Q J Med 2009; **102**:673–684
doi:10.1093/qjmed/hcp077 Advance Access Publication 25 June 2009

Review

QJM

Q fever: persistence of antigenic non-viable cell residues of *Coxiella burnetii* in the host—implications for post Q fever infection fatigue syndrome and other chronic sequelae

B.P. MARMION[1], O. SUKOCHEVA[2], P.A. STORM[1], M. LOCKHART[2], M. TURRA[1], T. KOK[1], J. AYRES[3], H. ROUTLEDGE[3] and S. GRAVES[2]

From the [1]Q fever Research Group, SA Pathology/Hanson Institute, Adelaide, South Australia, [2]Australian Rickettsial Reference Laboratory, Geelong, Victoria, Australia and [3]Environmental and Occupational Medicine, University of Birmingham, Birmingham, UK

Summary

Background: Our previous studies of persistence of *Coxiella burnetii* in humans after an initial acute Q fever infection revealed raised, maintained antibody levels and low levels of coxiella genomic DNA at the age of 5 years from onset in Australian patients and at 12 years in patients in the 1989 Birmingham UK Q fever outbreak. Attempts to isolate the coxiella in standard cell culture and susceptible mice by serial passage of PCR positive PBMC and bone marrow were negative.

Aim: To retest PCR positive patient samples by more sensitive methods for viable coxiellas and for the coxiella cell components of antigen and specific lipopolysaccharide (LPS). To re-interpret the previous results in the light of the new information. To review the pertinent literature for a concept of an immuno-modulatory complex generated by the current studies.

Design: Laboratory case study.

Methods: Stored patient samples were inoculated into SCID mice that were followed for 60 days. Mouse spleen and liver samples were then examined by PCR assay for targets in the COM1 and IS1111a sequences and for antigens by IFA with a polyclonal rabbit antiserum to *C. burnetii* Phase 1 and a monoclonal antiserum to Phase 1 LPS (details; O. Sukocheva *et al.*, unpublished data).

Results: All specimens, including a recently excised heart valve from a Birmingham patient with late developing endocarditis, were infection negative in SCID mice. Dilutions of SCID mouse spleen and liver homogenates titrated in PCR assays were negative at dilutions attained by control mice inoculated with an endpoint dilution of a viable prototype strain of *C. burnetii*. Sections of the spleens from all specimens showed a complex of coxiella antigen-LPS by IFA.

Discussion/Review: We advance a concept of long-term persistence of a non-infective, non-biodegraded complex of coxiella cell components with its antigens and specific LPS [so called Immunomodulatory complex (IMC)] associated with traces of genomic DNA that signalled its presence in our earlier studies. The IMC's survival in patients for at least 12 years, and in one patient for 70 years implies a capacity for serial passage in

Address correspondence to B. P. Marmion, Q fever Research Group, SA Pathology/Hanson Institute, Adelaide, Australia. email: bpmarmion@iprimus.com.au

macrophages with effective down-regulation of their biodegrading functions. The review assesses the compatibility of the IMC concept in relation to cogent literature on *C. burnetii* interactions with macrophage and cell-mediated immunity. Some remaining gaps in our knowledge of the organ sites and duration of carriage of viable coxiellas after initial infection are also identified.

Introduction

In 1996 research groups in Adelaide Australia and Birmingham UK identified an additional chronic sequel to acute Q fever—the post Q fever fatigue syndrome (QFS)—that differed from other recognized sequelae such as subacute endocarditis, recrudescence of viable coxiellas at parturition and granulomata in various organ systems.[1,2] Between 10% and 15% of patients who had had acute primary Q fever subsequently developed a seriously disabling post-infection fatigue syndrome (QFS), lasting for 5–10 years or longer and symptomatically closely similar to the idiopathic chronic fatigue syndrome (CFS). Subsequently QFS has also been recognized in Canada,[3] USA,[4] Croatia,[5] Japan[6]: also currently in the large goat-associated outbreak (~1300 cases) in the Netherlands (Gijs Limonard MD personal communication).

Further investigations of QFS in Australia and UK proceeded on the hypothesis that three interacting factors were involved. Namely, (i) a persisting antigenic challenge from *Coxiella burnetii* after the acute infection, (ii) failure in a subset of patients to down-regulate a continuing, raised but impaired cell-mediated immune (CMI) response to the antigen after the decline of the initial acute phase response of the primary Q fever and (iii) a failure of homeostasis in the QFS patient subset probably related to polymorphic variation in the immunogenetic control and response repertoire.

Studies of patterns of cytokine dysregulation[7] and of genetic polymorphism in QFS patients and other Q fever groups are described elsewhere.[8,9] Our earlier results,[10,11] on the search, long after initial acute Q fever, for persisting viable coxiellas based on antibody levels, detection of sequences in genomic DNA and inoculation of samples into cell culture and laboratory animals, while correct as original observations, now require review and reinterpretation in view of our current findings.

The ability of viable *C. burnetii* to persist in its host after an initial infection has been recognized since the 1940s—first in laboratory animals and domestic ruminants, and later in human beings (summary[10]). Until recently the carrier state has been defined mainly by detection of coxiellas as infective entities either by infection of laboratory animals, or by culture in eukaryotic cells or in the chick embryo yolk sac. Additionally, when coxiellas are present in large numbers, as in early stage florid endocarditis,[12] detection of viable and non-viable organisms with varying sensitivity and specificity is also possible with special histological stains (e.g. Giemsa, Gimenez, Macchiavello), with fluorochromes, by serological assays for antigen (CFT, EIA, immunofluorescence), or by electron microscopy.

Recently, PCR amplification of short target sequences in the *C. burnetii* genome has provided an indirect marker of the presence of very small numbers of coxiella cells, probably even when their DNA is fragmented. The increased sensitivity has revealed long-term persistence of small amounts of coxiella genomic DNA and by inference, the persistence of coxiella cell components in the host, as distinct from or in addition to infective organisms.

In 2000 and 2005, the Q fever Research Group in Adelaide in collaboration with colleagues in Australia and in England described the detection of *C. burnetii* cells by PCR amplification of DNA genomic sequences in bone marrow aspirates, buffy coats or peripheral blood mononuclear cells (PBMC), long after initial infection.[10,11] Thus with an Australian cohort of 29 QFS patients with laboratory evidence of previous Q fever, samples collected at varying periods from 9 months to 5 years after initial illness (mean period 37 months), were positive with primer/probe sets for targets in IS*1111*a, and Com1 *C. burnetii* genes. With the cohort of 149 cases from the 1989 Q fever outbreak[13] in Solihull, Birmingham UK, which followed a single exposure to infection, samples collected 12 years after acute illness from a subset of 92 patients were examined serologically, with uniformly positive results. Bone marrow aspirates and buffy coat/peripheral blood samples were positive with primer/probe sets for Com1 and 16S rRNA, but were either negative or gave only occasional borderline positives with IS*1111*a.[11] The last finding was in marked contrast to the Australian cohort sampled at a shorter time after initial infection. In both cohorts the PCR amplicons were shown to be correct sequence for the designated targets in the *C. burnetii* genes.

