

of MMP patients<sup>18</sup>. Passive transfer studies in newborn and adult mice have shown that polyclonal antibodies to human laminin-332, generated from rabbits, bind epidermal basement membrane and produce subepidermal blisters of skin and mucous membranes like those seen in patients with MMP<sup>6,19</sup>. In contrast, approximately 40 to 70% of patients with MMP show autoantibodies to multiple sites on COL17<sup>19</sup>. Rabbit antibodies, generated against the murine homologue of the NC16A domain of human COL17, induce subepidermal blisters when passively transferred into neonatal mice, and that resemble those seen in patients with BP and some forms of MMP<sup>19,20</sup>. The *in vivo* pathogenicity of autoantibodies to the C-terminal domain of COL17 has not yet been demonstrated<sup>21</sup>.

In contrast to BP, which is the most common autoimmune blistering disorder induced by autoantibodies against the NC16A domain of COL17, cutaneous involvement of MMP is usually limited to small areas<sup>22-24</sup>. To date, there have been only a few cases of MMP with widespread blisters<sup>23,24</sup>. Interestingly, these cases had autoantibodies against the NC16A domain of COL17<sup>23,24</sup>. Our patient also presented with widespread cutaneous blisters, and ELISA and immunoblot analysis revealed antibodies against the NC16A domain of COL17. These findings suggest that the presence of autoantibodies against

the NC16A domain of COL17 may be associated with the clinical involvement of wide spread blisters in MMP patients.

IgG is the main immunoglobulin subtype that has been confirmed as an autoantibody against laminin-332 and/or COL17 in sera from patients with MMP <sup>4,5</sup>. In addition, IgA autoantibodies against COL17 are another major immunoglobulin subtype found in sera from MMP patients, and the presence of both IgG and IgA anti-COL17 antibodies has been associated with more severe and persistent clinical features <sup>21,25,26</sup>. However, to the best of our knowledge, there are no reports of IgA autoantibodies against both laminin-332 and COL17 being detected in MMP sera <sup>5</sup>. In the previous study, a patient with IgA autoantibodies against laminin-332 had severe conjunctival involvement with multiple bullae on the extremities and was refractory to systemic steroid therapy <sup>5</sup>. Our patient was also intractable to steroid treatment and needed relatively high doses of oral prednisolone, azathioprine and diaphenylsulfone. The presence of IgA anti-laminin-332 might be associated with poor response to steroid treatment, although the correlation between clinical manifestations and the profile of autoantibody subtypes is difficult to determine due to limited number of patients.

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## Figure legends

### Fig. 1. Clinical manifestations and histopathological findings

Multiple erythema, bullae and erosions on the back (a) and forearm (b), and esophageal erosions (c) were observed at the initial visit. Refractory ulcers on the perianal area were also observed (d). After 3 months of treatment with systemic prednisolone, azathioprine and diaphenylsulfone, the skin lesions gradually healed with hyperpigmentation and scar formation (e, f). The esophageal erosions also slowly resolved (g).

A skin biopsy specimen obtained from the edge of blister on the back showed a subepidermal blister with inflammatory cell infiltrates, including eosinophils, lymphocytes and neutrophils (h). (Haematoxylin and eosin stain, original magnification x200)

### Fig. 2. Immunofluorescence studies

Direct immunofluorescence of the cutaneous lesions revealed *in vivo* linear IgG (a) and IgA (b) deposition at the BMZ (arrowheads). Indirect immunofluorescence microscopy of 1M NaCl-split normal human skin showed that circulating IgG (c) and IgA (d) anti-BMZ antibodies reacted with both epidermal and dermal sides (arrowheads). (original magnification x200; indirect immunofluorescence, serum dilutions were 1 : 40 (c) and 1 :

8 (d).)

**Fig 3. Immunoblot analysis using the recombinant NC16A and C-terminal domains of COL17, the 120-kDa soluble ectodomain of COL17 and purified laminin-332**

(a) The recombinant NC16A-COL17 and GST fusion protein (lane 1: control bullous pemphigoid (BP) IgG; lane 2: the patient's IgG). Circulating IgG autoantibodies reacted with NC16A-COL17 (arrow).

(b) BP915-COL17 (the recombinant protein of the C-terminal domain of COL17 amino acids spanning 1227 to 1532) and the GST fusion protein (lane 1: control BP IgG; lane 2: the patient's IgG). IgG against the C-terminal domain of COL17 was detected (arrow).

(c) The 120-kDa soluble ectodomain of COL17 (lane 1: control BP IgG; lane 2: the patient's IgG).

