Emerging Laboratory Safety and Health Issues

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Disclosure

• I have no financial conflicts.
• Off label use of medications will be discussed

UC System Occupational Health

• 10 campuses — Includes medical centers and campuses
• Office of the President
• UC Agriculture and Natural Resources
  • http://ucanr.edu/About_ANR/We_are_UC_ANR/
• 7 on-site Occupational Health Clinics
  • Campus workers compensation treatment
  • Laboratory occupational health
  • Institutional Biosafety Committee
  • Animal Care and Use Committee
  • Campus/MC Occupational Health and Wellness
University of California - Berkeley

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<tr>
<th>Title</th>
<th>FTE</th>
<th>Non-FTE</th>
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<tr>
<td>Faculty – Ladder Rank and Equivalent</td>
<td>1,677.3</td>
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University of California - Berkeley

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Reporting of Laboratory Accidents

- National Institutes of Health
- Office of Science Policy
- Office of Laboratory Animal Welfare
- OSHA
Laboratory Accidents
UCB 2016-17
Lab Accidents identified: 16
Total Injuries: 500-600/yr

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<td>Needlestick</td>
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<tr>
<td>Skin exp, with PPE</td>
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<tr>
<td>Splash with PPE</td>
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<td>Splash no PPE</td>
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In the news
• Boston College Student Hurt in Lab Accident – Boston Globe 2011
• Colorado College Lab Accident Hospitalizes 13 – The Denver Post 2013
• Death in the Lab – Discover 2015
• Dartmouth Faces Fine of $13,000 – Valley News 1997

Research at Berkeley
• World Class Research
• 8 current Nobel Laureates
• 144 members of National Academy of Sciences
• 235 Fellow of the American Academy of Arts and Sciences
• Innovative and Entrepreneurial
• Currently there are ~450 operating laboratories

UC Berkeley Faculty Health Programs
• Occupational Health Clinic
• Campus Employee Health
• Resource for Health in Research Laboratories
• MD 2.2 FTE
• NP 1.0 FTE
• AA 2.0 FTE
• MA 3.0 FTE
• Wellness Coordinator 1.0 FTE
• Registered Dietician 0.2 FTE
Rabies

Ancient Disease – Modern times

- A condition known for 4000 years
- Democritus in 4th Century BC, gave a clear description of rabies
- Aristotle (300 BC) notes that rabies as disease of dogs and any animal the dog bites
- Rabies: Latin “to rage” (rabere)
- 16th century, Girolamo Fracastoro discovered that rabies was a fatal disease affecting humans as well as animals, calling it “an incurable wound”
- Louis Pasteur a French biologist that created the first vaccine in 1885.

Canines – new WHO initiative (Jan 2018) to eradicate rabies (a neglected zoonotic disease) in developing countries
- Wild animals: carnivores, raccoons, skunks, foxes
- No rabies seen in Antarctica
- Current incidence in the U.S. is less than 2 per year (100 per year in the late 1800s to early 1900s)
- Canine vaccination very successful in the reduction of rabies in developing countries.
Neuroscience

- Imagine being able to choose one neuron and identify all of the neurons connected to that single neuron.
- This is felt to be key to fully understand the functioning of the brain.
- Scientists at the Salk Institute reported successfully turning the deadly rabies virus into a tool for neuroscience.

Modified Rabies Virus (RV)

- Single gene deletion – rabies Glycoprotein (G)
- This glycoprotein allows RV to move transynaptically from neuron to neuron from bite to the brain and then anterograde to the salivary glands. It is not involved in replication of the virus.
- If one deletes this gene, the rabies virus is marooned in the cell that has been infected.
- If you add the gene (G) via plasmid into the same cell, it will replicate and be able to transfect any synaptically connected neurons.
- However, the modified virus cannot spread further: monosynaptic

Pseudo-typing - A second modification

- In order to further customize the virus, the investigators pseudotyped the virus with an avian envelope protein (EnvA) to only recognize a specific receptor, (TLV).
- Changes the tropism of the virus. No longer recognized mammalian cells, but recognize avian cells.
- Can transfected specific neurons with plasmids with TLV gene, G gene and a fluorophore such as Green Fluorescent Protein (EGF), mCherry or DsRed2.
- SAD(G-EGFP/EnvA) is an example of a modified rabies virus. This modified version of the rabies virus forces neurons it infects to produce a green fluorescent protein.

