

MCP-I produced by keratinocytes is associated with leucocyte recruitment during elicitation of nickel-induced occupational allergic contact dermatitis

Weihua Mai¹, Dongqing Lu², Xingwei Liu³ and Ling Chen⁴

Abstract

To investigate the expression profile of monocyte chemoattractant peptide-I (MCP-I) by keratinocytes after nickel exposure and to identify its role for leucocyte migration during nickel-induced occupational allergic contact dermatitis (OACD), 26 workers diagnosed with nickel-induced OACD were enrolled. Skin biopsies from the positive nickel-challenged sites at different time points were assessed by immunohistochemistry (IHC) for MCP-I, CD68, CD45RO, and in situ hybridization (ISH) for MCP-I, using chronic periumbilical dermatitis as controls. The expressions of MCP-I in HaCaT cell culture after nickel treatment were quantified by enzyme-linked immunosorbent assay. The results showed that at positive nickel-challenged sites, strong expressions of MCP-I, both messenger RNA (mRNA) and protein, were detected in the basal keratinocytes during the early phase (24–48 h after nickel application), paralleled by the recruitment of CD68⁺ and CD45RO⁺ cells to the skin compartments. The expressions of MCP-I declined gradually in the late phase (72–96 h after nickel application). Treatment with nickel sulfate at noncytotoxic concentrations (0.01–100 µM) induced a concentration-related elevation of MCP-I expression by HaCaT cells compared to the untreated cells. The data indicated that a temporal expression pattern of MCP-I produced by keratinocytes after nickel exposure was involved in the complex process of mononuclear cell infiltration during elicitation of nickel-induced OACD. Targeting MCP-I might be a potential therapeutic strategy for OACD.

Keywords

Monocyte chemoattractant peptide-I, occupational allergic contact dermatitis, nickel, HaCaT keratinocyte, leucocyte migration

Received 6 April 2017; Revised 28 September 2017; Accepted 2 October 2017

Introduction

Occupational allergic contact dermatitis (OACD) due to chemical exposure in the workplaces is one of the most prevalent occupational skin diseases in many countries, with a prevalence of 15–25%, resulting in severe economic and social problems (Chang and Dong, 2011; Zuberbier et al., 2014). How to prevent and control OACD effectively has become a great health concern.

The OACD is a type IV delayed hypersensitivity response to haptens in contact with the skin. Contact hypersensitivity (CHS) induced by contact sensitizing agents is its experimental model. Up to now, more than 3000 contact allergens have been identified

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(Martin et al., 2011). Nickel, a widely used chemical in manufacturing, is a well-known allergen for OACD. The immunopathogenic mechanisms for allergic contact dermatitis (ACD) are complex. It is considered as a T-lymphocyte-mediated immune disorder, comprised of sensitization and elicitation phases. Chemokines are known to be important mediators in ACD. During elicitation of ACD, multiple chemokines were produced by skin resident cells in a sequential and coordinated manner, promoting the recruitment of monocytes and lymphocytes into inflammatory skin compartments (Girolomoni et al., 2001; Goebeler et al., 2001; Martín et al., 2003; Sebastiani et al., 2002a).

Monocyte chemoattractant peptide-1 (MCP-1) is a potent chemokine for many types of leucocytes including monocytes and T cells. It can be produced by fibroblasts, endothelial cells, mast cells, and keratinocytes when exposed to inflammatory stimuli (Sebastiani et al., 2002a). It is known that MCP-1 is an important mediator in various pathological conditions including allergic rhinitis (König et al., 2015), asthma (Giuffrida et al., 2017), and psoriasis (Zablotna et al., 2016). In addition, it is described as an early participant in elicitation of ACD. Elevated expression of MCP-1 has been detected in CHS model (Goebeler et al., 2001; Mitsui et al., 2003) and patients with ACD (Kaur et al., 2012, 2014). Besides, evidences show that pituitary adenylate cyclase-activating polypeptide, the γ -amino-butyric acid type B (GABA B) receptor agonist baclofen, and tetrahydrocannabinol can exert anti-inflammatory effects in ACD by reducing the expression/function of MCP-1 (Duthey et al., 2010; Gaffal et al., 2013; Kemény et al., 2010), whereas histone deacetylase 3 and falcarninol may increase MCP-1 expression and involve in the development of allergic skin inflammation (Kim et al., 2012; Leonti et al., 2010).

