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A systematic approach to evaluate humoral and cellular immune responses to *Coxiella burnetii* immunoreactive antigens

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INTRODUCTION

Coxiella burnetii is an obligate Gram-negative intracellular bacterium that causes acute Q-fever and chronic infections in humans [1]. Current diagnostic methods for human Q-fever are based on clinical presentation and supporting serological evidence of response against Nine Mile reference whole cell antigens (phase I and phase II), although these assays suffer from lack of uniformity and specificity. An effective formalin-killed whole cell vaccine (Q-Vax CSL Ltd Melbourne, Vic., Australia), in use in Australia, is administered to individuals who are skin test-negative and serologically negative. Vaccination can result in severe local or systemic adverse reactions [2], especially when administered to previously infected populations, and repeat vaccination can induce severe persistent reactions. Consequently, no vaccine is licensed in the USA. Although cellular immunity, especially as mediated by $CD4^+$ T-cells, is known to be critical for protective immunity[3], there is no satisfactory vaccine that can be administered without prior screening for immunity in populations at risk of potential exposure to the agent. Thus, identification of immunodominant antigens of *C. burnetii* with strong humoral and cellular immune responses after infection and vaccination should aid in the development of a safe and effective vaccine and reliable serodiagnostic tests. To achieve these goals, we developed a systematic platform to comprehensively analyse the humoral and cellular immune responses to a wide array of *C. burnetii* antigens in the context of *C. burnetii* infection or vaccination in animal models and humans.

MATERIALS AND METHODS

Human serum samples

Fifty-five immunofluorescent antibody analysis (IFA)-positive convalescent human sera were collected between 38 and 172 days after onset of clinical symptoms; they had phase II

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IFA titres ranging from 1 : 160 to 1 : 5120. Five chronic Q-fever sera were collected from endocarditis patients with persistent *C. burnetii* infection. Thirty two IFA-negative human sera were selected from our human serum library. Q-fever IFA responses were determined with a Q-fever IFA IgG Kit (FOCUS Diagnostic, Cypress, CA, USA), according to the manufacturer's instructions.

ELISA

Ninety-six-well microplates (Fisher Scientific, Pittsburgh, PA, USA) were coated with 100 μL of 2 μg/mL antigen. Fifty microlitres of diluted (1 : 50) human serum were tested by IgG indirect ELISA. The cut-off was determined as the mean of IFA-negative samples plus two standard deviations.

ELISPOT

C57BL/6 mice and human leukocyte antigen (HLA) DR4 molecule transgenic mice (C57BL/6-[KO]Abb-[Tg]DR-4) were vaccinated with 10 μg/mouse electron beam-inactivated *C. burnetii* Nine Mile, phase I (RSA493). Antigen-specific interferon (IFN)-γ recall was measured by ELISPOT using purified $CD4^+$ T-cells isolated at 12 days post-vaccination. The frequency of IFN-γ-producing cells was counted, and a stimulation index was calculated for each recombinant protein.

RESULTS

Six previously identified and five *C. burnetii* protein array proteins selected because of IgG responses with convalescent human sera were expressed as His-tag fusion proteins in *Escherichia coli* and purified by chromatography. Humoral and cellular immune responses to purified recombinant proteins were tested by ELISA and ELISPOT, respectively. The solubilized fraction of mechanically lysed whole cells of Nine Mile phase I was used as a positive control. Most purified recombinant proteins reacted strongly with a subset of convalescent human sera, and all recombinant proteins were able to differentiate a majority of IFA-positive sera from IFA-negative sera. No individual recombinant protein could detect all IFA-positive samples. The sensitivity and specificity for each recombinant protein were 25–52% and 78–100%, respectively (Table 1). All recombinant proteins reacted strongly with sera from endocarditis patients and reacted weakly with sera from vaccinated individuals. Cellular immune responses to recombinant proteins were evaluated by IFN-γ/ $CD4^+$ T-cell recall responses in vaccinated C57BL/6 and HLA-DR4 transgenic mice. Distinct antigen-specific $CD4^+$ T-cells were generated after vaccination in different mice. Seven and eight tested recombinant proteins induced antigen-specific IFN-γ/$CD4^+$ T-cell recall responses in vaccinated C57BL/6 and HLA-DR4 transgenic mice, respectively (Table 1).

