

Diminished Production of T Helper 1 Cytokines Correlates with T Cell Unresponsiveness to *Brucella* Cytoplasmic Proteins in Chronic Human Brucellosis

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This study evaluated the cellular immune response against *Brucella* species cytoplasmic protein (CP) in peripheral blood mononuclear cells (PBMC) of 25 patients with brucellosis. In vitro proliferation and cytokine gene expression and production were investigated. PBMC from 14 patients proliferated in response to CP (responder patients [RPs]) and cells from 11 patients did not (nonresponder patients [NRPs]). CP-specific interleukin (IL)-2 and interferon- γ were significantly induced in PBMC from RPs, compared with cells from NRPs. No significant differences were found in the production of IL-10 between the 2 groups. CP did not induce IL-4 production. A close relationship was observed between the clinical status of the patients and the T cell response against CP. Patient with acute infections responded to CP and induced production of T helper 1 (Th1) cytokines, whereas chronically infected patients did not. Diminished production of Th1 cytokines may contribute to T cell unresponsiveness in chronic human brucellosis.

Brucella species are gram-negative facultative intracellular bacteria that cause severe disease in both animals and humans. Brucellosis remains endemic in many developing countries, where it undermines animal health and productivity, causing important economic losses [1]. It also has a high human toll [2]. *Brucella* species often invade cells of the reticuloendothelial system and can be sequestered in infected macrophages at specific locations within the body (e.g., spleen, brain, heart, liver, and bone marrow) [3]. Among *Brucella* species, *B. melitensis*, *B. abortus*, *B. canis*, and *B. suis* are pathogenic for humans. After infection, most patients enter an acute phase with undulant fever that can progress either to recovery or to chronicity.

Chronic disease is characterized by mild fever, focal manifestations of the disease, and chronic fatigue syndrome.

Infection with *Brucella* species results in the activation of cell-mediated immune responses [4, 5]. In mice, passive transfer of immune cells indicates that protection against *B. abortus* infection is mediated by CD4⁺ and CD8⁺ T cells [5–7], although antibodies directed against the O chain of lipopolysaccharide (LPS) [5, 6] or against outer membrane proteins [8] appear to enhance resistance to infection, at least in certain host species. The role of cytokines in mediating resistance to *B. abortus* infection was underscored in the mouse model. The importance of interleukin (IL)-12 in inducing a Th1-type response modulated by interferon (IFN)- γ [9, 10] has been demonstrated. Endogenous IL-10 could also play a role by down-regulating protective immunity during *B. abortus* infection [11].

Although there is substantial information about the role of cytokines and T cells in murine brucellosis, knowledge of cell-mediated immune responses in humans is scarce [12, 13]. Most studies of T cell responses in brucellosis have used heat-killed whole bacteria or partially purified proteinaceous mixtures as antigens [9, 10, 12, 14, 15]. Because these preparations are often contaminated with bacterial LPS and DNA, the results derived from experiments conducted to evaluate antigen-specific responsiveness could be masked by the cellular responses elicited by these powerful stimulants [16].

The main goal of our laboratory has been to characterize proteinaceous antigens from *Brucella* organisms. We have obtained a preparation of cytoplasmic protein (CP; formerly LPS-free CYT) depleted of LPS by immunoadsorption [17]. CP was

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All subjects gave informed consent. Human experimentation guidelines of the US Department of Health and Human Services were followed.

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used as antigen in an indirect ELISA to test the reactivity of serum from different hosts. We found serum reactivity to CP in humans with brucellosis and in cows, sheep, goats, pigs, and dogs infected with different *Brucella* species [18–20], suggesting that many internal antigens are common to all *Brucella* species. We also have demonstrated that an 18-kDa *Brucella* cytoplasmic protein, which is present in all *Brucella* species [21], can be used for the serologic diagnosis of human and animal brucellosis [21, 22]. Functional studies have demonstrated that the 18-kDa antigen is an enzyme with lumazine synthase activity [23].

In the present study, we evaluated the cellular immune response against cytoplasmic proteins from *Brucella* organisms in peripheral blood mononuclear cells (PBMC) from patients with brucellosis. For this purpose, we used CP and recombinant *Brucella* lumazine synthase (rBLS) as model stimulants. The results are discussed in terms of the relevance of the cellular immune response against *Brucella* proteins during infection.

