The Journal of Laryngology & Otology

cambridge.org/jlo

Inflammatory cytokines and mononuclear cells in sudden sensorineural hearing loss

S H Yoon[1,†], M E Kim[2,†], H Y Kim[1], J S Lee[2] and C H Jang[1]

[1]Department of Otolaryngology, Chonnam National University Medical School, Gwangju and [2]Department of Life Science, BK21-plus Research Team for Bioactive Control Technology, Department of Biology, College of Natural Science, Chosun University, Gwangju, South Korea

Abstract

Objective. This study evaluated tumour necrosis factor-α, interleukins 10 and 12, and interferon-γ levels, peripheral blood mononuclear cells, and clusters of differentiation 17c and 86 expression in unilateral sudden sensorineural hearing loss.

Methods. Twenty-four patients with unilateral sudden sensorineural hearing loss, and 24 individuals with normal hearing and no history of sudden sensorineural hearing loss (who were attending the clinic for other problems), were enrolled. Peripheral blood mononuclear cells, and clusters of differentiation 11c and 86 were isolated and analysed. Plasma and supernatant levels of tumour necrosis factor-α, interferon-γ, and interleukins 10 and 12 were measured.

Results. There were no significant differences with respect to age and gender. Monocyte population, mean tumour necrosis factor-α level and cluster of differentiation 86 expression were significantly increased in the study group compared to the control group. However, interferon-γ and interleukin 12 levels were significantly decreased. The difference in mean interleukin 10 level was not significant.

Conclusion. Increases in tumour necrosis factor-α level and monocyte population might play critical roles in sudden sensorineural hearing loss. This warrants detailed investigation and further studies on the role of dendritic cells in sudden sensorineural hearing loss.

Introduction

Sudden sensorineural hearing loss (SNHL) is defined as acute SNHL of 30 dB or greater over a minimum of three contiguous frequencies in the pure tone audiogram, which develops in under 72 hours.[1] Vascular occlusion and viral infection are two feasible aetiological factors of sudden SNHL. An additional immune-mediated cause could be inflammation with abnormal sudden cellular stress in the cochlea.[2–5]

Experimentally, immunocompetent cells from the blood stream that pass through the blood–labyrinth barrier to the cochlea can induce an immune response. This recruited immune response can produce sudden degenerative changes in the organ of Corti, stria vascularis and spiral ganglion.[6,7] Autoimmune SNHL can rapidly progress between 30 and 60 years of age. However, sometimes it appears as sudden SNHL with bilateral involvement.[8] Clinically, unilateral sudden SNHL is more common than bilateral autoimmune SNHL. Merchant and colleagues reported that abnormal activation of cellular stress within the cochlea, especially activated nuclear factor kappa B that produces ancillary inflammatory cytokines, is a potential cause of sudden SNHL.[4,7]

Cytokines are small proteins secreted by cells. Cytokines exert specific effects on the interactions between cells. Cytokines are autocrine in nature, which means that the producer cells possess corresponding surface receptors. These receptors exist in cochlear cells.[9] Given the limited access to the cochlea and the difficulty in obtaining tissue samples, systemic inflammatory processes have been studied using peripheral blood to monitor possible pathogenetic mechanisms of sudden SNHL. Inflammatory cytokines and related proteins have been reported as potential markers in many cellular processes. However, to date, the role of inflammatory cytokines, specifically those in unilateral sudden SNHL, have not, to our knowledge, been extensively investigated. Few studies have discussed the involvement of inflammatory cytokines in sudden SNHL.[10–12]

This study investigated the levels of inflammatory cytokines, including tumour necrosis factor-α, interleukin (IL)-10 and IL-12, and interferon-γ, as well as peripheral blood mononuclear cells, and clusters of differentiation 11c and 86, in unilateral sudden SNHL.

Materials and methods

Participants

This study was given authoritative approval by the institutional review board of the tertiary university hospital (Chonnam National University Hospital; approval number CNUH-2013-042), and written informed consent was obtained from the participants.

Main Article

Dr C H Jang takes responsibility for the integrity of the content of the paper

[†]SH Yoon and ME Kim, both two authors contributed equally to this work.