Occupational Health Issues

- 2008 – First researcher at UCB requested to use this modified rabies virus.
- Only one gene different than wild-type RV
- Presented to Institutional Biosafety Committee, determined with input from the NIH that all researchers and persons potentially exposed to virus have pre-exposure prophylaxis for rabies infection.
- Mortality of RV infection approaches 100% in unvaccinated individuals. Vaccination offers close to 100% protection.
- Latency: an eclipse phase for days to months.
**Occupational Health Issues – G-deleted RV**

- Current recommendations are to have pre-exposure counselling for possibility of vaccination.
- Post-exposure: follow current guidelines appropriate for pre-vaccinated vs. unvaccinated individuals

**Sample SOP for G-deleted rabies**

- BL-2 containment: disposable gloves, gown and faceshield. Work to be done in dedicated BL-2 room
- All persons to be trained.
- Signage on doors
- Reduce level of sharps use. Proper sharps disposal
- Red biohazard bags for waste
- 10% bleach for decontamination of surfaces
- Animals injected with g-deleted RV remain housed in BL-2 cages but can be moved to Bl-1 rooms for housing.

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**Bats and Rabies**

Photos by Christie Ferrecchia, DVM
Research at UCB

• Field researchers
  • Museum of Vertebrate Zoology – specimen collection
  • Live trapping
  • Specimens from local health department
  • Teaching classes in basic field technique
  • Studying bat viruses and spillover events in Madagascar – new!

Rabies Vaccination – pre-exposure

• Recommended for:
  • Veterinarians
  • Persons who work with rabies virus
  • Frequent contact with animals known to be infected with rabies
  • Study bats, work in caves
  • Travel to countries with canine rabies

• Pre-exposure prophylaxis
  • IM vaccine at 0, 7, 21 – 28 days
  • No need for post-vx titer
  • Titer check every 1 or 2 years
  • Avoid vx in immune compromised

Rabies Post-Exposure Vaccine

• Decontamination of wound: thorough gentle cleansing with soap and H₂O₂. Povidone-iodine if available.
• If pre-vaccinated
  • Dose 1 – as soon as possible after decontamination of wound
  • Dose 2 – 3 days after first dose
• If not pre-vaccinated
  • Dose 1 – as soon as possible after decontamination of wound. HRIG, infiltrated around wound and the rest IM distant from vx site
  • Dose 2 – 3 days after first dose
  • Dose 3 – 7 days after first dose
  • Dose 4 – 14 days after first dose
  • Dose 5 (for immune compromised host) – 4 weeks after first dose

Viral Vectors

Biosafety Considerations for Research with Lentiviral Vectors
Viral Vectors

- Engineered viruses that take advantage of the infective and replicative qualities of the virus
- Introduce genetic material into the genome of cells, either in vitro or in vivo
- Risks include accidental exposure to laboratory workers to infectious viruses

Non-retroviral vectors

- Some vectors used to introduce genes into host cells, such as adenovirus or adeno-associated virus.
- Gene expression is typically transient because genes are not integrated into host genome, remain as plasmid
- Risks: may induce an immune response to viral proteins
- Risk seen in clinical trials
- Risk in research is theoretical. Adverse events have been documented in clinical trials of gene therapy
- No risk if bitten by an animal transfected with viral vector

Lentiviral Vectors

- HIV is a lentivirus in the genus Retroviridae
- HIV, as a viral vector inserts genetic material into host genome
- Can infect dividing and non-dividing cells
- Wild-type HIV is a safety issue for workers, so the virus has been engineered to achieve safe gene transfer
- HIV typically recognizes CD4 receptors, so has affinity for T-cells, macrophages, microglial cells. This is limiting in research.
- To increase tropism, the envelope protein is often replaced with vesicular stomatitis virus glycoprotein (VSV-G).
First generation lentiviral vector

Second generation lentivirus

Third generation
– genes necessary for expression on three plasmids

Packaging a 3rd gen lentivirus
Safety of Lentivirus – 3rd gen

- Non-replicative
- Requires several recombination events to revert to wild type HIV in the presence of HIV
- 4th generation
  - Creation of Self-Inactivating (SIN) LVV