In the Birmingham cohort,[11] both those patients who had made an asymptomatic recovery, and those who were still unwell with various morbidities —including QFS or developing, but covert subacute endocarditis—had closely similar geometric mean

levels of IgG class antibody to *C. burnetii* Phases 1 and 2 antigens by immunofluorescence assay (IFA). Again, counter intuitively but in line with the antibody studies, bone marrow samples from both asymptomatic, recovered patients and from those remaining ill, were positive in about the same proportions (85–90%) in PCR assays with primer and probe sets directed against COM1 or 16S rRNA genes. Significantly, subsequent observations showed that samples could rarely be diluted more than one in 10 before PCR reactivity was lost indicating presence of very few copies of DNA targets [This contrasted with the pattern found with the spleens of SCID mice inoculated with a viable *C. burnetii* (Henzerling) suspension at endpoint infectivity. The latter were PCR positive at dilutions 100–10 000 or more. (AQRG/ARRL: Unpublished 2005–2009)]

In our original reports, we canvassed various explanations including persistence of dormant non-replicating coxiella cells or their subcomponents surviving from the wide seeding during the original acute infection.

Eventually, swayed by the common belief that persistence implies the presence of viable infective organisms, we suggested a sequence of survival of very small numbers of viable coxiellas in the bone marrow, with low-level replication and periodic destruction, all under tight host control.

Nevertheless, serial passage of PCR positive bone marrow samples from one or other of our patient cohort in cultures of human lung fibroblasts (HEL),[14] or in A/J and interferonγ 'knockout' mice, failed to yield the postulated infective 'persisters'. It remained possible that the mouse lines and shell vial/HEL culture system that we used were not sensitive enough to detect the very small numbers thought to be present.[11] The recent demonstration[15] of the substantially greater susceptibility to *C. burnetii* infection shown by SCID versus immunocompetent mice led us to retest samples in the former.

Methods

SCID mice

Extracts of PCR positive marrow/peripheral blood cell homogenates from 10 Birmingham patients stored at –70°C were inoculated into groups of three SCID mice in parallel with a freshly excised optimally transported heart valve from one Birmingham patient with low-grade, late-stage endocarditis. Absence of Infection was judged by maintenance of weight gain and survival over an observation period of 60 days before euthanasia and autopsy (O. Sukocheva *et al.*, unpublished data).

PCR assays

SCID mouse spleen homogenates were tested over a range from undiluted to one in 100 by PCR with COM1 and IS*1111*a primers and probe sets.

Immunofluorescence assay for *C. burnetii* Phase 1 and 2 antigens

Valve tissue and SCID mouse spleen and liver samples were fixed in 10% v/v formal-saline and wax-embedded. Six microns sections were cut and taken to water then treated with acid citrate buffer pH 6.4 to elute bound host antibody globulin that might block rabbit antibody, followed by blocking with non immune goat serum. IFA staining was with a polyclonal rabbit antiserum to Phases 1 and 2 antigens followed goat anti-rabbit fluorescein conjugate. Control sera were negative serum from rabbit and an immune rabbit antiserum to influenza virus. A monoclonal antibody to Phase 1 antigen LPS was also used in the same format.

Results

Details of the weight changes in inoculated SCID mice, macro- and microscopic organ and tissue changes, PCR and immunofluorescence detection of antigen are detailed elsewhere (O. Sukocheva *et al.*, unpublished data).

In brief, infection was not detected in any of the SCID mice, which did not fail to gain weight. All PCR assays on their spleen or liver homogenates were negative at a one in 100 dilution thus excluding viable (i.e. replication competent) coxiella in the mice (AQRG/ARRL: Unpublished 2005–2009) although variable reactions remained in undiluted suspensions, presumably reflecting survival of the inocula. On the other hand, sections from the mouse spleens from all 11 Birmingham samples showed aggregates of coxiella antigen when stained by IFA with a rabbit polyclonal antiserum containing antibodies to Phases 1 and 2 antigens of *C. burnetii* but were negative with sera from an unimmunized rabbit (illustrated Figure 1B and E). Sections were also negative with a rabbit serum immunized with influenza virus but positive with a monoclonal antibody to *C. burnetii* Phase 1 antigen (O. Sukocheva *et al.*, unpublished data). The divergence between lack of infectivity on the one hand, and persistence of DNA fragments and antigen on the other, is illustrated with samples from three patients—one with

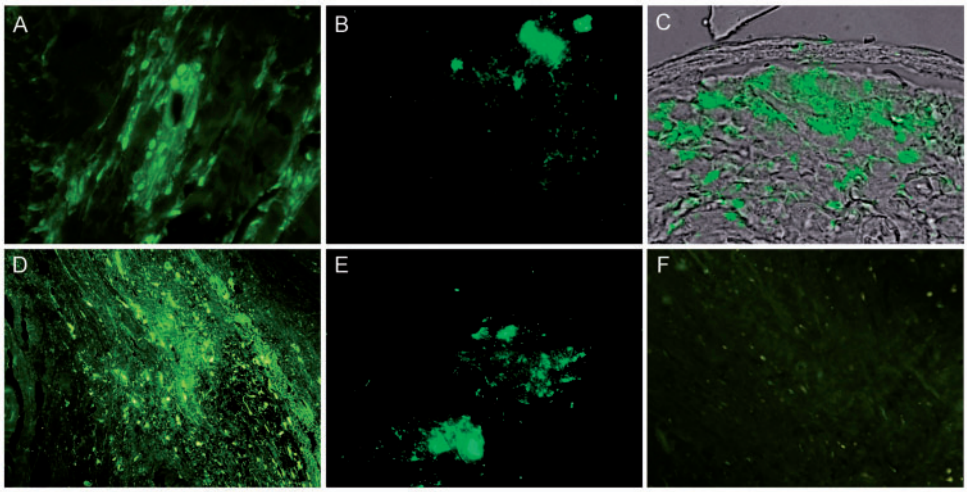

Figure 1. (A) Heart valve of Patient 1 ~400×. (B) Spleen of SCID mouse inoculated with heart valve P1 ~400×. (C) Superimposed images of co-located unstained spleen cells and fluorescent stained material in subcapsular region of SCID mouse in (B) and (D) Heart valve from Patient 2. ~400×. (E) Spleen (IFA only) from SCID mouse inoculated with bone marrow/buffy coat from Patient 3(44) QFS without endocarditis ~400×. (**F**) Control section for **A** with negative rabbit serum.

QFS and two with late stage endocarditis (Figure 1A–F, Box 1).

Discussion

The persistence of *C. burnetii* Phases 1 and 2 antigens and antibody, with the minor amounts of specific genomic DNA in the Birmingham patients, ⩾12 years after initial infection, coupled with the absence of infective coxiella cells in PBMC or bone marrow, adds a previously unsuspected dimension to the pathogenetic patterns of chronic Q fever.

