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RESEARCH NOTE

Release of granzymes and chemokines in Thai patients with leptospirosis

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ABSTRACT

The plasma concentrations of granzymes are considered to reflect the involvement of cytotoxic T-cells and natural killer cells in various disease states. Interferon (IFN)-γ-inducible protein-10 (IP-10) and monokine induced by IFN-γ (Mig) are members of the non-ELR CXC chemokine family that act on T-cells and natural killer cells. This study revealed that the plasma concentrations of granzyme B (but not granzyme A), IP-10 and Mig were higher in 44 Thai patients with definite or possible leptospirosis than in healthy blood donors. These data suggest that activation of cell-mediated immunity is part of the early host response to leptospirosis.

Keywords Cell-mediated immunity, chemokines, granzymes, host reponse, leptospirosis

Original Submission: 1 June 2006; **Revised Submission:** 24 August 2006; **Accepted:** 21 September 2006

Clin Microbiol Infect 2007; **13**: 433–436
10.1111/j.1469-0691.2006.01640.x

Leptospirosis is caused by pathogenic spirochaetes of the genus *Leptospira*, and is probably the world's most widespread zoonosis [1]. Cytotoxic T-cells

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Table 1. Patient characteristics upon admission[a]

	Definite leptospirosis $n = 28$	Possible leptospirosis $n = 16$
Age (years)	38 (15–63)	34 (15–51)
Gender (male/female)	18/10	13/3
Duration of symptoms (days)	4 (2–10)	5 (2–8)
Systolic blood pressure (mm Hg)	01 (74–165)	100 (50–139)
Temperature (°C)	38.2 (37.2–40.4)	38.6 (37.1–42.5)
Serum urea (mg/dL)	22 (8–102)	22 (4–110)
Serum creatinine (mg/dL)	1.5 (0.7–10.8)	1.2 (0.7–7.1)
Total serum bilirubin (mg/dL)	1.4 (0.3–23.2)	1.9 (0.4–24.5)
Direct serum bilrubin (mg/dL)	0.7 (0.1–14.2)	1.3 (0.1–11.9)
Haemoglobin (g/dL)	12.2 (7.0–16.1)	12.1 (7.3–14.3)
White blood cells $\times 10^9$/L	10.7 (4.7–32.6)	11.5 (4.3–44.0)
% Neutrophils	87 (45–96)	87 (62–95)
% Lymphocytes	9.5 (1–41)	10 (4–35)

[a]Values are given as medians and ranges. For definitions of definite and possible leptospirosis, see Materials and Methods. There were no significant differences in any of the parameters between the two groups.

and natural killer cells are the effector cells of cell-mediated immunity, collectively referred to as cytotoxic lymphocytes (CLs). CLs protect the host by lysing cells infected by viruses, intracellular bacteria or parasites [2]. Granzymes (Gr) are released during degranulation of CLs, and increased concentrations of soluble Gr are considered to reflect the involvement of cytotoxic T-cells and natural killer cells in various disease states [3]. Interferon-γ-inducible protein-10 (IP-10) and monokine induced by interferon-γ (Mig) are members of the non-ELR CXC chemokine family that share a common receptor, CXCR3, which is expressed only on natural killer cells and T-lymphocytes [4]. The present study sought to obtain a first insight into activation of CLs during leptospirosis.

The study formed part of a larger study of acute febrile illness in Udon Thani, Thailand [5,6]. EDTA-anticoagulated blood was collected from consecutive patients upon admission for acute febrile illness at Soon Udon Thani Hospital, Udon, northern Thailand. A diagnosis of definite leptospirosis ($n = 28$) was based on a positive blood culture for leptospira, or a four-fold rise in titre, or a single titre of >1:400, in the Microscopic Agglutination Test (MAT; NIH, Bangkok, Thailand) or the Indirect Immunofluorescence Test (IFA-IgM; NIH). A diagnosis of possible leptospirosis ($n = 16$) was made when a patient did not fulfil the above strict criteria, but had clinical symptoms compatible with the disease and a positive result with three or more of the following serological screening tests: Lepto Tek Lateral Flow (Organon Teknica, Boxtel, The Netherlands); Microscopic Capsular Agglutination Test (Japan Lyophilization Company, Tokyo, Japan); IgG/IgM ELISA (Panbio, Brisbane, Australia); Latex Agglutination Test (NIH); Dip-S-Tick Test (Panbio); or Multi-test (Panbio). Blood was also collected from 50 anonymous healthy blood donors from the same area. Informed consent was obtained from all patients. All mediators were measured by ELISA (GrA and GrB, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands; IP-10, R&D Systems, Abingdon, UK; Mig, Pharmingen, San Diego, CA, USA), with detection limits of 4.1, 3.3, 204.8 and 82.3 pg/mL, respectively. Values are given as medians and ranges. Differences between controls and patient groups were analysed by the Mann–Whitney *U*-test, with $p < 0.05$ considered to be statistically significant. Spearman's ρ was used to analyse correlations among variables.

