

## INCREASED SERUM LEVELS OF INTERFERON- $\gamma$ AND INTERLEUKIN-12 DURING HUMAN BRUCELLOSIS

KAMRUDDIN AHMED, KISMAT A. AL-MATROUK, GLENDA MARTINEZ, KAZUNORI OISHI,  
VINCENT O. ROTIMI, AND TSUYOSHI NAGATAKE

Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan; Department of Microbiology, Mubarak Al-Kabeer Hospital, Ministry of Health, Jabria, Kuwait; Department of Microbiology, Faculty of Medicine, Kuwait University, Jabria, Kuwait

**Abstract.** Brucellosis is a zoonotic disease that often becomes chronic with a high rate of recurrence. To understand the cytokines induced during this infection we determined the levels of interleukin (IL)-1 $\beta$ , IL-2, IL-4, IL-8, IL-12, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ ) in serum of patients with brucellosis and compared with those without brucellosis and controls. Control sera were taken from healthy persons residing in an area that was not endemic for brucellosis. The levels of IL-1, IL-2, IL-4, and TNF- $\alpha$  were not detectable in all the sera from patients. There was no difference in the level of IL-8 in patients with brucellosis and those without this disease. However, IL-8 was significantly higher in these two groups of patients compared with the controls. Significantly higher levels of IL-12 and IFN- $\gamma$  were found in the serum of patients with brucellosis compared with patients without brucellosis and controls. These data indicate that there is induction of Th1 type cytokines during human brucellosis.

Kuwait is an endemic area for brucellosis and for several years an increasing trend of brucellosis has been observed.<sup>1</sup> It continues to be one of the most widespread zoonoses in the world, causing serious health problems and economic losses. Intracellular survival of this microorganism in the reticuloendothelial system cells determines the chronic course of the disease, as well as the inability of antibiotic therapy to eradicate the infection completely.<sup>2</sup> Protective immunity against intracellular bacteria depends on the interplay between various T cell subsets and cytokines.<sup>3</sup> It has been established in a murine model that protective acquired immunity to brucellae involves T cell-dependent activation of macrophages in which both CD4<sup>+</sup> and CD8<sup>+</sup> T cells contribute to protection against *Brucella* infection.<sup>4,5</sup> Cell-mediated immunity to brucellosis, therefore, is likely to include the production of cytokines that activate macrophages and lymphocytes for production of anti-*Brucella* activities.

Recent studies have demonstrated central roles for interferon- $\gamma$  (IFN- $\gamma$ ) in inflammatory reaction associated with development of cellular immunity directed against a variety of intracellular pathogen. *In vitro* treatment of macrophages with recombinant IFN- $\gamma$ -enhanced killing of *Brucella abortus*<sup>6</sup> and other intracellular organisms.<sup>7,8</sup> Injection of recombinant IFN- $\gamma$  into mice enhanced resistance to *B. abortus*<sup>9</sup> and *Listeria monocytogenes*.<sup>10</sup> Unfortunately, no studies have been performed on human brucellosis to elucidate the role of cytokines. Therefore, to extend further our understanding of cellular immunity by identifying which cytokines are active during *Brucella* infection, we investigated the serum level of a panel of 7 cytokines in patients with brucellosis.

### PATIENTS AND METHODS

This was a retrospective study and no personal identifiers were used. The study was approved by the Ethical Committee of the Institute of Tropical Medicine at Nagasaki University. Between April 1996 and January 1997, 46 serum samples from patients with suspected brucellosis attending different hospitals in Kuwait were collected for this study. These sera were submitted at the microbiology laboratory of

Mubarak Al-Kabeer Hospital (Jabria, Kuwait) for the diagnosis of brucellosis. Standard procedures were followed for the diagnosis of this disease.<sup>11</sup> For the standard tube agglutination test (SAS febrile antigen; SA Scientific, Inc., San Antonio, TX and Bacto-*Brucella abortus* antigens and control antiserum; Difco Laboratories, Detroit, MI), an agglutination titer > 1:160 was considered a positive test result. Sera were also collected from 15 healthy adults residing in an area that was not endemic for brucellosis (Nagasaki, Japan) as a control. All serum was kept at -80°C until use.