One possible explanation for absence of infective cells might be attributed to neutralizing effects of human *C. burnetii* antibody in the inocula, however, note that while antibody modifies lesion development in immune-competent mice,[16] animals are nevertheless infected. Also for lesion prevention, co-operation of a competent cellular immune system is required: antibody does not prevent lesion development in congenitally athymic mice.[17] Overall, our results suggest the long-term survival of non-infective complexes of *C. burnetii* cell components including lipopolysaccharide (LPS) associated with small, variable amounts of coxiella genomic DNA sequences. The latter signalled the presence of the complexes in our original observations. These complexes now appear more likely to be a residue of the original heavy seeding during the bacteraemia of the acute infection ⩾12 years earlier rather than the product of an ongoing complex low-grade recurrent cycle of multiplication, destruction and renewal of infection as previously proposed.[11]

Clearly, in view of the regular recrudescence of infection in late pregnancy in animals and human beings, long-term low level survival of living coxiella is a reality (cf. [18]). Further study is being undertaken of the reproductive tract as a possible specialized locus (so called 'privileged site') for dormancy or low-grade coxiella infection after initial infection, given known infection of prostate, 'spontaneous' testicular granulomas, and prolonged excretion from the bovine mammary gland as well the differential pattern of survival of viable coxiella in the earlier Californian experimental inoculation of pregnant sheep (Lennette E. H. *et al.*, unpublished data: cited[10] and below),

Review

There appear to be key unanswered questions about the cellular state of persistent viable coxiellas in an immunologically competent host. This is despite much recent, highly illuminating *in vitro* and *ex vivo* model experiments with coxiella macrophage interactions. Our review explores the concept of an immunomodulatory complex (IMC) of antigen and LPS on the disease process as distinct from other modifiers coded by the coxiella genome.

How long do viable coxiellas persist in the human host after initial infection?

Derrick,[19] in reviewing the course of infection in 273 acute primary Q fever patients during the

Box 1 Notes on patients in Figure 1

(i) Patient 1 (#84) from the Birmingham cohort first presented as QFS after initial infection in 1989. Later he complained of progressive breathlessness on exertion and was found to have a low-grade endocarditis of the aortic valve. At 12 years after initial acute Q fever he remained antibody positive (*C. burnetii* Phase 1: 10; Phase 2: 160) but not with the 'classical' serological pattern seen in early florid Q fever endocarditis. His bone marrow was PCR positive with COM1 primer/probe set but not with IS*1111*a. At 17 years from onset his aortic valve failed and was replaced. It was distorted and fibrosed and a histological diagnosis of calcific aortic valve was offered. However an extract was positive by PCR with primer probe sets for IS*1111*a and COM1 in two different laboratories. *In vitro* culture in Vero, dog macrophage (DH82) and XTC-2 and L929 cells was negative. Inoculation of valve extract and bone marrow into SCID mice was also culture negative. IFA examination showed antigen was present in large amounts in sections from the heart valve as such, and also at lower levels in the subcortex of spleens of the SCID mice inoculated with valve homogenate and bone marrow even though a coxiella had not replicated in the mice (Figure 1A–C).

(ii) Patient 2, an unvaccinated Australian abattoir worker, had an aortic valve homograft (1990) after rheumatic fever in 1962. He then developed laboratory-proven acute Q fever (6/1992). After a period of QFS (1992–95) during which his Q fever serological tests (CFT) became negative; the tests slowly reverted to positive. When serological IFA tests (8/1997) were used antibody to Phases 1 and 2 antigens showed high titres in IgG and lower in IgM class (examples Phase 1: IgG: 5120; Phase 2: 10 240; Phase 1or 2: IgA class <10). PCMC samples (3/97 and 3/98) were PCR positive for coxiella DNA but isolation negative in A/J mice. At 9 years after the acute Q fever the valve was removed for mechanical reasons and was fibrosed and calcified. Surgical and tissue pathology diagnoses were, respectively, 'wear and tear', and calcific aortic valve with thrombotic vegetation. However a valve extract titrated out to 10^{-5} by PCR with primer/probe sets to IS*1111*a but even so a coxiella was not isolated in A/J mice. Electron microscopy showed lacunae in the valve with possible degenerate LCV and a few SCV. IFA examination showed plentiful focal staining probably corresponding to the lacunae. (Figure 1D).

(iii) Patient 3 (#44) from the Birmingham cohort after initial Q fever progressed to QFS without co-morbidity. At 12 years after infection IFA antibody tests showed IgG *C. burnetii* Phase 1 < 10; Phase 2: 320; IgM and IgA antibody negative. Bone marrow aspirate was positive in PCR with the COM1 primer probe set but negative with IS*1111*a sets. *Coxiella burnetii* was not isolated in HEL cells or in AJ or IFNγ ko mice. Later, bone marrow/buffy coat samples were also negative on inoculation of SCID mice. But the SCID mouse spleen and liver samples showed aggregates of antigen when stained with Phase 1 rabbit serum, but not with negative control sera (Figure 1E). PCR (COM1) primer set assays on mouse spleen showed variable positive ct values over a range of 1 in 1–10 dilutions but were clearly negative at 100.

pre-antibiotic era, concluded that virtually all blood samples were infective for guinea pigs when taken during the primary fever, from onset to ~Day 12. Likewise Japanese workers isolated *C. burnetii* in A/J mice from 13/18 (75%) of samples from children during the acute phase of serologically proven Q fever.[20] PCR assay detects the coxiella in serum during the same period and somewhat beyond.[21] However, during periods of 'secondary fever', after Day 15 from onset, Derrick did not isolate the coxiella from blood and only rarely from the urine. He surmised that the secondary fever was a side effect of the immune response ('inflammation'). More recently Musso and Raoult[22] in contrast with Derrick's findings and using shell vial cell culture had a low rate (17%) positive cultures from untreated acute Q fever before treatment. This also suggests a short 'window' of high titre bacteraemia after onset of illness.

Systematic SCID mouse and cell culture, and PCR examination of optimally collected and tested samples—taken at intervals beyond 10–12 days from onset of acute illness well into convalescence—are now required to document the duration of bacteraemia and the point of divergence between infectivity, on the one hand, and continuing positive assays for DNA sequences and antigens on the other.

Nevertheless strong hints remain that in general, levels of infective coxiella cells detected by culture of blood or tissues probably decline rapidly after an initial uncomplicated acute Q fever, apart from those patients who progress to subacute endocarditis, chronic granuloma or later recrudesce at parturition. [Such a sequence is illustrated e.g. by unpublished, systematic studies by Lennette's group in Berkeley, CA, 1951–53. They found that sheep inoculated with *C. burnetii* in early pregnancy

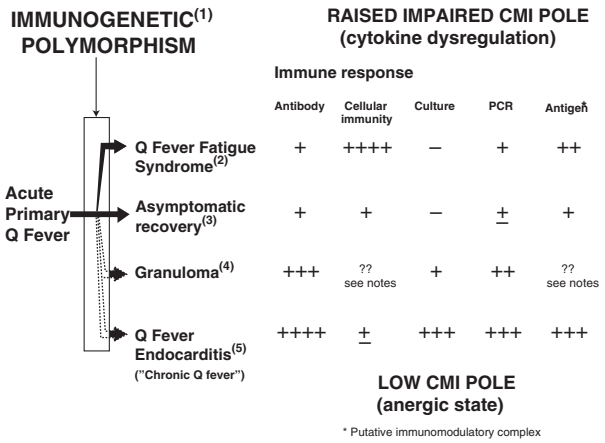

Figure 2. Provisional paradigm for further investigation of *C. burnetii* host–pathogen interactions (There is substantial variation between patients in antibody and CMI responses and also in assay sensitivity in different laboratories. This should be taken into account in viewing illustrative ranges of values given in Notes below).

initially had infective coxiellas (by hamster inoculation) in most organs through the body except in the developing placenta and fetus. After Week 13 the dispersed sites became non-infective. In contrast, placenta and fetus only became infective just before parturition at 19–20 weeks (cited[10])].