Patient characteristics are summarised in Table 1 (healthy blood donors remained anonymous). The plasma concentrations measured are shown in Fig. 1. GrA levels were significantly higher among controls (41.2, range ≤4.1–256.7, pg/mL) than among patients with definite leptospirosis (19.9, range ≤4.1–102.8, pg/mL, p 0.005) or possible leptospirosis (13.1, range ≤4.1–4487, pg/mL, p 0.028). GrB levels were lower among controls (3.3, range ≤3.3–153.0, pg/mL) than among patients with definite leptospirosis (28.7, range ≤3.3–191.6, pg/mL, $p < 0.0001$) or possible leptospirosis (43.8, range 7.9–4590, pg/mL, $p < 0.0001$). Gr levels did not differ among leptospirosis patients with and without positive blood cultures (data not shown). There was no correlation between GrA and GrB levels in any of the groups. IP-10 was detectable in only two of the healthy donors (range ≤204.8–1648 pg/mL). Plasma IP-10 levels among patients with definite leptospirosis (637.9, range ≤204.8–25 994, pg/mL, $p < 0.0001$) and patients with possible leptospirosis (1068, range ≤204.8–7783, pg/mL, p 0.0003) were higher than among controls. Mig was detectable in plasma in only one (2%) (range ≤82.3–332 pg/mL) of the controls, in 15 (54%) patients with definite leptospirosis (374.2, range ≤82.3–20 621, pg/mL) and in four (25%) patients with possible leptospirosis (82.3, range ≤82.3–24 987, pg/mL). Mig levels were higher among patients with definite leptospirosis than among controls ($p < 0.0001$). In patients with definite or possible leptospirosis, Mig correlated positively with IP-10 (ρ 0.60; $p < 0.0001$). There were no differences in the concentrations of these mediators among patients with and without positive blood cultures (data not shown).

