Reporting An Untoward Event

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Introduction

The National Institutes of Health (NIH), *Guidelines for the Use of Recombinant DNA Molecules,* requires that incidents involving a spill or release of recombinant DNA molecules are reported to the Office of Biotechnology Activities (OBA) within 24 hours in some cases or 30 days.

Introduction

This presentation will discuss the observations associated with an unexpected outcome in an experiment and the reporting of this incident in accordance with the NIH Recombinant DNA Guidelines.

The Experiment As Designed

Concept

Risks

Regulatory Basis

Purpose of the Experiment

- The experiment was part of a project that involved the immortalization of bone marrow macrophages.
- It was approved by the University of California-Berkeley (UCB) Institutional Biosafety Committee (IBC), known locally as CLEB (the Committee for Laboratory and Environmental Biosafety) at Biosafety Level 2.

The Experiment

- The investigator's research goals were to determine the molecular basis of the immunological response to pathogens.
- One phase of the experiment involved the production of immortalized mouse bone marrow macrophage cell lines by introducing mouse primary bone marrow cells to a retrovirus, called J2.

The Experiment

- This would result in the expression of v-raf/myc fusion oncogene.
- The virus was supposed to be replication deficient and ecotropic, infecting only mouse cells. However, the investigator noticed some activity suggesting otherwise.
- The concern was that was there an event that now allowed these viruses to infect mammalian cells.

Validation

- The investigator performed a validation test to verify if viral DNA was present.
  - With supernatant from the immortalized cell line, he filtered it through a 2 micron filter and then introduced it to fresh mouse bone marrow.
  - Result: the fresh bone marrow was immortalized which indicated that the cell line was producing infectious particles.

Validation

- The investigator performed a validation test to see if the supernatant could infect human cells.
- With supernatant from another J2 immortalized cell line, he introduced it to HEK 293 cells.
  - Result: Inconclusive-viral DNA was not observed in the human cells but sensitivity in detection may account for this.

Interpretation

- The J2 retrovirus had a viral RNA genome-the presence of any viral DNA would suggest the virus had entered the cells and reverse transcribed its genome.
- Was it a new viral genome generated by recombination? Unlikely-because the size of the v-raf/myc, gag and pol would exceed the size that could be efficiently packaged.

Interpretation

- What could have attributed to this observation?
  - Activation of endogenous helper virus through the expression of the raf/myc oncogene?
  - Cross-Contamination with viruses providing gag, pol for the retrovirus?
- Was the contaminant amphotrophic to allow infection of mammalian and possibly human cells.

Interpretation-worst case

- Since the J2 virus may have the ability to infect human cells, the original packaging cell line used to generate the virus may have been contaminated with amphotrophic helper virus.
- Less likely was the probability that murine bone marrow inherently had helper virus present.

The National Institutes of Health Requirements

The NIH Guidelines

Approval by the IBC
Condition of Approval

- UC Berkeley's biosafety applications have a condition of approval that all accidents and incidents involving recombinant DNA must be reported to CLEB must be reported within 8 working hours.

Approval by the IBC
Condition of Approval

- Any accidents must be reported:
  - National Institutes of Health, Guidelines for the Use of Recombinant DNA Molecules, Section IV-D-7-e-2

Approval by the IBC
Principal Investigator's Notification

- After observing the unaccounted for contamination, the investigator notified and filed a report with the Biosafety Officer.
- The Biosafety Officer and the Chair of CLEB recommended that the PI complete the NIH OBA incident reporting template and the Chair would send it to the NIH.

The Response By The NIH

What Should Your Institution Do?

- This incident did not involve an exposure to laboratory personnel nor did it result in an environmental release of recombinant DNA.
- The NIH Guidelines, Section IV-B-7-e-(2): Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities.

The NIH Response

- UC Berkeley received a letter from NIH OBA. In this correspondence:
  - Acknowledgement of the submitted report
  - Scientific interpretation of the report
  - Assessment of institutional actions
  - Recommendation for biological containment level
- Notification that the experiment can proceed.

Conclusion
Things to Remember

- Untoward research outcomes are an undesired event in any experiment-if one involves a potential safety risk, the IBC and Biosafety Officer should examine the data to determine if the experiment needs to be performed at a higher level of containment.
- If the experiment involves recombinant DNA, determine if a report to NIH/OBA is necessary.