Coxiella burnetii epitope-specific T-cell responses in chronic Q fever patients

Anja Scholzen[1], Guilhem Richard[2], Leonard Moise[2,3], Eva Hartmann[1], Chantal P. Bleeker-Rovers[4], Patrick M. Reeves[5], Susan Raju Paul[5], William D. Martin[2], Anne S. De Groot[2,3], Mark C. Poznansky[5], Ann E. Sluder[5], Anja Garritsen[1]

[1] Innatoss Laboratories B.V., Oss, The Netherlands; [2]EpiVax, Inc., Providence, USA, [3]University of Rhode Island, Providence, USA; [4]Radboud Expertise Center for Q Fever, Radboudumc, Nijmegen, The Netherlands; [5]Massachusetts General Hospital, Charlestown, USA

Background – Q fever

- **Neglected zoonotic disease** with world-wide prevalence, spread by ruminants
- **Outbreak in the Netherlands** (2007-2010) affected > 40,000 individuals (*Kampschreur et, 2013*)
- **Long-term complications** such as chronic fatigue and persistent infection are common (*Eldin et al., 2017*)
- Caused by the **obligate intracellular bacterium *Coxiella burnetii* (Cb)** – highly stable, contagious, and potential biothreat
- **T-cell produced IFN-γ key** in elimination of infection (*Ghigo et al., 2002*)
- **Whole-cell Q fever vaccine** (Q-VAX®) licensed in Australia, but **reactogenic** after prior exposure to Cb
- **Epitope-based subunit vaccines** show promise in murine studies and are likely **non-reactogenic** (*Xiong et al., 2014*)
- **Promiscuous MHC class II T-cell epitopes are highly antigenic in exposed individuals** *Scholzen et al., 2019*)
- These epitopes are **candidates for a novel human T-cell based Q fever vaccine** (*Scholzen et al., 2019*)

Objective

Determine antigenicity of Cb epitopes in chronic Q fever patients (persistent Cb infection) in comparison to individuals with resolved infection. Key questions are whether chronic Q fever patients:

- show greater reactivity to MHC class I epitopes given their more recent exposure
- recognize the same or a distinct set of MHC class II epitopes
- differ in their effector memory T-cell response profile

Approach and study subjects

- Enroll individuals diagnosed with and treated for probable or proven chronic Q fever
- Determine Cb serology (IFA, Focus Diagnostics) and cellular responses to heat-killed Cb (Q-detect IGRA)
- Analyze Cb epitope-specific T-cell responses by cultured ELISpot (central memory) and direct ELISpot (effector memory)
- Compare results to T-cell responses from individuals with past disease – *see Scholzen et al, 2019*

Subject	Gender	Age	Chronic Q fever category	Year of diagnosis	Site of focalized infection	Antibiotic treatment at inclusion	Phase I IgG titer	Phase II IgG titer	Cb-specific IFN-γ response (pg/ml)
1	M	75	proven	2017	vascular, bone	yes	>4096	>4096	10498
2	M	57	proven	2016	bone	yes	8192	4096	1288
3	M	74	proven	2014	vascular	yes	8192	8192	603
4	M	76	proven	2016	vascular	yes	2048	4096	5237
5	M	71	proven	2014	lung, possible vascular	no	4096	4096	4951
6	M	79	proven	2016	vascular	yes	2048	2048	499
7	M	79	proven	2010	vascular	yes	4096	2048	1042
8	M	72	proven	2009	vascular	no	512	1024	553
9	M	63	proven	2017	vascular, endocarditis	yes	2048	2048	4395
10	M	72	proven	2010	unknown (PCR+)	yes	8192	8192	2778
11	M	68	proven	2009	vascular	yes	8192	4096	5150
12	M	60	proven	2013	endocarditis	yes	16384	8192	4030
13	M	72	proven	2017	vascular	yes	32	512	470
14	M	74	proven	2016	vascular	yes	2048	4096	474
15	M	76	proven	2016	vascular	yes	16384	32768	34
16	M	71	proven	2011	vascular	no	4096	4096	3345
17	M	72	probable	2015	n.d.	yes	32768	32768	3804
18	M	59	probable	2015	n.d.	no	2048	2048	24
19	F	73	probable	2010	n.d.	no	1024	1024	51
20	M	67	probable	2016	n.d.	no	1024	2048	32
21	M	66	probable	2013	n.d.	no	1024	2048	4173
22	M	79	probable	2016	n.d.	no	4096	4096	118

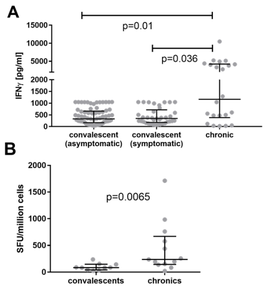


Figure 1: *IFNγ T-cell responses to whole cell Cb by (A) Q-detect IFNγ release assay (IGRA) and (B) direct ELISpot.*
Responses between groups were compared by Kruskal-Wallis test with Dunn's multiple comparison test (A) and Mann-Whitney test (B)

Results

1. Chronic Q fever patients show frequent central memory T-cell responses to Cb MHC class II, but not class I epitopes

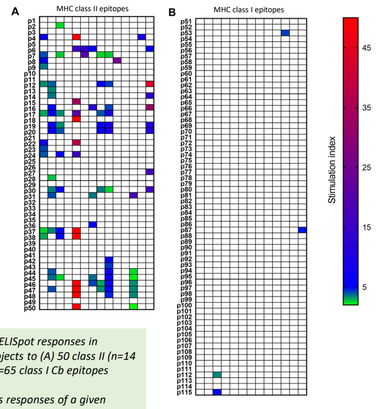


Figure 2: *Cultured ELISpot responses in chronic Q fever subjects to (A) 50 class II (n=14 subjects) and (B) n=65 class I Cb epitopes (n=13 subjects).*
Each column shows responses of a given human subject.

