

Hanna, Michael

From: Yongqun He [yongqunh@med.umich.edu]
Sent: Friday, August 07, 2009 4:41 PM
To: Hanna, Michael
Cc: Follo, Janet
Subject: Re: FW: ISATTAC follow up.

Hi Mike,

Here is my reply in terms of the three other Brucella strains: RA1, VTRM1, and VTRS1. RA1, VTRM1, and VTRS1 are stable attenuated rough mutants of virulent *B. abortus* strain 2308, *B. melitensis* strain 16M and *B. suis* strain 1330, respectively. They were generated by Tn5 element disruptions of their *wboA* genes (References: PMID: 10417145 and PMID: 8723881). My answers to the requested questions are shown as follows:

1. The committee would like more clarification/supporting evidence on wild type vs mutant strains, especially:

a. any data on spleen cultures and if they were alive or dead

ANSWER: Yes.

For RA1, in the reference PMID: 10417145

<http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=96661&blobtype=pdf>

The result is in Table 4.

BALB/c mice were injected with 1×10^8 to 2×10^8 CFU of either strain RA1 or strain RB51. The mice were killed at various intervals, and the bacterial CFU in their spleens were determined. All mice inoculated with strain RB51 were *Brucella* free at 4 weeks postinfection. All mice inoculated with strain RA1 had over 10^3 CFU/spleen at 5 weeks postinfection. Three of five mice were still infected at 7 weeks postinoculation. The bacterial CFU were low, though. Virulent strain 2308 can survive in mice spleen for more than 20 weeks (Reference: PMID: 8039890).

It is noted that another Brucella vaccine Strain 19 can survive in mouse spleen for more than 10 weeks (Reference: PMID: 8039890). Therefore, it is likely that RA1 is more virulent than RB51 but less virulent than Strain 19 and strain 2308.

Similar results are available for the other two strains: VTRM1 and VTRS1 (Reference PMID: 8723881).

VTRM1 and VTRS1 replicated extensively in the spleen during the first 3 weeks of infection, then decreased rapidly (Reference PMID: 8723881):

<http://www.ncbi.nlm.nih.gov/pubmed/8723881>

b. any data on positive vs negative controls

ANSWER: Yes. See the above. RB51, Strain 19, and Strain 2308 are used as controls for attenuated vaccine strains and virulent strain.

c. any comparative data on aerosolization experiments with RA1, VTRM1, and VTRS1

ANSWER: Not as I know.

d. information on lethality in tabular form

ANSWER: Yes. For RA1, see reference PMID: 10417145 Table 4:

<http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=96661&blobtype=pdf>

For VTRM1 and VTRS1, see reference PMID: 8723881. An electronic copy of this figure is not available.

2. IP injections

a. do you have data to support that IP is sufficient for predicting attenuation in the model

ANSWER: IP injection is the only method I know that is used in a persistence study to predict attenuation in the model. All the references I quoted above use IP injection.

b. have any other methods been tested

ANSWER: As far as I know, there is no other administration method that has been used to predict Brucella attenuation in the mouse model.

3. the BSL level: BSL-2 vs BSL3 is a NIH/OBA question

ANSWER: These three strains are currently available to my lab and stored in the BSL3 laboratory in the University of Michigan Medical School. If possible, it would be good to use them in a BSL2+ setting. This has to be approved.

I hope my reply is not too late. Thank you!

Sincerely,

--Oliver

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>>> "Hanna, Michael" <mhanna@bf.umich.edu> 8/4/2009 10:32 AM >>>

Oliver – I was able to discuss these ISATTAC questions over the phone with Pia this morning, and it all boils down to validation of assumptions. ISATTAC committee is comfortable with the current state of validation when it comes to the attenuation of RB51 strain of Brucella, but they still need some more supporting evidence from you that the three other strains handled outside of BL3 containment have the same level of attenuation. One aspect of their query is directed at the type of testing. What is the predictive value of the IP test? I suppose this is opposed to other potential virulence testing methodologies I know nothing about, but you might. There are other particulars in their query you can see below. They ask for response before this Friday if possible. Thanks, mgh

From: Shivdasani, Pia A. (CDC/COTPER/DSAT) (CTR) [mailto:hhj9@cdc.gov]
Sent: Tuesday, August 04, 2009 9:57 AM
To: Hanna, Michael
Subject: ISATTAC follow up.

Hi Mike,

ISATTAC needs some follow up information on the RA1, VTRM1, and VTRS1 strains of Brucella. I know this is short notice but if it is possible to get the information to us by Friday I can try and get it presented at the next committee meeting on the 18th. Please feel free to call me if you have any questions.

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 - b. any data on positive vs negative controls
 - c. any comparative data on aerosolization experiments with RA1, VTRM1, and VTRS1
 - d. information on lethality in tabular form

2. IP injections
 - a. do you have data to support that IP is sufficient for predicting attenuation in the model
 - b. have any other methods been tested

3. the BSL level: BSL-2 vs BSL3 is a NIH/OBA question

Thanks,

Pia Shivdasani
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