Cite this article: Yoon SH, Kim ME, Kim HY, Lee JS, Jang CH. Inflammatory cytokines and mononuclear cells in sudden sensorineural hearing loss. *J Laryngol Otol* 2019;**133**:95–101. https://doi.org/10.1017/S0022215119000100

Accepted: 24 September 2018
First published online: 11 February 2019

Key words:
Hearing Loss, Sudden;
Tumor Necrosis Factor-Alpha; Monocyte;
CD86 Antigen

Author for correspondence:
Dr Jun-Sik Lee,
Department of Life Science,
BK 21-plus Research Team for Bioactive Control Technology, Biology,
College of Natural Science, Chosun University,
Gwangju, South Korea
E-mail: junsiklee@chosun.ac.kr
Fax: +822308850

Dr Chul Ho Jang,
Department of Otolaryngology,
Chonnam National University Hospital,
Hakdong 8, Dongku, Gwangju, South Korea
E-mail: jchsavio@gmail.com
Fax: +82 6222 06776

Twenty-four patients with unilateral sudden SNHL were involved in the study between January 2013 and June 2014. Sudden SNHL was diagnosed based on clinical findings of SNHL exceeding 30 dB in three contiguous frequencies within 3 days. Patients with an objective cause of sudden SNHL, such as vestibular schwannoma or traumatic perilymph fistula, were excluded. Data on ototoxic mediation exposure, circulatory disease, and clinical and family history were obtained through self-reporting. Sudden SNHL patients with associated diseases, such as acute infection, systemic hypertension, hyperlipidaemia, diabetes mellitus, cardiovascular disease or liver disease, were also excluded.

Twenty-four normal-hearing patients, with no history of sudden SNHL, who were attending the clinic for other problems, were recruited to serve as the control group.

Blood samples

Blood samples were taken from the study group before corticosteroid treatment, to avoid the effects of corticosteroids on inflammatory cytokines or mononuclear cells. Peripheral blood mononuclear cells were separated using density gradient centrifugation of heparinised blood (Ficoll-Paque Plus; GE Healthcare Bio-Sciences, Piscataway, New Jersey, USA).

Flow cytometry

Two-colour cytometry analysis using fluorescence-activated cell sorting was performed using aliquots of 1×10^5 fixed cells and specific antibodies. The cells were incubated with staining buffer (phosphate-buffered saline containing 0.5 fetal bovine serum and 0.1 per cent sodium azide) containing anti-human cluster of differentiation 11c and anti-human cluster of differentiation 86 antibodies (both from BioLegend, San Diego, California, USA) for 30 minutes on ice. Cells stained using the applicable isotype-matched immunoglobulin G2b were considered as negative controls. After staining, the cells were processed using 1 per cent paraformaldehyde. Analysis was performed using a fluorescence-activated cell sorting Calibur instrument equipped with CellQuest software (BD Biosciences, San Diego, California, USA).

Enzyme-linked immunosorbent assay

The serum was separated from the blood samples of both groups and preserved at −20°C until analysis. Supernatant cytokine level was measured using human tumour necrosis factor-α, interferon-γ, interleukin (IL)-10 and IL-12 ELISA Max Deluxe enzyme-linked immunosorbent assay sets (BioLegend) according to the manufacturer's protocols. Absorbance was assessed by an enzyme-linked immunosorbent assay microplate reader at 405 nm and the results were expressed in pg/ml.

Statistical analyses

Statistical significance was analysed using the student's *t*-test for unpaired observations, and the differences were checked for statistical significance by one-way analysis of variance followed by Bonferroni's post-hoc test. A *p*-value of less than 0.05 was considered significant. Results are expressed as mean ± standard deviation or standard error of the total number of repeated measurements.

Table 1. Demographics of control and sudden SNHL groups

Parameter	Control group*	Sudden SNHL group†
Mean age (years)	43.12	46.91
Males (*n*)	12	15
Females (*n*)	12	9

*$n = 24$; †$n = 24$. SNHL = sensorineural hearing loss.

Table 2. Initial hearing level in sudden SNHL patients

Hearing loss (pure tone average)	Patients (*n* (%))
Mild (30–50 dB)	7 (29)
Moderate (51–70 dB)	3 (13)
Severe (71–90 dB)	7 (29)
Profound (>90 dB)	7 (29)

SNHL = sensorineural hearing loss.

Results

The sudden SNHL group was composed of 15 males (62.5 per cent) and 9 females (37.5 per cent). The control group was composed of 12 males (50 per cent) and 12 females (50 per cent). The mean age was 46.91 years in the sudden SNHL group and 43.12 years in the control group. No significant difference was found with respect to mean age (Table 1). The hearing severity of the sudden SNHL group is shown in Table 2.

The monocyte population percentage (mean ± standard error) was significantly higher in the sudden SNHL group (26.36 ± 4.3) than in the control group (14.32 ± 2.3) (Figure 1). The dendritic cell markers of cluster of differentiation 11c and cluster of differentiation 86 were identified in the sudden SNHL group (Figure 2). The cluster of differentiation 11c positive cell population was not significantly different between the groups. However, cluster of differentiation 86 expression was significantly higher in the sudden SNHL group than in the control group.

