ISSN: 3032-3134 (ONLINE)
InaJBCS Indonesian Journal of Biomedicine and Clinical Sciences

Potential biomarkers of IFN-γ, IL-2 and CXCL9 for diagnosis of Q fever disease

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https://doi.org/10.22146/inajbcs.v56i2.12991

Submitted: 2022-09-08
Accepted : 2023-07-07

ABSTRACT

The pathogen responsible for Q fever disease, *Coxiella burnetii*, is a zoonosis classified as a pathogen due to its airborne transmission. The *C. burnetii* infection could be both acute or chronic in humans. The main and most common entry of the pathogens to the body is through the breathing of polluted aerosols containing a resistant substance similar to *C. burnetii* spores. This small cell variant (SCV) or spore-like morphotype is extremely stress-resistant, therefore inadequate treatment causes serious effects even death. Due to the diversity of clinical manifestations of Q fever and the presence of less specific and sensitive diagnoses for other diseases, multiple platforms for exploring Q fever biomarkers are required. Apart from serological studies to determine a biomarker for Q fever, it will be prudent to concentrate on the more appropriate cell-mediated immune response. This article discusses *C. burnetii* causing *Q fever* disease and how the host develops humoral and cellular immunity, particularly IFN-γ, IL-2 and CXCL9, as potential biomarkers for the diagnosis of *Q fever* disease.

ABSTRACT

Coxiella burnetii penyebab penyakit Q fever merupakan zoonosis yang termasuk sebagai pathogen yang penularannya lewat udara. Infeksi *C. burnetii* dapat besifat akut atau kronis. Jalur utama dan umum masuknya patogen ke dalam tubuh adalah pernafasan aerosol yang terkontaminasi bahan resisten mirip spora *C. burnetii*. Varian sel kecil (*small cel variant*/SCV) atau morfotipe mirip spora ini sangat resisten sehingga pengobatan yang tidak tepat menyebabkan efek serius bahkan kematian. Manifestasi klinis Q fever yang bervariasi dan keberadaan diagnosis penyakit yang sudah ada masih kurang spesifik dan sensitif maka dibutuhkan berbagai platform untuk mengeksplorasi biomarker Q fever. Selain studi serologi sebagai biomarker Q fever, akan sangat bermanfaat juga berfokus pada respon imun yang dimediasi sel yang lebih relevan. Makalah ini memaparkan tentang *C. burnetii* sebagai agen penyebab Q fever dan bagaimana pejamu mengembangkan imunitas humoral dan seluler khususnya IFN-γ, IL-2 dan CXCL9, sebagai biomarker potensial untuk diagnosis penyakit Q fever.

Keywords:
biomarker;
Coxiella burnetiid;
immune response;
Q fever

INTRODUCTION

Q fever is caused by the zoonotic agent *Coxiella burnetii,* a bacterium that spreads through aerosol transmission.[1] These pathogens are isolated or clustered cells or spores that adhere to particulate matter or dust. As a result, *C. burnetii* can adhere to dust particles, which plays a significant role in wind dispersal.[2,3] Inhalation becomes the primary route of infection via contaminated aerosols containing an environmentally resistant material that resembles *C. burnetii* spores.[4] This spore-like morphotype is also known as the small cell variant (SCV), exhibiting exceptional resistance to environmental stresses (heat, drying, UV rays).[5] *Coxiella burnetii* is classified as a type B agent by the Centers for Disease

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Control and Prevention (CDC) due to its resistance to environmental factors, low infection doses, human transmission via aerosol, and previous use as a bioweapon.[5,6]

Acute infection of *C. burnetii* in humans shows nonspecific clinical symptoms until pneumonia or hepatitis develops. The other clinical manifestation, characterized by persistent infection of the heart valve, prosthesis, or vascular aneurysm, was reported in 1 to 5% of the individuals with chronic infection of Q fever.[7] High mortality will occur if not treated properly.[8-10] Impaired health status in more than 30% of Q fever patients despite following the prescribed antibiotic regimen reported in one 24-month cohort study.[10,11] Because chronic Q fever infection can be fatal, early diagnosis of different stages of this disease is essential for the prevention of this condition.[12] Clinical application and diagnosis have included immunoglobulin G (IgG) phase 1 of *C. burnetii* phase I, C-reactive protein, 18F-FDG-PET/computerized tomography scan, and PCR antibodies.[13]

