

*Short Communication***Interleukin-4 and interferon- γ levels in Epstein-Barr virus-associated infectious mononucleosis and nasopharyngeal carcinoma***Dyah R. Budiani^a, Sofia M. Haryana^b, Wihaskoro Sosroseno^{*c}***Abstract**

BACKGROUND: Cytokines have been suggested to participate in the pathogenesis of infectious mononucleosis (IM) and nasopharyngeal carcinoma (NPC).

METHODS: Serum levels and gene expression of interleukin-4 (IL-4) and interferon- γ (IFN- γ) were assessed by immunologic and PCR assays, respectively in patients with Epstein-Barr virus (EBV)-associated IM and NPC and EBV negative controls.

RESULTS: The serum levels of IFN- γ were elevated, but those of IL-4 were decreased in IM and NPC patients as compared with those of the control group ($p < 0.05$).

CONCLUSIONS: These results suggest that serum levels of IFN- γ may be predominant over those of IL-4 during the course of IM and NPC.

KEYWORDS: Interferon-Gamma, Interleukin-4, Infectious Mononucleosis, Nasopharyngeal Neoplasms.

JRMS 2011; 16 (1): 94-97

Infectious mononucleosis (IM), a syndrome of painful lymphadenopathy, sore throat and fatigue, and nasopharyngeal carcinoma (NPC), a tumor of epidermoid origin, are strongly associated with Epstein-Barr Virus (EBV), since the EBV genome has been found consistently in the specimens of both disorders.¹ However, the precise mechanism(s) by which EBV alters the host immune response and leads to the development of EBV-associated infections and/or malignancies remains to be further elucidated. Cytokines produced by the host immunocompetent cells and/or EBV-infected cells may play a crucial role in the progression of IM and NPC. For example, expression of T cell-derived cytokines such as interferon- γ (IFN- γ) and interleukin-4 (IL-4) has been observed in the biopsies of NPC and IM,² suggesting that both cytokines may participate during the course of EBV-

associated health disorders. Indeed, a serological observation also revealed increased levels of serum IFN- γ in patients with IM³ and NPC.⁴ On the other hand, the serum levels of IL-4 in IM patients were comparable with those of the healthy subjects,⁵ but those of NPC patients were slightly higher.⁴ Studies comparing directly the cytokine levels in patients with IM and NPC are, however, still lacking. The aim of this study was to determine the serum levels of IFN- γ and IL-4 and their gene expression in peripheral blood mononuclear cells in patients with EBV-associated IM and NPC.

Methods

After getting informed consents, peripheral blood and serum samples were obtained from 32 EBV-positive NPC and 5 EBV-positive IM patients who were under observation and treatment at Dr. Sardjito's General Hospital,

^a Lecturer, Immunology Unit, Department of Pathology, School of Medicine, Sebelas Maret University, Surakarta, Indonesia.

^b Professor, Department of Histology and Cell Biology, School of Medicine, Gadjah Mada University, Yogyakarta, Indonesia.

^c Professor, School of Dentistry, AIMST University, Semeling, Bedong, Kedah, Malaysia.

* Corresponding Author

E-mail: wsosroseno@yahoo.com

Yogyakarta, Indonesia. This study was approved by the ethical committee of the Faculty of Medicine, Gadjah Mada University. Ten EBV-seronegative subjects were used as the control. Serum anti-VCA and EBNA IgG assessed by a commercially available kit (Panbio, Sinnamon Park, Queensland, Australia) and were used to determine the positive or negative status of EBV in serum samples. The serum levels of IL-4 were determined by an ELISA kit (R&D System, Minneapolis, MN, USA). The serum levels of IFN- γ were detected by an immuno-dot blot assay using diluted anti-human IFN- γ antibodies (Boehringer Mannheim, Gaithersburg, Maryland, USA).⁶ The result of each sample subtracted from the optical density reading of the relevant internal control, was divided by 100 and expressed as densitometric units (DU).