Patients, Materials, and Methods

Study population. This study population comprised 25 consecutive patients (18 men and 7 women) with active brucellosis who attended the Section of Brucellosis of the Hospital F. J. Muñiz (Buenos Aires). Control subjects were 20 healthy persons (11 men and 9 women) with no history of *Brucella* infection. The ages of the patients and control subjects, respectively, were 14–59 years (mean \pm SD, 42.2 \pm 12.9 years) and 23–45 years (mean \pm SD, 31.25 \pm 7.13 years). Brucellosis was diagnosed on the basis of clinical, serologic, bacteriologic, and epidemiologic data. Disease activity was defined by the presence of typical signs and symptoms (e.g., fever, splenomegaly, lymphadenopathy, myalgia, arthralgia, and hepatic involvement). All patients but 1 showed positive results in classic serologic tests, including standard tube agglutination, 2-mercaptoethanol, Rose-Bengal, complement fixation, and Huddleson tests. In addition, we used ELISA to detect IgG antibodies to *Brucella* CP (see below). In positive blood cultures from 6 patients, we isolated *B. abortus* ($n = 4$) and *B. melitensis* and *B. canis* (1 each). Because classic serologic tests use smooth *Brucella* species as antigen, the patient from which *B. canis* (rough species) was isolated was the one whose classic serologic results were negative, as expected. Therapy with antimicrobials was initiated as soon as the diagnosis of brucellosis was established. The initial treatment was oral doxycycline (100 mg every 12 h for 42 days) plus parenteral streptomycin (1.0 g every 24 h for 21 days).

Antigens and mitogens. CP was prepared by immunoadsorption of the DNase/RNase-treated cytoplasmic fraction of *B. abortus*, as described elsewhere [17]. rBLS, obtained as described elsewhere [23], contained <0.05 endotoxin units per 100 μ g of protein, as assessed by limulus amoebocyte lysate analysis kit (Sigma Chemical). Mycobacterial purified protein derivative (PPD; obtained from the Instituto Nacional de Microbiología Dr. Carlos Malbran, Buenos Aires) and phytohemagglutinin (PHA; Sigma) were used in parallel control cultures. The protein concentration was determined by protein assay (Bio-Rad Laboratories). Final concentrations used in culture were 10 μ g/mL for CP, PPD, and PHA and 5 μ g/mL for rBLS. These concentrations were found previously to

provide optimal stimulation for proliferation and cytokine production by human PBMC in our laboratory.

Isolation of PBMC. Cells were isolated from preservative-free heparinized blood, as described elsewhere [24], and cultured at 2×10^6 PBMC/mL in RPMI 1640 medium supplemented with 25 mM HEPES buffer, 2 mM L-glutamine, 10% heat inactivated human AB serum (Sigma), 100 U/mL penicillin, 100 μ g/mL streptomycin, and 0.25 μ g/mL fungizone (complete medium). Viability of cells, as determined by trypan blue exclusion, was always >95%.

In vitro blastogenesis. Blastogenesis assays were performed in triplicate in round- or flat-bottom microtiter plates for antigen and mitogen cultures, respectively (Costar). To each well, we added 100 μ L of stimulant and 100 μ L of cell suspension (2×10^5 PBMC). Control cultures consisting of complete medium and cells were run simultaneously. Cultures were incubated at 37°C in a humidified atmosphere (5% CO₂ and 95% air) for 4 (PHA) or 6 (antigens) days. At 18 h before harvest, we added 1.0 μ Ci of [³H]thymidine (ICN Pharmaceuticals) in 25 μ L of complete medium to each well. Cells were harvested onto glass-fiber mats, washed with distilled water with a harvester (Skatron Instruments), and dried overnight at room temperature. The dried filters were placed in 3 mL of scintillation fluid, and radioactive incorporation was measured by liquid scintillation counter (Beckman Instruments). Results are expressed as stimulation indices (SIs) as follows: counts per minute of stimulated cultures/counts per minute of unstimulated cultures. SIs >2 were considered to be a specific response.

Stimulation of cytokine production. PBMC (2×10^6 cells) were cultured in round-bottom polypropylene tubes (Sarstedt) in the presence of RPMI 1640 medium supplemented with 25 mM HEPES buffer, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (Gibco BRL Life technologies), 100 U/mL penicillin, 100 μ g/mL streptomycin, and 0.25 μ g/mL fungizone (RPMI 1640 medium), CP, rBLS, PPD, or PHA in a 1-mL volume. Cultures were incubated at 37°C in a humidified atmosphere (5% CO₂) for 24 h (RNA expression) or 48 h (cytokine secretion) in different tubes. At the end of the incubation, cells were centrifuged (400 g at 4°C) and processed immediately for RNA extraction, whereas supernatants were aliquoted and stored at –70°C until assayed for cytokine production.