The mean tumour necrosis factor-α level was significantly higher in the sudden SNHL group (15.8 ± 9.3 pg/ml) than in the control group (12.4 ± 8.7 pg/ml). Interferon-γ and interleukin (IL)-12 levels were significantly lower in the sudden SNHL group compared to the control group. There was no significant difference between the two groups in IL-10 level (Figure 3).

Discussion

Pro-inflammatory cytokines and anti-inflammatory cytokines have potential roles in immune responses. Among the cytokines, tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-2, IL-8 and IL-12, and interferon-γ are proinflammatory cytokines, while IL-4, IL-6 and IL-10 are anti-inflammatory cytokines.[12] In the present study, we compared the cytokine levels and monocyte population between sudden SNHL and control groups. The TNF-α level, monocyte population and cluster of differentiation 86 expression were higher in the sudden SNHL group than in the control group.

Although the aetiology of sudden SNHL remains unclear, inflammation is reported to be one of the significant causative factors involved in sudden SNHL.[13] Monocytes constitute a subset of circulating blood cells. Extravasation of circulating

https://doi.org/10.1017/S0022215119000100 Published online by Cambridge University Press

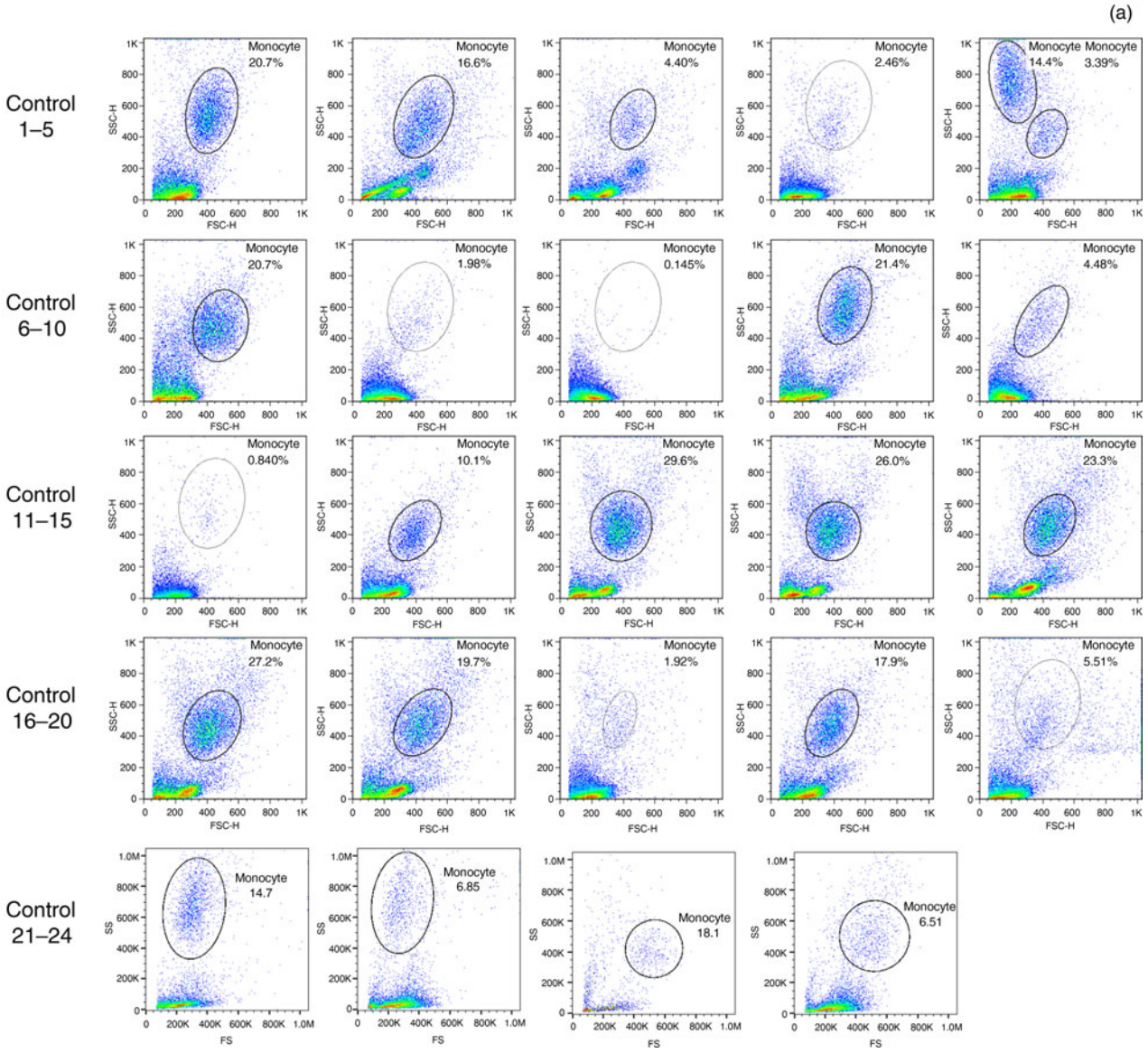

Fig. 1. Flow cytometry analysis of monocyte population in the (a) control group and (b) sudden sensorineural hearing loss (SNHL) group. (c) Monocyte population was significantly higher in the sudden SNHL group than the control group. SSC-H = side scatter height; FSC-H = forward scatter height; SS = side scatter; FS = forward scatter

monocytes in the bloodstream is activated by inflammatory mediators, such as proinflammatory cytokines, local growth factors and microbial products.[14] The monocytes subsequently migrate into tissue, and can differentiate into a range of tissue dendritic cells or macrophages.

