

Elevated levels of interleukin-13 and IL-18 in patients with dengue hemorrhagic fever

A.S. Mustafa^{a,*}, E.A. Elbishbishi^a, R. Agarwal^{b,1}, U.C. Chaturvedi^{a,b,1}

^a Department of Microbiology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait

^b Department of Microbiology, K.G. Medical College, Lucknow, India

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Abstract

Interleukin (IL)-13 is produced by T helper 2 (Th2)-type cells and inhibits the production of proinflammatory cytokines by activated monocytes, while IL-18 is a pleiotropic cytokine that induces interferon- γ and plays an important role in the development of Th1-type cells. Role of the shift from a Th1-type response to Th2-type has been suggested in the pathogenesis of dengue hemorrhagic fever (DHF). This study was undertaken to investigate the possible protective/pathogenic role of IL-13 and IL-18 in patients with DHF. Sera were collected from a total of 84 patients with various grades of dengue illness and 21 normal healthy controls and tested for IL-13 and IL-18 levels using commercial enzyme-linked immunosorbent assay kits. The results showed that very low levels of IL-13 (4 ± 3 pg ml⁻¹) and IL-18 (15 ± 4 pg ml⁻¹) were detected in the sera of healthy controls. In dengue patients, the levels of IL-13 and IL-18 were the highest in the patients with DHF grade IV (205 ± 103 pg ml⁻¹ and 366 ± 155 pg ml⁻¹, respectively) and the lowest in patients with dengue fever (22 ± 12 pg ml⁻¹ and 76 ± 50 pg ml⁻¹, respectively). Both the cytokines appeared (IL-13 = 20 ± 11 pg ml⁻¹ and IL-18 = 70 ± 45 pg ml⁻¹) during the first 4 days of illness and reached peak levels (IL-13 = 204 ± 96 pg ml⁻¹ and IL-18 = 360 ± 148 pg ml⁻¹) by day 9 onwards. The presence of high levels of IL-13 and IL-18 during severe illness and late phases of the disease suggests that both of these cytokines may contribute to the shift from a Th1- to Th2-type response and thus to the pathogenesis of DHF. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Interleukin (IL)-13 is produced by T helper 2 (Th2)-type cells and inhibits the production of proinflammatory cytokines by activated monocytes, induces B cell proliferation and differentiation including IgE production and upregulates major histocompatibility complex molecules on cell surfaces [1]. IL-13 shows significant structural and functional homology with IL-4 [2]. IL-18 is a pleiotropic cytokine and, like IL-12, it plays an important role in the development of Th1-type cells. Originally, IL-18 was known as interferon- γ (IFN- γ) inducing factor. IL-18 is produced by activated blood monocytes and tissue macrophages such as Kupffer cells, and acts primarily as a co-

stimulant for Th1 cells, inducing production of IFN- γ , IL-2 and GM-CSF, stimulating IL-2R α -chain expression and Th1 cell proliferation. Additionally, IL-18 enhances allo-specific CTL activity in vitro, and like IL-12 it augments cytotoxic NK cell activity and inhibits production of IL-10 (but not of IL-4) [3,4]. Although IL-18 and IL-12 share the capacity to induce IFN- γ production by activated Th1 cells, their induction pathways seem to be independent, since neutralizing antibodies to IL-12 do not block IFN- γ production induced by IL-18 and vice versa [5].

Dengue virus produces either a mild, self-limiting febrile illness, dengue fever (DF), or a severe, often fatal illness, dengue hemorrhagic fever (DHF). The characteristic pathological features of DHF are increased capillary permeability, cerebral edema, altered number and functions of leucocytes, increased hematocrit and thrombocytopenia [6]. Extensive plasma leakage into various serous cavities of the body may result in profound shock and death. Despite extensive studies, the pathogenesis of DHF is still not fully understood. We have reported a shift from a Th1-type cytokine response in DF to a Th2-type cytokine

* Corresponding author. Tel.: +965 (531) 2300 ext. 6505;
Fax: +965 (533) 2719/(531) 8454; E-mail: abuselim@hsc.kuniv.edu.kw