Detection of cytokine mRNA by semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR). RT-PCR for cytokine gene expression was done as described elsewhere [24]. Results are shown as fold increase over the mRNA levels of cells cultured in the absence of antigen. Fold increases >2 were considered to be an up-regulation of the investigated cytokine gene.

Cytokine ELISAs. IL-4, IL-10, and IFN- γ in culture supernatants were measured by sandwich ELISA by use of paired cytokine-specific monoclonal antibodies, according to the manufacturer's instructions (BD PharMingen).

CP ELISA. Serum IgG reactivities against CP were assayed by indirect ELISA, as described elsewhere [25]. Serum titer was established as the reciprocal of the last serum dilution yielding an optical density higher than the cutoff. Serum titers >100 were considered to be a specific response.

Statistical analysis. Cytokine secretion and increase in mRNA levels were compared by the nonparametric Mann-Whitney *U* test with InStat 2 software (GraphPad Software).

Results

rBLS- and CP-specific blastogenic response of patients with brucellosis. We assessed the in vitro cellular immune response against *Brucella* CP. CP induced T cell proliferation in PBMC from 14 of 25 patients with brucellosis (SI, >2; figure 1A). In contrast, cells from only 2 of 25 patients proliferated in response to rBLS (figure 1B). PBMC from healthy control subjects showed no response to CP and rBLS (SI, <2; figure 1A and 1B). The differences in proliferative responses were not due to differences in background responses, since the unstimulated (control) cultures did not differ significantly among the 3 groups (data not shown). PBMC from all subjects proliferated in response to the nonbrucellar antigen PPD and to the mitogen PHA, with no significant difference observed among the groups (figure 1C and 1D). Therefore, for further analysis, patients with brucellosis were grouped according to the ability of their PBMC to proliferate in vitro to CP. Fourteen patients were classified as responder patients (RPs; SI, >2), and 11 patients were classified as nonresponder patients (NRPs; SI, <2).

Expression of cytokine mRNA in CP-stimulated PBMC from Brucella-infected patients. To determine whether the differences in cellular responsiveness between RPs and NRPs were related to differences in cytokines profiles, we studied IL-2, IFN- γ , IL-4, and IL-10 mRNA expression. These cytokines were chosen because they regulate T cell responses in vitro [26]. CP induced a significant ($P < .001$) up-regulation of IFN- γ and

IL-2 transcripts (geometric mean fold increase [GMFI], 5.55 and 4.31, respectively) in cells from RPs, compared with PBMC from NRPs (GMFI, 1.22 and 0.92, respectively; figure 2A and 2B). No significant differences were found in the expression of the IL-10 gene between the 2 patient groups. IL-10 mRNA expression was induced in cells from 3 of 14 of the RPs (GMFI, 1.32) and in 2 of 11 NRPs (GMFI, 1.30; figure 2C). PBMC from RPs and NRPs did not induce IL-4 gene transcription (GMFI, 1.0; figure 2D). CP did not induce cytokine transcription in PBMC from healthy control subjects (figure 2). Cytokine gene transcription in response to PHA was detected in cells from all subjects. There was no significant difference among the 3 groups (data not shown). These results suggest the induction of a Th1 response in RPs.

Cytokine production in PBMC culture supernatants. As at the mRNA level, CP stimulation significantly increased ($P < .0001$) the secretion of IFN- γ in PBMC from RPs, whereas no detectable levels of this cytokine were measured in culture supernatants from NRPs. No differences in the CP-specific production of IL-10 were found between PBMC from RPs and NRPs. Under the same culture conditions, cells from RPs and NRPs did not secrete IL-4 (figure 3A, 3B, and 3C).

CP-stimulated PBMC from healthy control subjects did not produce detectable levels of the cytokines investigated. Cells from all subjects produced IL-4, IL-10, and IFN- γ in response to PHA and IL-10 and IFN- γ in response to PPD. There were