The serum levels of interleukin (IL)-1 $\beta$ , tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IFN- $\gamma$  (Otsuka Pharmaceuticals, Tokyo, Japan), IL-2, IL-4, IL-12 (Endogen Inc., Woburn, MA) and IL-8 (was kindly provided by Professor K. Matsushima, Department of Molecular and Preventive Medicine, School of Medicine, Tokyo University, Tokyo, Japan) were assayed by a plate ELISA method according to the instructions of the manufacturer's (Otsuka Pharmaceuticals, Endogen, Inc., and Professor K. Matsushima).

### RESULTS

Among the 46 samples, 29 were obtained from Mubarak Al-Kabeer Hospital and the rest were from Al-Qurain Hospital. The age of the patients ranged from 5 to 55 years; there were 24 males and 18 females and the sex of 4 cases was unknown. There were 7 males and 8 females with ages ranging from 24 to 35 years in the control group.

Twenty-seven sera were positive for brucellosis and 19 sera were negative. Interleukin-1 $\beta$ , IL-2, IL-4, and TNF- $\alpha$  were not detectable in all 46 serum samples. Table 1 shows the serum levels of cytokines in patients and controls. The level of IFN- $\gamma$  was significantly higher ( $P < 0.05$ ) in brucellosis-positive cases than in negative cases and controls. There was no significant difference in IFN- $\gamma$  levels between healthy persons and patients without brucellosis. Levels of IL-12 was also significantly higher ( $P < 0.05$  and  $P < 0.005$ ) in brucellosis cases compared with patients without brucellosis and controls. There was no significant difference in the serum levels of IL-12 in healthy persons compared with the brucellosis-negative cases. There was no significant

TABLE 1  
Levels of different cytokines in the serum of patients with and without brucellosis and in normal individuals\*

	Interferon- $\gamma$	Interleukin-12	Interleukin-8
Brucellosis (+)	165.5 $\pm$ 46.0 <sup>†</sup> (27)	327.5 $\pm$ 48.1 <sup>†</sup> (24)	184.7 $\pm$ 23.2 (24)
Brucellosis (-)	41.3 $\pm$ 12.3 (19)	130.9 $\pm$ 50.0 (17)	138.0 $\pm$ 13.6 (15)
Control	51.5 $\pm$ 8.6 <sup>‡</sup> (15)	45.7 $\pm$ 32.8 <sup>§</sup> (15)	4.4 $\pm$ 3.1 <sup>¶</sup> (15)

\* Values are the mean  $\pm$  SE in pg/ml. Values in parentheses are the number of samples.  
<sup>†</sup>  $P < 0.05$  between patients with and without brucellosis.  
<sup>‡</sup>  $P < 0.05$  between controls and brucellosis patients.  
<sup>§</sup>  $P < 0.005$  between controls and brucellosis patients.  
<sup>¶</sup>  $P < 0.0005$  between controls and patients with and without brucellosis.

difference in serum IL-8 level between brucellosis-positive and -negative cases. Compared with the controls, IL-8 levels were significantly higher ( $P < 0.0005$ ) in patients with and without brucellosis.

No correlation was found ( $r = 0.05$ , not significant) between the levels of IFN- $\gamma$  and IL-8 in cases of brucellosis. There was a positive correlation, although not significant ( $r = 0.22$ ), between levels of IL-8 and IL-12. A non-significant negative correlation ( $r = 0.25$ ) was found between the serum levels of IL-12 and IFN- $\gamma$  in brucellosis cases.

#### DISCUSSION

Significantly higher levels of IFN- $\gamma$  and IL-12 in serum from cases with brucellosis indicate that these cytokines are induced during this diseases in humans. Similar results have been found only in the mouse model of brucellosis.<sup>12,13</sup> Interferon- $\gamma$  is a product of T helper 1 (Th1) cells that inhibits Th2 cell proliferation and IL-4 function, and IL-4 and IL-10, which are products of Th2 cells, can down-regulate the Th1 cell response.<sup>14</sup> Similar to other intracellular pathogens, supplementing IFN- $\gamma$  can reduce the number of brucellae in mice<sup>15</sup> by inducing antimicrobial activity in macrophages.<sup>16</sup>

Interleukin-12 is produced predominantly by macrophages and B lymphocytes in response to a variety of stimuli,<sup>17</sup> and has been implicated in polarizing the maturation of T cells to the TH1 phenotype.<sup>18</sup> Secretion of IL-12 in response to microbial infection represent a major defense mechanism of the innate immune system against intracellular pathogens.<sup>19</sup> The central role of IL-12 in protection against intracellular pathogens, including *Listeria monocytogenes*, *Leishmania major*, and *Mycobacteria tuberculosis*, has been established.<sup>19</sup> In mouse model of brucellosis, IL-12 has been shown to be involved in resistance to infection.<sup>13</sup>