Peripheral blood mononuclear cells (PBMC) were isolated and the expression of IL-4 and IFN- γ mRNA was semiquantitatively assessed by a polymerase chain reaction (PCR)-colorimetric dot blot assay. Briefly, total RNA from PBMC was extracted and the resulting cDNA was amplified by PCR. The sequences of the primers used in the amplification of human IL-4 were as follow: 5'-CTGCAAATCGACACCTATTA-3' and 5'-GATCGTCTTTAGCCTTTC - 3' (product size: 0.44 kb). The sequences of the primers used in the amplification of human IFN- γ were as follow: 5'-CCATGGGCCCGCAGGCGGCAGC-3' and 5'-GAGGACGGAGAGCTGTTCTTCC-3' (product size: 0.49 kb). The PCR products were then immobilized on nitrocellulose membrane using a modified dot blot apparatus, hybridized, visualized and read at an absorbance of 546 nm. The result of each sample was subtracted from the optical density reading of the relevant internal control, divided by 100 and expressed as densitometric units (DU).

Data were statistically calculated by one-way analysis of variance followed by Fisher's least square difference using a statistical pack

age (SPSS Inc., Chicago).

Results

The serum levels of IL-4 in patients with IM and NPC were significantly lower than those of the control ($p < 0.05$) (Figure 1A). No significant difference between the serum levels of this cytokine in patients with IM and those with NPC was observed ($p > 0.05$) (Figure 1A). However, the serum levels of IFN- γ in patients with NPC were significantly higher than those in the control and patients with IM ($p < 0.05$) (Figure 1B). Slightly increased serum levels of IFN- γ in patients with IM as compared to those of the control could be detected ($p < 0.05$) (Figure 1B). Interestingly, the levels of IL-4 and IFN- γ mRNA expression in patients with IM and NPC were not significantly different from those of the control ($p > 0.05$) (Figure 2).

Discussion

The present study showed that the serum levels of IL-4 in IM and NPC patients were lower, but the serum levels of IFN- γ in these patients were higher than those of the healthy subjects, suggesting predominant IFN- γ -driven immunity in IM and NPC patients which is in agreement with previous reports.³⁻⁵ It has been believed that increased levels of IFN- γ may be associated with decreased levels of IL-4 during viral infections and vice versa.⁷ One may assume, therefore, that IM and NPC seen in the present study might be predominantly associated with IFN- γ -associated immunity which might, in turn, down-regulate the activation of IL-4-associated immunity. However, this speculation remains to be further investigated.

Interestingly, the present study also found that the serum levels of IFN- γ in NPC were much higher than those in IM. The exact reason of this finding is not well understood. Moreover, the previous studies have reported much lower levels of IFN- γ gene expression in infected lymphoid tissues of IM patients compared to those in the tissues of NPC.^{8,9} Since