¹ Present address: Industrial Toxicology Research Centre, Lucknow, India.

response in DHF that correlates with increasing severity of the illness [7]. A similar cytokine response is observed in dengue virus-infected human peripheral blood leucocyte cultures [8]. The levels of IL-12 (an inducer of Th1-type cytokines) were the highest in patients with DF, but this cytokine could not be detected in the sera of patients with DHF grades III and IV [9]. While the levels of transforming growth factor β 1 (TGF- β 1) (an inhibitor of Th1-type and enhancer of Th2-type cytokines) in patients with dengue correlated with the severity of the disease and showed an inverse relationship with IL-12 [10]. This indicated a possible role of Th1-type cytokines in protection and Th2-type cytokines in pathogenesis of DHF [11]. IL-4 and IL-13 have similar biological activities and are characteristic of cytokines expressed by Th2-type cells. On the other hand, IL-12 and IL-18 are strong cofactors for development of Th1-type cells. To further understand the role of cytokine-mediated effects, the present study was undertaken to investigate the possible protective/pathological role of IL-13 and IL-18 in patients with dengue disease.

2. Materials and methods

2.1. Patients

For this study, a total of 84 patients with laboratory-

confirmed diagnosis of dengue virus infection were selected as described earlier [7,12]. Depending upon the severity of the illness they were classified as DF, the mildest, to DHF grades I, II, III or IV, the most severe, according to the criteria of the World Health Organization [12,13]. As controls, 21 normal age-matched healthy individuals, without history of any febrile or other illnesses in the previous 3 months, were included. Among the patients, 24 were classified as DF, 10 as DHF grade I, 24 as DHF grade II, and 13 each as DHF grade III and grade IV. Sera collected from the patients and the controls were divided into aliquots (to avoid repeated freezing and thawing) and quickly frozen and stored at -60°C . For the cytokine study sera were transported to Kuwait on dry ice and stored at -70°C until tested.

2.2. Assay of IL-13 and IL-18

Commercial enzyme-linked immunosorbent assay (ELISA) kits (purchased from R&D Systems, Minneapolis, MN, USA) were used to measure IL-13 and IL-18 levels in the sera of patients and controls according to the instructions of the manufacturer. All the tests were set up in duplicate and the data were analyzed by Genesis Windows Software for microplate-based assays (Labsystems, Finland). By this assay, the minimum detectable concentrations of IL-13 and IL-18 were 1.5 pg ml^{-1} and 12.5 pg ml^{-1} , respectively. The mean value of each cytokine in the