Several countries also reported outbreaks of Q fever, including the United States, Spain, Australia, Japan, and Israel exemplifying how widespread *C. burnetii* infection is worldwide.[14] However, with the world's largest outbreak of Q fever in the Netherlands,[7] it is necessary to identify and test biomarkers that can better discriminate against the different stages of Q fever infection.[13]

In another bacterial infection, *Mycobacterium tuberculosis*, measuring particular IL-2 production in addition to IFN-γ distinguishes between active and latent infection in tuberculosis. In this instance, active infection is characterized by high specific IFN- and low IL-2 production, while both high IFN- and high IL-2 production imply latent tuberculosis.[15,16] During active tuberculosis treatment, a change from T cells secreting only IFN- and IFN- /IL-2 to T cells secreting IFN- /IL-2 and only IL-2 has been documented.[17]

Measurement of specific antibodies is still the standard detection method for Q fever.[18] The detection approach through the measurement of T-lymphocyte immunity can be an alternative and provides advantages based on cellular immunity as a defense against these intracellular pathogens, including macrophage activation mediated by interferon-gamma (IFN-γ).[19] This paper describes *C. burnetii* causing Q fever diseases, how the immune system reacts to *C. burnetii* and identifies the immune system's response as potential biomarkers for enhancing diagnostic tools against *C. burnetii* infection.

DISCUSSION

Coxiella burnetii

Coxiella burnetii includes small, gram-negative bacteria, ranging from 0.2-0.4 μm in width and 0.4-1 μm in length, forming a *coccobacillus*. Originally classified in the order *Rickettsiales* because of their presence in ticks and intracellular morphology.[20] It is stated that *C. burnetii* can last up to 10 mo at temperature 15 to 20°C, and if kept in the cold storage, can last more than one month in meat and at room temperature in skim milk could be stored for more than 40 mo.[3] Studies continue using experimental animals to determine virulence factors[21,22] and genetic mechanisms in LPS.[23] Phase I (nine-mile phase I/NM I) that cause Q fever in humans has complete and smooth lipopolysaccharide, very virulent and pathologically challenges laboratory animals. A virulent called Phase II, or nine-mile phase II (NM II), does not have the O-side chain of LPS, is rough, and shows growth impairment in immunocompetent animals.[22,24]

Pathogenesis

In contrast to other intracellular obligate pathogens, *Coxiella's* stability in environmental stresses includes

enhancement of temperature, ultraviolet light, and osmotic pressure.[25] It has a biphasic development cycle with characteristics of small cell variant (SCV) forms, and the form that is more metabolically active and replicative than these organisms is the large cell variant forms (LCVs).[26] The main mode of infection in humans is inhalation of contaminated aerosols. *Coxiella* can replicate in the most inhospitable compartments of host cells, i.e., phagolysosomes, indicating its extracellular stability.[25] In the intracellular cycle of *C. burnetii*, after interacting with macrophages, *C. burnetii* is absorbed passively by binding to αvβ3 integrin with an actin-dependent phagocytosis process.[27] Newborn *Coxiella*-containing vacuole (CCV) will combine with the autophagosome after absorption, then mature. In addition to joining the cellular lysosomes, the mature CCV also lowers the pH to 4.5. It is stated that the transition from SCV to a metabolically active morphotype, namely, LCVs occurs after inhalation.[25,28]

Protein synthesis by *Coxiella* is required to start the initial interactions of autophagosome to promote the cessation of the VP that has matured. Type IV secretion system (T4SS) in *Coxiella* is the same as in *Legionella*, known as Dot/Icm T4SS system. The Dot/Icm of T4SS plays an important role in forming the *Legionella* vacuole replication, and T4SS is used for gram-negative pathogen advantage by translocating proteins that can modulate certain mechanisms in host bodies.[27,28] An acute Q fever pathological condition may progress to chronic Q fever, different tissues show granulomatous lesions in different varieties including, but not limited to, the lung, liver, and spleen are common in *C. burnetii infection.* In contrast, granuloma formation in the chronic condition of Q fever is declared reduced.[29]