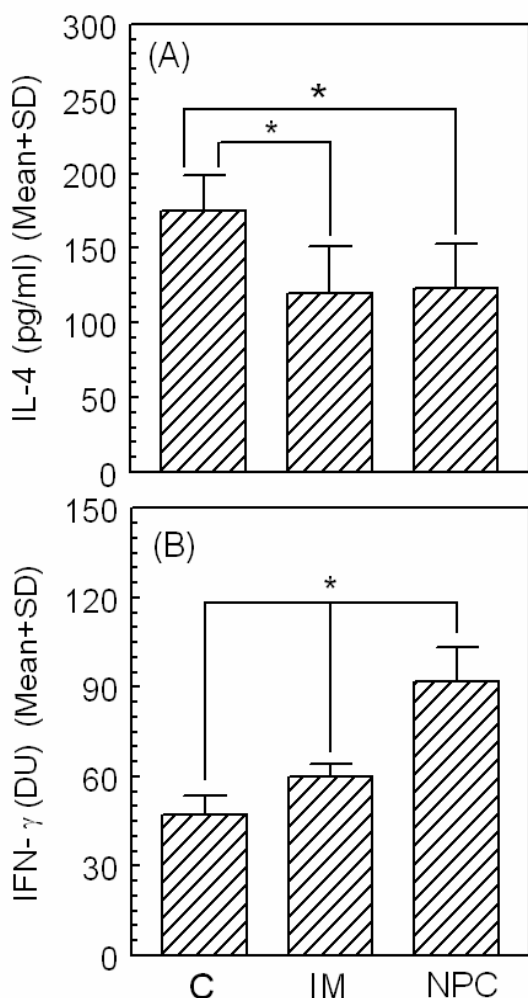


Figure 1. Serum levels of IL-4 (A) and IFN- γ (B) in patients with infectious mononucleosis (IM) and nasopharyngeal carcinoma (NPC). * $P < 0.05$ is considered as significant; C: controls.

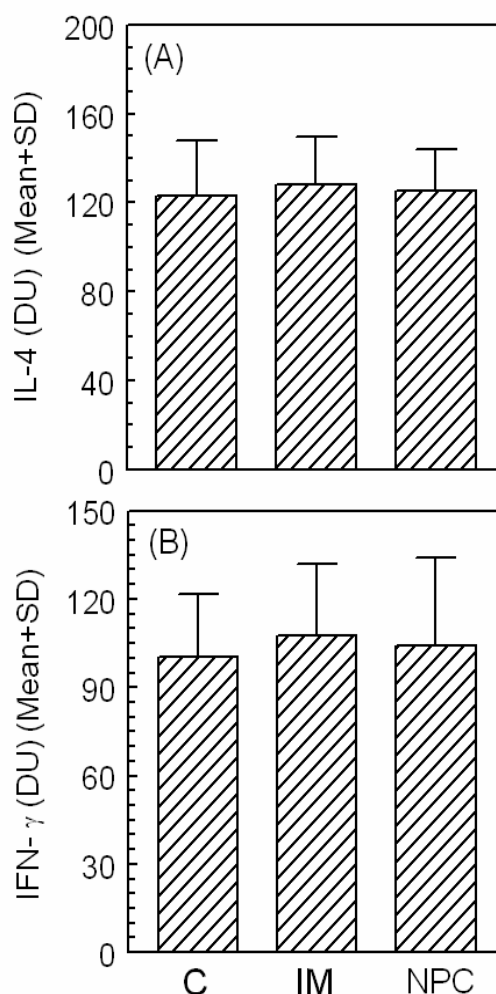


Figure 2. The levels of IL-4 (A) and IFN- γ (B) mRNA in the unstimulated peripheral blood mononuclear cells of patients with infectious mononucleosis (IM) and nasopharyngeal carcinoma (NPC). C: controls.

increased levels of IFN- γ are indicators of a more active cellular immunity in NPC,¹⁰ a possibility that IFN- γ -driven cellular immune response in NPC may be more intense than that in IM cannot be ruled out.

Surprisingly, IL-4 and IFN- γ mRNA levels in PBMC of IM and NPC patients were comparable with those of the control. One of the possibilities to explain this result is that the present study has used unstimulated PBMC from these patients, making detection of altered cytokine gene expression difficult. Support for this notion is the fact that elevated

cytokine production by peripheral blood cells from IM patients has been observed only after activation of the cells by mitogens¹¹ or T cell receptor ligation.¹² It seems plausible, therefore, that unstimulated PBMC from IM and NPC patients may not be the preferable source of cells to detect EBV-altered cytokine gene expression. If so, one may speculate further that detectable serum level of IL-4 and IFN- γ in both IM and NPC patients seen in the present study might be driven from the EBV-infected tissues.

The extrapolation of the present study in the management of IM and NPC remains

speculative. If increased serum levels of cytokines seen in the present study originate from the infected or tumor tissues, it would seem that the host immune response in both IM and NPC is predominated by IFN- γ -driven cellular immunity which is much more intense in the latter than the former disorder. Subsequently, it remains to be further investigated whether the serum levels of IFN- γ may be used as one of the markers to assess the management of IM and NPC. The fact that the serum level of this cytokine has been used in IM patients to monitor the progression of disease and follow the treatment^{3,5} suggests that monitoring of IFN- γ serum levels may be worthy to detect the progression of NPC as well.

