Identification of candidate *Coxiella burnetii* T-cell epitopes for a novel human Q fever vaccine

Anja Scholzen[1]*, Guilhem Richard[2]*, Leonard Moise[2,3], Laurie A. Baeten[4], Patrick M. Reeves[5], William D. Martin[2], Timothy A. Brauns[5], Christine M. Boyle[2], Susan Raju Paul[5], Richard Bucala[6], Richard A. Bowen[4], Anja Garritsen[1], Anne S. De Groot[2,3], Ann E. Sluder[5], Mark C. Poznansky[5];

***authors contributed equally**

[1] Innatoss Laboratories B.V., Oss, Netherlands; [2]EpiVax, Inc., Providence, USA, [3]University of Rhode Island, Providence, USA; [4]Colorado State University, Ft. Collins, USA; [5]Massachusetts General Hospital, Charlestown, USA; [6]Yale University, New Haven, USA

Background – Q fever

- Zoonosis primarily spread by small ruminants (goat, sheep) and cattle
- Neglected disease with world-wide prevalence; poor surveillance, studies indicate substantial contribution to "fever & infectious endocarditis of unknown origin"
- Caused by the obligate intracellular bacterium *Coxiella burnetii* (Cb) – infects macrophages
- Highly stable and contagious (potential biothreat agent)
- Long-term complications common (chronic fatigue, persistent infection = chronic Q fever)
- Outbreak in the Netherlands (2007-2010) affected more than 40,000 individuals
- T-cell produced IFNg key in inducing phagosome/lysosome fusion to eliminate infection
- Current whole-cell Q fever vaccine (Q-VAX®) only licensed in Australia and reactogenic in individuals previously exposed to Cb
- Epitope-based subunit vaccines are expected to be non-reactogenic and show promise in murine studies

Objective & Approach

Identify target epitopes to develop a non-reactogenic T-cell-targeted vaccine to prevent Q fever disease in humans

T-cell epitopes were predicted from

- Cb ***type IV secretion system (T4SS) substrates***, expected to enter the class I antigen processing pathway and thus to trigger CD8+ T cell responses
- Cb ***sero-reactive proteins*** known to elicit antibody responses in mice and humans

Epitopes were down-selected for cross-strain conservation, strong HLA binding, broad HLA coverage and low cross-reactivity with human/microbiome proteins. HLA binding was assessed in vitro, immunogenicity in HLA-DR3 transgenic mice and antigenicity in naturally exposed volunteers. Reactogenicity was excluded using a sensitized guinea pig model (data not shown).

In silico epitope selection

Results:
65 HLA class I epitopes (10 per HLA I supertype allele) and 50 promiscuous class II epitope clusters were down-selected (no more than 2 epitopes / source Ag)

HLA Class	Class I		Class II
Antigen source	T4SS Effector	Sero-reactive	Sero-reactive
Antigen count	53	40	40
Epitopes predicted	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**

In vitro HLA binding

Results:
In vitro competition assays confirm that

- HLA class II peptides are promiscuous binders
- 89% of class I and 75% of class II predictions are correct

Peptides have potential for stimulating *Cb*-specific immune responses across a broad range of HLA types.

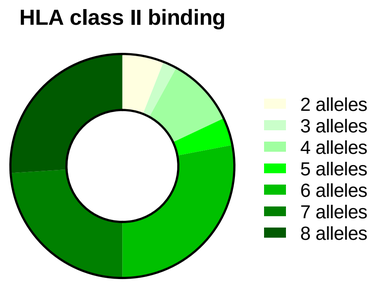


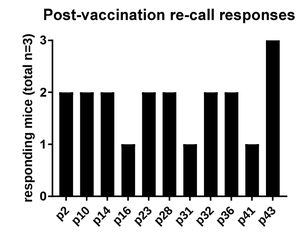
Figure 1: *HLA class II peptide binding was assessed to the main supertypes (DRB1*0101, *0301, *0401, *0701, *0801, *1101, *1301, *1501 – each peptide against each allele)*

In vivo immunogenicity

Results:
11/50 HLA class II peptides were immunogenic in tgHLA-DR3 mice. All 11 were predicted to bind HLA-DR3, and all except one (p36) also showed binding to DRB1*0301 *in vitro*.

Peptides show capacity to elicit de novo responses *in vivo*.