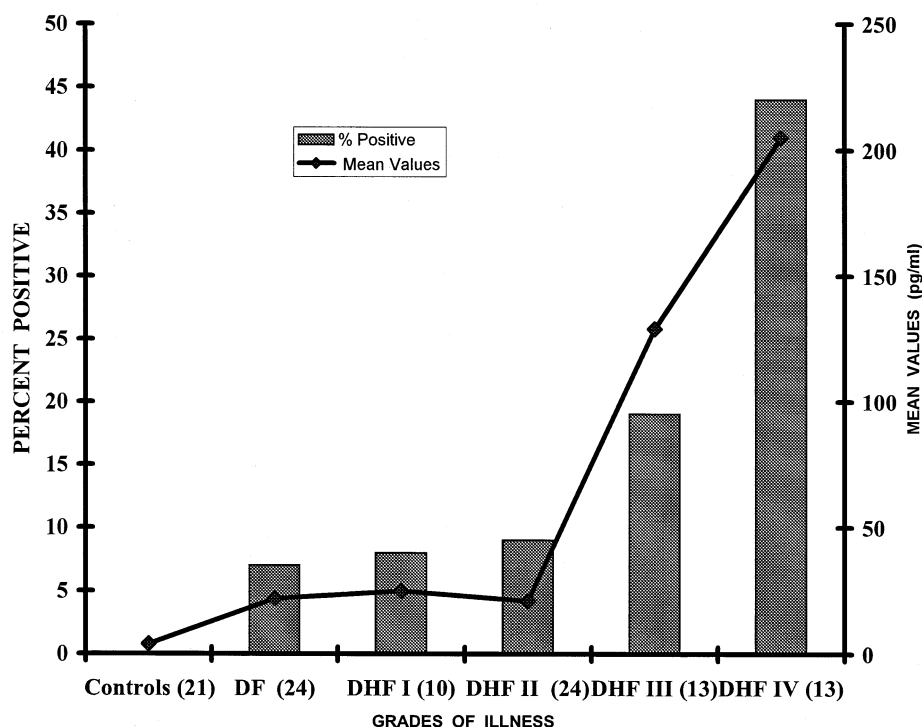


Fig. 1. Levels of IL-13 in patients with dengue. Sera collected from the patients with various grades of the illness were screened for IL-13 concentration by sandwich ELISA using commercial kits. The mean values (line) of the data (pg ml^{-1}) and the percentages of the patients positive for IL-13 (columns) have been presented. The figures in parentheses represent the total number of the cases in each group.

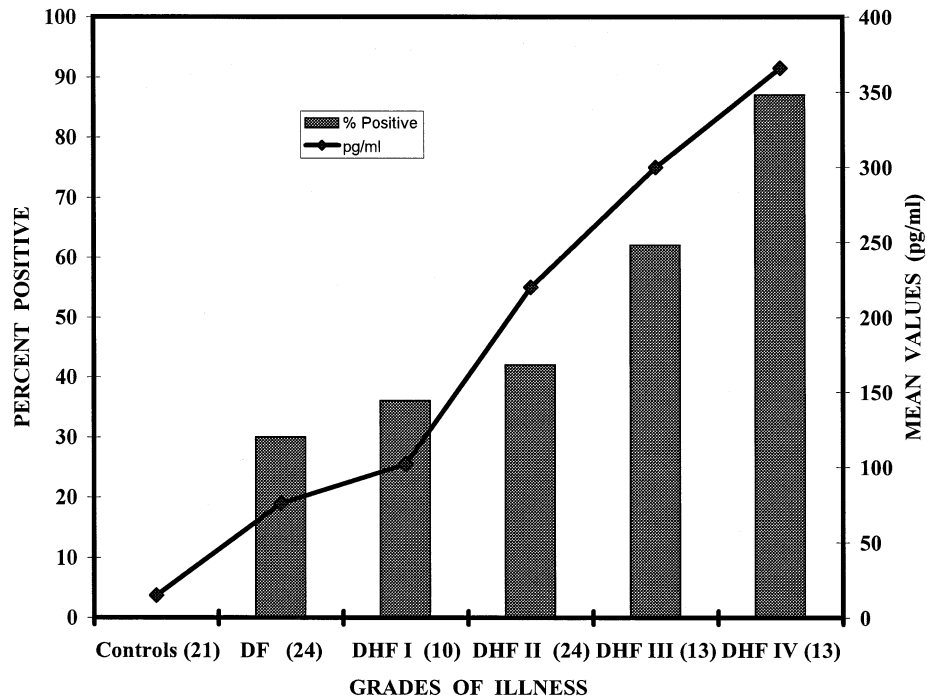


Fig. 2. Levels of IL-18 in patients with dengue. Sera collected from the patients with various grades of the illness were screened for IL-18 concentration by sandwich ELISA using commercial kits. The mean value (line) of the data (pg ml^{-1}) and the percentages of the patients positive for IL-18 (columns) have been presented. The figures in parentheses represent the total number of the cases in each group.

control sera plus 3 S.D. was used as 'cut-off' value for designation of patient sera as positive or negative. The data were analyzed using Student's *t* test. A *P* value of less than 0.05 was considered significant. The findings are presented as mean value \pm S.D. and percentage of cytokine positive patients.