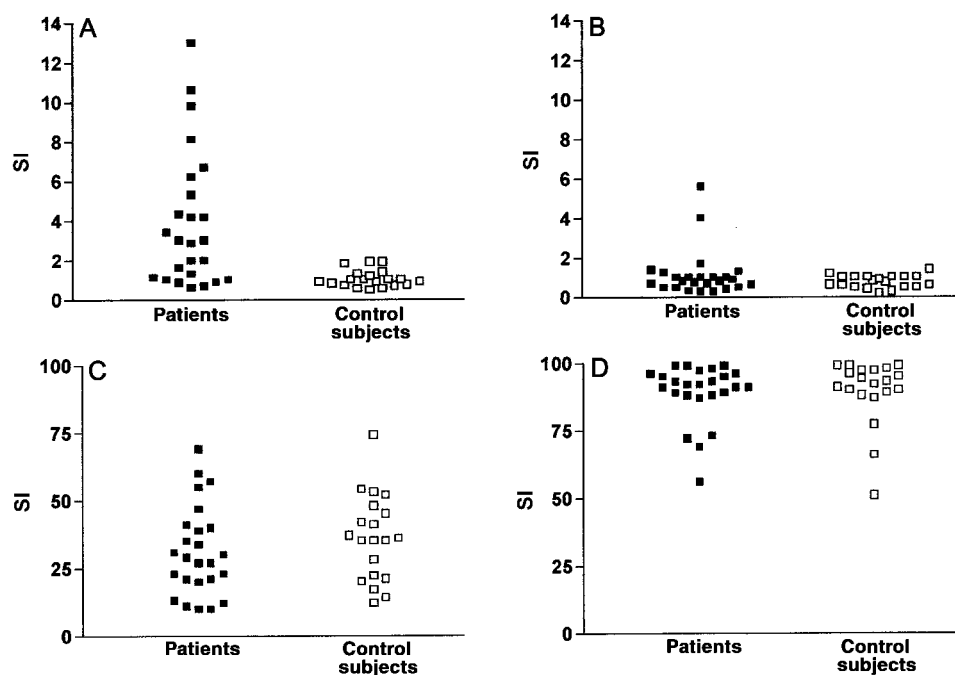


Figure 1. In vitro proliferation of peripheral blood mononuclear cells (PBMC) from patients with brucellosis. PBMC (2×10^5 /well) from patients and healthy control subjects were stimulated with *Brucella* cytoplasmic proteins (10 $\mu\text{g}/\text{mL}$; A), recombinant *Brucella* lumazine synthase (5 $\mu\text{g}/\text{mL}$; B), purified protein derivative (10 $\mu\text{g}/\text{mL}$; C), and phytohemagglutinin (5 $\mu\text{g}/\text{mL}$; D). Results are shown as stimulation indices (SIs) and were calculated as follows: cpm of stimulated cultures/cpm of unstimulated cultures. Each symbol represents the SI of PBMC of 1 subject.

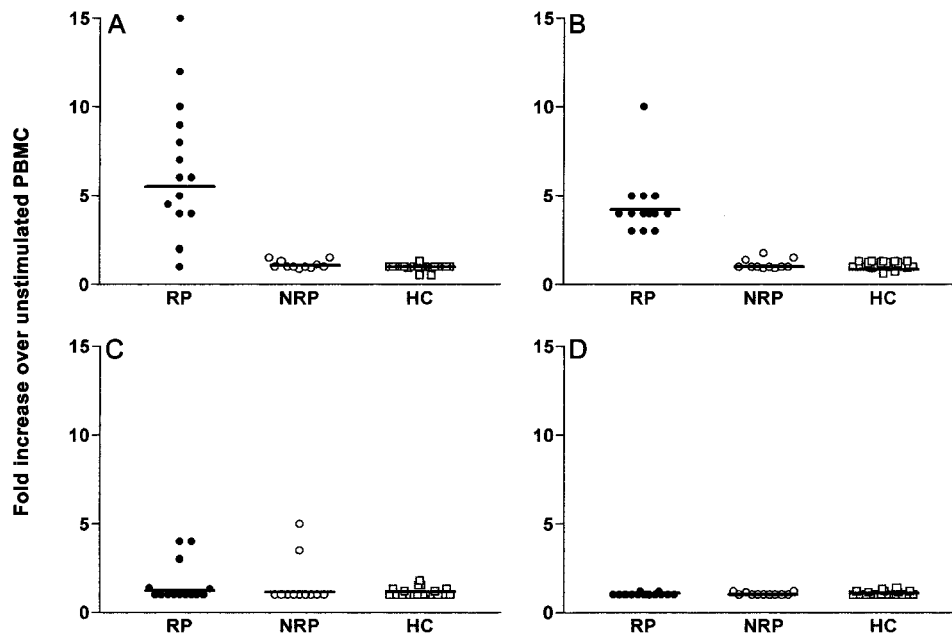


Figure 2. *Brucella* cytoplasmic proteins (CP)-induced cytokine mRNA expression in peripheral blood mononuclear cells (PBMC) from patients with brucellosis. PBMC (2×10^6 /mL) from responder patients (RPs), nonresponder patients (NRPs), and healthy control subjects (HCs) were stimulated with CP ($10 \mu\text{g/mL}$) for 24 h. The induced mRNA levels of interferon- γ (A), interleukin (IL)-2 (B), IL-10 (C), and IL-4 (D) were determined by reverse-transcriptase polymerase chain reaction. Responses are shown as fold increase over unstimulated PBMC. Each symbol represents the PBMC response of 1 person. Horizontal lines, geometric means. All values were normalized with respect to glyceraldehyde-3-phosphate dehydrogenase mRNA levels.

no significant differences among the 3 groups (figure 3A, 3B, and 3C).