This event induces inflammation and activates the host-defence immune system.[15] Proinflammatory cytokines, such as TNF-α, induce the differentiation of human monocytes into mature dendritic cells. The TNF-α-dependent dendritic cells elicit T helper 1 and 17 cell responses.[15] Moreover, T helper 1 and 17 cell responses are essential for autoimmune SNHL.[16] To our knowledge, this is the first study to evaluate the role of dendritic cells in sudden SNHL. In the present study, we identified the presence of dendritic cell markers, namely, clusters of differentiation 11c and 86. Our results showed that the cluster of differentiation 86 level was significantly higher in the sudden SNHL group than in the control group. Although we did not clarify the correlation between dendritic cells and cytokines, we suggest that TNF-α can enhance the differentiation of monocytes into mature dendritic cells. Further studies are planned to clarify the mechanism of dendritic cells in sudden SNHL.

The role of TNF-α in sudden SNHL has rarely been reported.[9,17] Furthermore, different studies have reported different levels of TNF-α. One study showed that sudden SNHL patients had lower levels of TNF-α compared to the control group.[17] However, another study reported higher levels of TNF-α in sudden SNHL patients, with steroid-treated non-responders showing significantly higher levels of TNF-α post-treatment.[9] In the present study, we did not compare TNF-α levels before and after treatment with steroids. The patients included in our study had unilateral sudden SNHL. None had bilateral symmetrical sudden SNHL with any history of autoimmune disease.

In this study, IL-12 and interferon-γ levels in the sudden SNHL group were lower than those in the control group. Interferon-γ is not a toxin designed to poison a key molecule in the cell. Normally, interferon and similar ILs mediate continuous interactions between cells related to growth and defence. Interferon-γ is produced by T-lymphocytes when stimulated with antigens or

https://doi.org/10.1017/S0022215119000100 Published online by Cambridge University Press