3. Results

3.1. Serum levels of IL-13

The mean value of IL-13 in control sera was $4 \pm 3 \text{ pg ml}^{-1}$ with a range of $0\text{--}8 \text{ pg ml}^{-1}$. The findings presented

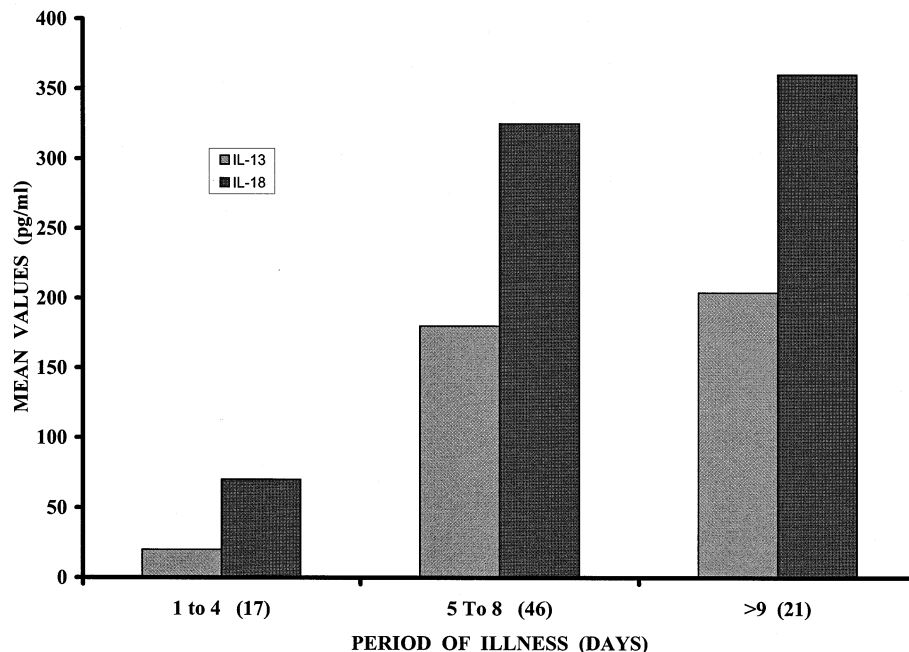


Fig. 3. Amount of IL-13 and IL-18 in patient sera (pg ml^{-1}) as a function of the stage of dengue illness. The figures in parentheses represent the total number of patients in each group.

in Fig. 1 show that IL-13 was present in the sera of 7–9% patients with DF, DHF grade I and DHF grade II, while it was present in 19% patients with DHF grade III and in 44% patients with DHF grade IV. As compared to patients with DF (mean value of 22 ± 12 pg ml⁻¹), significantly higher levels of IL-13 were detected in the sera of patients with DHF grade III (129 ± 76 pg ml⁻¹; $P < 0.001$) and DHF grade IV (205 ± 103 pg ml⁻¹; $P < 0.001$).

The patients were grouped according to the day of the illness at the time of collection of the sera, as between days 1 and 4, between days 5 and 8 and day 9 onwards. When the data were analyzed with respect to the days of illness, it was observed that IL-13 levels were lowest (20 ± 11 pg ml⁻¹) during the first 4 days of illness, the levels increased to 180 ± 70 pg ml⁻¹ on days 5–8 and peaked to 204 ± 96 pg ml⁻¹ on day 9 onwards (Fig. 3); the difference from the initial period was significant ($P < 0.001$).

3.2. Serum levels of IL-18

The mean value of IL-18 in the control sera was 15 ± 4 pg ml⁻¹. As compared to the patients with DF (mean value 76 ± 50 pg ml⁻¹), significantly higher concentrations of IL-18 were detected in the sera of patients with DHF grade III (300 ± 110 pg ml⁻¹; $P < 0.001$) and grade IV (366 ± 155 pg ml⁻¹; $P < 0.001$) (Fig. 2). Among patients with DHF, 35%, 40%, 62% and 87% patients with DHF grade I, grade II, grade III and grade IV, respectively, were positive, while only 30% of patients with DF were positive for IL-18 (Fig. 2). One of the DHF grade IV patients had the highest IL-18 serum concentration of 568 ± 21 pg ml⁻¹. The levels of IL-18 during different time periods of the illness are presented in Fig. 3. The data show that serum levels of IL-18 were lowest (70 ± 45 pg ml⁻¹) during 1–4 days of the illness and reached the highest level (360 ± 148 pg ml⁻¹; $P < 0.001$) on day 9 onwards.

