Promiscuous *Coxiella burnetii* CD4 epitope clusters associated with human recall responses are candidates for a novel T-cell targeted multi-epitope Q fever vaccine

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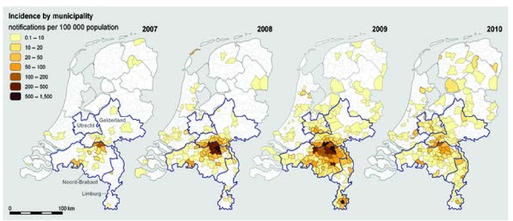
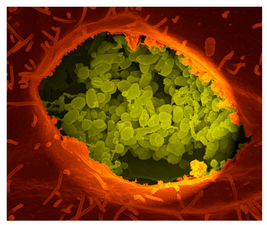
Background

- *Coxiella burnetii* (Cb), the causative agent of Q fever, is a Gram-negative intracellular bacterium transmitted via aerosol.
- >50% of Q fever patients are asymptomatic. >20% of symptomatic patients develop Q fever fatigue syndrome (flu-like symptoms).
- An outbreak of Q fever in the Netherlands from 2007 to 2011 affected ~ 40,000 individuals.



Incidence of notified Q fever patients by municipality in 2007, 2008, 2009 and 2010. Dijkstra *et al.* FEMS Immunol Med Microbiol. 2012 Feb;64(1):3-12



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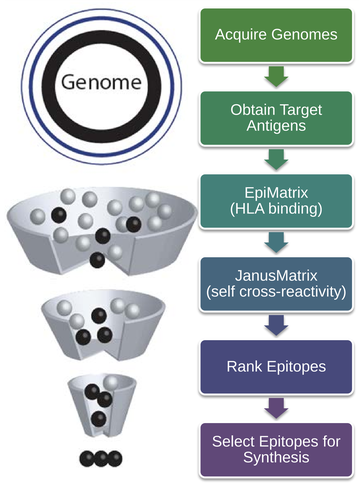
- The U.S. Centers for Disease Control & Prevention (CDC) considers Q fever a category B (the second-highest priority) bioterrorism agent.
- The whole-cell Q fever vaccine (Q-VAX®) is highly reactogenic in pre-exposed individuals.
- Current antibiotic treatments against Q fever are effective, but treated patients may still suffer debilitating side effects that can last for up to several years.

1. *In silico* Identification of *Coxiella burnetii* Epitopes



- Selected source Cb antigens for epitope prediction were derived from published T4SS effector and sero-reactive Cb antigens.
- Antigen sequences were analyzed with the iVAX platform to identify candidate T cell epitopes.

Candidate Epitope Triage

HLA Class	Class I		Class II
Antigen source	T4SS Effector	Sero-reactive Cb antigens	
Antigen count	53	40	40
Epitopes	8,643	5,100	282
Conserved across Cb	3,971	4,578	188
Highly immunogenic	1,710	1,945	153
Different from human	1,511	1,558	98
W/o synthesis issues	1,108	1,163	81
Selected	**30**	**35**	**50**

2. HLA Binding of Predicted *Coxiella burnetii* Epitopes

HLA Class I

- 65 peptides, 65 peptide-allele pairs
- **86% overall agreement with iVAX predictions**

Allele	N=	TP	FP	TN	FN	Accuracy
A*0101	11	8	3	-	-	73%
A*0201	11	11	0	-	-	100%
A*0301	10	10	0	-	-	100%
A*2402	11	11	0	-	-	100%
B*0702	11	8	3	-	-	73%
B*4403	11	8	3	-	-	73%
Total	**65**	**56**	**9**	**-**	**-**	**86%**

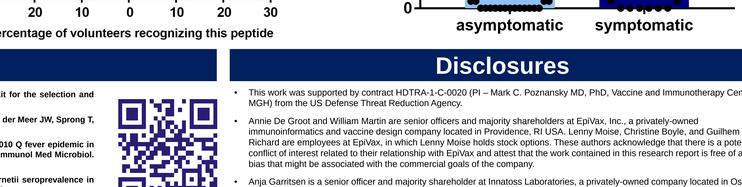
HLA Class II

- 50 promiscuous peptides, 400 peptide-allele pairs
- **75% overall agreement with iVAX predictions**