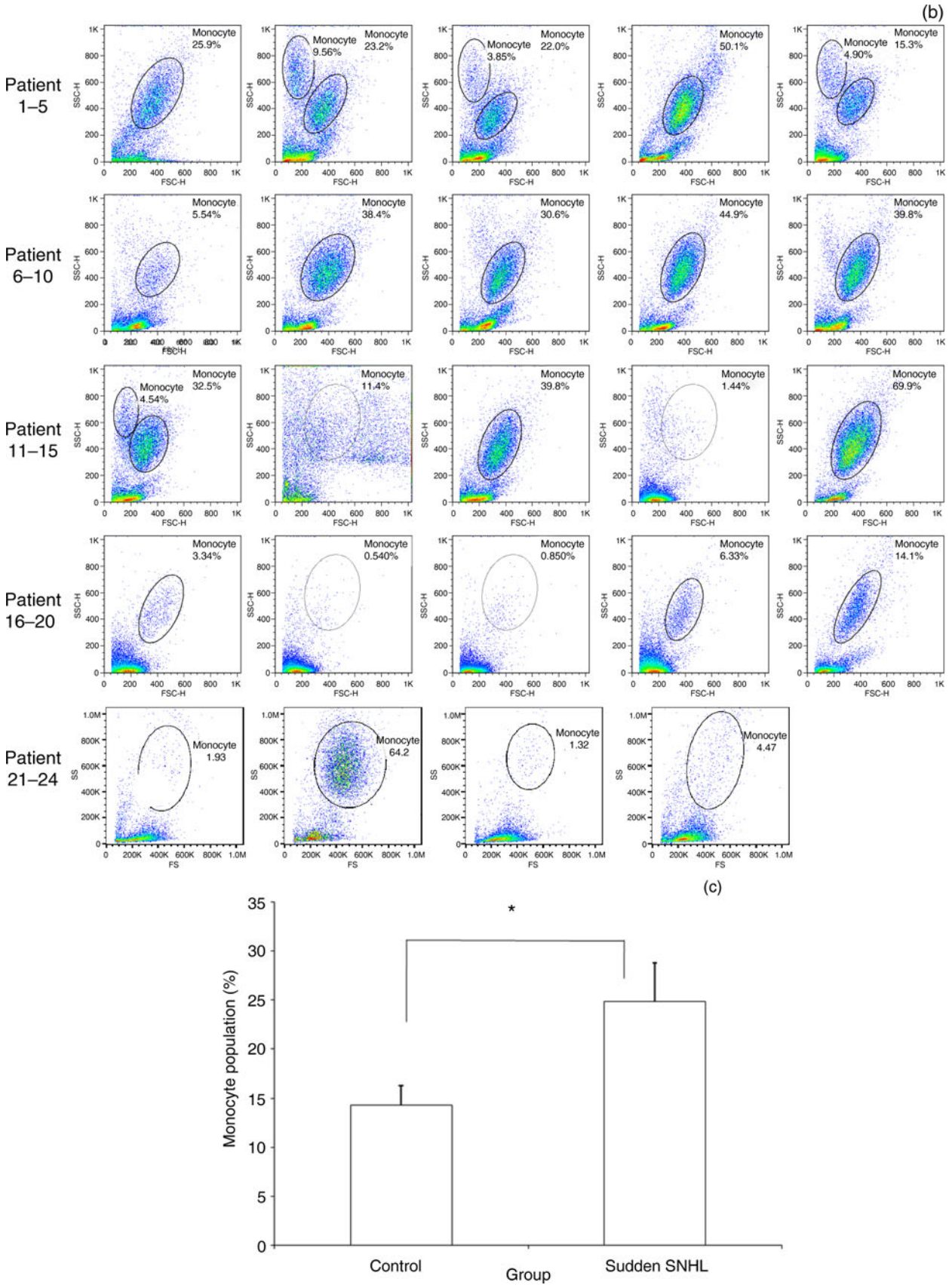

Fig. 1. (*Continued*)

https://doi.org/10.1017/S0022215119000100 Published online by Cambridge University Press