Interleukin-8 is produced *in vitro* by a variety of cells in response to lipopolysaccharide and proinflammatory cytokines and acts as a potent chemoattractant and activator of monocytes, lymphocytes, and neutrophils, respectively.<sup>20</sup> Production of IL-8 *in vivo* may therefore provide a set of signals responsible for activating a broad spectrum of cellular host defense. A continued presence of an agonist is necessary for the prolonged expression of IL-8.<sup>21</sup> Therefore, statistically nonsignificant a trend towards higher IL-8 levels in the brucellosis patients indicates the role of a possible agonist in human brucellosis that deserves further investigation.

The TH1 cells, through production of IL-2 and IFN- $\gamma$ , orchestrate the cellular immune response.<sup>18</sup> Interleukin-2 has been reported to act as a cofactor in the activation of macrophages, as well as in decreasing the growth of *Brucella* in macrophages.<sup>17</sup> Moreover, IL-2 has been demonstrated to induce expression of TNF in human peripheral blood monocytes.<sup>22</sup> Tumor necrosis factor- $\alpha$  and IL-1 induce IL-8, which is assumed to be a secondary proinflammatory cytokine.<sup>23</sup> Interestingly, levels of IL-1, IL-2, IL-4, and TNF- $\alpha$  were not detectable in serum from patients with brucellosis. We assume that due to their short half-lives,<sup>24</sup> IL-2 and TNF- $\alpha$  were not detectable in serum of patients with brucellosis. Levels of IL-1 are low and difficult to detect because a significant amount of proIL-1 remains inside the cell and IL-1 also binds to large proteins such as  $\alpha$ -2-macroglobulin, complement, and the soluble type II IL-1 receptor.<sup>25</sup>

Taking into consideration the high levels of IFN- $\gamma$  and IL-12 and undetected level of IL-4 in this study, we may assume that mainly Th1 cells are involved during human brucellosis. The induction of the Th1 response in brucellosis has been demonstrated in mice.<sup>26</sup> Our results are the first to elucidate the involvement of Th1 cytokines during human brucellosis. Further studies are needed to clarify the role of these cytokines in the pathogenesis of *Brucella* infection in humans.

Acknowledgments: We are indebted to Dr. Christina Cheers (Department of Microbiology, University of Melbourne, Melbourne, Australia) for critical comments and valuable advice. We are also indebted to Dr. Wafa Y. Jamal (Department of Microbiology, Mubarak Al-Kabeer Hospital) for collecting patients data. We thank all technicians and the microbiologist of the Department of Microbiology (Mubarak Al-Kabeer Hospital) for cooperation.

Authors' addresses: Kamruddin Ahmed, Glenda Martinez, Kazunori Oishi, and Tsuyoshi Nagatake, Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto-machi, Nagasaki 852-8102, Japan. Kismat A. Al-Matrouk, Department of Microbiology, Mubarak Al-Kabeer Hospital, PO Box 43787, Hawally 32052, Kuwait. Vincent O. Rotimi, Department of Microbiology, Faculty of Medicine, Kuwait University, PO Box 24923, Safat 13110, Kuwait.

Reprint requests: Kamruddin Ahmed, Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto-machi, Nagasaki 852-8102, Japan.

#### REFERENCES

1. Lulu AR, Araj GF, Khateeb MI, Mustafa MY, Yusuf AR, Fenech FF, 1988. Human brucellosis in Kuwait: a prospective study of 400 cases. *Q J Med* 66: 39-54.

