Circulating Cytokines in Patients with Cat Scratch Disease

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Levels of circulating interleukin (IL)-2, IL-6, and IL-10, measured by enzyme-linked immunosorbent assay, were significantly higher in patients with cat scratch disease (CSD) than in healthy control subjects; no induction of IL-12 was observed, and levels of interferon-γ and IL-4 were generally not detectable. This is the first report showing increased circulating cytokine levels in patients with CSD. The induction of these mediators can partly explain some clinical and pathological features of the disease.

Cat scratch disease (CSD) is one of the most common causes of regional lymphadenitis in children [1]. Although it is a generally benign, self-limited disease, its prolonged clinical course, the limited usefulness of antimicrobial therapy, and occasional systemic involvement [2] may lead to considerable morbidity. Specific diagnosis of the causative microorganism, Bartonella henselae, can now be determined by culture, immunofluorescence, ELISA, and PCR [3], enabling a better understanding of CSD epidemiology [4]. However, the mechanisms involved in the pathogenesis of the disease are still not completely understood.

The involvement of cellular immunity is strongly suggested by the delayed-type hypersensitivity reaction to CSD antigen with skin testing, which as been used for many years as a major diagnostic criterion [5]. However, only a few studies have assessed lymphocyte responses in CSD, all of them by means of antigen-mediated lymphocyte proliferation [6–8]. Although specific lymphocyte proliferation confirms the significant role of cell-mediated immunity in CSD, it offers little information about the mechanisms of this process, which include an increasingly complex network of cytokines, chemokines, surface antigens, and more. The study of such mediators can reveal new potential therapeutic targets, which are certainly needed for management of CSD.

The first attempt at such an approach was recently reported; it involved an experimental model with mice [9], in which induction of IFN-γ from splenocytes of B. henselae-infected animals was observed. We hypothesized that a vigorous cellular immune response in patients with CSD would result in significant changes of pro-inflammatory and/or T cell regulatory cytokines that could be detected in the serum of patients with CSD. In order to confirm this hypothesis, we examined the levels of IL-2, IL-4, IL-6, IL-10, IL-12, and IFN-γ in serum samples obtained from patients referred to our laboratory for CSD diagnosis.

Methods. Serum samples obtained from 21 patients with a clinical diagnosis of CSD were sent to our laboratory for detection of antibodies to B. henselae. The patients were 13 boys and 8 girls (mean age ± SD, 9.2 ± 2.1 years). They all had regional lymphadenopathy, fever (temperature, >38°C), a history and/or objective evidence of recent contact with a cat, and negative findings on workups for other possible causes of lymphadenopathy. Serum samples were obtained from all patients after week 2 and before week 4 after the onset of the disease. Antigen skin testing and/or lymph node biopsies were not performed for these patients for reasons that are commonly discussed in the literature (namely, safety and potential complications) [3, 4]. The control group consisted of 9 children (5 boys) who were admitted to our hospital for minor elective surgery; the mean age (± SD) was 8.7 ± 3.3 years. Blood samples were obtained from the venipuncture performed for scheduled diagnostic tests, prior to any other medical manipulation, and informed consent was obtained from the parents in all cases.

Slides coated with B. henselae antigen were obtained from MRL Diagnostics. For the detection of IgM, serum samples were serially diluted at 1:64–1:512 in phosphate-buffered saline (PBS; Sigma-Aldrich LTD). The diluted serum was incubated on the substrate slide for 30 min at room temperature. For the detection of IgG antibodies, serum samples were diluted at 1:20 in PBS containing goat anti-human IgG hyperimmune serum (Pharmalex) to precipitate IgG. The dilution was incubated for 15 min at room temperature, followed by further serial dilutions in PBS up to 1:80. The incubation period of the substrate slide for IgM antibodies was 1 h. Slides were then washed twice with PBS, and either fluorescein isothiocyanate–conjugated goat anti-human IgG or goat anti-human IgM...
titer was by 2 observers. Samples were considered positive if the IgG mounted and the end point titer was determined independently at room temperature. After 2 more washes, the slides were

Commercially available kits (Biosource) were used according to the manufacturer’s instructions. Each sample was assayed in duplicate. The sensitivity of the assays were 5 pg/mL, for IL-2; 0.27 pg/mL, for IL-4; 2 pg/mL, for IL-6; 5 pg/mL, for IL-10; 1 pg/mL, for IL-12; and 4 pg/mL, for IFN-γ.

Results are presented as mean values ± standard error of the mean. The Student’s t test was used for the comparison of means, and regression analysis was used to evaluate any correlation between different cytokine levels. A P value of <.05 was considered significant.

Results. Seventeen (81%) of 21 patients tested positive for antibodies to B. henselae: 4 had only IgM (titer, ≥1:20), 5 had only IgG (titer, ≥1:256), and 8 had both IgM and IgG. All children in the control group tested negative. The concentration of IL-2 in the serum samples of patients with CSD was significantly higher than that in the serum samples of control subjects (85.2 ± 14.2 pg/mL, within range of 27–221 pg/mL, vs. 39.8 ± 5.1 pg/mL, within range of 28–78 pg/mL; P < .05; figure 1A). Serologically confirmed cases had somewhat higher IL-2 levels than did antibody-negative cases (94.3 ± 12.3 pg/mL vs. 46.4 ± 2.1 pg/mL); however, this difference was not statistically significant (P = .2).