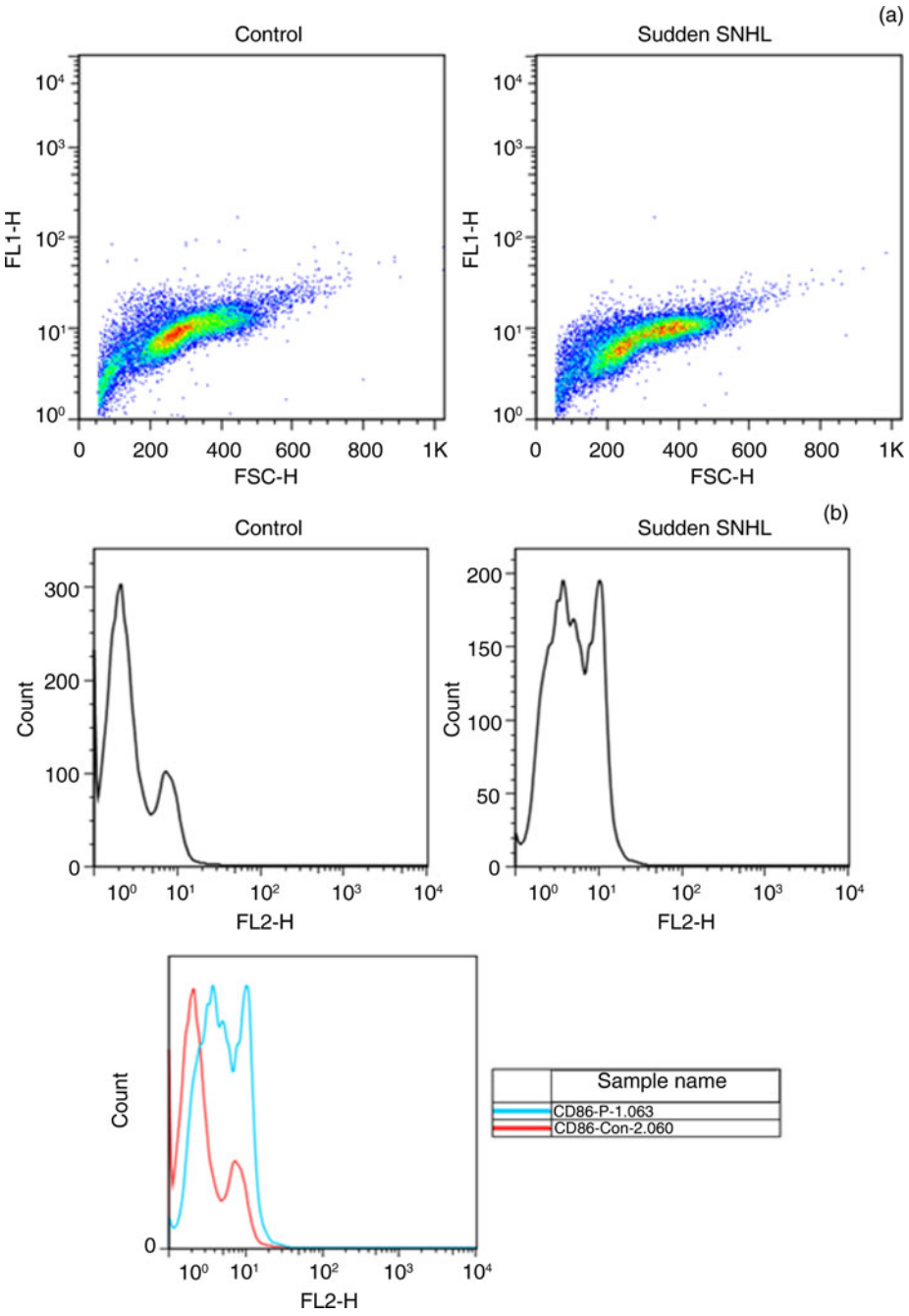

Fig. 2. (a) Representative fluorescence-activated cell sorting analysis of cluster of differentiation 11c showed no difference between the two groups. (b) Cluster of differentiation 86 (CD86) expression was significantly higher in the sudden sensorineural hearing loss (SNHL) group than the control group. Histograms demonstrate the sudden SNHL group (red colour) and control (red colour) group. FL1/2-H = fluorescence pulse height; FSC-H = forward scatter height

mitogens. Interleukin-12 is a central player in the generation of T helper 1 cells. Interleukin-12 is a regulatory cytokine that stimulates the production of interferon-γ, IL-2, IL-6 and granulocyte-macrophage colony-stimulating factor.[18]

- Increses in tumor necrosis factor-α level and monocyte popluation might play critical roles in sudden sensorineural hearing loss
- Further studies of role of dendritic cells in sudden sensorineural hearing loss are necessary

Interleukin-10 is an important anti-inflammatory cytokine, which inhibits the production of proinflammatory cytokines (interferon-γ and TNF-α) by T helper 1 cells.[9,19] In the present study, we did not find any significant differences in IL-10 production between the two groups. The observed IL-10 levels might not be adequate to counteract the strong proinflammatory response that occurred in the patients prior to our study.

Recently, TNF-α inhibitors have been used for sudden SNHL treatment. Demirhan *et al.* suggested that increased TNF-α level can inhibit the response to steroid treatment, and insisted that TNF-α inhibitor plays an important role in sudden SNHL.[9] Syrakic *et al.* also found that steroid-treated non-responders had a higher TNF-α level than control participants.[20] In this study, we did not analyse the prognosis of sudden SNHL with regard to TNF-α levels because peripheral blood was collected only once, before steroid treatment. Although the underlying pathology of sudden SNHL is not fully understood, disturbed cochlear blood flow is a major cause.[21,22] Recent *in vivo* experiments showed that TNF-α inhibitor can reverse the decreased cochlear blood flow induced by TNF-α.[23]

https://doi.org/10.1017/S0022215119000100 Published online by Cambridge University Press

