Study on Experimental Infection in Sprague-Dawley Rats with *Brucella abortus* Biotype 1

By

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ABSTRACT

Brucella abortus (B. abortus) is an intracellular bacterium. Upon invasion into the host it is taken up by the phagocytic cells and undergoes proliferation and disseminates into the organs of the reticuloendothelial system such as spleen, liver, kidney and bone marrow. *B. abortus* causes hematological abnormalities in humans such as pancytopenia through bone marrow suppression and splenomegaly. Bacterial as well as hematological profiles have not been studied in rats after experimental infection with *B. abortus*.

In chapter 1, the bacterial and hematological profiles in SD rats were measured during acute and subacute brucellosis caused by *B. abortus* biotype 1. A total of 40 SD rats received an intraperitoneal dose of 1×10^{10} CFU of *B. abortus* biotype 1 isolated from cattle in Korea. 4 rats were used as uninfected control rats (0). Blood, spleen, liver and kidney were collected from the 4 randomly selected rats at 0, 3, 7, 14, 21, 28, 35, 42, 60, 90 and 120 days after infection for bacteriological and hematological examinations. The persistence of *B. abortus* in blood was noted for short time, up to 5 weeks of infection. Bacterial persistence was recorded in the spleen throughout the experiment. All of the infected rats manifested splenomegaly. Hematological analysis revealed significant decreased of WBC, RBC, PLT, HGB and HCT in the infected rats in compared to uninfected control rats ($p < 0.05$). Analysis of blood films of acutely and subacutely infected rats by WG stain revealed abnormal shapes of RBC, with extensive to moderate infiltration of the lymphocytes, neutrophils and macrophages. These changes of hematological parameter are the clear indication of pancytopenia.

In chapter 2, the IgG specific immune responses as well as antigen recognition were investigated during the acute and subacute stages of *B. abortus* biotype 1 infection in SD rats. The IgG specific immune responses in sera were measured at 0, 3, 7, 14, 21, 28, 35, 42, 60, 90 and 120 days after infection against LPS, WCA, OMP, PP, CP and CBP by IELISA. The IgG specific antibody response was detectable at 3 days after infection against these antigens. The peak serum IgG antibody titers were recorded against LPS, CP and PP at 28 days after infection. On the other hand, the highest serum IgG antibody titers were recorded at 42 days after infection against WCA and CBP. The highest serum IgG antibody titer was recorded at 90 days after infection only against OMP. Data of this study indicated that LPS, WCA, PP, CP and CBP of *B. abortus* could be useful for diagnosis of acute and subacute brucellosis in SD rat model. On the contrary, OMP of *B. abortus* could be useful for differential diagnosis of subacute brucellosis. Recognition of immunodominant antigens in WCA, OMP, PP, CP and CBP of *B. abortus* were performed by WB assay using infected rat sera collected at 0, 3, 7, 14, 21, 28, 35, 42, 60, 90 and 120 days after infection. WB assay of the sera revealed a wide array of protein bands between molecular weight of 10 and 98 kDa for WCA, 13 and 95 kDa for OMP, 20 and 65 kDa for PP, 12 and 85

kDa for CP and 19 and 125 kDa for CBP. Proteins bands of approximately 10, 13, 20, 24, 46 and 76 kDa for WCA; 28, 35, 39, 85, and 95 for OMP; 20, 30, 40, 43, 46 and 65 kDa for PP; 12, 23, 68 and 85 for CP; 125, 105, 82, 66, 54, 46, 32, 24, 22, 21 and 19 kDa for CBP were intensively recognized by the sera of infected rats during the course of infection. These antigens should be considered useful for the diagnosis of *B. abortus* infection.

In chapter 3, total serum level of IgA, IgG1, IgG2a against LPS, WCA, OMP, PP, CP and CBP of *B. abortus* as well as in vivo and in vitro production of INF-γ and IL-10 against CBP were measured in the SD rats at 0, 3, 7, 14, 21, 28, 35, 42, 60, 90 and 120 days after infection with *B. abortus* biotype 1 by ELISA. A very low level and short persistence of IgA antibody were recorded in this experiment. The low level and short persistence of IgA antibody suggest that this antibody isotype might not be protective against brucellosis in rats. Both Th1 and Th2 specific immune responses were recorded in this study with the production of IgG2a and IgG1 antibody isotypes, respectively. A significant dominant IgG2a antibody response over IgG1 responses were recorded throughout the experiment ($p < 0.001$) against LPS and OMP. A mixed Th1 and Th2 dominant immune responses mediated by IgG2a and IgG1 antibody isotypes were observed against WCA, PP, CP, and CBP. Data of this study suggest that IgG2a dominant responses in the early stages of disease play the main role in conferring protection against brucellosis and with the progress of disease IgG1 dominant responses were elicited.

The INF-γ and IL-10 levels in the sera of the infected rats were detectable by ELISA at 3 days after infection. A statistically significant higher serum level of INF-γ was recorded at 3 and 7 days after infection ($p < 0.001$) as compared to the IL-10 level. Statistically significant higher serum IL-10 levels were recorded at 21 and 28 days after infection as compared to the serum INF-γ levels ($p < 0.001$). The INF-γ levels in the spleen supernatant were significantly increased at 7 and 14 days after infection as compared to the IL-10 ($p < 0.001$). Then IL-10 responses were found to be significantly increased at 21, 28, 35 and 42 days after infection as compared to INF-γ ($p < 0.001$). The in vivo and in vitro cytokine profiles in this study indicated that *B. abortus* infection in rats initially elicit Th1 dominant immune response followed by Th2 dominant immune response.

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Keywords : (*Brucella abortus* biotype 1, Infection, Sprague-Dawley rat, Western blot assay, Cytokine profiles)