Clinical symptom and detection

Conxiella burnetii is a highly contagious intracellular obligate γ-proteobacterium. Flu-like pneumonia often becomes an acute clinical manifestation in humans.[30] Other symptoms include dry cough, malaise, myalgia, fever, and chills, usually appears within 2-3 weeks of *C. burnetii* exposure.[31] Most cases typically resolve on their own; following primary infection, between 1% and 5% of patients develop a chronic condition of Q fever, the manifestation of which can occur several years after the initial infection.[7,32] The development of chronic condition Q fever is affected by risk factors including heart valve disease, aortic aneurysms, immune disorders, pregnancy, and vascular grafts.[33,34]

If left untreated, chronic Q fever will cause significant morbidity and mortality, up to 60% and requires long-term treatment with antibiotics such as hydroxychloroquine and doxycycline.[35] Diagnosis of chronic condition Q fever is still quite a challenging task. The difficulty of diagnosis is due to several things, including culturing *C. burnetii*; which takes time, requires a laboratory with security level 3, and less sensitive.[36] Currently, with samples from blood or tissue, the polymerase chain reaction (PCR) is still a potential tool for serology and detection of DNA is used to confirm the chronic condition of Q fever. Detection of *C. burnetii* DNA in the sample from tissue or blood by PCR without acute infection was expressed as chronic Q fever although the sensitivity of this technique is low.[33]

The antigenic variation of *C. burnetii* forms the basis of serological diagnosis.[37] The first detected in the time of acute infection is antibodies related to phase II antigen, then the antibodies against phase I antigen.[37] High levels of long-lasting antibodies to phase I and phase II antigens are regarded as an indicator of the chronic condition of Q fever, which is interpreted as the result of persistent antigen stimulation. The limit determination for IgG virulence (phase I) of serological from in-house immunofluorescence assay (IFA) is

≥1: 800; this value has been accepted internationally to chronic diagnosis condition of Q fever. However, this test yields a high number of false positives.[33]

Conxiella burnetii infection has significantly impacted the health of humans and domesticated livestock and the sustainability of human food.[1,5] This condition showed in the epidemic occurrence of acute cases of Q fever over four years (2007-2011) in the Netherlands, reported over 4,000 acute Q fever cases occurred.[1,7,34] Test consistency is critical with cases reported throughout the Netherlands as of 2007, during the large Q fever outbreak. Moreover, the result test interpretation also impacts on improving patient care.[33] Because there is still much uncertainty in the algorithms used by diagnostic tools for the chronic condition of Q fever.[33] It is very important to carry out further research in the development of biomarkers that can be used for early detection of Q fever.[13]

Host immune response against *C. burnetii* infection

Innate immune response

Neutrophils, monocytes, and macrophages

In a study conducted by Elliott *et al.*[38] neutrophils are initially recruited to the lungs during the early infection stage due to cytokine production from infected macrophages. That role can be seen from the decrease in neutrophils using the lowering antibody RB6-8C5, resulting in a more severe infection in mice.[38] The elimination of *C. burnetii* is associated with the formation of reactive oxygen species (ROS), followed by reactive nitrogen species (RNS) in collaboration with other immune cells via the production of cytokines.[39] Neutrophils also enhance the ability of macrophages in killing phagocytic bacteria. Both of these play a significant part in the elimination of this bacterium.[39] However, one study showed that the ability to inhibit protein transfer from this bacterium is needed in ROS production into CCV.[25]

It is interesting that *C. burnetii* targets not only monocytes but also macrophages.[24,40] A subversion mechanism from the microbicidal properties of these cells makes *C. burnetii* persistently reside and carry out intracellular trafficking. The drastic changes of the actin cytoskeleton, activation of the protein tyrosine kinase pathway, and cytokine production occur after this bacteria interaction through αvb3 integrins with macrophages and monocytes.[39] This functional polarization occurs as a result of monocyte/macrophage interaction with *C. burnetii*.[24,40] Currently, the resting phase of the monocyte, which this bacteria exist but does not multiplicate, results in the induction of M1 polarization. After being triggered in response to infectious agents or IFN-γ, these M1-polarized cells contribute to the host's microbicidal function.[24,40] The *C. burnetii* replication occurs in macrophages and induces M2 polarization-related gene expression. This leads us to the conclusion that *C. burnetii* controls its intracellular life by polarization M1 or M2-like phenotype of macrophages.[24,40]

Professional antigen presentation cells and TLRs and NOD-like receptors (NLRs)