It is proposed that different contact allergens may activate different types of cells, leading to distinct chemokine expression. The MCP-1 production in pleural mesothelial cells has been shown to be enhanced after nickel nanoparticle exposure (Glista-Baker et al., 2012). But the expression pattern of MCP-1 induced by nickel and its role in leucocyte recruitment during OACD has not been well elucidated. In this study, we sought to investigate the expression profile of MCP-1 by keratinocytes after nickel exposure in vitro and in vivo and to identify its correlation to the infiltration of mononuclear cells during elicitation of OACD. We supposed that MCP-

1 produced by keratinocytes early after nickel application was involved in the complex process of leucocyte infiltration and contributed to the development of OACD. The knowledge of the role of MCP-1 for nickel-induced OACD will help to develop novel therapeutic strategies in the future.

Methods

Study subjects

Twenty-six workers diagnosed with nickel-induced OACD at the Fifth Affiliated Hospital of Sun Yat-Sen University, with positive patch tests to 5% nickel sulfate, were recruited. The study group demographics were: 20 male cases, 6 female cases, average age: 30.7 ± 1.26 years, and nickel-exposure period: 6.5 ± 2.98 years. All enrolled patients were not accompanied with other inflammatory/autoimmune diseases, neither did they receive immunosuppressant/glucocorticoid medication at least three months prior to the study. This study was approved by the Local Ethical Committee of the Fifth Affiliated Hospital of Sun Yat-Sen University (Approval Number: 2014L30-1). The procedures were performed with informed consent of all subjects.

Patch test and skin biopsy

Patch test was performed on each enrolled patient with a standard technique (Bilcha et al., 2009) using occlusive Finn Chambers (IQ-Ultra, Chemotechnique Diagnostics, Vellinge, Sweden) and the Chinese Screening Series (CH-1000) comprising 20 allergens (cobalt chloride, mercapto mix, imidazolidinyl urea, *p*-phenylenediamine, *N*-cyclohexyl sulfur peptide lactone, potassium dichromate, ethylenediamine dihydrochloride, colophony, formaldehyde, epoxy resin, 2-bromo-2-nitropropane-1,3-diol, thiuram mix, paraben mix, nickel sulfate, sesquiterpene lactone mix, fragrance mix, Cl+Me-isothiazolinone, black rubber mix, carba mix, and quaternium-15). Results were graded as – (absent), + (mild), ++ (moderate), or +++ (intense). “Positive” or “negative” reaction was determined by the patch test appearance and the pattern. Skin biopsies were taken from the positive nickel-challenged sites at different time points, such as 24 h ($n = 8$), 48 h ($n = 9$), 72 h ($n = 9$), and 96 h ($n = 10$) after allergen application with the method described before (Lee et al., 2016), using chronic periumbilical dermatitis lesions ($n = 5$) as controls. Cryostat sections (6 μ m) were mounted

on 3-aminopropyltriethoxysilane (APES; AR0001, BOSTER, Wuhan, China)-coated slides and stored until use.