Anti-CP humoral responses in *Brucella*-infected patients. To investigate the humoral immune response elicited against *Brucella* proteins, CP-specific IgG antibodies were detected by indirect ELISA in serum samples from infected subjects and healthy control subjects. High titers of CP-specific IgG antibodies (titers ≥ 800) were detected in serum samples of 12 of 25 patients. In contrast, the remaining 13 patients developed a marginal IgG response (titers ≤ 200) against *Brucella* CP (figure 4A). Moreover, the 4 patients who had no IgG anti-CP were also negative for IgM-specific antibodies (data not shown). Healthy control subjects did not produce anti-CP antibodies.

Because both antibody and T cell responses to CP were dichotomic in patients with brucellosis, we decided to further investigate the relationship between humoral and cellular responses. Therefore, we compared the anti-CP IgG titer and SI of CP-stimulated PBMC in infected patients. No correlation ($r^2 = 0.014$) was found between the CP-specific humoral and cellular responses. Although some patients with a high SI had low antibody titers, others developed strong humoral responses with no cellular proliferation (figure 4B).

Correlation between Th1 responses and clinical status of patients with brucellosis. To learn which factors were possibly associated with the lack of cellular proliferation and Th1-type cytokine production in response to CP in NRPs, we compared

some parameters between the RP and NRP groups. We found no significant differences between the groups for mean age or sex. Antibiotic regimens were distributed evenly among groups. Other factors that could potentially influence the immune response did not seem to be associated with the dichotomy of CP-specific cellular responses. No patient had an intercurrent disease or a clinical condition that could affect the immune response. In addition, no patient was taking immunosuppressive agents or any drug known to modify leukocyte counts. The mRNA levels of the costimulatory molecules CD80, CD86, and CD40 ligand and the immunomodulatory cytokines IL-18 and transforming growth factor (TGF)- β were not significantly different in CP-stimulated PBMC from RPs and NRPs (data not shown).

Retrospective analysis of clinical records allowed us to re-group the patients by acute or chronic disease duration, as defined by Young [27]. We classified 13 patients as having acute disease (duration of illness, 28–300 days; median, 60 days) and 12 with chronic disease (duration of illness, 400–1200 days; median, 675 days). Of interest, the majority of RPs (9 of 14) were in the acute category, whereas most NRPs (7 of 11) were in the chronic category, although the difference was not statistically significant. In agreement with this trend, the expression of the IFN- γ and IL-2 genes, which is closely associated with the blastogenic response, was significantly up-regulated ($P < .05$) in PBMC from patients with acute disease (GMFI, 4.08