Through a study, virulent types of this bacterium can replicate and infect human dendritic cells without inducing a response to inflammatory cytokines.[25] Differs from a virulent *C. burnetii* resulting in the induction of dendritic cells. This is related to the virulence properties of the *Coxiella's* complete virulent lipopolysaccharide (LPS) related to the ability of the molecule to protect the outer membrane.[25] In avirulent organisms, *C. burnetii* or phase II *C. burnetii* is easily eliminated through the complement system's membrane attack complex (MAC). In addition to inhibiting the interaction of *Coxiella*

with the CR3 receptor (αMβ2 integrin) from macrophages,[41] the *Coxiella* toll-like receptor (TLR) ligand is also masked by phase I LPS, so can't recognize this bacteria.[42] Chemically identical parts and molecules from LPS lipid in phase I and phase II apart not only fail to bind to TLR-4, but is also different to TLR-4 signalling by another LPS.[42]

In response to *C. burnetii* infection in humans, a study by Ammerdorffer *et al.*[42] demonstrated that stimulation of cytokines required receptor recognition occurs via heterodimers with NOD-like receptors as well as TLR1/TLR2.[42] The absorption process of *C. burnetii* bacteria during infection indicates that the ability of TLR2 receptors to form a heterodimer with either TLR1 or TLR6 greatly assists the function of TLR2 receptors compared to TLR4.[42] It also appears that the signalling of lipid A from *C. burnetii* via TLR2 is important in producing inflammatory cytokines.[42]

Adaptive immune response

Humoral response

The paradigm that protection against intracellular pathogens is solely cell-mediated has been challenged through research trials. In one experiment,[43] wild-type mice were protected from the challenge by passive transfer of antibodies to *C. burnetii*, and another study demonstrated that the antibodies act by neutralizing *C. burnetii*.[44] In acute infection, a potential role for B cells during the primary immune response is demonstrated. One study discovered that mice lacking B cells had more severe histopathological damage to their liver and spleen than mice with intact B cells.[43]

In laboratory experiments with *C. burnetii* phase I vaccination in cell-deficient mice showed no protection. However, the protection remained under other conditions even though the mice had $CD4^+$ T cell deficiency compared to wild-type mice. Under these conditions, we can see that IgM production, which can take place independently of T cells, mediates the protection. In contrast, IgG-mediated protection requires the role of $CD4^+$ T cells. As a result, humoral immunity appears to play a role; in the immunological response to this bacterial infection.[45]

According to Fournier *et al.* in Shannon, *et al.*[46] on acute Q fever, antibodies to phase II antigen acquire in less than 3 until 4 weeks of disease progression.[46] Acute Q fever is detected by combining anti-phase II antibodies with a low dose with anti-phase I antibodies directed primarily against LPS. If a patient has an anti-phase I titer greater than 800, they are diagnosed with chronic Q fever.[47] Given that nearly all effective vaccines are antibody-dependent, a better understanding of antibody-mediated immunity to *C. burnetii* will aid in vaccine development.[46]

Chemokines and cytokines

During the early phases of infection with intracellular pathogens, IFN-γ is an essential cytokine. In both phagocytic and non-phagocytic cells that are infected, IFN-γ interacts synergistically with bacterial products to trigger a range of bacteriostatic or bactericidal effector pathways.[48] The real presence of IFN-γ in eradicating intracellular infections was confirmed by antibody-mediated neutralization of IFN-γ *in vivo* and subsequent confirmation using mice with the IFN-γ knockout (KO) gene. The severity of infection with *C. burnetii* was elevated in these mutant mice.[49] TNF-α is also involved in the cytokines response to *C. burnetii* infection.[49] When IFN-γ and TNF-α are combined, macrophages and/or other target cells become more capable of controlling growth and/or killing certain intracellular organisms.[14,48] Although the contribution of the cytokine IL-10 in chronic infection with *C. burnetii* is not clear, data suggest an increase in IL-10 in patients with chronic infection.[29]