The IHC

The IHC was performed as previously described (McManus et al., 1998). Briefly, after inactivation of endogenous peroxidases and blockage of nonspecific binding, slides were incubated overnight at 4°C with mouse anti-CD45RO mAb at 1:100 (Clone UCHL1, M074201-2, DAKO, Denmark), mouse anti-CD68 mAb at 1:100 (Clone PG-M1, M087601-2, DAKO, Denmark), and mouse anti-MCP-1 mAb at 1:100 (BM1255, BOSTER, Wuhan, China). Isotype-matched immunoglobulins were used as negative controls. After washing with phosphate-buffered saline (PBS), the slides were incubated with biotin-conjugated goat anti-mouse immunoglobulins at 1:200 (SABC-POD kit, SA1021, BOSTER, Wuhan, China) for 20 min at 20°C. After that, the slides were incubated with streptavidin–biotin complex (SABC-POD kit, SA1021, BOSTER, Wuhan, China) at 20°C for 20 min. The reaction cascade was visualized by incubation with DAB (AR1022, BOSTER, Wuhan, China) as a substrate, followed by counterstain with hematoxylin (AR1180-1, BOSTER, Wuhan, China). The slides were counted in duplicate on five adjacent fields at 200× magnification. Cells expressing MCP-1, CD45RO, or CD68 were stained as brown. The expressions of MCP-1⁺, CD45RO⁺, and CD68⁺ cells were evaluated according to the following criteria: (A) the percentage of stained cells: 0 score (<20%), 1 score (20–50%), and 2 score (>50%); (B) cell staining: 0 score (*no brown staining*), 1 score (*stained light brown*), and 2 score (*stained dark brown*). Based on the total scores of these two indexes, the expressions were classified as follows: 0 score (–), 1 score (–/+), 2 score (+), 3 score (++), and 4 score (+++).

In situ hybridization

DIG-11-dUTP-labeled MCP-1 messenger RNA (mRNA) probe was generated by in vitro transcription, using the primers: 5'-TGAGTGGGGCGTT GATTGCATCTGGCTGAG-3' and 5'-AGCTTCTT TGGGACACTTGCTGCTGGTGAT-3'. In situ hybridization (ISH) was performed with MCP-1 ISH kit (MK1404, BOSTER, Wuhan, China) according to the manufacturer's instructions, using the method as previously described (Zhao et al., 2015). All reagents used in ISH were included in the MCP-1 ISH kit.

Briefly, after dewaxed and rehydrated in graded ethanol, slides were fixed in 4% paraformaldehyde/0.1 M PBS (pH 7.4) containing 1/1000 diethyl pyrocarbonate (DEPC) at room temperature for 20 min, followed by treatment with 3% hydrogen peroxide for 10 min to inactivate endogenous peroxidase. After washing, slides were treated with pepsin in 3% citric acid for 1 min at 37°C, followed by postfixed in 4% paraformaldehyde/0.1 M PBS (pH 7.4) containing 1/1000 DEPC for 10 min. Then, slides were incubated with prehybridization solution at 40°C for 4 h. The ISH was performed overnight at 40°C with sense or antisense probes diluted in hybridization solution. After hybridization, the sections were washed with standard saline citrate (2 × SSC) for 5 min at 37°C, followed by two additional washes in 0.5 × SSC and 0.2 × SSC for 15 min. After treated with blocking buffer for 30 min at 37°C, followed by biotin-conjugated anti-digoxin antibody for 60 min at 37°C, slides were incubated with streptavidin–biotin complex for 20 min at 37°C. The reaction cascade was visualized by incubation with DAB as a substrate. Cells expressing MCP-1 mRNA were stained as brown. MCP-1 mRNA expressions were evaluated according to the following criteria: (A) the percentage of stained cells: 0 score (<2.5%), 1 score (2.5–10%), and 2 score (>10%); (B) cell staining: 0 score (*no brown staining*), 1 score (*stained light brown*), and 2 score (*stained dark brown*). Based on the total scores of these two indexes, MCP-1 mRNA expressions were classified as follows: 0 score (–), 1 score (–/+), 2 score (+), 3 score (++), and 4 score (+++).