The termination of the bacteraemia appears to be mediated by the developing adaptive CMI and antibody responses—nowadays probably assisted by antibiotic therapy. Apoptotic changes are induced in infected host macrophages and other cells via CMI and cytokine responses of TNFα and IFNγ[23] to *C. burnetii* epitopes presented on the infected macrophage surface.

The delayed onset of the adaptive CMI response so long after initial infection requires explanation. The incubation period of Q fever is around 3 weeks depending on the infective dose. Yet during this period it appears that the coxiella is able to multiply, initially in alveolar macrophages, and then spread through the body to other cells essentially unchecked by the repertoire of innate immune responses (latter as summarized by Lydyard and Gross.[24]). *Coxiella burnetii* is poorly cytolytic in *in vitro* cell culture and forms phagolysosome-bounded microcolonies taking up much of the cell cytoplasm. It may be that the apoptotic changes induced by the adaptive CMI serve to liberate the nascent coxiellas from the infected host cells, signalling the onset of the bacteraemia and febrile phase of the clinical disease as a by-product of the cellular immune responses.

What survives after the bacteraemia?

The residua from the containment of the infection by killing the coxiella-infected host cell appear to include incompletely biodegraded non-replicating coxiella cells with small amounts of DNA sequences. Presumably the acid hydrolases of activated macrophages cut the coxiella genomic DNA yet leaves fragments still detectable by PCR with closely spaced primers. At present it is uncertain whether the different targets (IS*1111*a, COM1, 16S rRNA) detected by our assays are together on the same coxiella genome, or on subgenomic fragments from different coxiellas. It is also possible that some DNA sequences—e.g. insertion sequence genes (IS*1111*a)—present in variable copy numbers—are more sensitive over time to host nuclease degradation than constituent genes such as COM1 (cf. low rate of IS*1111*a reactors compared with COM1 reactors in Birmingham outbreak samples compared with those from the cohort of Australian patients sampled earlier after infection).

Electron microscopy[25] and Patient #2 Figure 1, suggests both large cell variants (LCV) and small cell variants (SCV) may survive as morphological entities, the latter presumably retaining their LPS and protein antigens. The outcome is most easily seen in endocarditis valves where the remains of microcolonies can be visualized in electron micrographs, by Giemsa, Macchiavello or by IFA staining as reported by numerous authors in the 1950s and 1960s [summary,[11,26,27] and by PCR and IFA (Figure 1A and D)].

The elimination of the coxiellas as infective entities may vary in efficiency depending on the polymorphism of the individual host's cellular immunogenetic repertoire[8,9] and Figure 2. In subacute endocarditis and some granulomatous complications living coxiella survive but in a lower proportion of the total number of coxiellas visualized by other means. For example in the prototype florid early case described by Andrews and Marmion[12] aortic valve vegetations titrated to 10^{-6} in guinea pigs but the morphological material detected by Giemsa stains indicated more numerous extracellular coxiella. In late stage endocarditis cases, the discrepancy widens between numbers of coxiellas detected by animal inoculation or cell culture and those detected by PCR or histological staining[27] and Figure 1A and D, Patients 1 and 2).

In the great majority (>80%) of patients with acute Q fever there is a symptom-free recovery with homeostatic control of the level of antigenic stimulation from the postulated persisting undegraded coxiella cell debris. The covert stimulation is signalled only by long lasting or slowly declining

antibody levels and the heightened cellular immunity detected in humans by positive intradermal skin tests in pre-vaccination 'screen' tests,[28] or in lymphocyte stimulation assays with PBMC and *C. burnetii* antigen.[29]

In a small minority (10–15%) of patients with a different (partly defined) immunogenetic background,[8,9] who develop post QFS it appears that there is a failure of control of the continuing CMI response to the persistent antigens with development of raised, impaired cell mediated and cytokine responses (IL-6 in particular) on stimulation of PBMC in short term culture with Q fever antigens;[7] see also Figure 2.

Survival and replication of *C. burnetii* in the macrophage

Factors influencing survival are (i) the highly bactericidal environment of the phagolysosome in an activated macrophage (ii) innate and adaptive immune responses, particularly in the cell-mediated compartment (iii) polymorphism in host immunological control and response genes. In relation to (i), recent studies[30–32] of the interaction of live *C. burnetii* with macrophage lineage cells in short-term culture revealed the profound modifications of macrophage function coded by the coxiella, which completes its whole life cycle in the phagolysosome[30] and whose enzyme systems operate optimally at the pH of phagolysosome.[33–35] *Inter alia*, the normal autophagic functions of the macrophage are subverted to provide metabolic precursors for the synthesis and replication of the coxiella LCV. Expression of Type-IV secretion proteins from *C. burnetii* genes,[32] analogous to virB of Brucella and Icm/Dot of *Legionella* spp., modifies and enlarges the phagolysosome to a favourable environment for growth—the so-called parasitophorous vesicle. Inhibition of host cell apoptosis by coxiella gene product is another recently identified survival mechanism.[36]

However, even given these remarkable diversions of macrophage function in models *in vitro*, in the whole animal innate immune mechanisms and the developing adaptive CMI response remain as barriers to coxiella survival and replication. The LCV, the putative replicating unit, lacks the robust cell wall of the SCV and has an open membrane structure, presumably evolved to allow intake of metabolic precursors from the autophagic products of the macrophage, and a nuclear configuration to allow DNA transcription. It might be expected that its genomic DNA and ribosomes would be highly vulnerable to the enzymes and reactive radicals in the phagolysosome of a macrophage activated by IFNγ—e.g. from NK cells of the innate immunity or later from the adaptive CMI response. But as already noted, the fact that multiplication occurs during the long incubation period indicates a highly effective down-regulation of at least the mechanisms of innate immunity until fever signals the onset of illness and the increasing dominance of the adaptive immune response.

Many facets of modification of macrophage function by *C. burnetii* have also been explored by Mege and colleagues (Rickettsial Unit, University of Marseilles). These have covered cytokine responses in acute Q fever, in subacute Q fever endocarditis and the interactions of *C. burnetii* with monocytes/macrophages *ex vivo* from healthy persons, Q fever endocarditis patients or, *in vitro,* with a continuous macrophage cell line (TPH1)[37–49]: see also notes for Figure 2). Aspects pertinent to the present review are (i) coxiella LPS stimulates TNFα production by human monocytes via cell receptor alpha (v) β3 integrin. (ii) IL-10, but not TGFβ, down-regulates TNFα production allowing *C. burnetii* survival and replication in monocytes. (iii) viable *C. burnetii* survives longer in monocytes from Q fever endocarditis patients than in monocytes from healthy uninfected persons—see also below. (vii) *C. burnetii* LPS is involved in phagocytosis by macrophages, reorganization of actin filaments and inflammatory responses via Toll-like receptor 4. Overall these studies highlight the pivotal role of *C. burnetii* LPS in modulation of macrophage functions. Note also the central role of Phase 1 LPS as a protective epitope in Q fever vaccine for generation of immunity to block macrophage down-regulation.[50]

Coxiella burnetii LPS–protein complex as a modulator of macrophage function

We advance a concept of immunologically mediated disease from impaired function of the various cells in the monocyte/macrophage or dendritic lineage from carriage of incompletely degraded coxiella cell components with Phase 1 LPS as an alternative additional regulator to persisting viable coxiella cells; a postulate foreshadowed by some previous reports viz.