CONCLUSIONS

Humoral and cellular immune responses to 11 recombinant proteins were evaluated in this study. Although none of the individual antigens provided complete detection of all positive serum samples, one or more antigens reacted with each serum, indicating that combinations of two or more antigens could increase sensitivity. In accordance with previous studies showing that murine and human MHCs recognize different epitopes [4], we found that antigen-specific $CD4^+$ T-cells were generated differently in HLA-DR4 transgenic mice and wild-type mice; this confirms that the HLA transgenic mouse is a more relevant model for screening human T-cell antigens. Most proteins with strong antibody responses also strongly induced IFN-γ/recall responses in purified $CD4^+$ T-cells of vaccinated mice, which means that immunoreactive antigen screening based on serology testing, such as protein

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microarray, can aid in the discovery of T-cell antigens. We have developed a sensitive, high-throughput approach for screening immunoreactive *C. burnetii* antigens for strong humoral and cellular immune responses. This platform will be used for the rational design of effective subunit vaccines and serodiagnostic tools.

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REFERENCES

1. Maurin M, Raoult D. Q fever. Clin Microbiol Rev 1999;12:518–553. [PubMed: 10515901]
2. Marmion BP, Ormsbee RA, Kyrkou M, et al. Vaccine prophylaxis of abattoir-associated Q fever. Lancet 1984;ii:1411–1414. [PubMed: 6151039]
3. Andoh M, Zhang G, Russell-Lodrigue KE, Shive HR, Weeks BR, Samuel JE. T cells are essential for bacterial clearance, and gamma interferon, tumor necrosis factor alpha, and B cells are crucial for disease development in *Coxiella burnetii* infection in mice. Infect Immun 2007;75:3245–3255. [PubMed: 17438029]
4. Engelhard VH. Structure of peptides associated with Class I and Class II MHC molecules. Annu Rev Immunol 1994;12:181–207. [PubMed: 7516668]

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Table 1

ELISA sensitivity, specificity and interferon-γ recall responses in C57BL/6 and HLA-DR4 transgenic mice using *Coxiella burnetii* recombinant proteins

ID	Molecular mass (kDa)	Predictive function	Specificity (%)	Sensitivity (%)	Presented by murine MHC II	Presented by HLA DRA
NMI BBS			87.5	85	√	√
CBU0383	24	DNA-3-methyladenine glycosidase I	87.5	31.6	√	√
CBU0612	19	Outer membrane protein OmpH, putative	81.2	51.6		√
CBU0718	10.4	Hypothetical membrane-associated protein	78.1	45	√	
CBU0781	38.5	Ankyrin repeat domain protein	81.3	40	√	√
CBU0891	34.4	Hypothetical exported membrane protein	80.6	41.6	√	
CBU0311	29	Major outer membrane protein porin (P1)	78.1	43.3	√	√
CBU0395	25	5 Lipoprotein, putative	90	28.3		√
CBU0952	28	Outer membrane protein AdaA	100	25		
CBU1221	22	Lipoprotein, putative	81.2	48.3		√
CBU1716	35	Glycine cleavage system T-protein	90	46.6	√	√
CBU1910	27	Outer membrane protein (Com1)	90	50	√	√

HLA, human leukocyte antigen; MHC, major histocompatibility complex.

Sensitivity was calculated using number of ELISA-positive samples from the IFA-positive pool divided by the total number of IFA-positive samples. Specificity was calculated using the number of ELISA-negative samples from the IFA-negative pool divided by the total number of IFA-negative samples. A tick mark (√) indicates a stimulation index higher than three (stimulation index was calculated using number of spot-forming cells in vaccinated mice divided by number of spot-forming cells in naïve mice). NMI BBS was prepared from a solubilized fraction of mechanically lysed Nine Mile phase I (RSA493).