2. Ariza J, 1996. Brucellosis. *Curr Opin Infect Dis* 9: 126–131.
3. Kaufmann SHE, 1997. Antibacterial vaccines: impact of antigen handling and immune response. *J Mol Med* 75: 360–363.
4. Araya LN, Elzer PH, Rowe GE, Enright FM, Winter AJ, 1989. Temporal development of protective cell-mediated and humoral immunity in BALB/c mice infected with *Brucella abortus*. *J Immunol* 143: 3330–3337.
5. Pavlov H, Hogarth M, McKenzie IFC, Cheers C, 1982. *In vivo* and *in vitro* effects of monoclonal antibody to Ly antigens on immunity to infection. *Cell Immunol* 71: 127–138.
6. Jones SM, Winter AJ, 1992. Survival of virulent and attenuated strains of *Brucella abortus* in normal and gamma-interferon-activated murine peritoneal macrophages. *Infect Immun* 60: 3011–3014.
7. Flesch IEA, Kaufmann SHE, 1988. Attempts to characterize the mechanisms involved in mycobacterial growth inhibition by gamma-interferon-activated bone marrow macrophages. *Infect Immun* 56: 1464–1469.
8. Rook GAW, Steele J, Ainsworth M, Champion BR, 1986. Activation of macrophage to inhibit proliferation of *Mycobacterium tuberculosis*: comparison of the effects of recombinant  $\gamma$ -interferon on monocytes and murine peritoneal macrophages. *Immunology* 59: 333–338.
9. Stevens MG, Pugh Jr GW, Tabatabai LB, 1992. Effects of gamma interferon and indomethacin in preventing *Brucella abortus* infections in mice. *Infect Immun* 60: 4407–4409.
10. Kiderlen A, Kaufman S, Lohmann-Matthes M, 1984. Protection of mice against the intracellular bacterium *Listeria monocytogenes* by recombinant immune interferon. *Eur J Immunol* 14: 964–967.
11. Malik GM, 1998. Early clinical response to different therapeutic regimens for human brucellosis. *Am J Trop Med Hyg* 58: 190–191.
12. Zhan Y, Cheers C, 1993. Endogenous gamma interferon mediates resistance to *Brucella abortus* infection. *Infect Immun* 61: 4899–4901.
13. Zhan Y, Cheers C, 1995. Endogenous interleukin-12 is involved in resistance to *Brucella abortus* infection. *Infect Immun* 63: 1387–1390.
14. Golding B, Zaitseva M, H. Golding H, 1994. The potential for recruiting immune responses toward type 1 and type 2 T cell help. *Am J Trop Med Hyg* 50 (suppl 4): 33–40.
15. Baldwin CL, Jiang X, Fernandes DM, 1993. Macrophage control of *Brucella abortus*: influence of cytokines and iron. *Trends Microbiol* 1: 99–104.
16. Jiang X, Baldwin CL, 1993. Effects of cytokines on intracellular growth of *Brucella abortus*. *Infect Immun* 61: 124–134.
17. Trinchieri G, 1994. Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood* 84: 4008–4027.
18. Curfs JHAJ, Meis JFGM, Hoogkamp-Korstanje JAA, 1997. A primer on cytokines: sources, receptors, effects, and inducers. *Clin Microbiol Rev* 10: 742–780.
19. Ladel CH, Szalay G, Riedel D, Kaufmann SHE, 1997. Interleukin-12 secretion by *Mycobacterium tuberculosis*-infected macrophages. *Infect Immun* 65: 1936–1938.
20. Oppenheim JJ, Zachariae CO, Mukaida N, Matsushima K, 1991. Properties of the novel pro-inflammatory supergene “intercrine” cytokine family. *Annu Rev Immunol* 9: 617–648.
21. Strieter RM, Standiford TJ, Rolfe MW, Kunkel SL, 1993. Interleukin-8. Kelley J, ed. *Lung Biology in Health and Disease: Cytokines of the Lung*. Volume 61. New York: Marcel Dekker, Inc., 281–305.
22. Strieter RM, Remick DG, Lynch JP, Spengle RNR, Kunkel SL, 1989. IL-2 induced TNF expression in human alveolar macrophages and blood monocytes. *Am Rev Resp Dis* 139: 335–342.
23. Matsukawa A, Yoshimura T, Miyamoto K, Ohkawara S, Yoshinaga M, 1997. Analysis of the inflammatory cytokine network among TNF- $\alpha$ , IL-1 $\beta$ , IL-1 receptor antagonist, and IL-8 in LPS-induced rabbit arthritis. *Lab Invest* 76: 629–638.
24. Markham RM, 1992. Cell-mediated immunity. Gorbach SL, Bartlett JG, Blacklow NR, eds. *Infectious Diseases*. Second edition. Philadelphia: W.B. Saunders Company, 66–82.
25. Dinarello CA, 1996. Biologic basis for interleukin-1 in disease. *Blood* 87: 2095–2147.
26. Fienkelman FD, Katona ID, Mosmann TR, Coffman RL, 1988. IFN-gamma regulates the isotypes of Ig secreted during *in vivo* humoral responses. *J Immunol* 140: 1022–1027.