In the 1980s, Schramek *et al.*[51] studied LPS from a killed (1% phenol) suspension of *C. burnetii* Nine Mile strain in Phase 1. After chloroform–methanol treatment the LPS was extracted from the coxella by the phenol–water method. Analysis of the purified LPS revealed two unusual 3-carbon-branched sugars designated virenose and dihydro-hydroxystreptose, respectively, linked into the LPS in the pyranose and furanose configurations. Methylation analysis suggested that the two sugar units occupied terminal

positions in the LPS chain. Mild acid hydrolysis of the LPS with 1% acetic acid progressively decreased specific Phase 1 antigen potency in coating sheep erythrocytes exposed to *C. burnetii* Phase 1 antiserum in a passive haemolysis system, while gradually revealing activity with the underlying lipid A with antiserum to *Salm.minnesota*. LPS from Phase 2 coxiella cells did not contain the unusual sugars. The two unusual sugars offer a possible basis for specific engagement with macrophage receptors e.g. via a complex with CD14-Toll receptor 2 or 4 and the expression of the cytokines, prostaglandins and other mediators that modulate macrophage and T cell activation.[52]

Koster *et al*.[53] recorded modulation of cellular immune responses by *C. burnetii* via a suppressor T-cell monocyte circuit involving prostaglandin inhibition of T lymphocyte function. They drew attention to the role of the mechanism in the anergy of Q fever endocarditis.[54] Significantly, the inhibition of cellular immunity was specific for *C. burnetii* as that to Candida spp. was not impaired. The specificity of the inhibition of Q fever immune responses appeared to originate from the coxiella–monocyte/macrophage interaction.

Izzo and Marmion,[55] in parallel observations, found that the interaction of separated monocytes and killed suspensions of *C. burnetii* with Phase 1 antigen generated immune mediators, including prostaglandin PGE2. The latter down-regulated IL-2 and IFNγ production by T lymphocytes. The inhibition of IFNγ was reversed by addition of a small unitage of IL-2 and also by Piroxicam, an inhibitor of prostaglandin synthesis. The immmodulation was not produced by coxiellas with Phase 2 antigen, or by a Phase 1 coxiella suspension treated with potassium periodate to open and ablate the sugar rings of the Phase I LPS 'O' chain, thereby creating an artificial Phase 2 antigen. In this context note earlier observations underlining the role of Phase 1 antigen as a protective immunogen by sero-prophylaxis in immunocompetent mice,[16] in challenge experiments in guinea pigs with inactivated Phases 1 and 2 vaccines.[28] Also experiments in mice with an inactivated Phase 1 *C. burnetii* vaccine that showed its protective effect in mice was removed by treatment with potassium periodate to modify the LPS sugar units.[50]

Waag and Williams[56] described 'an immunosuppressive complex' associated with *C. burnetii*. The immunomodulatory capacity of formalin-inactivated whole cell (wc) *C. burnetii* preparations and derived fractions was measured in endotoxin non-responder C57BL/10ScN mice inoculated with the test materials and killed 14 days later. Spleen weights were measured and 'nucleated spleen cells' were then assayed for their *in vitro* mitogenic responses to concanavalin A and pokeweed mitogen. Marked splenic enlargement and a (variable) suppression of mitogenesis were induced by inactivated coxiella Phase 1 wc but significantly less by Phase 2 cells.

Extraction of Phase 1 cells with chloroform-methanol produced a cell residue (CMR) retaining its LPS phase specificity, and a supernatant extract (CME). When the two fractions were recombined the inhibitory effects of the original wc preparation were reproduced. Pre-treatment of CMR cell component with potassium periodate destroyed the effect, as did lysozyme and neuraminidase to a lesser extent; lipase or proteinase were inactive. (The chemical basis of the effect of recombining CMR and CME is perhaps puzzling and does not suggest a chemical bonding. When taken with the ability of lipids such as cholesterol and cardiolipin to substitute for CME in restoring suppressive activity it may indicate enhanced presentation of hydrophilic LPS components as a mixed lipid micelle thereby increasing binding of critical epitopes to macrophage receptors—see role of 'auxiliary lipids': Rapport.[57]).

Dellacasagrande *et al*.[38] observed that monocytes from healthy control subjects held in culture were able to reduce the infectivity of a challenge dose of Phase 1 *C. burnetii* cells by 75% in 3 days whereas monocytes from active Q fever endocarditis patients did not alter infectivity levels of the challenge inoculum over 6 days. In this first stage of their experiment, the factor down-regulating activation of the endocarditis monocytes was not identified. The cells as taken from the endocarditis patients apparently did not contain infective coxiellas. The possibility remains that non-infective IMC was present and responsible for down-regulating activation and retarding destruction of the challenge dose of *C. burnetii*.

Finally, Shannon *et al*.[58] found that inactivated *C. burnetii* Phase1 but not Phase 2 coxiella cells inhibited maturation of macrophage-derived dendritic cells. Modification of Phase1 cells by removal of LPS layer with trichloroacetic acid removed the inhibition of dendritic maturation.

The IMC of *C. burnetii*

Overall, the above reports, and our current analysis and results, support the concept of an 'IMC' of an antigenic residue from coxiella cells that can persist in the mononuclear phagocyte cell department and in dendritic cells. Cells in the former lineage include e.g. fixed macrophages and promonocyte stages in the bone marrow, circulating monocytes and fixed tissue macrophages, and a range of specialized cells

such as alveolar macrophages, Kupffer cells, synovial A cells, microglia, osteoclasts and juxtaglomerular macrophage complex, etc.

The wide distribution in the body of such potential host cells able to take up IMC and be stimulated would facilitate delivery of cytokines and other biological modulators to the proximity of specialized organ parenchymal cells with other functions.

The long-term persistence of the IMC and the limited half life of mononuclear phagocytes seem to require that IMC are shed from pyknotic or apoptotic cells and recycled into fresh phagocytes. Note that non-infective, undegradable silica particles ingested by macrophages induce cytokine expression and apoptotic change: liberated particles are recycled through fresh macrophages: Subra *et al.*[59]

Remarkably, *C. burnetii* Phases 1 and 2 antigens were detected in Patients 12–17 years after Q fever in the Birmingham outbreak. Persistence for over 70 years is the longest period after infection known to us (see Box 2).

It postulated that although no longer infective, the residua of coxiella cells or subcellular elements are not finally degraded because they carry the Phase 1 LPS determinants that modify cellular immune function and that of macrophage and dendritic cells. The coxiella cell components constitute the IMC. However although in our experiments the material concentrated in SCID mouse spleens from clinical specimens reacted specifically with a polyvalent Q fever Phase1 or 2 antiserum and also with a monoclonal antibody to Phase 1 antigen (latter data not shown, O. Sukocheva *et al.*, unpublished data), its capacity to stimulate or down-regulate the cellular immune system also needs to be tested directly. This is being tested in various model systems. Thus far reactive lesions have been produced by the material in the skins of Q fever hyperimmune guinea pigs (assay model after Ascher *et al.*,[62,63] but not in unimmunized control animals (O. Sukocheva *et al.*, unpublished data). The regulatory effect on cytokine responses and immunosuppression provoked by the IMC is also under investigation.

