the discussion day for that experiment. At 2 times during the semester, your lab notebooks will be picked up and graded. There will also be a final lab notebook grading. Lab notebooks should only be written in using ink.

Attendance and Participation

All students are expected to be present each week in lab. (This includes being on time.) If you do not provide the instructor with a valid Georgia Tech excused absence or tardiness (see the bylaws) within 24 hours of missing a lab, it will count against you. Participation will be based both on contribution to class discussions and cooperation during lab activities. During the discussion sessions, participation will be scored based on the quality (not correctness) of answering questions and if you ask questions that allow for forward movement of the discussion. During lab activities there will be incubation times and other periods where you may not be actively conducting an experiment. This is still considered lab time. The participation grade will be a collaborative grade between the TAs and the instructor. There will be an additional component of a group/peer evaluation.

Grading

Lab notebooks and assignments/take home quizzes	20%
Lab reports, Experiments 1-3	30% (10% each)
Attendance and Participation	10%
Final Presentation	10%
Final cumulative report	30% (Covers labs 1-5)

Final scores will be rounded to the nearest whole number, and grades will be assigned according to the following scale: 90-100% A; 80-89% B; 70-79% C; 60-69% D; <60% F

SCHEDULE (subject to change)

August 24:	Introduction to the cell biology laboratory, Objectives and format Cáceres <i>et al</i> , 2010 Take home quiz 1 Lab Safety
August 31:	In depth discussion of paper Statistics exercise and tutorial Take home quiz due
September 7:	Procedures used in cell culture Cell culture exercise Preparation for Experiment 1

- September 14: **EXPERIMENT 1: Viability and activation of KDO2-Lipid A treated RAW264.7 cells**
You will examine the effects of KDO2-LipidA dose on cell viability using two different assays: trypan blue exclusion and marker enzyme activity (WST-1).
Experiment 1 quiz due in class
- September 21: Discussion of Experiment 1
Preparation for Experiment 2
- September 28: **EXPERIMENT 2 Part 1: The effect of KDO2-LipidA on RAW264.7 cell phagocytic function**
In this experiment, you will use light and fluorescence microscopy and flow cytometry to examine the effects of KDO2-Lipid A on RAW264.7 cell morphology, culture density and phagocytosis relative to a negative control.
Report for Experiment 1 due
Experiment 2 quiz due in class
- October 5: **EXPERIMENT 2 Part 2: Flow cytometry**
- October 12: Discussion of Experiment 2
Preparation for Experiment 3
- October 19: **EXPERIMENT 3: Expression of MMP-9 mRNA during KDO2-Lipid A-induced activation**
You will use a quantitative reverse-transcription PCR (RT-PCR) assay to measure relative amounts of MMP-9 mRNA in treated and untreated cells.
Report for Experiment 2 due
Experiment 3 quiz due in class
- October 26: Discussion of Experiment 3
Preparation for Experiment 4
Experiment 5 grant proposals due
- November 2: **EXPERIMENT 4: Changes in MMP-9 enzymatic activity (Type IV collagenase/gelatinase) during cellular activation**
You will determine the relative activity of MMP-9 protein in stimulated and unstimulated RAW264.7 cells using an in situ gel electrophoresis (zymography) assay.
Report for Experiment 3 due
Experiment 4 quiz due in class
- November 9: Discussion of Experiment 4
Preparation for Experiment 5
- November 16: **EXPERIMENT 5: Original Experiments**
In this final experiment, the class will use the findings from Experiments 1-4 to derive hypotheses that will be tested by experiment(s) designed by the students.
- November 23: **Thanksgiving – No Lab**
- November 30: Discussion of Experiment 5 and course wrap-up
Group Presentations
- December 7: **Final (cumulative) lab report due (by noon)**