(d) The purified laminin-332 (lane 1: ponceau S stain sample; lane 2: the patient's IgG; lane 3: the patient's IgA). Both IgG and IgA from the patient reacted with the 105-kDa,  $\gamma$ 2 subunit of laminin-332 (arrow).

## Lichen Planus in Childhood Showing Various Cutaneous Features

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Lichen planus (LP) in childhood is less common than in adulthood, with cases in individuals under 14 years of age estimated to account for no more than 10% of cases of LP (1). We describe here a child with LP who showed various cutaneous presentations.

### CASE REPORT

A 5-year-old boy presented with papules on his right leg lasting for 6 months, which had gradually progressed to his trunk and extremities. He had a history of cytomegalovirus (CMV) hepatitis. His general condition was good and he was not taking any medications. On physical examination, keratotic papules and plaques with a peachblow-colour were observed on his legs. On the right leg, the papules were arranged linearly (Fig. 1a). On the dorsum of the right foot, hyperkeratosis and hypertrophy of the eruptions was conspicuous, showing a verrucous appearance (Fig. 1b). Miliary, lustrous eruptions were scattered on his abdomen, most of which were folliculo-centred (Fig. 1c). The oral mucosae and nails were normal.

Blood tests showed a small number of atypical lymphocytes (1% of 5,200/mm<sup>3</sup> white blood cells (WBC)) and liver dysfunction (aspartate transaminase (AST) 99 IU/l, alanine transaminase (ALT) 80 IU/l). Cytomegalovirus-immunoglobulin G (CMV-IgG) and antibodies for Epstein-Barr (EB) virus (EB nuclear antigen (EBNA)-IgG and viral capsid antigen (VCA)-IgG) were positive, although CMV-IgM and VCA-IgM were

negative. Antibodies for the hepatitis B virus (HBV) and the hepatitis C virus (HCV) were negative.

Histopathological examination of a skin biopsy specimen taken from a verrucous papule on the dorsum of the right foot showed epidermal ortho-hyperkeratosis, wedge-shaped hypergranulosis, serrated change in the epidermal rete ridge, and band-like infiltration of lymphocytes in the superficial dermis. In addition to above-mentioned features, a skin biopsy specimen taken from a papule on the abdomen showed marked vacuolar degeneration of the epidermis and many Civatte bodies.

### DISCUSSION

This case presented various clinical phenotypes of LP, including linear LP, hypertrophic LP and follicular LP. Linear LP and hypertrophic LP each has been reported as accounting for 10% of cases of childhood LP, and follicular LP as accounting for approximately 4% of cases of childhood LP (2). Linear LP is less common in adult (<1% of all LP) (2) and is considered a characteristic feature of childhood LP. The simultaneous existence of different phenotypes of LP in a single patient has been reported in several papers (3); however, to our knowledge, no report has shown simultaneous presentation of linear, hypertrophic and follicular LP, which were shown in our case.

LP is often induced by drugs, dental metals, chronic liver disease, viral infection, etc. The association of HBV or HCV infection with LP has been given particular focus (4). In this case, laboratory data of liver dysfunction together with the finding of atypical lymphocytes suggested the reactivation of EBV or CMV, which might be related to the aetiology of the LP.

Topical tacrolimus was applied as a treatment, and the eruptions gradually subsided within 6 months. Several reports have shown the usefulness of topical tacrolimus against LP, especially against LP in the oral cavity (5). This case showed a favourable clinical course during the topical treatment with tacrolimus.

*The authors declare no conflicts of interest.*



Fig. 1. Clinical appearance of (a) linear keratotic papules and plaques on the right leg and (b) verrucous plaque on the dorsum of the right foot. (c) Miliary, lustrous papules distributed almost consistently with hair follicles on the abdomen.

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# Conversion from human haematopoietic stem cells to keratinocytes requires keratinocyte secretory factors

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## Summary

**Background.** Recent studies have reported that bone-marrow-derived stem cells (BMSCs), including haematopoietic stem cells (HSCs) and mesenchymal stromal cells, differentiate in order to regenerate various cellular lineages. Based on these findings, it is known that BMSCs can be used clinically to treat various disorders, such as myocardial infarction and neurotraumatic injuries. However, the mechanisms of HSC conversion into organ cells are incompletely understood. The mechanism is suspected to involve direct cell–cell interaction between BMSCs, damaged organ cells, and paracrine-regulated soluble factors from the organ, but to date there have been no investigations into which of these are essential for keratinocyte differentiation from HSCs.

**Aim.** We tried to elucidate the mechanism and necessary conditions for HSC differentiation into keratinocytes *in vitro*.

