

Identification of *Brucella abortus* virulence proteins that modulate the host immune response

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Brucellosis is an important zoonotic disease of almost worldwide distribution. One significant immune phenomenon of this disease is the ability of the pathogen to hide and survive in the host, establishing long lasting chronic infections. *Brucella* was found to have the ability to actively modulate the host immune response in order to establish chronic infections, but the mechanism by which the pathogen achieves this remains largely unknown. In our screening for protective antigens of *Brucella abortus*, three proteins (BAB1_0597, BAB1_0917 and BAB2_0431) were found to induce significantly higher levels of gamma interferon (IFN γ) in splenocytes of PBS immunized mice than those immunized with S19. This finding strongly implied that these three proteins inhibit the production of IFN γ . Previous studies have shown that LPS, PrpA and Btp1/TcpB are three important immunomodulatory molecules with the capacity to interfere with host immune response. They have been shown to have the ability to inhibit the secretion of IFN γ , or to increase the production of IL-10. Due to the role of these proteins in virulence and immunomodulation, they likely offer significant potential as live, attenuated *Brucella* vaccine candidates. Understanding the mechanisms by which these proteins modulate the host immune responses will deepen our knowledge of *Brucella* virulence and provide important information on the development of new vaccines against Brucellosis.

Brucella spp is a Gram-negative, facultative, intracellular bacterium that causes abortion in domestic animals and undulant fever, endocarditis, arthritis and osteomyelitis in humans.¹ Immunity against *Brucellae* requires cell-mediated mechanisms, in particular a T helper 1 (Th 1) immune response characterized by the production of gamma interferon (IFN γ), which is associated with protective immunity.² Therefore, proteins which present T-cell epitopes to the host could be protective antigen candidates. Previous work in our laboratory led to the identification of *Brucella* protective antigens, proteins associated with *Brucella* pathogenesis were expressed in *E. coli* and their abilities to induce T-cell responses were tested.³ The purified proteins were used to stimulate the splenocytes of S19 or PBS (negative control) immunized mice and IFN γ secretions were quantified. Only those proteins that stimulate significantly higher levels of IFN γ in S19 immunized mice than those immunized with PBS were considered to induce cellular immune responses. Fortunately, a number of proteins were found to stimulate stronger IFN γ responses. These proteins were used to immunize BALB/c mice and the protective efficacies against virulent *B. abortus* infection were assessed. At last, two proteins were found to induce protective immune responses in mice.³

Unexpectedly, we also found that three proteins (BAB1_0597, BAB1_0917 and BAB2_0431) induced significantly higher

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Table 1. Proteins that inhibited the secretion of IFN γ in mice immunized with S19

Locus	Protein name	The location of Protein	Description
BAB1_0597	-	Unknown	Hypothetical cytosolic protein
BAB1_0917	Tig	Cytoplasmic	Trigger factor
BAB2_0431	-	Cytoplasmic	D-galactarate dehydratase

levels of IFN γ in splenocytes of PBS immunized mice than those of S19 immunized ones (Table 1). This is a very interesting phenomenon which was not observed in our previous studies on *Yersinia pestis*, where all tested proteins induced higher or identical levels of IFN γ in a vaccine strain immunized mice than control mice.⁴ We assumed that this phenomenon might be the result of interference of these proteins with the host immune responses; prompting us to carefully analyze the ability of Brucella to interfere with the host immune responses.

As an intracellular bacterial pathogen, the virulence of Brucella depends on its survival and replication properties within host cells. One significant immune phenomenon observed during brucellosis is the ability of the pathogen to hide and survive in the host, establishing long lasting chronic infections.⁵ In general, microbial pathogens with the ability to establish chronic infections have evolved strategies to actively modulate the host immune response. Indeed, significant evidence exists to suggest that Brucella has the ability to avoid and/or interfere with host innate and acquired immune responses.⁶

One strategy used by Brucella to subvert the host innate immune system is via modification of pathogen-associated molecular LPS. Unlike enterobacterial LPS, *B. abortus* lipid A contains a much longer fatty acid residue C28 other than C12–C16, and this modification greatly reduces its endotoxic properties.⁷ The low endotoxic *B. abortus* LPS dramatically reduces and delays inflammatory response in the infected hosts compared with the endotoxins from other Gram-negative bacteria.⁸ In addition, due to the particular function and structure of Brucella LPS, which avoids the activation of the macrophage-killing systems and confers resistance to the pathogen against the microbicidal action of antibiotics, Brucella are able to survive and multiply inside phagocytic cells without provoking their

apoptosis.^{9,10} Furthermore, Brucella LPS was described in vitro as a downregulator of CD4 T cell activation.¹¹ Taken together, all these studies indicate that Brucella LPS plays a central role in the immunosuppression observed upon Brucella infection.