2. Chronic Q fever patients show a similar breadth of central memory T-cell responses to Cb MHC class II epitopes, but are less frequently non-responders than convalescents

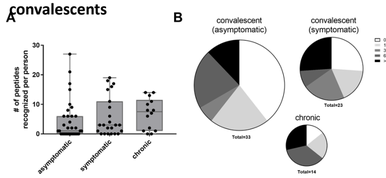


Figure 3: *Number of HLA class II peptides recognized by convalescents and chronic Q fever and subjects, shown (A) per subject and (B) as proportion of individuals per group.*

3. Chronic Q fever patients show a similar response pattern of Cb MHC class II epitopes, but recognize 6 peptides more frequently than convalescents

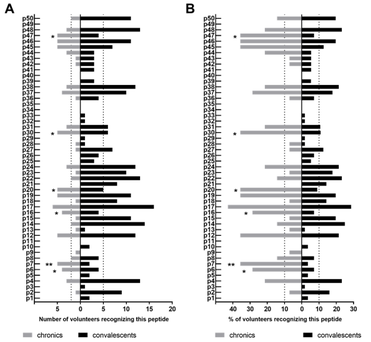
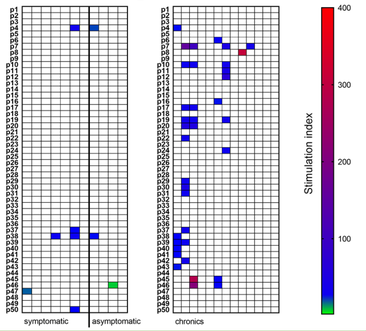


Figure 4: *(A) Number and (B) proportion of chronic Q fever subjects and convalescents recognizing the 50 analyzed HLA class II peptides; asterisks indicate significant differences between groups by Fisher's exact test* p<0.05; ** p<0.01*

- 15/22 previously identified highly antigenic peptides (>10% responders in convalescents) are also highly antigenic in chronics
- Additional 6 peptides expand previous set of vaccine candidate epitopes

4. Chronic Q fever patients show more frequent *ex vivo* effector memory T-cell responses to Cb MHC class II epitopes than convalescents

Figure 5: *Direct ELISpot responses in (A) individuals with a history of symptomatic (n=7) or asymptomatic (n=4) Q fever infection and (B) chronic Q fever subjects (n=13) to 50 MHC class II Cb epitopes.*
Each column shows responses of a given human subject.



- p7, p10 and p19 are the peptides most commonly recognized by chronic subjects in direct ELISpot

Summary & Conclusions

- Virtually **no reactivity to MHC class I** epitopes is present in chronic Q fever patients, despite more recent and prolonged exposure
- Chronic Q fever patients show **central memory T-cell responses** to a set of epitopes that is **largely overlapping** with that of **convalescents**
- Six peptides identified as highly antigenic in this cohort **expand the previous set of vaccine candidate epitopes**
- There is **no unique set of target peptides** which would justify consideration for a therapeutic vaccine
- Chronic subjects shows **more frequent ex vivo responses** to individual peptides, in line with a stronger ex vivo response to whole cell Cb
- To **evaluate the diagnostic potential** of peptide-specific direct *ex vivo* responses, further research in larger cohorts is necessary

References

Eldin C, Melenotte C, Mediannikov O, et al. 2017. **From Q Fever to *Coxiella burnetii* Infection: a Paradigm Change**. Clin Microbiol Rev 30:115-190.
Kampschreur LM, Hagenaars JC, Wielders CC, et al. 2013. **Screening for *Coxiella burnetii* seroprevalence in chronic Q fever high-risk groups reveals the magnitude of the Dutch Q fever outbreak**. Epidemiol Infect 141:847-51.
Ghigo E, Capo C, Thung CH et al.. 2002. ***Coxiella burnetii* survival in THP-1 monocytes involves the impairment of phagosome maturation: IFN-gamma mediates its restoration and bacterial killing**. J Immunol 169(8):4488-95.
Xiong X, Qi Y, Jiao J, et al. 2014. **Exploratory study on Th1 epitope-induced protective immunity against *Coxiella burnetii* infection**. PLoS ONE 9:e87206.
Scholzen A, Richard G, Moise L, et al. 2019. **Promiscuous *Coxiella burnetii* CD4 epitope clusters associated with human recall responses are candidates for a novel T-cell targeted multi-epitope Q fever vacc**ine. Front Immunol 10:207.

Ethics statement

The human study was reviewed and approved by the Medical Ethical Committee Brabant (Tilburg, Netherlands, NL51305.028.15) and all donors provided written informed consent.

Disclosures

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A Garritsen is CEO and A Scholzen is senior scientist at Innatoss Laboratories B.V.; AS de Groot and WD Martin are senior officers and majority shareholders and L Moise is scientific director at EpiVax , Inc.