**Methods.** We cultured human (h)HSCs under various conditions to try to elucidate the mechanism and necessary conditions for hHSCs to differentiate into keratinocytes.

**Result.** hHSCs cocultured with mouse keratinocytes induced expression of human keratin 14 and transglutaminase I. Only 0.1% of the differentiated keratinocytes possessed multiple nuclei indicating cell fusion. Coculture of hHSCs with fixed murine keratinocytes (predicted to stabilize cellular components) failed to induce conversion into keratinocytes. Conversely, keratinocyte-conditioned medium from both human and mouse keratinocytes was found to mediate hHSC conversion into keratinocytes.

**Conclusions.** Human HSCs are capable of differentiation into keratinocytes, and cell fusion is extremely rare. This differentiation is mediated by the plasma environment rather than by direct cell–cell interactions.

## Introduction

Recent studies have reported that bone marrow-derived stem cells (BMSCs), including haematopoietic stem cells (HSCs) and multipotent mesenchymal stromal cells

(MSCs), differentiate into various cellular lineages.<sup>1–3</sup> Based on these findings, BMSCs have been used to treat several disorders in animal models, including myocardial infarction, Parkinson's disease and neurotraumatic injuries.<sup>4–6</sup> We previously used a murine bone-marrow transplantation model to show that HSCs can differentiate into functional keratinocytes *in vivo*.<sup>7,8</sup> Cutaneous T-cell-attracting chemokine (CTACK)/CCL27 was found to be the major regulator involved in the migration from bone marrow of keratinocyte precursor cells, which expressed CCR10, the receptor for the CTACK ligand.<sup>7</sup> In addition, we reported that bone marrow-derived MSCs also convert into keratin 14-positive keratinocytes *in vivo* and *in vitro*.<sup>9</sup>

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1 The factor or mechanism governing the differentia-  
2 tion of HSCs into injured organ cells is been fully  
3 understood. Possible mechanisms include direct cell–cell  
4 interaction between peripheral haematopoietic progen-  
5 itor cells and damaged organ cells, and involvement of  
6 paracrine-regulated soluble factors from the organ.  
7 Requirement of feeder cells such as 3T3 cells when  
8 culturing keratinocytes implies that direct cellular  
9 interactions play a major role in keratinocyte differ-  
10 entiation, proliferation and homeostasis. Previous papers  
11 have reported that use of secretory factors from dam-  
12 aged liver tissue enables HSCs to take on many  
13 characteristics of liver cells.<sup>10</sup>

14 We cultured human (h)HSCs under various condi-  
15 tions to elucidate the mechanism and necessary condi-  
16 tions of hHSC differentiation into keratinocytes.

## 17 Methods

18 The study was approved by the ethics committee of  
19 Hokkaido University Graduate School of Medicine, and  
20 volunteers signed consent forms approved by the  
21 Hokkaido University Graduate School of Medicine and  
22 the Hokkaido Red Cross Blood Centre Committee for the  
23 Protection of Human Subjects.<sup>11</sup> All animal procedures  
24 were conducted according to guidelines provided by the  
25 Hokkaido University Institutional Animal Care and Use  
26 Committee under an approved protocol.

## 27 Cells

28 Human peripheral blood CD34-positive cells, which are  
29 considered to be hHSCs, were collected. Recombinant  
30 human granulocyte colony-stimulating factor (G-CSF;  
31 Chugai Pharmaceutical Co. and Kyowa Hakko Pharma-  
32 ceutical Co., Tokyo, Japan) was administered to the  
33 healthy subjects. Mobilized hHSCs were then isolated  
34 from peripheral blood using immunomagnetic beads with  
35 an antibody against CD34 as described previously.<sup>11,12</sup>

36 Pam 212, a murine keratinocyte cell line, was derived  
37 from spontaneously transformed BALB/c keratinocytes.  
38 Murine dermal fibroblasts were obtained from the dorsal  
39 skin surface of C57BL/6 mice. Normal human epider-  
40 mal keratinocytes (NHEKs; Cambrex, East Rutherford,  
41 NJ, USA) were used as controls.