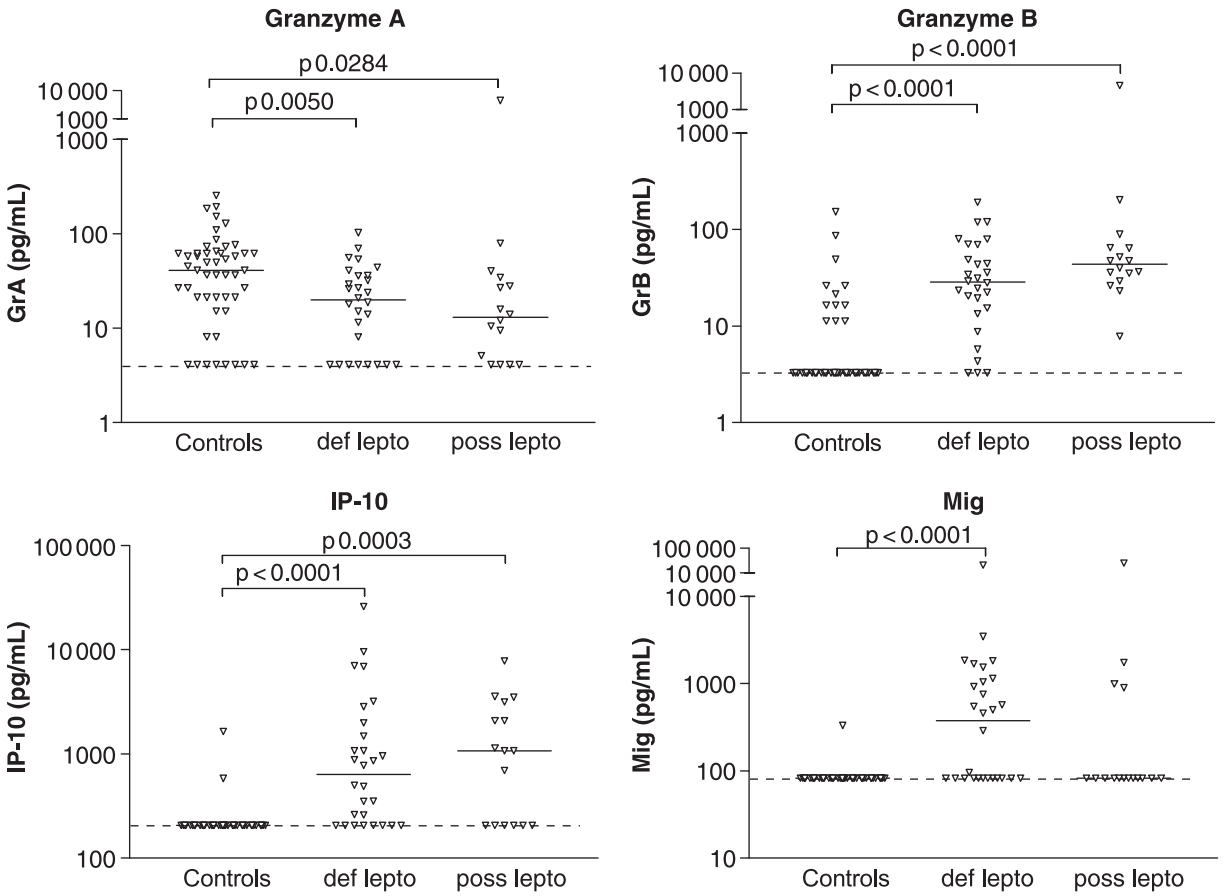

Fig. 1. Plasma concentrations of granzyme A, granzyme B, interferon (IFN)-γ-inducible protein 10 (IP-10) and monokine induced by IFN-γ (Mig) in patients with definite (def) or possible (poss) leptospirosis (for definitions see Materials and Methods), and healthy blood donors. Horizontal lines represent medians; dotted lines represent detection limits of the assays. The p values reflect differences between patient groups according to the Mann–Whitney *U*-test.

Host defence against extracellular organisms is predominantly humoral in nature, whereas intracellular organisms are contained by a cellular immune response [7]. Since leptospires are extracellular organisms, a humoral immune response has been considered previously to contribute most to host defence against this pathogen [8]. The present study investigated the role of cell-mediated immunity in response to infection with *Leptospira* by studying the activation of CLs. Previous studies have documented elevated levels of Gr among patients with viral infections, scrub typhus and melioidosis [6,9–11]. Gram-positive and Gram-negative bacteria can induce the secretion of Gr *in vitro* [11]. In the present study, GrB levels were found to be elevated among patients with definite or possible leptospirosis. Surprisingly, GrA levels were lower in both patient groups compared with controls, which contrasts with earlier findings for scrub typhus and melioidosis [6,11]. In addition, GrA and GrB levels did not show a correlation. Together, these data suggest that the release of GrA and GrB is regulated by different mechanisms, and that leptospirosis does not trigger GrA release.

IP-10 and Mig, two chemokines that are induced in response to interferon-γ, bind specifically to CXCR3 [4]. It has been suggested that enhanced production of IP-10 and Mig may be important for the activation and attraction of $CXCR3^+$ T-helper 1 cells to the site of infection, which can contribute to host defence by the production of additional type 1 cytokines. In particular, expression of IP-10 and/or Mig has been found among patients suffering from diseases associated with a type 1 immune response, including leishmaniasis, tuberculosis, tuberculoid leprosy, *Chlamydia* infections, and scrub typhus [6,12–14]. The present findings indicate that increased production of these mediators of a

cellular immune response is not limited to intracellular infections. In agreement with these results, a number of Gram-negative and Gram-positive bacteria are capable of inducing IP-10 and Mig release in human whole blood *in vitro* [15].

To our knowledge, this is the first study in which the circulating levels of GrA, GrB, IP-10 and Mig have been studied in a large series of patients with leptospirosis. The observation of elevated levels of GrB, IP-10 and Mig in these patients suggests that activation of a cellular immune response is part of the early host defence against *Leptospira*.

ACKNOWLEDGEMENTS

We are grateful to N. Boonyarueng and all the staff of the Department of Medicine, Udon Thani General Hospital, Udon Thani, Thailand, for help in collecting specimens and clinical data. This study was part of the Wellcome Trust-Mahidol University, Oxford Tropical Medicine Research Programme funded by The Wellcome Trust of Great Britain.

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RESEARCH NOTE

Sexual behaviour and *Chlamydia trachomatis* infections in German female urban adolescents, 2004

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ABSTRACT

This ad-hoc observational study, conducted in the metropolitan area of Berlin during 2004, revealed that the prevalence of *Chlamydia trachomatis* (CT) infections in female urban adolescents self-presenting at their gynaecologist without (n = 397) or with (n = 124) symptoms of CT infection was 5.5% (95% CI 3.7–8.2%) and 9.7% (95% CI 5.6–16.2%), respectively. The prevalence of CT infection was significantly dependent on the number

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