OBA GENE TRANSFER PROTOCOL REGISTRATION: 0301-570

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Study Goals

- The short term goal of this protocol is to identify novel antigens and pathogenicity factors that are expressed *in vivo* by *Vibrio cholerae* in human volunteers.
- Approach:
  - *in vivo* expression technology (IVET)
  - Human cholera challenge model

RAC Concerns

1. Safety and pre-clinical data involving the specific IVET approach and the genomic *V. cholerae* library to be used.
2. Rationale of the experimental questions
3. Safety, ethics, and approach of the human volunteer challenge model for development and testing of cholera vaccines.

Recombinant organisms

- Unlikely to be more virulent than the parent vaccine CVD 110.
- Gene transfer is not planned or likely.
- Eradication of the ingested organisms → unlikely to be enduring concerns about the transfer of genetic material or release of the vector into the environment.

Immunity to Cholera

- Cholera ⟶ severe dehydrating diarrhea
- Immunity against cholera toxin (CT) and vibrio organism
  - components of *V. cholerae* against which the vibriocidal antibody response are directed have not been fully defined
  - Vibriocidal immunity is a good but not perfect predictor of immunity

Vibriocidal Antibody Protection

- CCHMC volunteer immunized with cholera vaccine CVD 103-HgR
- Strong anti-toxin response and vibriocidal antibody titer:
  - 1:20,480 at 9 days
  - 1:280 at 3 months
- Challenged with virulent *V. cholerae*
  - 6.8L of diarrhea
- An exception that proves the rule?

Vibriocidal antibody response

- *V. cholerae* is a non-invasive organism
- No disruption of the intestinal epithelium during cholera
- Why would a serum complement-fixing antibody response (vibriocidal antibodies) have any activity during mucosal infection with *V. cholerae?*
- Vibriocidal response = imperfect surrogate marker of immunity
- We do not know what is responsible for immunity to cholera.

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- Identify additional antigens that are uniquely expressed during *in vivo* infection and are not produced during *in vitro* growth.
- These antigens may provide additional insights into the protective immune responses to *V. cholerae* infection and to subsequent targets for immune surveillance and vaccine development.

Human cholera challenge model

1. General properties of cholera
2. Clinical features of the model and specific features of the pathogen
3. Selection of volunteers
4. Study design considerations
5. Ethical concerns

General properties of cholera:

- Short & predictable incubation period: 24-48 hrs
- Effective treatment: Tetracycline or ciprofloxacin quickly eradicates the organism

General properties of cholera:

- Volunteers can be adequately monitored in an inpatient setting and supportive care (ORS or IVF) can be promptly given to minimize the effects of severe cholera.
  - Diagnosis is known, treatment is available, staffing is adequate. These are not field conditions.
  - The investigators are highly experienced in the clinical management of cholera.

Clinical features of the model and specific features of the pathogen:

Why CVD 110 for the IVET study in humans?

1. O1 El Tor is the most prevalent *V. cholerae* serogroup and biotype in the world today.
2. CVD 110 studied in human volunteers. It causes mild to moderate diarrhea but is otherwise safe.
3. CVD 110 colonizes human and mouse intestine well.

V. cholerae (N1961) vs CVD 110:

Organism	***V. cholerae* (N1961)**	**CVD 110**
Dose	10^5	10^8
Attack rate	85%	70%
Mean stool weight	3416 g	861 g
Mean cholera excretion cfu/gram stool	3.9×10^7	2.0×10^7

Clinical features of the model and specific features of the pathogen:

- No good animal model of cholera.
- These characteristics permit evaluation of the endpoints described in this study with a minimal number of volunteers.
- Use of an attenuated strain means less toxicity than using wild type organisms.

Use of 3-5 Volunteers

- Although the scientific rationale for using 3-5 volunteers is not precisely stated as it might be for a vaccine study, because of the high attack rate and high colonization rate (10^7/g), interpretable data will likely result from the use of a small number of individuals.
- Limited number of volunteers = strength of the approach.

Use of 3-5 Volunteers

- Results based on this initial pilot group could be evaluated using already obtained and stored sera from previously challenged volunteers
- Additional volunteers in US and endemic areas could be challenged *only if needed*.

Selection of volunteers:

- Volunteers are carefully screened and must return for serial outpatient visits to confirm interest in the study.
- Volunteers must pass a written exam. Both at CVD and at CCHMC protocol specific examinations are reviewed by the local IRB for language and content.

Selection of volunteers:

- Appropriate safeguards and psychological evaluations are in place to identify volunteers who can comply with the restrictions of the study design e.g., an inpatient stay on an isolation ward.
- Appropriate exclusion criteria are used to avoid volunteers who might have increased risk of a complication related to fluid loss.

Study design considerations:

- The vector will be “validated” in an infant mouse model.
- Many factors relating to infectious dose and incubation time, quorum sensing, and host differences that make people variably responsive to cholera infection may be different in the mouse model.
- The infant mouse model will be useful to validate that the library is complete and robust and gives reproducible results.

Study design considerations:

- It would be quite useful if true, but remains to be shown that there is a good correlation between the *in vivo* gene expression in the mouse (or any animal) and the human volunteers.
- This is not a serious weakness of the protocol but argues for human experimentation rather than extrapolation to humans without this comparative data.

Study design considerations:

- Concerns have been raised about the appropriateness of the challenge model as a test for vaccine efficacy
  - CVD-103 HgR was shown to be protective in a North American volunteer challenge model
  - but not protective in a large scale study in Indonesia.

Study design considerations:

- Possible reasons for lack of efficacy of CVD-103HgR in the field:
  - Vaccine confers short term but not long term immunity
  - Low incidence of cholera in first 4 months of the study: no statistical difference vs. control
  - Host differences between Indonesian and NA subjects

Host differences

- We have previously shown that the challenge model may not give identical quantitative and qualitative results in volunteers from a cholera endemic area.
- Therefore, it will eventually be necessary to test any hypothesis derived from this protocol in such a diverse group.

Host differences

- However, it is not feasible to stratify volunteers by all of the known (and unknown) variables.
- Until the true nature of immunity to cholera is known, stratification would be on the basis of speculation.

Study design considerations:

- Moreover, in the North American volunteer challenge model there are important unknown host factors that determine who will get severe diarrhea vs. mild diarrhea vs no diarrhea with exposure to an identically administered dose of virulent *V. cholerae*.
- This is similar to the “field condition.”

Study design considerations:

- Thus the North American human volunteer challenge model is an appropriate first step in identifying unrecognized antigenic targets and pathogenicity factors of clinical relevance to cholera infection.

Ethical concerns:

- The ethics of paying volunteers for participation in more than minimal risk studies have been debated, although most agree that it is acceptable as long as the compensation is not so much as to affect the volunteer's assessment of the risks.

Ethical concerns:

- The proposed compensation uses a standard wage scale employed at CVD for volunteer compensations and is:
  - less than that approved by our local IRB for a similar protocol at CCHMC,
  - less than the going rate for phase I drug studies at commercial clinical research establishments in Cincinnati and
  - equal to approximately $3.13 per hour for the duration of the inpatient stay.