HaCaT cell culture and stimulation

The human keratinocyte HaCaT cell line was kindly provided by Laboratory of Immunodermatology in China Medical University, the Key Laboratory of the Ministry of Health. After thawing, HaCaT cells were cultured in RPMI 1640 medium (Gibco, 11875-093, ThermoFisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco, 26140-079, ThermoFisher Scientific, Waltham, MA, USA), 1 mM pyruvate (Gibco, 11360-070, ThermoFisher Scientific, Waltham, MA, USA), 2 mM L-Glutamine (Gibco, A2916801, ThermoFisher Scientific, Waltham, MA, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco, 15140122, ThermoFisher Scientific, Waltham, MA, USA), incubated at 37°C in 5% CO₂/95% O₂. For nickel stimulation, cells were seeded in 24-well plates at a density of 2×10^5

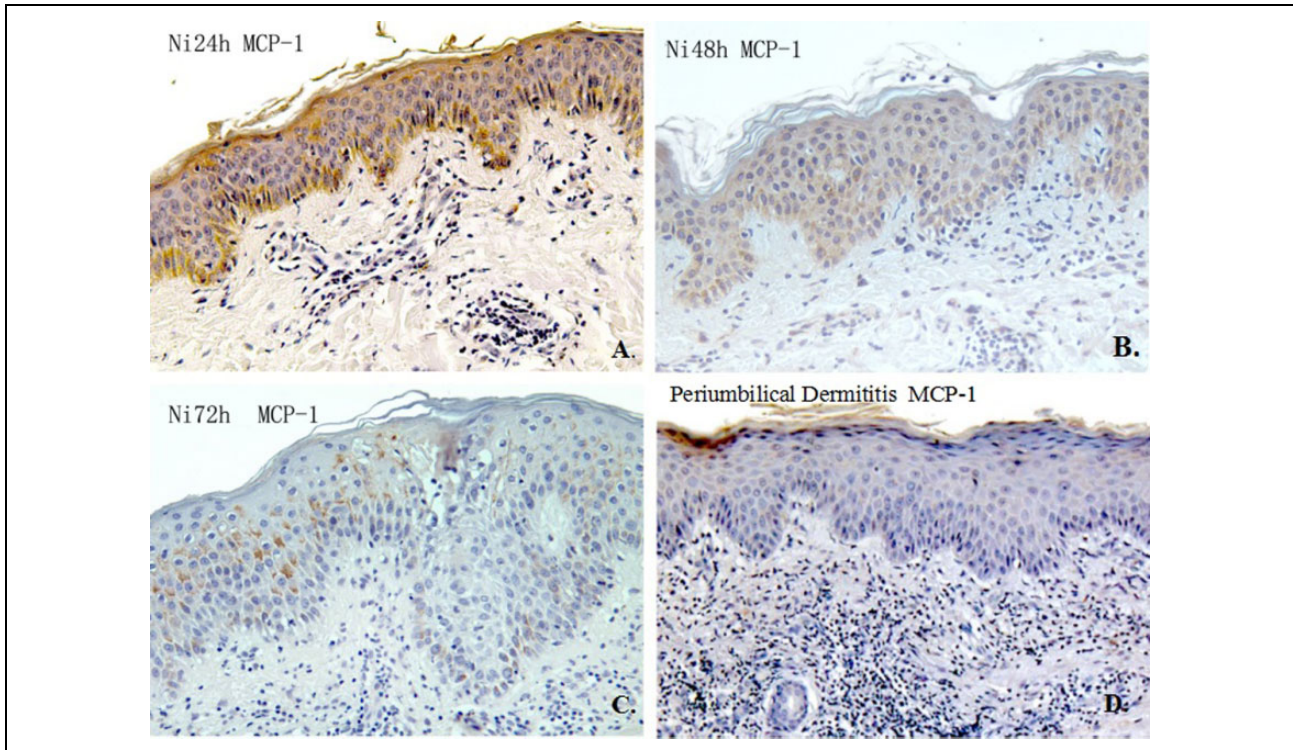


Figure 1. Expression profile of MCP-1 during elicitation of CHS in patients with OACD. Skin biopsies obtained from positive nickel-challenge sites at different time intervals (24, 48 and 72 hrs after allergen application) were immunohistologically labeled with anti-MCP-1 pAbs, using chronic periumbilical dermatitis as controls. Cells expressing MCP-1 were stained as light or dark brown. The amounts and staining intensity of MCP-1⁺ cells were compared. A strong expression of MCP-1 was presented in the basal keratinocytes at 24 and 48 hrs (A, B), whereas the expression of MCP-1 declined at 72 h (C). Non-immunoreactivity for MCP-1 was detectable in chronic periumbilical dermatitis (D). Magnification ratio: $\times 200$.