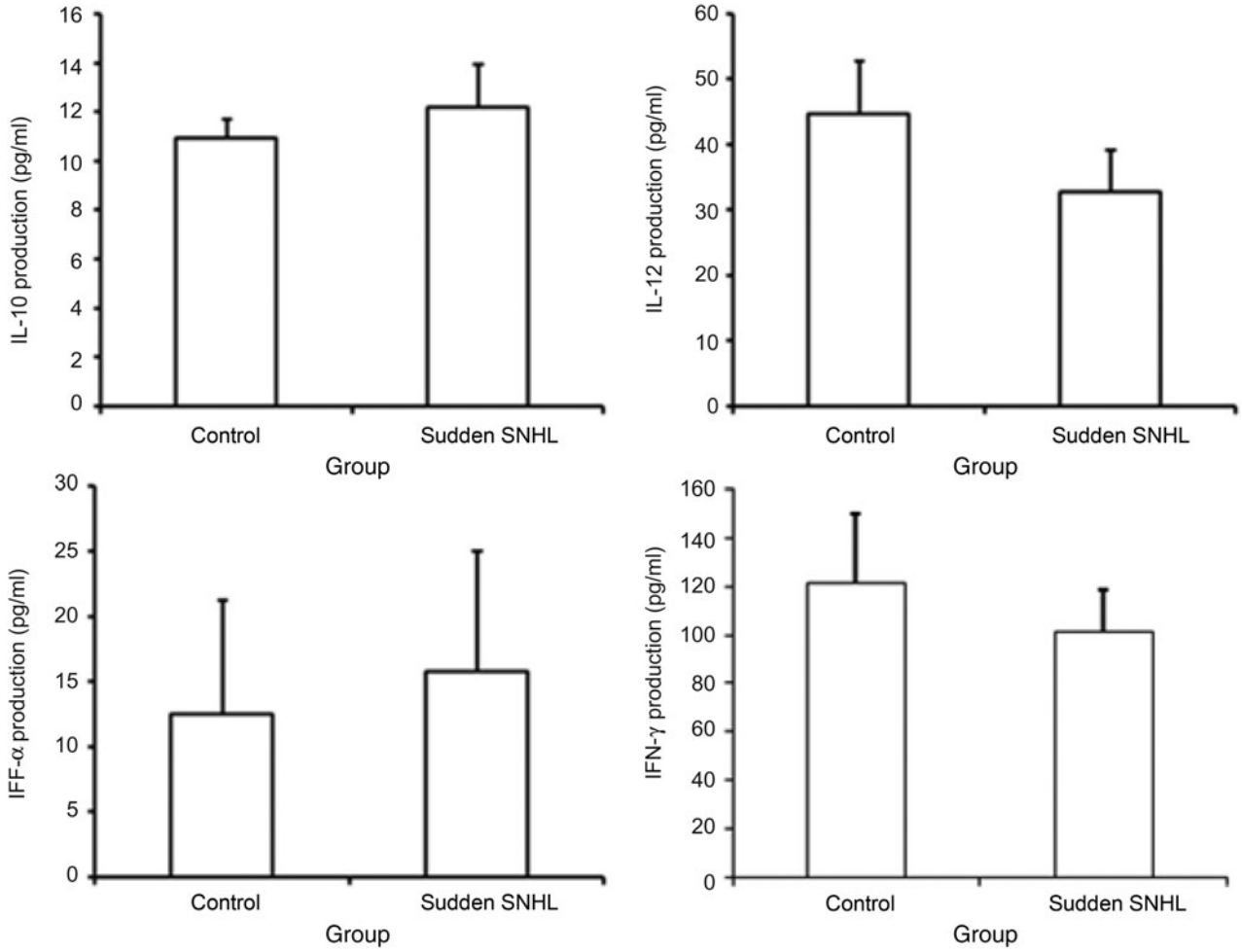

Fig. 3. Interleukin (IL)-10, IL-12, tumour necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) in serum samples analysed by enzyme-linked immunosorbent assay. The mean TNF-α level in the sudden sensorineural hearing loss (SNHL) group (15.8±9.3 pg/ml) was significantly higher than that in the control group (12.4±8.7 pg/ml). Interferon-γ and IL-12 levels were significantly lower in the sudden SNHL group compared to the control group. There was no significant difference between the two groups in IL-10 level.

Thus, our results show that increased TNF-α level, monocyte population and cluster of differentiation 86 play important roles in sudden SNHL. Further studies are necessary to clarify the significance of TNF-α, monocyte population and cluster of differentiation 86 in the pathogenesis of sudden SNHL.