Conclusions: implications of IMC for *C. burnetii*–host interactions

For acute and chronic Q fever and for Q fever fatigue syndrome

For example, in QFS it identifies a mechanism for the maintenance of antibody and cell-mediated immunity long after an acute attack and in the absence of demonstrable viable organisms. The pattern of systemic symptoms in QFS may reflect the wide distribution of parasitized mononuclear phagocytes. The common—but not invariable—lack of response of QFS to broad-spectrum antibiotics, effective against living organisms in the early infective bacteremic stage of the acute illness becomes understandable. The finding that QFS follows a clinically overt infection (heavy seeding), but rarely that of a subclinical infection is also illuminated.

The matter of infectivity, antigen persistence, IMC and its relation to QFS need to be integrated into a broader paradigm for the coxiella–host interaction in other chronic sequelae, in particular Q fever endocarditis. The broad elements of a suggested paradigm are shown in Figure 2 and associated notes (Box 3). The paradigm also tallies with current, evolving views on levels of macrophage differentiation and differences in Type 1 and Type 2 cytokine-polarized macrophages—so called M1 and M2 states.[69] It includes the concept that the polarity of the response is influenced by the immunogenetic background of the host in addition to the numerous functions specified by the coxiella.

Implications for other intracellular bacterial infections

The concept of an IMC may have application to a range of facultative or obligate intracellular bacteria that interact with the macrophage and are associated with prolonged post-infection fatigue syndromes. Some examples are Brucella spp., *Chlamydophilia pneumoniae*, Rickettsias.[64,65] Additionally, disease states with immunologically mediated tissue reactions suspected to follow an infection but with either few or no demonstrable bacteria (e.g. Crohn disease; Rheumatoid arthritis: Sarcoidosis) might repay investigation for 'candidate' prokaryotic antigens in addition to a search for their infective cells and specific nucleic acid sequences.

Acknowledgements

Animal experimentation followed protocols agreed with the IMVS Animal Ethics Committee. All inoculated animals were held in Level 3 biocontainment accommodation. Grateful acknowledgement is made of assistance from Dr T Kuchel and staff of the SA Pathology Biocontainment level 3 Suite; Dr John Stenos of ARRL; Mrs Jayne Groves Heartlands Hospital Birmingham UK for co-ordinating sample collection in the UK. And to Drs Jonah Smith and Rajiv Jayasena CSL Ltd. Melbourne Vic for reagents.

Box 2

Professor Robin Cooke (Brisbane[60] during a historical study of Derrick's laboratory records, met a survivor—JJ, Case 5—of the group of eight acute primary Q fever patients originally described by Derrick.[61] Mr J.J., a part-time abattoir worker, had primary Q fever in May 1936. He made a slow recovery from his acute Q fever with loss of ~2 stone in weight and with joint and muscle pains for many months and at intervals since. In October1989, after a cholecystectomy, he was found to have anaemia and neutropenia. A bone marrow trephine examination showed decreased cellularity and an increased proportion of atypical lymphocytes. His spleen was removed in January 1991 and showed little abnormality except a few areas of subendothelial lymphocyte accumulation. A definitive diagnosis was not reached. A TURP operation for prostatic enlargement was performed in mid 2007. Wax embedded blocks of spleen and prostatic tissues were PCR positive for *C. burnetii* genomic sequences and for *C. burnetii* antigen. A serological test in 2001 showed by IFA on coxiella microdots: IgG antibody to *C. burnetii* Ag Phase 1: 160; Phase 2: 640; IgM Phases 1 and 2: <10; IgA Phase 1: 640; Phase 2: <10.

Box 3 Notes on Figure 2

[1] For differences in HLA DR types and other genes in QFS, asymptomatic Q fever, Q fever endocarditis and in control population panels, see Helbig *et al.*[8,9]

[2] **QFS** (a) IF antibody ranges. IgG; Ph 1: 10→80. Ph 2: 40→1280. Ph 1/2: IgM/A < 10. see Marmion *et al.*[11]
(b) CMI; raised IL-6: reduced IL-2, IFNγ variable, IL-10 reduced. -see Penttila *et al.*[7]

[3] **Asymptomatic convalesents**
(a) IF antibody ranges; IgG: Ph 1, 10→160:Ph 2, 10→640: IgM/A <10: -see Marmion *et al.*[11]
(b) CMI; cytokine release patterns IL-2, IFNγ and Il-6 values significantly lower than in QFS ($P < 0.01$) see Penttila *et al.*[7]

[4] **Granuloma**
(a) IF antibody ranges; IgG: Ph 1, 500→2000+: Ph 2, 1000→10 000+; IgM Ph 1, 500→2000. Ph 2: 1000→10 000+. IgA Ph 1: < 10, Ph 2, 10→40. Limited data. Examples from Peacock *et al.*[66] Nourse *et al.*[67]and AQRG unpublished.
(b) CMI data on *in vitro* cytokine release patterns not available. Tissue pathology patterns suggest intense CMI reaction.

[5] **Q fever Endocarditis**
Early stage
(a) IF antibody ranges[a]. IgG: Ph 1, 5000→10^5; Ph 2, 5000→10^7. IgM Ph1/2: < 10→500[a]:IgA Ph1: 500→5000: Ph2: 10→3000.
(b) CMI: note poor cytological reaction in valve tissue and the evidence of down regulation of CMI (Koster *et al.*[54] Dellacasagrande *et al.*[38] Honstette *et al.*[45] Lepedi *et al.*[12,27,68]).
Late stage 'Burnt out' *cases*. In contrast to the early florid cases above, late cases with fibrosed, calcified valves may have IFA IgG Ph 1 and 2 antibody in the range 100→500 although *C. burnetii* may sometimes be isolated and the material is PCR and antigen positive.[16]

[a]Rheumatoid factor, prozone effect and differences in test sensitivity between laboratories influences titres and Ig class reactions. Values given are relative and illustrative only.

Funding

Financial support was provided by the Mason Foundation for CFS and Alzheimer Disease, by the Alison Hunter Foundation for ME/CFS and for several years by Meat and Livestock LTD Australia.

Conflict of interest: None declared.

References

1. Marmion BP, Shannon M, Maddocks I, Storm PA, Pentilla IA. Protracted debility and fatigue after Q fever. *Lancet* 1996; **347**:977–8.
2. Ayres JG, Flint N, Smith EG, Tunnicliffe WS, Fletcher TJ, Hammon K, *et al.* Post infection fatigue syndrome following acute Q fever; follow-up study of patients involved in the

1989 outbreak in the West Midlands. *Q J Med* 1998; **91**:105–23.