Conflict of Interests

Authors have no conflict of interests.

Author's Contributions

DRB and SMH designed, conducted and analyzed the study. WS designed and analyzed the study and prepared the paper. All authors have read and approved the content of the manuscript.

References

1. Middeldorp JM, Brink AA, van den Brule AJ, Meijer CJ. Pathogenic roles for Epstein-Barr virus (EBV) gene products in EBV-associated proliferative disorders. *Crit Rev Oncol Hematol* 2003;45(1):1-36.
2. Huang YT, Sheen TS, Chen CL, Lu J, Chang Y, Chen JY, et al. Profile of cytokine expression in nasopharyngeal carcinomas: a distinct expression of interleukin 1 in tumor and CD4+ T cells. *Cancer Res* 1999;59(7):1599-605.
3. Dukers DF, Jaspars LH, Vos W, Oudejans JJ, Hayes D, Cillessen S, et al. Quantitative immunohistochemical analysis of cytokine profiles in Epstein-Barr virus-positive and -negative cases of Hodgkin's disease. *J Pathol* 2000;190(2):143-9.
4. Corsi MM, Ruscica M, Passoni D, Scarmozzino MG, Gulletta E. High Th-1 type cytokine serum levels in patients with infectious mononucleosis. *Acta Virol* 2004;48(4):263-6.
5. Li J, Zeng XH, Mo HY, Rolén U, Gao YF, Zhang XS, et al. Functional inactivation of EBV-specific T-lymphocytes in nasopharyngeal carcinoma: implication for tumor immunotherapy. *PLoS ONE* 2007;2(11):e1122.
6. Attarbaschi T, Willheim M, Ramharter M, Hofmann A, Wahl K, Winkler H, et al. T cell cytokine profile during primary Epstein-Barr virus infection (infectious mononucleosis). *Eur Cytokine Netw* 2003;14(1):34-9.
7. Panigrahi D, Burks M, Hariharan H, Finkelstein RA. Evaluation of immuno-dot-blot assay for detection of cholera-related enterotoxin antigen in *Salmonella typhimurium*. *J Clin Microbiol* 1987;25(4):702-5.
8. Estcourt MJ, Ramshaw A, Ramsay AJ. Cytokine responses in virus infections: effects on pathogenesis, recovery and persistence. *Curr Opin Microbiol* 1998;1(4):411-8.
9. Setsuda J, Teruya-Feldstein J, Harris NL, Ferry JA, Sorbara L, Gupta G, et al. Interleukin-18, interferon- γ , IP-10, and Mig expression in Epstein-Barr virus-induced infectious mononucleosis and posttransplant lymphoproliferative disease. *Am J Pathol* 1999;155(1):257-65.
10. Tang KF, Chan SH, Loh KS, Chong SM, Wang D, Yeoh KH, et al. Increased production of interferon-gamma by tumor infiltrating T lymphocytes in nasopharyngeal carcinoma: indicative of an activated status. *Cancer Lett* 1999;140(1-2):93-8.
11. Hornef MW, Wagner HJ, Kruse A, Kirchner H. Cytokine production in a whole-blood assay after Epstein-Barr virus infection in vivo. *Clin Diagn Lab Immunol* 1995;2(2):209-13.
12. Andersson J, Andersson U. Characterization of cytokine production in infectious mononucleosis studied at a single-cell level in tonsil and peripheral blood. *Clin Exp Immunol* 1993;92(1):7-13.

Conclusions

The present study shows that the serum levels of IFN- γ , but not IL-4, elevate in IM and particularly NPC patients. However, the gene expression of both cytokines in unstimulated PBMC of IM and NPC patients is similar to those of the controls. These results suggest that the levels of IFN- γ may be predominant over those of IL-4 during the course of IM and NPC.

Acknowledgements

This work was supported by DCRG-URGE (Grant batch III, 1996/1997), from The Ministry of National Education, The Indonesian Government. The authors gratefully thank Drs M. Tjokronagoro and T. Saleh for their assistance.