Risks of Lentivirus and Transgene exposure

- Recombination and reversion to wild-type HIV
- Oncogenic potential when using LVV
  - Activation of oncogenes or inactivation of tumor suppressor genes
  - Insertional mutagenesis
  - Gene transduction of an oncogenic transgene
  - LVVs might target multiple genes which could silence or inactivate tumor suppressor activity
  - Unknown functions of some transgenes may promote oncogenesis or other toxic effects
- Transduction of a toxin gene

Clinical gene therapy


Proposed Post-exposure protocol

- Discussed at recent professional conferences: OBA, Eagleson Institute
- Journal of Occupational and Environmental Medicine
- Off label use of FDA approved medications
- Re-framing treatment for researchers who have been convinced that use of late generation LVVs are “safer”
Pre-Exposure preparations

- Proposed recommendations
  - Review biological hazard protocols for lentiviral exposure
    - Consider nature of transgene
      - Does it silence a tumor suppressor gene?
      - Does it express an oncogene? A toxin?
    - Expanded host range (pseudotyped?)
    - Large volumes?
- Create a post-exposure protocol for each lab
- Train each lab member on the procedures for decontamination, notification and the need for immediate care.
- Use of medical cards for providers

Post-exposure first aid

- Call 911 if needed for injury requiring emergency treatment
- Begin decontamination:
  - Intact Skin Exposure: immediately wash area with copious amounts of running water to dilute, cleanse and flush
  - Nonintact Skin Exposure: immediately wash area with generous amounts soap and water to dilute, cleanse and flush
  - Mucous Membrane Exposure: immediately flush the area with running water for at least 15 minutes
  - Droplet exposure: see above depending on area of exposed
- Contact the institution’s Biosafety Officer

Post-exposure prophylaxis

- Integrase inhibitor plus nucleoside reverse transcriptase inhibitor
- Initiate ASAP, within 2 hours, but no later than 72 hours (optimally)
- 7 day course
- Non-FDA approved
- A suggested regimen
  - Raltegravir (Isentress) 400 mg po BID
  - tenofovir (Truvada)– 300 mg po once daily (Descovy may be used instead)
- Follow-up for medication side effects
- Any sequela from exposure may have long latency, and truly unknown effects.
PEP Considerations for LVV exposure

- Off label use of medications
  - However safety profile of meds fairly well known
- Recommendations based on HIV PEP
- Risk of insertional mutagenesis or oncogene promotion is not quantifiable
- Integration thought to occur in 1-2 hours. PEP in time?
- Exposure registry?

Other antiviral PEP protocols

- HIV exposure – PEP
  - Isentress and Descovy for 28 days
- B virus (macaque herpes B virus 1)
  - Valacyclovir or Acyclovir for 28 days

CRISPR/Cas9

CRISPR= Clustered Repeating Interspaced Short Palindromic Repeats
What is CRISPR/Cas9

- First identified in E. coli
- An “immune system” for bacteria against viruses
- Palindromic DNA interspaced with:
  - Spacer DNA
  - Match up with viral DNA
  - When a virus attempts to infect the bacteria, a copy of the spacer DNA is created
- Cas proteins are also made and form into a complex
  - Function is to unwind DNA (helicase) and cut DNA (nuclease) using the spacer genes to identify the viral genes

CRISPR technology and Ethics

- Can edit any cell
- Use in gene therapy
- Inexpensive compared to previous methods of creating transgenes or genetically modified animals.
- Will Cas9 stop cutting? AKA “brake failure”
- Gene drives – controversial
Vaccinations

- Diphtheria toxin (0.1 µ/kg body weight) – used to transport large proteins across cell membranes
- Pertussis toxin – interrupt cell signaling.
- Seasonal influenza
  - Work with Swine
  - Work with influenza virus (not mouse adapted)
  - Association of Dengue virus pathogenesis and H1N1
- Anthrax
  - Field research with elephants, vultures and zebras in Namibia

Question 1. G-deleted rabies has all of the following qualities except:

A. Has a single gene deletion
B. Can move from cell to cell transynaptically until it reaches the brain
C. Can transfect any synaptically connected neuron if one adds the gene for G protein on a plasmid
D. Can be used as a tracer gene by adding jelly fish genes
Question 2. PEP for LVV exposure includes

A. Acyclovir
B. Valacylovir
C. Integrase inhibitor
D. Protease inhibitor

Question 3. The risk of LVV exposure includes all but which answer?

A. Insertional mutagenesis
B. Insertion of an oncogene
C. Recombination to wild-type HIV1
D. Dengue Fever