Competing interests. None declared

References

1 Wilson H, Alderson DJ. Sudden sensorineural hearing loss: when is it idiopathic? *J Laryngol Otol* 2010;**124**:690–3
2 Yamasoba T, Kikuchi S, Higo R, O'Uchi T, Tokumaru A. Sudden sensorineural hearing loss associated with slow blood flow of the vertebrobasilar system. *Ann Otol Rhinol Laryngol* 1993;**102**:873–7
3 Masuda M, Kanzaki S, Minami S, Kikuchi J, Kanzaki J, Sato H *et al.* Correlations of inflammatory biomarkers with the onset and prognosis of idiopathic sudden sensorineural hearing loss. *Otol Neurotol* 2012;**33**:1142–50
4 Merchant SN, Durand ML, Adams JC. Sudden deafness: is it viral? *ORL J Otorhinolaryngol Relat Spec* 2008;**70**:52–60; discussion 2
5 Seo YJ, Jeong JH, Choi JY, Moon IS. Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio: novel markers for diagnosis and prognosis in patients with idiopathic sudden sensorineural hearing loss. *Dis Markers* 2014;**2014**:702807
6 Garcia-Berrocal JR, Trinidad A, Ramirez-Camacho R, Lobo D, Verdaguer M, Ibanez A. Immunologic work-up study for inner ear disorders: looking for a rational strategy. *Acta Otolaryngol* 2005;**125**:814–18
7 Merchant SN, Adams JC, Nadol Jr JB. Pathology and pathophysiology of idiopathic sudden sensorineural hearing loss. *Otol Neurotol* 2005;**26**:151–60
8 Xenellis J, Nikolopoulos TP, Stavroulaki P, Marangoudakis P, Androulakis M, Tsangaroulakis M *et al.* Simultaneous and sequential bilateral sudden sensorineural hearing loss: are they different from unilateral sudden sensorineural hearing loss? *ORL J Otorhinolaryngol Relat Spec* 2007;**69**:306–10
9 Demirhan E, Eskut NP, Zorlu Y, Cukurova I, Tuna G, Kirkali FG. Blood levels of TNF-alpha, IL-10, and IL-12 in idiopathic sudden sensorineural hearing loss. *Laryngoscope* 2013;**123**:1778–81
10 Haubner F, Martin L, Steffens T, Strutz J, Kleinjung T. The role of soluble adhesion molecules and cytokines in sudden sensorineural hearing loss. *Otolaryngol Head Neck Surg* 2011;**144**:575–80
11 Keles E, Sapmaz E, Godekmerdan A. The role of allergy in the etiopathogenesis of idiopathic sudden sensorineural hearing loss. *Eur Arch Otorhinolaryngol* 2013;**270**:1795–801
12 Singh AK, Dutta M, Chattopadhyay R, Chakravarty B, Chaudhury K. Intrafollicular interleukin-8, interleukin-12, and adrenomedullin are the promising prognostic markers of oocyte and embryo quality in women with endometriosis. *J Assist Reprod Genet* 2016;**33**:1363–72
13 Hiramatsu M, Teranishi M, Uchida Y, Nishio N, Suzuki H, Kato K *et al.* Polymorphisms in genes involved in inflammatory pathways in patients with sudden sensorineural hearing loss. *J Neurogenet* 2012;**26**:387–96
14 Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. *Nat Rev Immunol* 2011;**11**:762–74
15 Iwamoto S, Iwai S, Tsujiyama K, Kurahashi C, Takeshita K, Naoe M *et al.* TNF-alpha drives human CD14+ monocytes to differentiate into CD70+ dendritic cells evoking Th1 and Th17 responses. *J Immunol* 2007;**179**:1449–57
16 Mijovic T, Zeitouni A, Colmegna I. Autoimmune sensorineural hearing loss: the otology-rheumatology interface. *Rheumatology (Oxford)* 2013;**52**:780–9

https://doi.org/10.1017/S0022215119000100 Published online by Cambridge University Press

17 Suslu N, Yilmaz T, Gursel B. Utility of anti-HSP 70, TNF-alpha, ESR, antinuclear antibody, and antiphospholipid antibodies in the diagnosis and treatment of sudden sensorineural hearing loss. *Laryngoscope* 2009;**119**:341–6
18 Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* 2003;**3**:133–46
19 Joss A, Akdis M, Faith A, Blaser K, Akdis CA. IL-10 directly acts on T cells by specifically altering the CD28 co-stimulation pathway. *Eur J Immunol* 2000;**30**:1683–90
20 Svrakic M, Pathak S, Goldofsky E, Hoffman R, Chandrasekhar SS, Sperling N *et al*. Diagnostic and prognostic utility of measuring tumor necrosis factor in the peripheral circulation of patients with immune-mediated sensorineural hearing loss. *Arch Otolaryngol Head Neck Surg* 2012;**138**:1052–8
21 Muzzi E, Zennaro B, Visentin R, Soldano F, Sacilotto C. Hyperbaric oxygen therapy as salvage treatment for sudden sensorineural hearing loss: review of rationale and preliminary report. *J Laryngol Otol* 2010;**124**:e2
22 Narozny W, Sicko Z, Przewozny T, Stankiewicz C, Kot J, Kuczkowski J. Usefulness of high doses of glucocorticoids and hyperbaric oxygen therapy in sudden sensorineural hearing loss treatment. *Otol Neurotol* 2004;**25**:916–23
23 Ihler F, Sharaf K, Bertlich M, Strieth S, Reichel CA, Berghaus A *et al*. Etanercept prevents decrease of cochlear blood flow dose-dependently caused by tumor necrosis factor alpha. *Ann Otol Rhinol Laryngol* 2013;**122**:468–73

https://doi.org/10.1017/S0022215119000100 Published online by Cambridge University Press