4. Discussion

The findings of the present study show, for the first time, that both the cytokines, IL-13 and IL-18, are associated with the severity of the dengue illness and are at peak levels in patients with DHF grade IV. Our previous studies on the same group of dengue patients showed a shift from the predominant Th1-type cytokine response observed in 66% cases of mild illness (DF) to a Th2-type response in 71% of the patients with severe DHF grade IV, which have a high fatality rate [7]. These findings are supported by studies carried out in the mouse model [6,14]. During dengue virus infection a unique cytokine, cytotoxic factor (hCF), is also produced by CD4⁺ T cells, which is pathogenesis-related and plays a key role in the development of DHF [6,11,15]. Recent studies have shown the presence of hCF autoantibodies in sera of patients with

dengue illness. The high hCF autoantibody levels in patients with DF are associated with the low levels of hCF. On the other hand, high hCF levels in patients with DHF grade IV are associated with low levels of hCF autoantibodies [16]. These findings support the view that presence of high levels of hCF autoantibodies, that are neutralizing, may protect against development of severe DHF [16].

With respect to macrophage cytokines, our previous studies with DHF patients have shown that the presence of IL-12 is associated with Th1-type response and its absence with the Th2-type response [9], while the reverse is true for TGF- β 1 [10]. On the basis of these results, it has been proposed that dengue virus induces the production of a cytokine cascade that shifts a Th1-dominant response to a Th2-biased response resulting in an exacerbation of dengue disease and possible death of the patients [11]. The findings of the present study with respect to the highest levels of IL-13 (a Th2-type cytokine) in DHF grade IV patients are in line with the above scheme. Moreover, Hoshino et al. [17] have recently reported that IL-18 acts as a potent coinducer of IL-13 in T cells and NK cells. Whether this is true in patients with severe dengue disease remains to be investigated.

IL-18 is produced by activated monocytes/macrophages and acts primarily as a co-stimulant with IL-12, for Th1-type cells, inducing production of IFN- γ , IL-2 and GM-CSF, stimulating IL-2R α -chain expression and Th1 cell proliferation [4]. It has also been shown that IL-18 acts synergistically with influenza A virus-induced IFN- α and IFN- β to induce IFN- γ production [18]. But the finding of increased levels of IL-18 in patients with severe dengue disease, similar to Th2-type cytokines, was unexpected. However, recent studies show that cytokines may not follow the expected path in different circumstances. For example IL-12 is not essential for the generation of Th1-type cytokine response in mouse hepatitis virus [19] and lymphocytic choriomeningitis virus [20] infections. Marshall et al. [21] have reported complete restoration of IL-12 production by Th2-associated cytokines while other reports have suggested that IL-18 may act as a strong coinducer of Th2-type cytokines depending upon the nature of stimulation and the target cell type [22,23].

Macrophages are the primary source of IL-12, TGF- β 1 and IL-18 that are important immunoregulatory cytokines and have multiple roles to play in the pathogenesis of viral diseases. Macrophages are also the principal cells to replicate dengue virus and present its antigen to immunologically competent cells (reviewed in [6]). It is possible that a selective induction to produce IL-18 occurs during dengue virus infection of macrophages. Similar induction of macrophages to produce IL-18 has been reported through activation of caspase-1 in Sendai virus infection [24]. Further, on one hand IL-18 has been shown to have an antiviral effect in a mouse model of vaccinia virus infection [25] and on the other, IL-18 is associated with the pathogenesis of autoimmunity [4], arthritis [26], and sepsis

[27] etc. Therefore, the findings of increased levels of IL-18 in patients with severe dengue disease, similar to Th2-type cytokines, although unexpected, may in fact be contributing to the pathogenesis of severe DHF.