cells/well. When HaCaT cells grew to 90% confluence, the original medium was removed and the cells were washed with PBS twice. Then, HaCaT cells were incubated in serum-free RPMI 1640 medium and treated with nickel sulfate for 24 h at a range of concentrations (0.01, 0.1, 1, 10, 100 μM), using untreated cells as controls. Each experiment was performed in quintuplicate ($n = 5$). After nickel treatment, the supernatants of cultured cells were collected for assessment of MCP-1.

Enzyme-linked immunosorbent assay

MCP-1 expressions in the supernatants of cultured HaCaT cells were quantified by enzyme-linked immunosorbent assay kit (BMS281, Invitrogen, ThermoFisher Scientific) according to the manufacturer's instructions. The concentrations of MCP-1 were read at 450 nm in a Model 550 Microplate Reader (Bio-Rad, Hercules, CA, USA), using Microplate Manager version 4.0 software.

Statistical analysis

All statistical procedures were performed using the statistical package of the social sciences 16.0 (SPSS16.0). The MCP-1 expression rates in skin biopsies from positive patch test sites at different time points were compared by crosstabs chi-square tests (Fisher's exact test), while the expressions of MCP-1 induced by different concentrations of nickel in HaCaT cell culture were compared by one-way analysis of variance (ANOVA). $p < 0.05$ was considered to be statistically significant.

Results

Expression profile of MCP-1 induced by nickel during elicitation of CHS in patients of OACD

Skin biopsies obtained from positive nickel-challenged sites at different time points after allergen application were analyzed by IHC, using chronic periumbilical dermatitis as control. As shown in Figure 1, a strong expression of MCP-1 was detected in the

Table 1. Expression of MCP-I during elicitation of CHS.^a

	MCP-I expression		
	0 score (-)	1-2 score (-/+ ~ +)	3-4 score (++ ~ ++++)
24 h after elicitation of CHS	0 (0%)	1 (12.5%)	7 (87.5%) ^b
48 h after elicitation of CHS	0 (0%)	1 (11.1%)	8 (88.9%) ^b
72 h after elicitation of CHS	0 (0%)	7 (77.8%)	2 (22.2%)
Chronic periumbilical dermatitis	5 (100%)	0 (0%)	0 (0%)

MCP-I: monocyte chemoattractant peptide-I; CHS: contact hypersensitivity.

^aAccording to the amounts and staining intensity of MCP-I⁺ cells by immunohistochemistry, MCP-I expressions were classified as follows: 0 score (-), 1 score (-/+), 2 score (+), 3 score (++), and 4 score (+++), as described in the Methods section.

^b $p < 0.05$, compared with the rate of intensive MCP-I expression (++ ~ ++++) at 72 h after elicitation of CHS.

basal keratinocytes at 24 and 48 h, whereas MCP-I expression began to decline at 72 h. Nonimmunoreactivity for MCP-I was detectable in chronic periumbilical dermatitis.

The rates of intensive MCP-I expression (++ ~ ++++) at 24 and 48 h were higher than that of 72 h (87.5 vs. 22.2% Fisher's exact two-sided $p = 0.015 < 0.05$; 88.9 vs. 22.2%, Fisher's exact two-sided $p = 0.015 < 0.05$), but there was no significant difference between 24 and 48 h (87.5 vs. 88.9%, Fisher's exact two-sided $p = 1.000 > 0.05$). The rates of MCP-I expression at 24, 48, and 72 h were all significantly higher than that of chronic periumbilical dermatitis (100 vs. 0%, Fisher's exact two-sided $p = 0.001$, 0.000, 0.000 < 0.01). The data are shown in Table 1.