Allele	N=	TP	FP	TN	FN	Accuracy
DRB1*0101	50	43	3	1	3	88%
DRB1*0301	50	21	16	9	4	60%
DRB1*0401	50	33	13	1	3	68%
DRB1*0701	50	40	3	1	6	82%
DRB1*0801	50	25	15	7	3	64%
DRB1*1101	50	39	6	1	4	80%
DRB1*1301	50	34	6	3	7	74%
DRB1*1501	50	43	4	0	3	86%
Total	**400**	**278**	**66**	**23**	**33**	**75%**

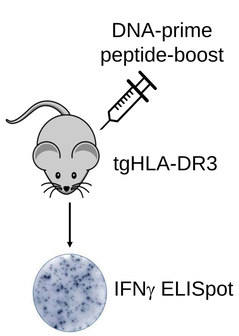
Blue: Predictions agree with in vitro findings; Red: Predictions disagree with in vitro findings

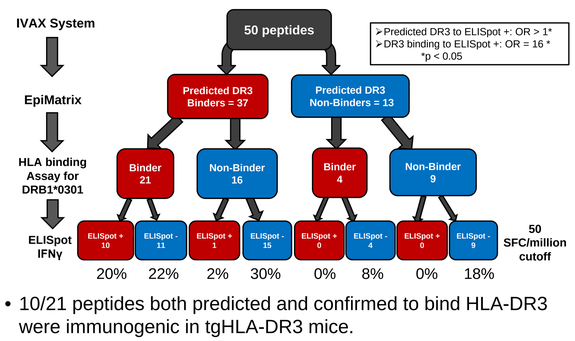
Accuracy of Class I and Class II predictions



3. Immunogenicity Analysis of Predicted *Coxiella burnetii* Class II Epitopes

Immunogenicity in tgHLA-DR3 mice





- 10/21 peptides both predicted and confirmed to bind HLA-DR3 were immunogenic in tgHLA-DR3 mice.

Human Donors

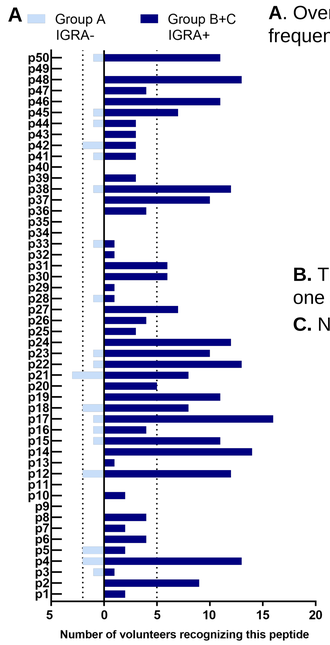
- 77 volunteers from the village Herpen (NL) were screened for antigenicity against the selected Class II epitopes. This region was at the epicenter of the recent Dutch Q fever outbreak.
- Cellular reactivity was determined using the Q-detect™ assay (Cb-induced **I**FN-**g**amma **r**elease **a**ssay in whole blood, IGRA) and Cb serology was assessed based on IFA and Western Blot.

Group	Description	N	Q-detect™ (IGRA)	Anti-Cb Antibodies	Clinical Disease
A	No evidence of previous infection	21	–	–	–
B	Asymptomatic Infection	33	+	+	–
C	Symptomatic Infection	23	+	+	+

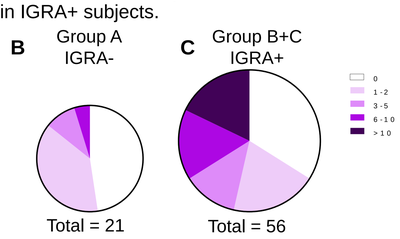
- Cultured ELISpot was performed in 2016-17 on fresh PBMCs (~10 years after the latest Q fever outbreak).

