



We will be studying the response 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 tsquare 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.

### **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 for each exp't)
- 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 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.

### **Quizzes**

Quizzes will be held throughout the semester on non-experiment days. These quizzes will cover the necessary background for you to perform and understand the experiment that will follow the next week. Also, these quizzes may cover material that you have already done so stay prepared. The information that the quiz covers can be found on tsquare under each individual experiment folder. These will NOT be open note. Do note that these quizzes are not considered pop quizzes. MAKE- UP EXAMS WILL BE ADMINISTERED AT THE END OF THE SEMESTER ONLY FOR "UNIVERSITY APROVED EXCUSE ABSENCES"

### **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.

### **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 (see the bylaws) within 24 hours of missing a lab, it will count against you. Participation will be based mainly on contribution to class discussions. 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.

### **Grading**

Quizzes and assignments	20%
Lab reports, Experiments 1-3	30% (10% each)
Attendance and Participation	10%
Final Presentation	10%
Final cumulative report	30%

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 20: Introduction to the cell biology laboratory  
Objectives and format  
Introduction of the paper  
Distribution and discussion of take home quiz  
Lab Safety discussion  
Photograph each student for class directory

August 27: In depth discussion of paper  
Statistics exercise and tutorial  
**Take home quiz due**

September 3: Procedures used in cell culture  
Cell culture exercise  
Experiment 1 quiz  
Preparation for Experiment 1

September 10: **EXPERIMENT 1: Viability and activation of LPS-treated RAW264.7 cells**  
You will examine the effects of LPS dose on cell viability using two different assays: trypan blue exclusion and marker enzyme activity (WST-1).

September 17: Discussion of Experiment 1  
Experiment 2 quiz  
Preparation for Experiment 2

September 24: EXPERIMENT 2 Part 1: The effect of LPS on RAW264.7 cell phagocytic function  
In this experiment, you will use light and fluorescence microscopy and flow cytometry to examine the effects of LPS on RAW264.7 cell morphology, culture density and phagocytosis relative to a negative control.  
**Report for Experiment 1 due**

October 1: EXPERIMENT 2 Part 2: Flow cytometry

October 8: Discussion of Experiment 2  
Experiment 3 quiz  
Preparation for Experiment 3

October 15: EXPERIMENT 3: Expression of MMP-9 mRNA during LPS-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**

October 22: Discussion of Experiment 3  
Experiment 4 quiz  
Preparation for Experiment 4  
**Experiment 5 grant proposals due**

October 29: 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**

November 5: Discussion of Experiment 4  
Preparation for Experiment 5

November 12: 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 19: Discussion of Experiment 5 and course wrap-up  
**Group Presentations**

November 26: **Thanksgiving – no lab**

December 3: **Final (cumulative) lab report due (by noon)**