mRNA expression pattern of MCP-I induced by nickel during elicitation of CHS in patients of OACD

To assess the in situ mRNA expression pattern of MCP-I, skin biopsies obtained from positive nickel-challenged sites at 48 and 96 h after hapten application were hybridized with anti-sense probes of MCP-I. As shown in Figure 2, a strong expression of MCP-I mRNA appeared in the basal keratinocytes at 48 h, whereas the expression of MCP-I mRNA declined at 96 h. The rate of intensive MCP-I mRNA expression (++ ~ ++++) at 48 h was significantly

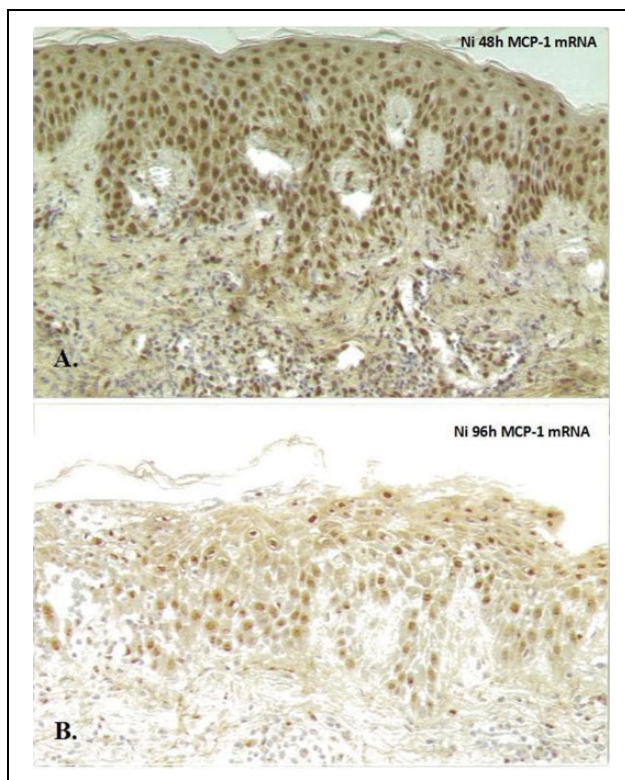


Figure 2. In situ mRNA expression of MCP-I during elicitation of CHS in patients with OACD. Skin biopsies obtained from positive nickel-challenged sites at 48 and 96 hrs after allergen application were hybridized with anti-sense probes of MCP-I. Cells expressing MCP-I mRNA were stained as light or dark brown. The amounts and staining intensity of MCP-I mRNA⁺ cells, as well as their location were compared. A strong expression of MCP-I mRNA was detected in the basal keratinocytes at 48 h (A), whereas the expression of MCP-I mRNA declined at 96 h (B). Magnification ratio: $\times 200$.

Table 2. In situ mRNA expression of MCP-I during elicitation of CHS.^a

	MCP-I mRNA expression		
	0 score (-)	1-2 score (-/+ ~ +)	3-4 score (++ ~ ++++)
48 h after elicitation of CHS	0 (0%)	2 (22.2%)	7 (77.8%) ^b
96 h after elicitation of CHS	0 (0%)	8 (80%)	2 (20%)

MCP-I: monocyte chemoattractant peptide-I; CHS: contact hypersensitivity; mRNA: messenger RNA.

^aAccording to the amounts and staining intensity of MCP-I mRNA⁺ cells by in situ hybridization, MCP-I mRNA expressions were classified as follows: 0 score (-), 1 score (-/+), 2 score (+), 3 score (++), and 4 score (+++), as described in the Methods section.

^b $p < 0.05$, compared with the rate of intensive MCP-I mRNA expression (++ ~ ++++) at 96 h after elicitation of CHS.