No less important mediators in the resistance of intracellular pathogens are chemokines. Early chemokine induction is not only necessary for recruiting professional antigen-presenting cells (APC), but it is also necessary for maintaining the primary role of specialist phagocytic cells and other target cells.[48] Through the defensin pathway, several chemokines that induce IFN-γ like (MIG or CXCL9), IFN-γ induced by IP-10 or CXCL10, a protein with a size of 10 kDa and IFN-γ induced chemoattractant T cells (CXCL11) can directly neutralize microbes.[48] Throughout BALB/c infections associated with *C. burnetii* during acute infection, they show high production of the specific antigen CXCL10. However, elevated CXCL10 is more likely to be a marker of inflammation than a specific marker of acute Q fever.[13]

Lymphocyte T CD4^{+} and CD8^{+}

The importance of $CD8^{+}$ and $CD4^{+}$ T cells in this bacterial infection was demonstrated clearly in a study,[50] in which both SCID and nude mice lacked T cells exhibiting severe infection that could be fatal. When $CD8^{+}$ T cells were combined with $CD4^{+}$ T cells or observed alternately in this study, they demonstrated a greater protective ability than $CD4^{+}$ T cells. This finding may be explained by lymphocyte T-$CD8^{+}$ ability to produce IFN-γ, a cytokine that $CD4^{+}$ Th1 T cells also produce.[45] Inactivated bacteria in phase I have been shown to elicit robust Th1 protection and response, although inactivated bacteria in phase II elicit a weak Th1 response.[51] This identical death rate documented in *C. burnetii* infections of the IFN-$\gamma^{-/-}$ mouse model demonstrates the critical role of IFN-γ in *C. burnetii* protection.[51]

Potential biomarkers

Humoral immune response

It is important to carry out further studies to identify the humoral response to *C. burnetii* with a powerful tool. A comprehensive study of the antigen profile using protein microarrays has been reported that new seroreactive antigens have been identified.[52] Through the capacity to determine specific antigens against specific IgM antibodies and subsequent IgG antibodies,[52] it is possible to determine better when a person is exposed to the pathogen, determine the progression of infection, and the therapeutic response. In the diagnosis of infectious diseases, detection of IgM needs to be done early.[52] A study conducted by Vigil *et al*.[52] used protein microarrays to profile the antibody repertoire generated in response to infection. It is important to detect IgM immune response in infectious disease early diagnosis. That IgM production will continue by the development of IgG antibodies, the ability to determine when an individual was exposed to a pathogen, and potentially the progression of infection and therapeutic response by selecting antigen-specific IgM antibodies and subsequent IgG antibodies. This study identified a significant reactive protein capable of discriminating between acute and chronic conditions of Q fever, namely the CBuK 1974 protein. CBuK 1974 is a 63-amino acid small protein discovered in the genome of *C. burnetii* strains isolated from people with endocarditis.[52]

It is stated in the literature study conducted by Kowalczewska *et al*.[53] that some proteins have been found by proteomic analysis as potential marker candidates and have been cross-validated. CBU 0952 (acute disease antigen A), CBU 0236 (elongation factor Tu/tuf-2), and CBU 0092 (tol-pal system protein/Ybgf) are proteins that have the potential to be a significant markers in acute Q fever conditions.[53] The protein rpoA (CBU_0263) and the universal stress family (CBU_1916) are potential markers for chronic Q fever.[53]

INF-γ and IL-2

Even though anti-phase I IgG has only a minor protective role against *C. burnetii*, it is still used to diagnose and monitor Q fever's chronic condition. Anti-phase I IgG is also used to detect infections and complications. It is a manifestation of the humoral response to specific B cells and CRP and IL-6-mediated inflammatory products.[13] However, with the dominance of the IFN-γ-mediated T-helper 1 response as a protective immune response against *C. burnetii*, the existing markers have not accurately reflected disease progression.[50]

It is critical to investigate whether IFN-γ is a cytokine that plays a critical role in determining whether chronic Q fever progresses from a previously cured infection. Measurement of other cytokines that also confirm IFN-γ is important to evaluate.[19] Type I interferon, IL-12, IL-18, and IL-23 were inhibited in the presence of IFN-γ.[54] When M2 macrophages are polarized in vitro, we observe increasing production of IL-10[29] and an increase in the regulation production of receptor antagonists IL-1 (IL-1Ra) and IL-6 as a decrease in the production of TNF-α.[19] Proliferation and development of lymphocytes in the memory response are also critical, as is the role of the IL-2 cytokine in the induction process.[55] To protect the host cell from *C. burnetii*, the combined effort of IFN-γ producing T cells and natural killer cells stimulates the microbicidal activity of the macrophage.[40]