Another strategy used by Brucella to thwart an effective immune response might be to induce expansion of interleukin-10-producing B cells.⁶ In the early stages of acute human brucellosis the predominant response is Th1, with IFN γ production by T cells and natural killer (NK) cells.¹² However, in chronic human brucellosis, the cell-mediated immunity, mainly Th1, is transiently immunosuppressed in comparison to the antibody response.^{5,12,13} Previous studies have shown that *B. abortus* also induces the anti-inflammatory cytokine interleukin-10 (IL-10) in addition to an early Th1 response.^{14,15} IL-10 can inhibit the microbicidal activity of macrophages against Brucella, as well as antagonizing the activity of IFN γ .¹⁴ A *B. abortus* proline racemase, PrpA, was shown to induce a T cell-independent B cell-nonspecific polyclonal activation concomitant with the secretion of IL-10.¹⁶ B cell polyclonal activation induced by different virus, bacteria and parasites has been strongly associated to immune suppression and PrpA is a T-independent B cell mitogen first identified in Brucella. The protein plays an important role in the modulation of the host immune response during Brucella infection. Whereas the wild type (WT) and the complemented *prpA* mutant strains induce a temporal restricted unresponsive status in the mouse, the *prpA* mutant is unable to achieve this.¹⁶

Besides, several studies have shown a Brucella protein called Btp1 (also known as TcpB), which bears significant homology with the Toll/Interleukin-1 receptor (TIR) domain present in Toll-like receptors (TLRs) and adaptor molecules, also has immunomodulatory properties.¹⁷⁻¹⁹ TLRs play essential roles in the activation

of innate immune responses against microbial infections. Btp1/TcpB has been shown to inhibit TLR2 and TLR4 mediated NF- κ B activation.¹⁸ Therefore, Brucella could subvert TLR signaling pathways to suppress host immune responses to benefit their survival and persistence.

From the above discussion, we can conclude that LPS, PrpA and Btp1/TcpB, three important immunomodulatory molecules which could interfere with host immune responses, are able to inhibit the secretion of IFN γ , similarly with the three proteins (BAB1_0597, BAB1_0917 and BAB2_0431) found in our study. Our results suggest BAB1_0597, BAB1_0917 and BAB2_0431 may have the ability to interfere with host immune responses. But the definite role of these virulence proteins in immune response regulation needs to be further experimentally verified.

Due to the role of these proteins in virulence and immunomodulation, they may have significant potential as live, attenuated Brucella vaccine candidates. To date, development of live, attenuated Brucella vaccines that are safe for use in humans has focused on the deletion of important genes required for survival. However, mutations may over attenuate the organism, so that the level of protection induced is insufficient. Thus, the level of attenuation requires fine-tuning to provide a protective immune response while maintaining safety.²⁰ As these virulent proteins have the ability to inhibit the secretion of IFN γ , which is associated with protective immunity, their mutation may have less impact on the protection efficiency. In fact, in our laboratory, a *prpA* deletion mutant of S19 was constructed and its protective efficacy was analyzed. We observed that the mutant was attenuated in mice and elicited higher levels of protection when compared with the S19 strain (data not published). Furthermore, PrpA is a better diagnostic antigen that could be used to differentiate vaccinated and infected animals by serological diagnosis (data not published). These results appear to indicate that PrpA is a good candidate for constructing live attenuated Brucella vaccines. In addition, our studies have demonstrated that BAB1_0917 and BAB2_0431 are better diagnostic antigens of Brucella (data not published), but whether they are ideal

live attenuated vaccine candidates needs to be further verified. Understanding the mechanisms by which these three proteins modulate the host immune responses will help us to develop efficient vaccines against brucellosis.

In conclusion, *Brucella* is an intracellular bacterial pathogen with the

capacity to establish a chronic infection. In the past 10 years, the study of *Brucella* pathogenicity has been focused mainly on identifying factors that affect the intracellular trafficking and multiplication of the bacterium within the host cell. However, little is known about the molecular factors associated with

chronicity and immunomodulation of the host. Our findings provide important clues in this regard. Understanding these mechanisms can be useful not only for the study of host immune regulation by *Brucella*, but also for the development of new vaccines or therapeutic agents against Brucellosis.

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