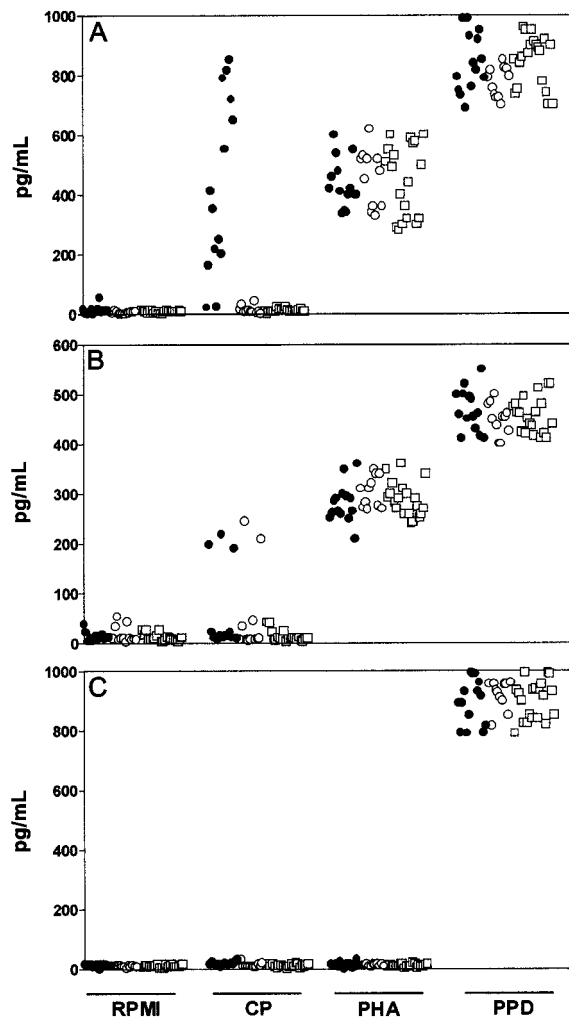


Figure 3. *Brucella* cytoplasmic proteins (CP)-induced cytokine production in peripheral blood mononuclear cells (PBMC) from patients with brucellosis. PBMC (2×10^6 /mL) from responder patients (●), nonresponder patients (○) and healthy control subjects (□) were stimulated with RPMI 1640 medium, CP (10 μ g/mL), purified protein derivative (PPD; 10 μ g/mL), and phytohemagglutinin (PHA; 5 μ g/mL) for 48 h. Interferon- γ (A), interleukin (IL)-10 (B), and IL-4 (C) in the supernatant was determined by antibody capture ELISA. Each symbol (the mean of duplicate determinations) represents the concentration of cytokine in the culture supernatant of PBMC of 1 person.

and 2.97, respectively), compared with cells from patients with chronic disease (GMFI, 1.68 and 1.60, respectively; figure 5A and 5B). No differences were found in IL-4 and IL-10 expression between PBMC from patients with either acute or chronic disease (figure 5C and 5D). Similar results were obtained when IFN- γ production in culture supernatants was evaluated (data not shown). Thus, these results show that the CP-specific T cell cytokine patterns observed in patients with brucellosis differ between the acute and chronic phases of the disease. They also suggest that diminished production of Th1 cytokines may con-

tribute to the T cell unresponsiveness to *Brucella* CP observed in chronic human brucellosis.

Discussion

Brucella species usually replicate within reticuloendothelial cells, and immunity depends on a suitable cell-mediated response by the host [5, 6]. This response relies on the expression of bacterial proteinaceous antigens, in association with molecules of the major histocompatibility complex, for recognition of the infected cell by different T cell subsets. Because the T cell receptor recognizes proteolytic fragments, it is not necessary for T cell antigens to be exposed on the surface of intact bacteria and, therefore, *Brucella* cytoplasmic proteins could also stimulate T lymphocytes. This prompted us to investigate the cellular immune response against *Brucella* cytoplasmic antigens in humans with brucellosis.

Our results suggest a close relationship between a patient's clinical status and the T cell response against cytoplasmic anti-

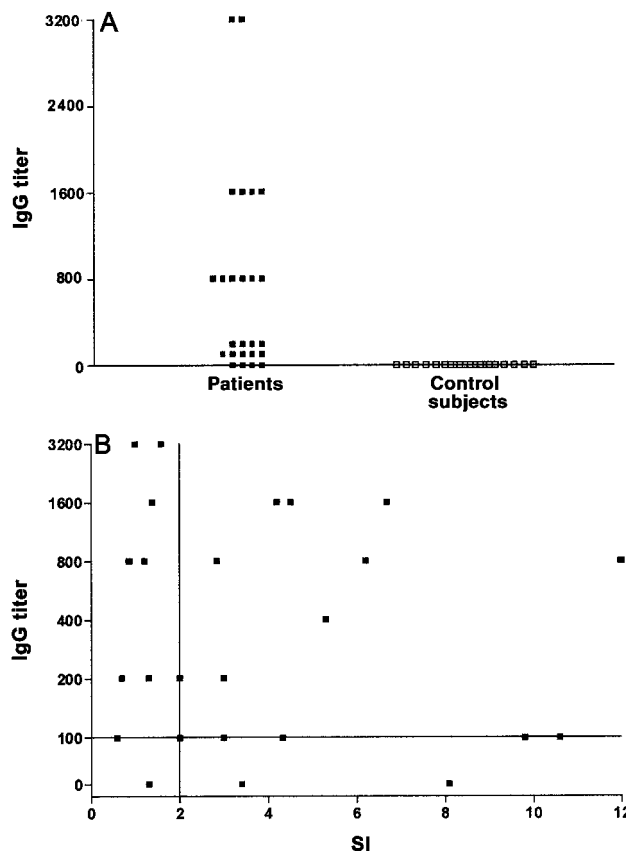


Figure 4. A, Antibody IgG titers to *Brucella* cytoplasmic proteins (CP) in patients with brucellosis and control subjects. Serum IgG reactivities against CP were assayed by indirect ELISA. Each symbol represents a serum sample from 1 subject. B, Comparison of cellular (stimulation index [SI], X-axis) vs. humoral (IgG titer, Y-axis) responses in patients with brucellosis.