Schoffelen *et al*.[19] demonstrated that an IFN-γ greater than eleven compared to IL-2 had a specificity of 96% and a sensitivity of 79% for diagnosing chronic Q fever, respectively. These study results demonstrate that the effector response and memory of $CD4^+$ T cells play a role; the high ratio IFN-γ compared to IL-2 is indicative of the dominance of T-cell effectors and T-cell memory effectors as a result of the prolonged stimulation of the infecting organism. Two distinct populations of T-cell memory effectors and T-cell memory centers are important in our understanding of the functioning of memory T lymphocytes. T cell effectors and T cell memory effectors will produce IFN-γ, while T cell memory centers will produce IL-2 (Figure 1).[56] As a result, subsequent studies examining the ratio IFN-γ to IL-2 found a significant reduction in chronic Q fever patients who had recovered from treatment.[57]

Another study by Schoffelen *et.al*[58] in 2013 regarding the specific detection of IFN-γ for previous *C. burnetii* infection through Bayesian analysis, with sensitivity and (87.0% and 90.2%, respectively,) comparable to the results of serology and ST. The concordance between IFN-γ detection and a combination of serology and ST measurements was moderate (84% concordance; = 0.542).[58]

It is noteworthy that Schoffelen *et al.*[19] found that the IFN-γ/IL-2 value among test subjects with chronic Q fever did not meet the criteria established by Raoult D. or the Dutch consensus.[33,59] These subjects would have phase I IgG without a definitive clinical, PCR, or imaging diagnosis of chronic *C. burnetii* infection. This low ratio of IFN-γ to IL-2 could indicate the absence of infection with *C. burnetii* or infection on a small scale. The sensitivity of PCR and imaging techniques, on the other hand, affects this condition. Thus, in such difficult circumstances, an elevated IFN-γ to IL-2 ratio may be a useful marker for diagnosing chronic Q fever.[19] The wide difference in IFN-γ to IL-2 ratios among study participants necessitates further research to formulate solid guidelines for using these biological markers. However, this study establishes a potentially important impact on the IFN-γ to IL-2 output profile as a biomarker of chronic Q fever.[19]

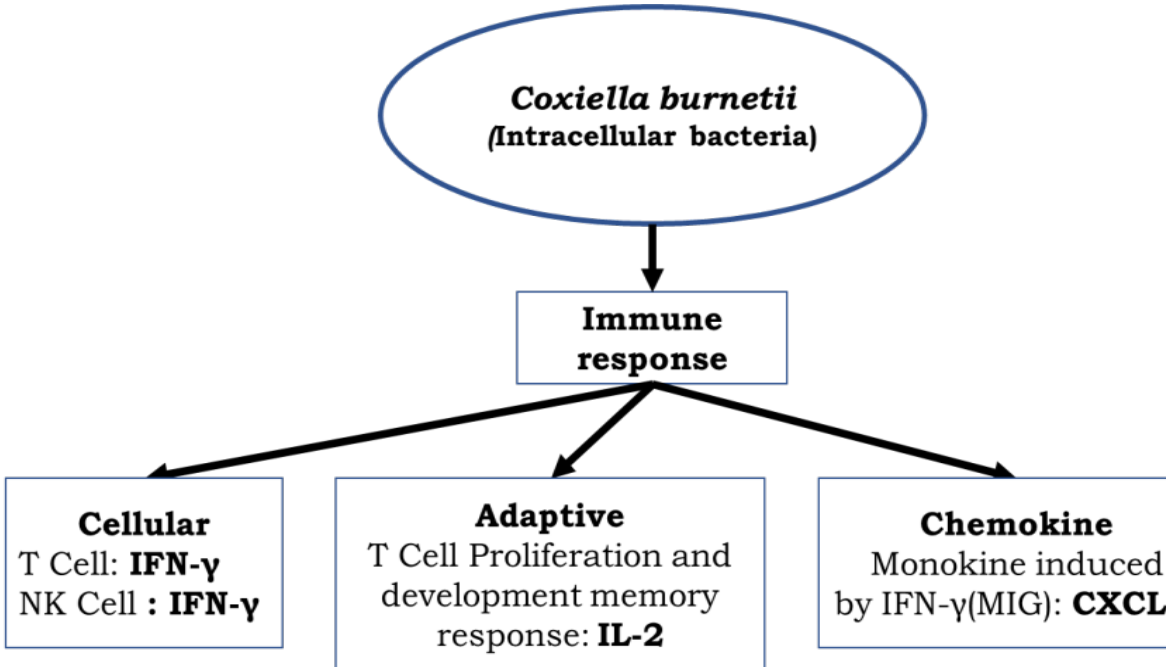

FIGURE 1. Schematic overview of potential biomarkers from the immune response toward *C. burnetii* that cause Q fever disease.