Figure 2: *5 groups of mice (n=3 each) were immunized with 10 epitopes each by heterologous DNA/DNA/peptide/ peptide prime-boost immunizations. Re-call responses were assessed by IFNg ELISpot. Response were defined positive when spot counts reached (i) a stimulation index of at least 2 above the matched negative control wells (ii) >50 spot/million splenocytes (above background) and were (iii) statistically higher (p<0.05) than in mock immunized mice (Student's t-test)*



Human cohort

Antigenicity of the peptides was assessed in naturally Cb-exposed individuals from the Dutch village of Herpen. Volunteers were grouped by clinical Q fever history (2007-2010 outbreak) and cellular responses to whole cell Cb.

Group A: IFA and IGRA-; no clinical Q fever history
Group B: IGRA+, no clinical Q fever history (asymptomatic)
Group C: IGRA+, past symptomatic Q fever episode

Two partially overlapping subgroups of n=77 individuals each were selected for class I and class II peptide screening by cultured ELISpot.

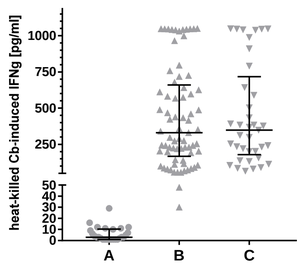


Figure 3: *Cellular responses to whole cell heat-killed Cb (strain Cb2009-02629) were assessed for 136 volunteers from Herpen in a whole blood IFNg release assay (Q-detect™ IGRA)*

Peptide re-call responses in exposed human volunteers

Results:

- HLA class I peptides recall only a few infrequent responses
- HLA class II peptides recall a wide range of responses, particularly amongst IGRA+ volunteers
- 21 of the 50 HLA class II peptides are highly antigenic = recognized by 10-28% of IGRA+ volunteers
- Amongst these 21 peptides are 5 epitope pairs where both peptides originate from the same source antigen
- Q-detect™ IGRA responses to whole Cb correlate with number of Cb peptides recognized per volunteer (Spearman p=0.026)
- Past Q fever asymptomatic and symptomatic volunteers do not differ in their cumulative peptide response

Promiscuous HLA class II peptides are antigenic and recall long-lasting memory responses from natural exposed IGRA+ volunteers following, irrespective of their clinical Q-fever history.