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References

- [1] de Vries, J.E. and Zurawski, G. (1995) Immunoregulatory properties of IL-13: its potential role in atopic disease. *Int. Arch. Allergy Immunol.* 106, 175–179.
- [2] Zurawski, G. and de Vries, J.E. (1994) Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol. Today* 15, 19–26.
- [3] Ushio, S., Namba, M., Okura, T., Hattori, K., Nukada, Y., Akita, K., Tanabe, F., Konishi, K., Micallef, M., Fujii, M., Torigoe, K., Tanimoto, T., Fukuda, S., Ikeda, M., Okamura, H. and Kurimoto, M. (1996) Cloning of the cDNA for human IFN- γ -inducing factor, expression in *Escherichia coli*, and studies on the biologic activities of the protein. *J. Immunol.* 156, 4274–4279.
- [4] Gillespie, M.T. and Horwood, N.J. (1998) Interleukin-18: Perspectives on the newest interleukin. *Cytokines Growth Factor Rev.* 9, 109–116.
- [5] Kohno, K., Kataoka, J., Ohtsuki, T., Suemoto, Y., Okamoto, I., Usui, M., Ikeda, M. and Kurimoto, M. (1997) IFN- γ -inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. *J. Immunol.* 158, 1541–1550.
- [6] Chaturvedi, U.C., Dhawan, R. and Mukerjee, R. (1997) Immunosuppression and cytotoxicity of dengue infection in the mouse model. In: *Dengue and Dengue Haemorrhagic Fever* (Gubler, D.J. and Kuno, G., Eds.), pp. 289–309. CAB International Press, Wallingford, Oxon.
- [7] Chaturvedi, U.C., Raghupathy, R., Pacsa, A.S., Elbishbishi, E.A., Agarwal, R., Nagar, R., Misra, A., Kapoor, S., Mathur, A., Khan, M.A.Y. and Azizieh, F. (1999) Shift from a Th1-type response to Th2-type in dengue haemorrhagic fever. *Curr. Sci.* 76, 63–69.
- [8] Chaturvedi, U.C., Elbishbishi, E.A., Raghupathy, R., Nagar, R., Tandon, R., Younis, O.I. and Azizeh, F. (1999) Sequential production of cytokines by dengue virus infected human peripheral blood leukocyte cultures. *J. Med. Virol.* 59, 335–340.
- [9] Pacsa, A.S., Agarwal, R., Elbishbishi, E.A., Chaturvedi, U.C., Nagar, R. and Mustafa, A.S. (2000) Interleukin-12 in patients with dengue haemorrhagic fever. *FEMS Immunol. Med. Microbiol.* 28, 151–155.
- [10] Agarwal, R., Elbishbishi, E.A., Chaturvedi, U.C., Nagar, R. and Mustafa, A.S. (1999) Profile of transforming growth factor β 1 in patients with dengue haemorrhagic fever. *Int. J. Exp. Pathol.* 80, 143–149.
- [11] Chaturvedi, U.C., Agarwal, R., Elbishbishi, E.A. and Mustafa, A.S. (2000) Cytokine cascade in dengue haemorrhagic fever: implications for pathogenesis. *FEMS Immunol. Med. Microbiol.* 28, 183–188.
- [12] Agarwal, R., Kapoor, S., Nagar, R., Misra, A., Tandon, R., Mathur, A., Misra, A.K., Srivastava, K.L. and Chaturvedi, U.C. (1999) A clinical study of the patients with dengue haemorrhagic fever during the epidemic of 1996 at Lucknow, India. *Southeast Asian J. Trop. Med. Public Health* 30, 735–740.
- [13] Nimmannitya, S. (1993) Clinical manifestations of dengue/dengue haemorrhagic fever. In: *Monograph on Dengue/Dengue Haemorrhagic Fever* (Thongcharoen, P., Ed.), pp. 48–54. WHO-SEARO, 22, New Delhi.
