In Vitro Lecture--Table Discussion Leader Instruction Guide

Focus of the discussion:

In vitro systems are important models for identifying effects and mechanisms by which xenobiotics produce toxicity. The complexity of *in vitro* culture systems can be increased with addition of a relevant cell type, which might help identify mechanisms more similar to that observed *in vivo*.

Background on Dr. Kaminski's models:

B cells produce antibodies after they receive signals from T cells

Co-culture model 1 is T cell-B cell co-culture

Co-culture model 2 is a surrogate T cell expressing a protein critical for the T-B cell interaction so model 2 is CD40L-expressing cells-B cell interaction

Importantly in co-culture model 2, the surrogate cells are irradiated, which allows them to provide the stimulation, but they should otherwise be unresponsive

Thought questions (provided to students at the tables):

1) **Does using more than one cell type in an *in vitro* co-culture model more closely mirror biological processes seen *in vivo*? Why or why not?**

Most tissues are comprised of several cell types so using two cells that can interact might provide more information than just focusing on one cell

Toxicant effects on a target cell might be the result of an alteration in an adjacent cell type so this could be identified in this system

Most tissues are comprised of several cell types so using two cells will likely not mimic ALL the cellular interactions in the tissue

2) **What controls should be considered for experiments using two different cell types in toxicology? How does choosing the correct control influence your data interpretation?**

Cell controls: each cell type alone versus the co-culture

Solvent controls: vehicle or solvent in which toxicant is delivered

Toxicant controls: concentration response for toxicant of interest
positive control if needed

Timing controls: short exposures? longer exposures?
consideration for viability of both cell types

Cell concentrations: consider the density of each cell alone versus having both present in the culture in different ratios

Timing of cell/xenobiotic addition: add xenobiotic to one cell first then use second cell type to stimulate the initial cell type versus adding stimulator cells first then adding xenobiotic

In the case of using an irradiated stimulator cell, verify that toxicant does not produce any effects on the stimulator cell

3) How does the co-culture system described by Dr. Kaminski allow researchers to apply the 3Rs (reduce, refine, replace) of animal research?

Can replace or reduce use of whole animals with use of cells only, especially if the co-culture system is comprised of immortal cell lines

Can obtain mechanistic information through focusing only on two cell types; this can help identify *in vivo* endpoints

Can use the *in vitro* co-culture system to refine techniques before using the techniques for *in vivo* endpoints

Additional questions if time allows:

4) If the endpoint to be measured is a protein that both cell types can produce, how could you identify that the xenobiotic affected your target cell?

Perform an isolation at the end of the culture period using cell sorting, magnetic sorting, columns, differential centrifugation

Conduct the single cell controls to see if your protein is affected in both cell types

Can design studies in which the two cell types are co-cultured in the presence of a membrane that only allows soluble mediators to pass, but prevents cell-cell interactions; then the target protein could be characterized in the separated, but co-cultured cells. This could also help characterize the mechanism of toxicity if it is compared to co-culture system in which the cells are allowed to interact

5) **What are differences between using cells derived from primary tissues as opposed to cell lines? How could these differences alter your data interpretation?**

Cell lines are usually immortal, providing unlimited cells

It is usually easier to overexpress proteins in cell lines versus primary cells so if want to use a stimulator cell line, as in model 2 above, cell lines are often easier

Use of cell lines exclusively replaces animal use; use of one cell line and one primary cell at least reduces animal use

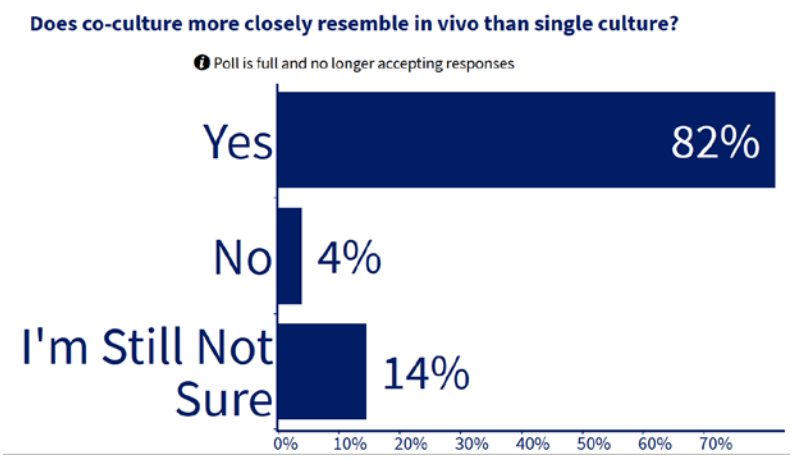
Cell lines are usually immortal, so might not always reflect the response of a primary cell

Take home message to provide to the students near end of discussion:

Single or multi cell *in vitro* systems are excellent options that reduce, refine or replace animals, while still providing a means to identify xenobiotic effects and mechanisms.

Report out polling questions:

1) Does co-culture more closely resemble in vivo than single culture? (Audience response below)



2) Which types of controls might be important for co-culture toxicology experiments?
3) What are the 3R's?

Based on what you learned today, what are the 3R's?

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