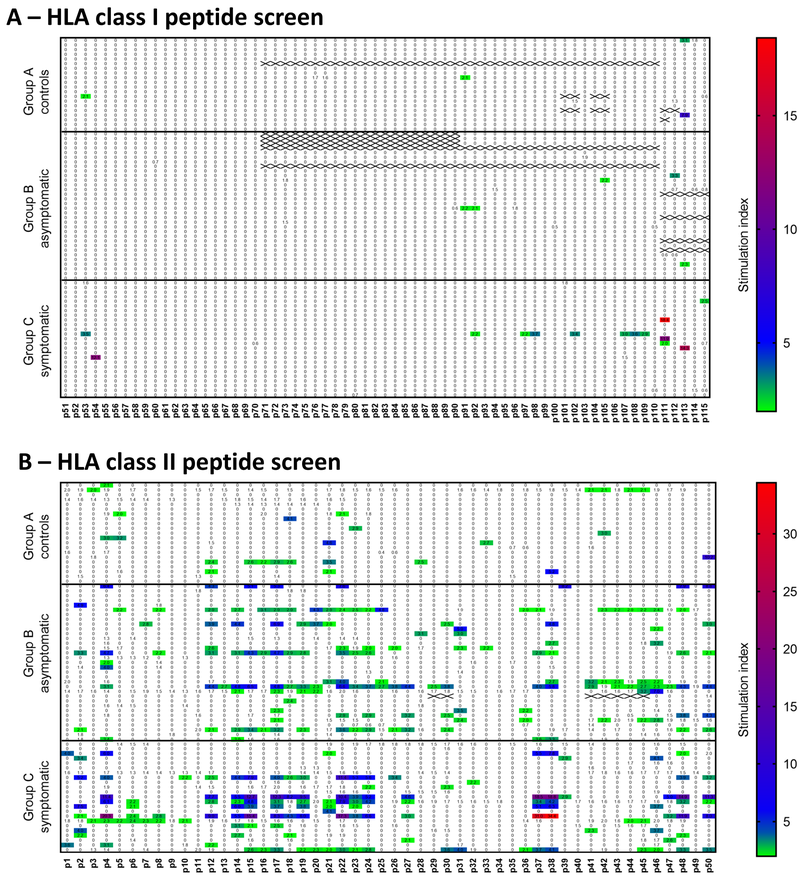


Figure 4: *Re-call responses to HLA class I (A) and II peptides (B) assessed by cultured ELISpot for group A, B and C volunteers.*
Responses were defined positive when (i) significantly higher than spot counts in matched negative control wells from the same expansion culture by one-way ANOVA with Holm-Šídák multiple comparison correction post-hoc test, reached (ii) a stimulation index of at least 2 above the matched negative control wells and (iii) an absolute cut-off of >10 SFU/well.

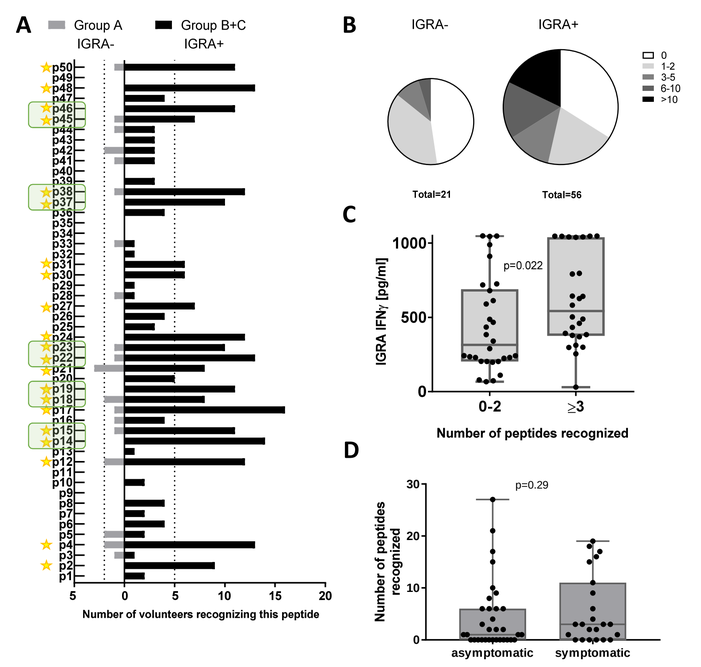


Figure 5: *Re-call responses to HLA class II peptides:*
(A) Proportion of responding volunteers amongst group A controls (n=21) and group B+C IGRA+ volunteers (n=56)
Yellow stars *in (A) indicate highly antigenic peptides recognized by >10% of IGRA+ volunteers, and* ***green boxes*** *indicate epitope pairs from the same source antigen (for highly antigenic peptides only).*

(B) Number of peptides recognized by individual volunteers in both groups.

(C) IGRA responses to whole cell heat-killed Cb for volunteers recognizing none or few (0-2) or a larger number of peptides (≥3).

(D) Number of peptides recognized by individual volunteers compared for those IGRA+ volunteers that experienced either asymptomatic Cb infection or a clinical Q fever episode during the 2007-2010 epidemic.

Discussion / Conclusions

- Sero-reactivity guided screening approach is very efficient in identifying relevant source antigens for HLA class II (21/50 = 42% highly antigenic)
- Natural exposure to Cb induces long-lived responses to immunodominant, promiscuous and conserved HLA class II T-cell epitope clusters
- Weak responses to T4SS HLA class I epitopes
  - *Greater number of binding opportunities for class II clusters than for individual class I epitopes*
  - *CD4 repertoire larger than CD8 repertoire?*
  - *CD8 responses more short-lived?*
- **Class II epitope clusters are key candidates for an epitope-based Q fever vaccine to elicit sustained T-cell memory that can be boosted and recalled by natural exposure**

Disclosures

This work was supported by contract HDTRA1-15-C-0020 from the US Defense Threat Reduction Agency. 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.

Ethics statement

Animal research protocols for studies with HLA-DR3 transgenic mice performed by EpiVax were reviewed and approved by TGA Sciences Incorporated Institutional Animal Care and Use Committee (P07-10R20-EV69, P07-10R20-EV71). Animal research protocols for guinea pig experiments were reviewed and approved by the Colorado State University Institutional Animal Care and Use Committee (14-5305A, 16-6844A). 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.