- [14] Chaturvedi, U.C., Tandon, P., Mathur, A. and Kumar, A. (1978) Host defence mechanisms against dengue virus infection of mice. *J. Gen. Virol.* 39, 293–302.
- [15] Agarwal, R., Chaturvedi, U.C., Misra, A., Mukerjee, R., Kapoor, S., Nagar, R., Tandon, R. and Mathur, A. (1998) Production of cytotoxic factor by peripheral blood mononuclear cells (PBMC) of the cases of dengue haemorrhagic fever. *Clin. Exp. Immunol.* 112, 340–344.
- [16] Chaturvedi, U.C., Elbishbishi, E.A., Agarwal, R. and Mustafa, A.S. (2001) Cytotoxic factor-autoantibodies: possible role in the pathogenesis of dengue haemorrhagic fever. *FEMS Immunol. Med. Microbiol.* 30, in press.
- [17] Hoshino, T., Wiltrot, R.H. and Young, H.A. (1999) IL-18 is a potent coinducer of IL-13 in NK and T cells: a new potential role for IL-18 in modulating the immune response. *J. Immunol.* 162, 5070–5077.
- [18] Sereniva, T., Matikainen, S., Kurimoto, M. and Julkunen, I. (1998) Influenza A virus-induced IFN- α/β and IL-18 synergistically enhance IFN- γ gene expression in human T cells. *J. Immunol.* 160, 6032–6038.
- [19] Schijns, V.E.C.J., Haagsmans, B.L., Wierda, C.M.H., Kruithof, B., Heijnen, I.A.F.M., Alber, G. and Horzinek, M.C. (1998) Mice lacking IL-12 develop polarized Th1 cells during viral infection. *J. Immunol.* 160, 3958–3964.
- [20] Oxenius, A., Karrer, U., Zinkernagel, R.M. and Hengartner, H. (1999) IL-12 is not required for induction of type 1 cytokine responses in viral infections. *J. Immunol.* 162, 965–973.
- [21] Marshall, J.D., Robertson, S.E., Trinchieri, G. and Chehimi, J. (1997) Priming with IL-4 and IL-13 during HIV-1 infection restores in vitro IL-12 production by mononuclear cells of HIV-infected patients. *J. Immunol.* 159, 5705–5714.
- [22] Hoshino, T., Yagita, H., Ortaldo, J.R., Wiltrot, R.H. and Young, H.A. (2000) In vivo administration of IL-18 can induce IgE production through Th2 cytokine induction and up-regulation of CD40 ligand (CD154) expression on CD⁺ T cells. *Eur. J. Immunol.* 30, 1998–2006.
- [23] Leite-De-Moraes, M.C., Hameg, A., Pacilio, M., Koezuka, Y., Taniguchi, M., Van Kaer, L., Schneider, E., Dy, M. and Herbelin, A. (2001) IL-18 enhances IL-4 production by ligand-activated NKT lymphocytes: a pro-Th2 effect of IL-18 exerted through NKT cells. *J. Immunol.* 166, 945–951.
- [24] Pirhonen, J., Sareniva, T., Kurimoto, M., Julkunen, I. and Matikainen, S. (1999) Virus infection activates IL-1 β and IL-18 production in human macrophages by a caspase-1-dependent pathway. *J. Immunol.* 162, 7322–7329.
- [25] Tanaka-Kataoka, M., Kunikata, T., Takayama, S., Iwaki, K., Ohashi, K., Ikeda, M. and Kurimoto, M. (1999) In vivo antiviral effect of interleukin 18 in a mouse model of vaccinia virus infection. *Cytokine* 11, 593–599.
- [26] Wei, X., Leung, B.P., Arthur, H.M., McInnes, I.B. and Liew, F.Y. (2001) Reduced incidence and severity of collagen-induced arthritis in mice lacking IL-18. *J. Immunol.* 166, 517–521.
- [27] Endo, S., Inada, K., Yamada, Y., Wakabayashi, G., Ishikura, H., Tanaka, T. and Sato, S. (2000) Interleukin 18 (IL-18) levels in patients with sepsis. *J. Med.* 31, 15–20.