Chemokine CXCL-9

Recognizing the critical role of biomarkers in the early detection of Q fever, Jansen *et al.*[13] discovered the potential of the chemokine CXCL9 (FIGURE 1) as a biomarker through transcriptome analysis of peripheral mononuclear cells (PBMC) from chronic Q fever sufferers induced to *C. burnetii*. Transcriptome analysis of serum from sick people with Q fever and a validated comparison group revealed the presence of four chemokines stimulated by IFN-γ in high concentrations in responding to inactive *C. burnettii* after heating.[13] The fact that CXCL9 and CXCL11 serum levels have increased in individuals with persistent Q fever than in patients who have had Q fever demonstrates that CXCL9 and CXCL11 can aid in diagnosing chronic Q fever.[13] Then, more conclusive results demonstrated that the chronic condition of Q fever patients had a greater increase in CXCL9 than patients who had been exposed to Q fever.[13]

The chemokine found in the study of Jansen *et al.*[13] is a small chemotactic protein with characteristic features, including four cysteines with the amino acid variable 'X' dividing it.[60] The four CXCL chemokines are CXCL10, CXCL11, CXCL8, and CXCL9. IFN-γ induces all four chemokines, but only CXCL10, CXCL11, and CXCL8 can be stimulated by type 1 interferon as well as by TNF-α. CXCL9 can be expressed by macrophages, endothelial cells, fibroblasts, and peripheral blood mononuclear cells (PBMCs). Although the chemokines CXCL9, CXCL10, and CXCL11 share CXCR3 receptor ligands on the same cells, their affinity for CXCR3 varies, as does their expression, which is stimulus and time-dependent, indicating critical production as well as functional differences.[60,61] The presence of the CXCR3 chemokine also varies and has been used as a surrogate marker for active and latent tuberculosis conditions, treatment monitoring, and, of course, this is related to the immune system response of *C. burnetii*, another intracellular bacterium.[13] According to Van den Steen *et al.*[60] in Jansen *et al.*[13], different active concentrations of MMP-8 (metalloproteinase-8) or MMP-9 affect

the level as well as the functionality of CXCL9 and CXCL10.[13]

Given that elevated CXCR3 ligand levels are not increased specifically through the chronic condition of Q fever, careful consideration is warranted in further interpretation.[62] Likewise, the increase in the concentrations of CXCL9 and CXCL10 in heart failure patients, which, if observed, were still lower when compared to when patients were in chronic Q fever conditions.[62,63] Some lack in the study of Jansen *et al.*[13] the specificity of CXCL9 in chronic infection by *C. burnetii* in comparison with any other pathogen that also elicits a T helper-1 response, which of course also induces an increase in the serum concentration of the CXCR3 ligand.[13] In addition, transcriptome analysis is very helpful, but this test without validation from other tests makes it less than optimal. Similarly, the number of samples analyzed will significantly impact the representativeness of the condition of patients with persistent Q fever.[13]

CONCLUSION

The varied clinical manifestations of Q fever and a diagnosis that is often based solely on systematic consideration have been a barrier to the assessment and diagnosis of Q fever. Therefore, the availability of other relevant biologic markers may be helpful. The diagnosis of Q fever is less specific and sensitive in different stages of the disease through serological approaches and PCR techniques, makes it necessary to have multiple platforms to explore biomarkers of Q fever. Combining more than one potential biomarker could be done to obtain a more precise diagnosis. Cell-mediated immune responses, particularly IFN-γ, IL-2, and CXCL9, have shown potential biomarkers for diagnosing Q fever disease, but validation with a large number of samples is needed.

ACKNOWLEDGEMENTS

We would like to thank the Master Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia, and the Indonesian Research Center for Veterinary Science for the guidance and support.

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