An increased concentration of IL-6 was also observed in patients with CSD (33.6 ± 9.3 pg/mL, within a range of 0–189 pg/mL, vs. 3.3 ± 2.2 pg/mL, within a range of 0–17 pg/mL, for control subjects; P < .01; figure 1B). Antibody-negative CSD cases had higher IL-6 concentrations than did antibody-positive cases, but once again this difference was not significant (61.1 ± 35.2 pg/mL vs. 27.1 ± 6.8 pg/mL; P = .15).

Furthermore, IL-10 was significantly increased in patients with CSD (9.5 ± 1.3 pg/mL, within a range of 4–30 pg/mL, vs. 4.8 ± 0.4 pg/mL, within a range of 4–7 pg/mL, for control subjects; P < .05; figure 1C). B. henselae antibody–positive and –negative serum samples displayed identical values for this parameter (9.5 ± 1.6 pg/mL vs. 9.2 ± 1.1 pg/mL).

When all the above comparisons were made for only the patients with serologically proven cases versus control subjects, statistical significances remained unchanged. No differences between the groups were observed with regard to IL-12 levels (figure 1D). IL-4 and IFN-γ were not detectable in these serum samples, with the exception of 1 sample that tested positive for IL-4 and 4 samples that tested positive for IFN-γ, all of which were from patients with CSD.

When regression analysis was performed between levels of different cytokines or B. henselae antibody titers, IL-6 was found to correlate significantly with IL-10 (r = 0.78; P < .001; figure 2), but no other significant correlation was observed (data not shown).

Discussion. Activation of cellular immunity in patients with CSD has been postulated on the basis of early reports of the disease, when a delayed-type hypersensitivity skin reaction to the CSD antigen was observed [10]. It is surprising that only few studies reported in the literature have attempted to confirm this observation in vitro or ex vivo. In fact, the first of such studies failed to show any specific lymphocyte proliferation responses to CSD antigen in patients with CSD [6]; this was opposed 10 years later, with the use of a more elaborate CSD preparation [7]. Nevertheless, yet another decade later, the meaning of the term “cellular immunity” has expanded considerably, including myriad soluble mediators, cell subtypes, and surface antigens. It is therefore necessary to define the specific components of T cell immunity involved in CSD, not only in the context of a deeper understanding of the disease.
process but also—and more importantly—for the delineation of novel therapeutic targets.

In this context, we sought to determine levels of circulating pro-inflammatory and/or T cell regulatory cytokines in patients with CSD, because this issue has not been previously addressed. According to our findings, IL-2, IL-6, and IL-10 are significantly upregulated in the serum of CSD patients. Upregulation of IL-2 can thus play a considerable role in the induction and/or augmentation of a T cell response in CSD. Furthermore, it can help explain the increased susceptibility of immunocompromised individuals, such as patients with AIDS, to *B. henselae* [1].

IL-6 is a multipotent cytokine that is critical to the induction of inflammation. Its levels correlate well with clinical scores in patients with a variety of diseases, such as multiple myeloma, Kawasaki syndrome, and pneumonia [11]. It has been proposed that IL-6 might be useful as an inflammation marker, and that it might be superior to leukocyte counts or C-reactive protein [12]. It has also been proposed that IL-6 has an important role in the induction of an effective immune response to *Mycobacterium tuberculosis* and *Francisella tularensis* [13], which produce pathological characteristics similar to those of *B. henselae*. Therefore, the observed upregulation of IL-6 in patients with CSD stimulates further interest in the role of this cytokine in the pathogenesis of CSD.

On the contrary, IL-10 is a potent anti-inflammatory mediator [14]. Therefore, it may have a key role in controlling the vigorous cellular immune response to CSD. It is of interest that IL-10 levels correlated strongly with IL-6 levels in our experiments. A similar finding was recently reported with regard to patients with community-acquired pneumonia, which led the authors to hypothesize that IL-10 might control the cytokine cascade, perhaps as a protective mechanism [14]. This is further supported by studies showing low levels of IL-10 in patients who did not survive sepsis, in contrast to levels in survivors [15].

In our experiments, the presence of antibodies to *B. henselae* was noted in 81% of patients with clinically suspected CSD, which is consistent with percentages reported in the literature [4]. The cytokine profile of serologically negative cases was slightly—although not significantly—different from serologically confirmed CSD cases. Even though *B. henselae* is currently believed to be the main cause of CSD, the same clinical syndrome can be caused by *Bartonella quintana*, *Bartonella claridgeiae*, or *Afipia felis* [16]. Lymphadenopathy due to other causes, in which history of contact with a cat might have been coincidental, is also possible. In any case, our results did not differ whether or not serologically negative cases were included in the analysis.

In conclusion, this is the first study to show an upregulation of circulating cytokines, namely, IL-2, IL-6, and IL-10, in patients with CSD. The induction of these mediators, which are critical in the regulation of inflammation as well as cellular immunity, can partly explain some clinical and pathological features of the disease. Further studies to evaluate the consequences of these observations are now needed.

References