3. Hatchette TF, Hayes M, Merry H, Schlech WF, Marrie TJ. The effect of *C. burnetii* infection on the quality of life of patients following an outbreak of Q fever. *Epidemiol Infect* 2003; **130**:491–5.
4. Leung-Shea C, Danaher PJ. Q fever in members of the United States Armed Forces returning from Iraq. *Clin Infect Dis* 2006; **43**:77–82.
5. Ledina D, Bradaric N, Milas I, Ivic I, Brncic N, Kuzmicic N. Chronic fatigue syndrome after Q fever. *Med Sci Monit* 2007; **13**:CS8–92.
6. Arashima Y, Kato K, Komiya T, Kumasaka, Murakami M, Takahashi K, *et al.* Improvement of chronic non-specific symptoms by long term monocycline treatment in Japanese patients with *Coxiella burnetii* infection considered to have post infection fatigue syndrome. *Intern Med* 2004; **43**:49–54.
7. Pentilla IA, Harris RJ, Storm PA, Haynes D, Worswick DA, Marmion BP. Cytokine dysregulation in the post Q fever fatigue syndrome. *Q J Med* 1998; **91**:549–66.
8. Helbig KJ, Heatley SL, Harris RJ, Mullinghan PG, Bardy PG, Marmion BP. Variation in immune response genes and chronic Q fever. Concepts: preliminary test with post Q fever fatigue syndrome. *Genes and Immun* 2003; **4**:82–5.
9. Helbig K, Harris RJ, Ayres J, Dunckley H, Lloyd A, Robson J, *et al.* Immune response genes in the post Q fever fatigue syndrome, Q fever endocarditis and uncomplicated acute primary Q fever. *Q J Med* 2005; **98**:565–74.
10. Harris RJ, Storm PA, Lloyd A, Arens M, Marmion BP. Long term persistence of *Coxiella burnetii* in the host after primary Q fever. *Epidemiol Infect* 2000; **124**:543–9.
11. Marmion BP, Storm PA, Ayres JG, Semendric L, Matthews L, Winslow W, *et al.* Long-term persistence of *Coxiella burnetii* after acute primary Q fever. *Q J Med* 2005; **98**:7–20.
12. Andrews PS, Marmion PB. Chronic Q fever 2 morbid anatomical and bacteriological findings in a patient with endocarditis. *Brit Med J* 1959; **11**:983–8.
13. Hawker JI, Ayres JG, Blair I, Evans MR, *et al.* (14 authors). A large Q fever outbreak in the West Midlands: wind borne spread into a metropolitan area? *Common Dis Pub Health* 1989; **1**:180–7.
14. Raoult D, Vestris G, Enea M. Isolation of 16 strains of *Coxiella burnetii* from patients using a sensitive cell culture system and establishment of strains in HEL cells. *J Clin Microbiol* 1990; **28**:2482–4.
15. Andoh M, Nagauawa T, Holta A, Yamaguchi T, Fukushi H, Masegi T, *et al.* SCID mouse model for lethal Q fever. *Infec Immun* 2003; **71**:4717–32.
16. Abinanti FR, Marmion BP. Protective or neutralising antibody in Q fever. *Am J Hyg* 1957; **66**:173–95.
17. Kishimoto RA, Rozmiarek H, Larson EW. Experimental Q fever in congenitally athymic mice. *Infect Immun* 1978; **22**:69–71.
18. Syrucek L, Sobeslavsky O, Gutwirth I. Isolation of *Coxiella burnetii* from human placentas. *J Hyd Epidemiol Microbiol Immunol* 1958; **2**:29–35.
19. Derrick EH. The course of infection with *Coxiella burnetii*. *Med J Aust* 1973; **1**:1051.
20. Nagaoka H, Akltama M, Susieda M, Nishio T, Hattor H, Ho T, *et al.* Isolation of *Coxiella burnetii* from children with influenza-like sysmptoms in Japan. *Microbiol Immunol* 1996; **40**:147–51.
21. Turra M, Chang G, Whybrow D, Higgins G, Qiao M. Diagnosis of acute Q fever by PCR on sera during a recent outbreak in rural South Australia. *Ann NY Acad Sci* 2006; **1078**:566–9.
22. Musso D, Raoult D. *Coxiella burnetii* blood cultures from acute and chronic Q fever patients. *J Clin Microbiol* 1995; **33**:3129–32.
23. Dellacasagrande J, Capo C, Raoult D, Mege J-L. IFNγ mediated control of *Coxiella burnetii* survival in monocytes: role of cell apoptosis and TNF. *J Immunol* 1999; **162**: 2259–65.
24. Lydyard PM, Gross CE. Cells, Tissues and Organs of the Immune System. In: Roit I, Brostoff J, Male D. eds. Immunology 6th edn, Chapter 2. London, Mosby 2001; 15–21.
25. McCaul TF, Dale AJ, Gannon JP, Galbraith AJ. *In vivo* endogenous spore formation by *Coxiella burnetii* in Q fever endocarditis. *J Clin Pathol* 1994; **4**:978.
26. Mitchell R, Grist NR, Bazaz G, Kenmuir ACF. Pathological, rickettsiological and immunofluorescence studies of Q fever endocarditis. *J Path Bact* 1966; **91**:317–23.
27. Marmion BP, Higgins F, Bridges JB, Edwards AT. A case of subacute rickettsial endocarditis with a survey of cardiac patients for this infection. *Brit Med J* 1960; **ii**:1264.
28. Ormsbee RA, Marmion BP. Prevention of *Coxiella burnetii* infection: vaccines and guidelines for those at risk. In: Marrie T, ed. *Q fever 1 The Disease*. Ch 12. Baton Rouge Flo., CRC Press, 1990:225–48.
29. Izzo AA, Marmion BP, Worswick DA. Markers of cell mediated immunity after vaccination with an inactivated whole-cell Q fever vaccine. *J Infect Dis* 1988; **157**:781–9.
30. Voth DE, Heinzen RA. Lounging in a lysosome: the intracellular lifestyle of *Coxiella burnetii*. *Cell Microbiol* 2007; **9**:829–40.
31. Romano PS, Gutterez MG, Beron W, Rabinovitch M, Colomb MI. The autophagic pathway is actively modulated by phase II *Coxiella burnetii* to efficiently replicate in the host. *Cellular Microbiol* 2007; **9**:891–909.
32. Zamboni DS, McGrath S, Rabinovitch M, Roy CR. *Coxiella burnetii* express type IV secretion system proteins that function similarly to components of the *Legionella pneumophilia* Dot/1 cm system. *Mol Microbiol* 2003; **49**:11965–76.
33. Ormsbee R, Peacock M. Metabolic activity in *Coxiella burnetii*. *J Bacteriol* 1964; **88**:1205–10.
34. Hackstadt T, Williams JC. Biochemical stratagem for obligate parasitism of eukaryotic by Coxiella burnetii. *Proc Soc Natl USA* 1981; **78**:3240–4.
35. Maurin M, Benoliel AM, Bongrand P, Raoult D. Phagolysomes of Coxiella burnetii-infected cell lines maintain an acid pH during persistent infection. *Infect Immun* 1992; **60**:5013–6.
36. Voth DE, Heinzen RA. Sustained activation of Akt and Erkn $\frac{1}{2}$ is required for *Coxiella burnetii* anti-apoptotic activity. *Infect Immun* 2009; **77**:205–13.
37. Dellacasagrande J, Ghigo E, Hammami SM, Toman R, Raoult D, Capo C, *et al.* Alpha (v) beta 3 integrin and bacterial lipopolysaccaride are involved in *Coxiella*

burnetii—stimulated production of tumour necrosis factor by human monocytes. *Infect Immun* 2001; **68**:5673–8.