Epitope-Specific Human IFNγ Responses

- 44/50 (88%) Class II peptides elicited a response in at least one donor.
- Responses detected to 27/29 source antigens (93%).
- 21 HLA class II epitopes recalled T cell IFNγ responses in 10-28% of IGRA+ subjects.

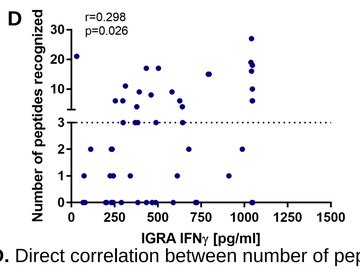


A. Overall, peptide response per individual are more frequent in IGRA+ subjects.



B. The large majority of IGRA- individuals recognized zero, one or two peptides.

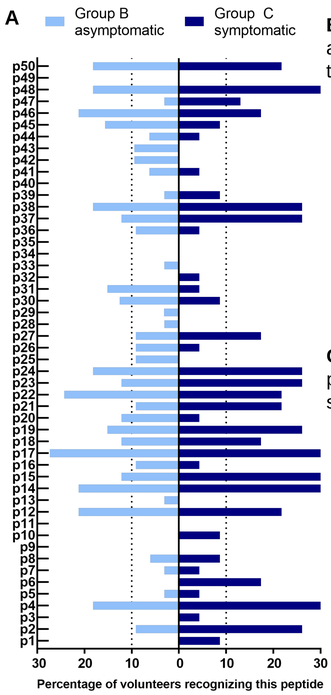
C. Nearly half of IGRA+ recognize three or more peptides.



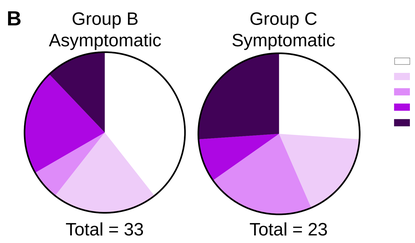
D. Direct correlation between number of peptides recognized and IFNγ responses in IGRA.

Comparison of responses in Asymptomatic vs. Symptomatic Donors

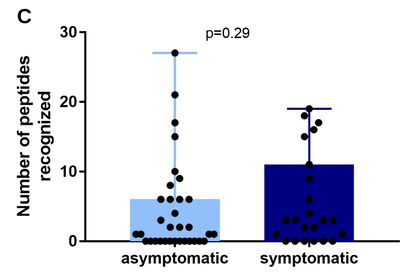
A. Antigenic peptides were more frequently recognized among symptomatic donors.



B. Proportionally, there are more high responders (>10) and fewer non-responders (0) in symptomatic individuals than asymptomatic donors.



C. There is no significant difference overall in number of peptides recognized between asymptomatic and symptomatic donors



Conclusions

- **Immunoinformatic methods efficiently identify HLA binding, immunogenic and human antigenic class II epitopes among T4SS effector and sero-reactive Cb antigens.**
- **Natural exposure to Cb induces long-lived responses to promiscuous and conserved HLA class II T cell epitopes.**
- **Cb T cell epitope peptides are not reactogenic in a guinea pig model of exposure-primed delayed-type hypersensitivity (not shown)**
- **Class II epitopes are candidates for a T cell epitope-based Q fever vaccine.**

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Disclosures

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- Annie De Groot and William Martin are senior officers and majority shareholders at EpiVax, Inc., a privately-owned immunoinformatics and vaccine design company located in Providence, RI USA. Lenny Moise, Christine Boyle, and Guilhem Richard are employees at EpiVax, in which Lenny Moise holds stock options. These authors acknowledge that there is a potential conflict of interest related to their relationship with EpiVax and attest that the work contained in this research report is free of any bias that might be associated with the commercial goals of the company.
- Anja Garritsen is a senior officer and majority shareholder at Innatoss Laboratories, a privately-owned company located in Oss, the Netherlands. Anja Scholzen is an employee at Innatoss. These authors acknowledge that there is a potential conflict of interest related to their relationship with Innatoss and attest that the work contained in this research report is free of any bias that might be associated with the commercial goals of the company.