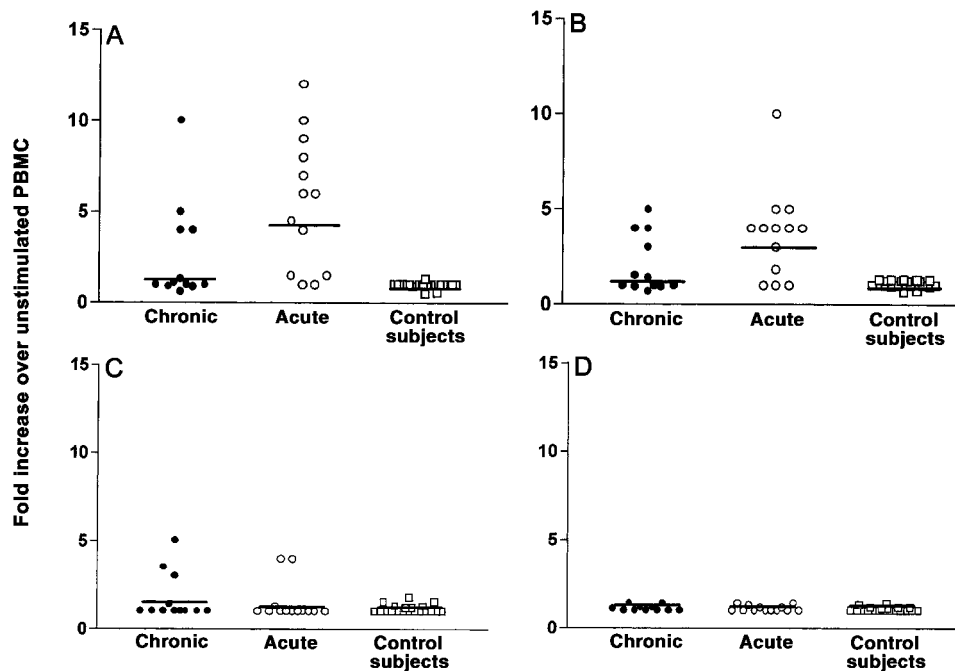


Figure 5. *Brucella* cytoplasmic proteins (CP)-specific cytokine mRNA levels in patients with acute and chronic brucellosis. Peripheral blood mononuclear cells (PBMC) ($2 \times 10^6/\text{mL}$) from healthy control subjects and patients with acute and chronic brucellosis were stimulated with CP ($10 \mu\text{g}/\text{mL}$) for 24 h. The induced mRNA levels of interferon- γ (A), interleukin (IL)-2 (B), IL-10 (C), and IL-4 (D) were determined by reverse-transcriptase polymerase chain reaction. Responses are shown as fold increase over unstimulated PBMC. Each symbol represents the response of PBMC of 1 person. Lines, geometric means. All values were normalized with respect to glyceraldehyde-3-phosphate dehydrogenase mRNA levels.

gens from *Brucella*. PBMC from patients with acute brucellosis responded to CP and induced the production of Th1 cytokines, whereas lymphocytes from patients with the chronic form of the disease did not. Polarization of cellular immune responses have been similarly observed in infectious diseases caused by parasites, such as filariasis [28] and Chagas' disease [29], and by intracellular bacteria that cause leprosy [30, 31] and tuberculosis [32], where distinct T cell cytokine profiles appear to control the clinical and immunologic spectrum of the diseases.

Several mechanisms could account for the decreased Th1 response toward *Brucella* cytoplasmic antigens in patients with chronic brucellosis. The concept that the endogenous production of IL-10, a Th2 cytokine that down-regulates IFN- γ production and/or increases the susceptibility to a number of bacterial [33, 34] and parasitic [35, 36] infections, could actively down-modulate Th1 responses in brucellosis has gained favor in the murine model of the disease [11]. PBMC from a small number of patients with both acute and chronic disease produced IL-10 in response to CP. Therefore, our data do not support a major role for IL-10 in the CP-specific T cell unresponsiveness observed in chronic brucellosis, at least in the cohort of patients under study.