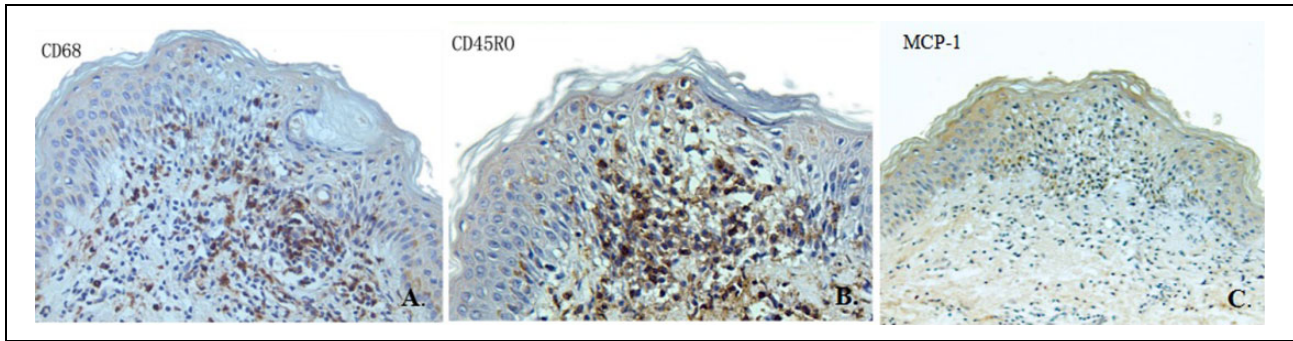


Figure 3. Correlation between MCP-1 expression and recruitment of CD68⁺ and CD45RO⁺ cells during elicitation of CHS. Skin sections obtained from positive nickel-challenged sites at 48 h after allergen application were immunohistologically labeled with anti-MCP-1, anti-CD68 or anti-CD45RO antibodies. Cells expressing MCP-1, CD68 or CD45RO were stained as light or dark brown. The expression of MCP-1 in the basal layer (C) was overlapping with the infiltration of CD68⁺ cells (macrophages) and CD45RO⁺ cells (memory T lymphocytes) in the dermis (A and B). Magnification ratio: $\times 200$.

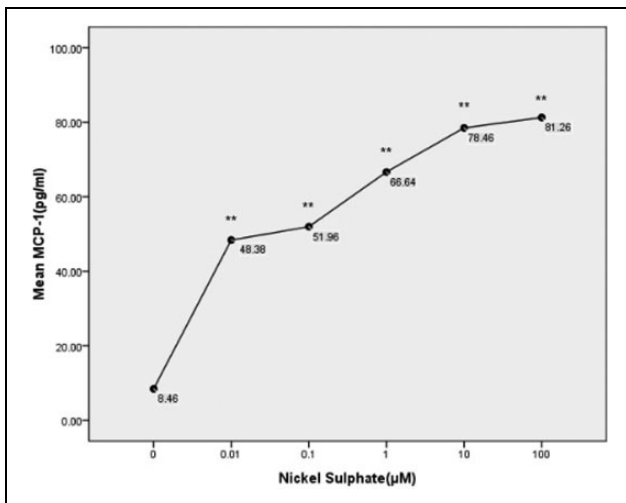


Figure 4. Up-regulation of MCP-1 induced by nickel in HaCaT cells. After exposed for 24 h to nickel sulphate at a range of concentrations (0.01 ~ 100 μM) under serum-free conditions, the supernatants of cultured HaCaT cells were collected for MCP-1 assessment by ELISA. Values were given as the means of five experiments ($n=5$).

** $p = 0.000 < 0.01$, compared with untreated control cells.

higher than that of 96 h (77.8 vs. 20%, Fisher's exact two-sided $p = 0.023 < 0.05$). The data were presented in Table 2.

Correlations between MCP-1 expression and recruitment of CD68⁺ and CD45RO⁺ cells.

As presented in Figure 3, the expression of MCP-1 in the basal layer by IHC was overlapping with the infiltrating CD68⁺ (macrophages) and CD45RO⁺ cells (memory T lymphocytes) into the dermis at 48 h after elicitation of CHS.