38. Dellacasagrande J, Ghigo E, Capo C, Raoult D, Mege JL. *Coxiella burnetii* survives in monocytes from patients with Q fever endocarditis: involvement of tumour necrosis factor. *Infect Immun* 2000; **68**:160–4.
39. Ghigo E, Capo C, Raoult D, Mege JL. Interleukin 10 stimulates *Coxiella burnetii* replication in human monocytes through tumour necrosis factor down-regulation: role in microbicidal defect of Q fever. *Infect Immun* 2001; **69**:2345–52.
40. Ghigo E, Capo C, Tung CH, Raoult D, Gorvel JP, Mege JL. *Coxiella burnetii* survival in TP1 monocytes involves impairment of phagosome maturation. INF gamma mediates its restoration and bacterial killing. *J Immunol* 2002; **169**:4488–95.
41. Ghigo E, Imbert G, Capo C, Raoult D, Mege JL. Interleukin 4 induces *Coxiella burnetii* replication in human monocytes but not in macrophages. *Ann NY Acad Sci* 2003; **990**:450–9.
42. Ghigo E, Capo C, Tung CH, Raoult D, Gorvel JP, Mege JL. *Coxiella burnetii* survival in IPE monocytes involves impairment of phagsome maturation. INF gamma mediates its restoration and bacterial killing. *J Immunol* 2002; **169**:4488.
43. Dellacasagrande J, Ghigo E, Raoult D, Capo C, Mege JL. IFN-gamma induced apoptosis and microbiocidal TNF and homotypic cell adherence. *J Immunol* 2002; **169**:6309–15.
44. Ghigo E, Imbert G, Capo C, Raoult D, MegeJ-L. Interleukin 4 induces *Coxiella burnetii* replication in human monocytes but not in macrophages. *Ann NY Acad Sci* 2003; **990**: 450–409.
45. Honstettre A, Imbert G, Ghigo E, Gouriet F, Capo C, Raoult D, *et al.* Dysregulation of cytokines in acute Q fever: role of interleukin-10 and tumour necrosis factor in chronic evolution of Q fever. *J Infect Dis* 2003; **187**:956–62.
46. Honstette A, Ghigo E, Moyault A, Capo C, Toman R, Akira S, *et al*. Lipopolysaccharide from *Coxiella burnetii* is involved in bacterial phagocytosis, filamentous actin reoganisation and inflammatory responses through Toll-like receptor 4. *J Immunol* 2004; **172**:3695–703.
47. Ghigo E, Honstette A, Capo C, Gorvel JP, Raoult D, Mege JL. Link between impaired maturation of phagosomes and defective *Coxiella burnetii* killing in patients with chronic Q fever. *J Infect Dis* 2004; **190**:1767–72.
48. Honstette A, Megharis S, Nomes JA, Lepidi H, Raoult D, Olive D, *et al.* Role for CD28 molecule in control of *Coxiella burnetii* infection. *Infect Immun* 2006; **74**:1800–8.
49. Benoit M, Ghigo E, Capo C, Raoult D, Mege JL. The uptake of apoptotic cells drives *Coxiella burnetii* replication and macrophage polarization: a model for Q fever endocarditis. *J Immunol* 2008; **181**:3733–99.
50. Marmion BP. Q fever: the long journey to control by vaccination. *Med J Aust* 2007; **186**:164–6.
51. Schramek S, Radziejewska-Lebrecht J, Mayer H. 3-C branched aldoses in lipopolysaccharide of Phase I *Coxiella burnetii* and their role as immunodominant factors. *Eur J Biochem* 1985; **148**:455–61.
52. Zamboni DS, Campos MA, Torrechilhas AC, Kiss K, Samuel JE, Golenbrock DT, *et al.* Stimulation of Toll-like receptor 2 by *Coxiella burnetii* is required for macrophage production of pro-inflammatory cytokines and resistance to infection. *J Biol Chem* 2004; **279**:54405–15.
53. Koster FT, Williams JC, Goodwin JS. Cellular immunity in Q fever: modulation of responsiveness by a suppressor T cell-monocyte circuit. *J Immunol* 1985; **135**:1067–72.
54. Koster T, Williams JC, Goodwin JS. Cellular immunity in Q fever: specific lymphocyte unresponsive in Q fever endocarditis. *J Infect Dis* 1985; **152**:1283–9.
55. Izzo AA, Marmion BP. Variation in interferon gamma responses to *Coxiella burnetii* antigen with lymphocytes from vaccinated or naturally infected subjects. *Clin Exp Immunol* 1993; **94**:507–15.
56. Waag DM, Williams JC. Immune modulation by *Coxiella burnetii*: characterisation of a Phase I immunosuppressive complex differentially expressed among strains. *Immunopharmacol Immunotoxicol* 1988; **10**:231–60.
57. Rapport MM. Structure and specificity of the lipid haptens of animal cells. *J Lipid Res* 0000; **101**:25.
58. Shannon JG, Howe D, Heinzen RA. Virulent *Coxiella burnetii* does not activate human dendritic cells: role of lipopolysaccharide as a shielding molecule. *Proc Natl Acad Sci USA* 2005; **102**:8722–7.
59. Subra JF, Renier G, Tollis F, Boivinet R, Schwartz P, Chevailler A. Lymphopenia in occupational pulmonary silicosis with or without autoimmune disease. *Clin Exp Immunol* 2001; **126**:540–4.
60. Cooke RA. Q fever. Was Edward Derrick's contribution under valued? *Med J Aust* 2008; **189**:800–82.
61. Derrick EH. ''Q'' fever, a new fever entity: clinical features, diagnosis and laboratory investigation. *Med J Aust* 1937; **2**:281–99.
62. Ascher MS, Berman MA, Parker D, Turk JL. Experimental model for dermal granulomatous hypersensitivity in Q fever. *Infect Immun* 1983; **39**:388–93.
63. Asher MS, Williams JC, Berman MA. Dermal granulomatous hypersensitivity in Q fever: comparative studies of the granulomatous potential of whole cells of *Coxiella burnetii* Phase I and subfractions. *Infect Immun* 1983; **42**:887–9.
64. Unsworth N, Graves S, Nguyen C, Kemp G, Graham J, Stenos J. Markers of exposure to spotted fever rickettsiae in patients with chronic illness, including fatigue, in two Australian populations. *Q J Med* 2008; **101**:269–74.
65. Watts M, Benn R, Hudson B, Graves S. A case of prolonged fatigue following an acute rickettsial infection. *Q J Med* 2008; **101**:591–3.
66. Peacock MG, Philip RN, Williams JC, Faulkner RS. Serological evaluation of Q fever in humans: enhanced Phase 1 titers of immiunoglobulin G and A are diagnostic for G fever endocarditis. *Infect Immun* 1983; **41**:1089–98.
67. Nourse C, Allworth A, Jones A, Horvath R, Mc Cormack J, Bartlett J, *et al.* Three cases of Q fever osteomyelitis in children and a review of the literature. *Clin Infect Dis* 2004; **39**:61–6.
68. Lepedi H, Houpikian P, Liang Z, Raoult D. Cardiac valves in patients with Q fever endocarditis; microbiological molecular and histologic studies. *J Infect Dis* 2003; **187**:1097–106.
69. Celada A, Grage-Griebennow E, Kayasu S Leibermann DA, Steiniger B. Macrophage differentiation. *Trends Immunology* UK, Ellsevier Science Ltd, 2002.