The mRNA levels of TGF- β , another immunoregulatory cytokine [37, 38], were not preferentially induced in cells from patients with chronic brucellosis. Another mechanism that could

be implicated in the hyporesponsiveness observed in chronically infected patients is the induction of CP-specific anergy due to deficient costimulatory signals. However, this seemed to be unlikely, because the expression of CD80, CD86, and CD40 ligand mRNA in CP-induced PBMC was not significantly different between groups. However, the expression of other costimulatory molecules that boost IFN- γ and IL-2 production and proliferation, such as the signaling lymphocytic activation molecule (SLAM; CD150) [39], CD28 [40], and the inducible costimulator [41], were not investigated. Recently, SLAM expression was correlated with the polarization of the cellular immune response against the intracellular pathogen *Mycobacterium leprae* [42]. Studies are currently underway in our laboratory to determine the role of these costimulatory molecules in the polarization of the cellular immune response in human brucellosis. It is also possible that the differences observed in the cellular responsiveness against CP were due to differences in the size of the bacterial inoculum or in particular features of the infecting species that could influence the immune response.

Evidence obtained in our laboratory suggests that many internal antigens are common to all *Brucella* species [18–20]. Therefore, it seems difficult to attribute the differences in cellular anti-CP responses to antigenic differences among infecting species. Nevertheless, the cellular response could be influenced by different pathogenic characteristics of species. Unfortu-

nately, because of the few positive cultures in our cohort, we cannot draw any conclusion on this issue.

The differences in the T cell responses are puzzling, and it is difficult to know whether a change in the pattern of cytokines occurs at some point during the disease or whether these differences arise from the beginning of the disease. Nevertheless, although it is difficult to establish the pathogenic implications of this immunologic alteration, we hypothesize that a down-regulation of the Th1 response could be implicated in the intracellular survival of the bacterium during the chronic phase of the disease.

Bertotto et al. [43] described a dramatic increase in the number of activated $\gamma\delta$ T lymphocytes in patients with acute *B. melitensis* infection. The same study showed that the expansion of these cells (which can be stimulated by *Brucella* antigens to proliferate and rapidly secrete large amounts of IFN- γ and tumor necrosis factor- α [44]) was greatly diminished during convalescence. The preferential Th1-type cytokine secretion in patients with acute brucellosis observed in our study could be due to a selective overexpansion of IFN- γ -producing $\gamma\delta$ T cells, followed by a decrease of these cells during the chronic phase of the disease. A follow-up study on acute patients would help address this question.

Moreno-Lafont et al. [13] demonstrated that PBMC from chronically ill patients with brucellosis proliferated in response to a sonicated bacterial suspension rich in internal antigens, whereas cells from patients with acute brucellosis did not. Although this may appear to contradict our findings, their results were obtained with an antigenic preparation that contained bacterial LPS and DNA and, therefore, the inherent ability of these contaminants to induce cellular proliferation could have masked the protein-specific immune response. Persons with acute brucellosis have diminished proliferative responses [45] and defective IFN- γ production [46]. However, this hyporesponsiveness was not specific, because PBMC were stimulated with the polyclonal mitogen PHA. Of note, the differences in all of the responses we investigated (cell proliferation, cytokine mRNA transcription, and protein production) are CP-specific, since there was no significant difference in these responses from subjects when PBMC were stimulated with the mitogen PHA or with the nonbrucellar antigen PPD.

Although a T cell dichotomy could be observed when patient PBMC were stimulated with CP, cells from only 2 patients responded to rBLS in vitro. However, most patients in our study developed anti-rBLS antibodies (data not shown). rBLS is present in the CP antigenic preparation [21]. Therefore, our results suggest that this antigen displays immunodominant B cell epitopes rather than T cell epitopes. The lack of T cell response against rBLS is not restricted to human infection, since dogs and mice infected with *Brucella* species show a marked T cell hyporesponsiveness against rBLS in the presence of high titers of specific antibodies (authors' unpublished data).

Finally, our findings demonstrate that, during human *Bru-*

cella infection, there is a polarization of the T cell response against cytoplasmic antigens. This polarized response seems to be related to the patient's clinical status. Although in vitro findings do not necessarily mirror the in vivo situation, our results indicate that patients with acute brucellosis display a Th1-type response with cell proliferation and production of IFN- γ and IL-2, whereas patients with the chronic form of the disease do not. The results also suggest that the CP antigen could be a useful tool for investigation of antigen-specific T cell responses in different host. This antigen has a unique characteristic of representing a pool of cytoplasmic proteins common to all *Brucella* species, free of the most common contaminant of all *Brucella* antigenic preparation, LPS.

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