MCP-1 modulation by nickel in cultured HaCaT cells

To analyze the in vitro effects of nickel on MCP-1 expression in human keratinocytes, HaCaT cells were treated with nickel sulfate at a range of subtoxic concentrations for 24 h under serum-free conditions. As shown in Figure 4, treatment with nickel sulfate at concentrations from 0.01 μM to 100 μM induced an elevation of MCP-1 expression by keratinocytes, compared to the untreated cells ($p = 0.000 < 0.01$). Moreover, with the increase of nickel concentrations, nickel-induced MCP-1 expressions were upregulated as well.

Discussion

During ACD, leucocyte migration to inflammatory skin lesion is a complex multistep event in which multiple chemokines are involved. Previous studies have implicated MCP-1, a potent chemokine for the elicitation of ACD (Gaffal et al., 2013; Ishimoto et al., 2008; Kaur et al., 2012). But the expression pattern of MCP-1 induced by nickel and its role for leucocyte recruitment during OACD has not been well defined.

In this study, skin biopsies obtained from sensitized OACD workers at distinct time intervals after allergen application were analyzed by IHC and ISH. The results showed that at positive nickel-challenged sites, strong expressions of MCP-1 were detected in the basal keratinocytes during early phase (24–48 h after nickel application), paralleled by the infiltration of macrophages and memory T lymphocytes to the skin compartments. In the late phase (72–96 h after nickel application), the expressions of MCP-1 declined

gradually. In addition, the expressions of MCP-1 mRNA in the basal keratinocytes were elevated at 48 h, but declined at 96 h. This was consistent with the previous findings that MCP-1 mRNA appeared early after nickel exposure, further increased up to 48 h and declined after 72 h (Goebeler et al., 2001; Sebastiani et al., 2002b). All these data suggested a temporal expression pattern of MCP-1 induced by nickel challenge, which was associated with the migration of monocytes and lymphocytes during elicitation of OACD.

To further confirm whether keratinocytes were the major contributors to MCP-1, the effects of nickel exposure on the expression of MCP-1 in human keratinocytes (HaCaT cells) were analyzed in vitro. Because of the fact that nickel at concentrations higher than 100 μ M became cytotoxic in keratinocytes (Ermolli et al., 2001), probably mediated by cell cycle arrest and induction of apoptotic cell death (D'Antò et al., 2012; Shih et al., 2015), HaCaT cells were treated with nickel sulfate at subtoxic concentrations ranging from 0.01 μ M to 100 μ M for 24 h in this study. As a result, we found that under serum-free culture conditions, nickel sulfate at noncytotoxic concentrations induced a concentration-related elevation of MCP-1 production by HaCaT cells, indicating that keratinocytes were the major producing cells for MCP-1 during elicitation of OACD. As to the mechanisms for nickel-induced MCP-1 expression, it was suggested that at least three independent pathways were participating in it, including IKK β /I κ B/NF- κ B pathway, the p38 MAP kinase cascade, and PC-PLC-dependent pathways (Lamberti et al., 2004), while to the nickel toxicity in keratinocytes, phosphoglycerate kinase and annexin II were possibly implicated in it (Acevedo et al., 2001).

In conclusion, the data demonstrated that a temporal expression pattern of MCP-1 induced by nickel exposure was associated with the migration of monocytes and lymphocytes during CHS, suggesting an initial involvement of the nonspecific MCP-1-induced proinflammatory signal during elicitation of nickel-induced OACD. Keratinocytes were the major contributors to MCP-1. Targeting MCP-1 might be a potential therapeutic strategy for OACD.

Acknowledgments

The authors would like to thank the Laboratory of Immunodermatology in China Medical University, for providing the human keratinocyte cell line HaCaT.

Declaration of Conflicting Interests

The author(s) declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed the receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Science and Technology Planning Project of Zhuhai city, China (Grant NO: 2014D0401990013) and Natural Science Foundation of Guangdong Province, China (Grant NO: 2014A030313028).

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