## 42 Coculture of hHSCs

43 To distinguish the differentiated HSC-derived keratino-  
44 cytes and cocultured keratinocytes, we chose hHSCs  
45 and murine keratinocytes Pam 212 to coculture. PAM  
46 212 and murine dermal fibroblasts were grown

separately on eight-well culture slides to 80% conflu-  
ence in DMEM (Invitrogen, Grand Island, NY, USA), and  
NHEKs were grown in Konjac glucomannan medium  
(KGM, Lonza Walkersville, Walkersville, MD, USA).  
Each cell was washed twice with phosphate-buffered  
saline (PBS) and then  $1 \times 10^5$  hHSCs were added to  
each well and cocultured for 5 days in RPMI medium  
(Invitrogen). Each experiment was repeated three times. ■

## 47 Paraformaldehyde fixation of keratinocytes

In coculture with fixed cells, Pam 212 cells or NHEKs  
were grown on eight-well culture slides to 80% conflu-  
ence, and then fixed with 0.5% paraformaldehyde for  
15 min at 25 °C. Each well was washed twice with PBS,  
and  $1 \times 10^5$  hHSCs per well were cultured with fixed  
keratinocytes for 5 days with RPMI.

## 48 Preparation of conditioned medium

Pam 212 cells or NHEKs at 80% confluence were washed  
twice with PBS, and cultured for 48 h with RPMI. The  
conditioned media were centrifuged at 2500 g for  
10 min, filtered through 0.22- $\mu$ m filters (Millipore, Bed-  
ford, MA, USA), and stored at – 80 °C until use. These  
conditioned media were then used to culture  $1 \times 10^5$   
hHSCs on eight-well culture slides with the conditioned  
media collected from the Pam 212 cells or NHEKs.

## 49 Culture with secreted factors

The hHSCs ( $1 \times 10^5$ ) were plated onto eight-well slides  
as before, and cultured in keratinocyte basal medium  
(Invitrogen) containing 0.5 nmol/L bone morphoge-  
netic protein-4 (R&D Systems, Minneapolis, MN, USA),  
keratinocyte growth factor (KGF; Invitrogen) or inter-  
leukin-1-induced growth factor (IGF; Invitrogen). After  
48 h of culture, hHSCs were stained to investigate their  
differentiation into keratinocytes.

## 50 Immunocytochemistry

Skin samples were embedded in optimal cutting temper-  
ature compound (Sakura Finetek Japan, Tokyo, Japan),  
then cut on a cryostat into 5  $\mu$ m sections, which were  
placed onto microscope slides. The slides were used for  
indirect immunofluorescence using the following pri-  
mary antibodies were used: human cytokeratin (CK)5  
(catalogue no. RCK103) and human CK14 (LL002) (both  
Santa Cruz Biotechnology, Santa Cruz, CA, USA), human  
transglutaminase 1 (B.C1) and human involucrin (rabbit  
polyclonal (both Biomedical Technologies, Stoughton,

MA, USA), human N-cadherin (GC4; Sigma-Aldrich, St. Louis, MO, USA), anti-pankeratin goat polyclonal, human  $\alpha 6$ -integrin (GoH3) and human HLA-ABC (G46-2.6) (all BD Biosciences Pharmingen), and human nuclei (235-1; Millipore, Billerica, MA, USA).

Fluorescence staining was investigated using a confocal laser scanning fluorescence microscope (Laser Scanning Confocal Imaging System MRC 1024; Bio-Rad, Richmond, CA, USA). Cells that stained positive to human (human nuclei or HLA-ABC) and keratinocyte markers were counted as positive for bone marrow-derived keratinocytes.

#### Statistical evaluation of results

Statistical analysis of differences in the means for each experimental group was carried out using the Student *t*-test, with significance set at  $P < 0.05$ .

### Results

#### Human nuclei-positive and cytokeratin-positive cells are derived from human haematopoietic stem cells

No cells stained positively with antibodies against pankeratin, human CK5 or CK14, human transglutaminase I or human involucrin. Furthermore, culture of hHSCs in RPMI medium for 5 days did not result in any cells positive for any of these five antibodies. Spontaneous conversion of hHSCs to keratinocytes seldom happened.

Next, we investigated whether coculture with keratinocytes mediates hHSC differentiation into keratinocytes. Using a specific antibody against human nuclei,<sup>13</sup> we detected human nuclei+/cytokeratin+ cells (hNCs) after coculture of hHSCs and Pam 212 cells for 5 days (Fig. 1a). We also found human nuclei+/cytokeratin 14+ cells and human cytokeratin 5+ cells (Fig. 1b,c). These hNCs expressed HLA-ABC as a human origin marker (Fig. 1d). Furthermore, hNCs expressed human transglutaminase 1 and involucrin as other specific markers of keratinocytes (Fig. 1e). The number of hNCs increased relative to the coculture time, from  $0.03 \pm 0.002\%$  at 6 h to  $0.46 \pm 0.11\%$  at 48 h (Fig. 1f). However, coculture for 5 days did not introduce any colonies of hHSCs or hNCs.

#### Human haematopoietic stem cells cultured with fixed keratinocytes do not express keratinocyte markers

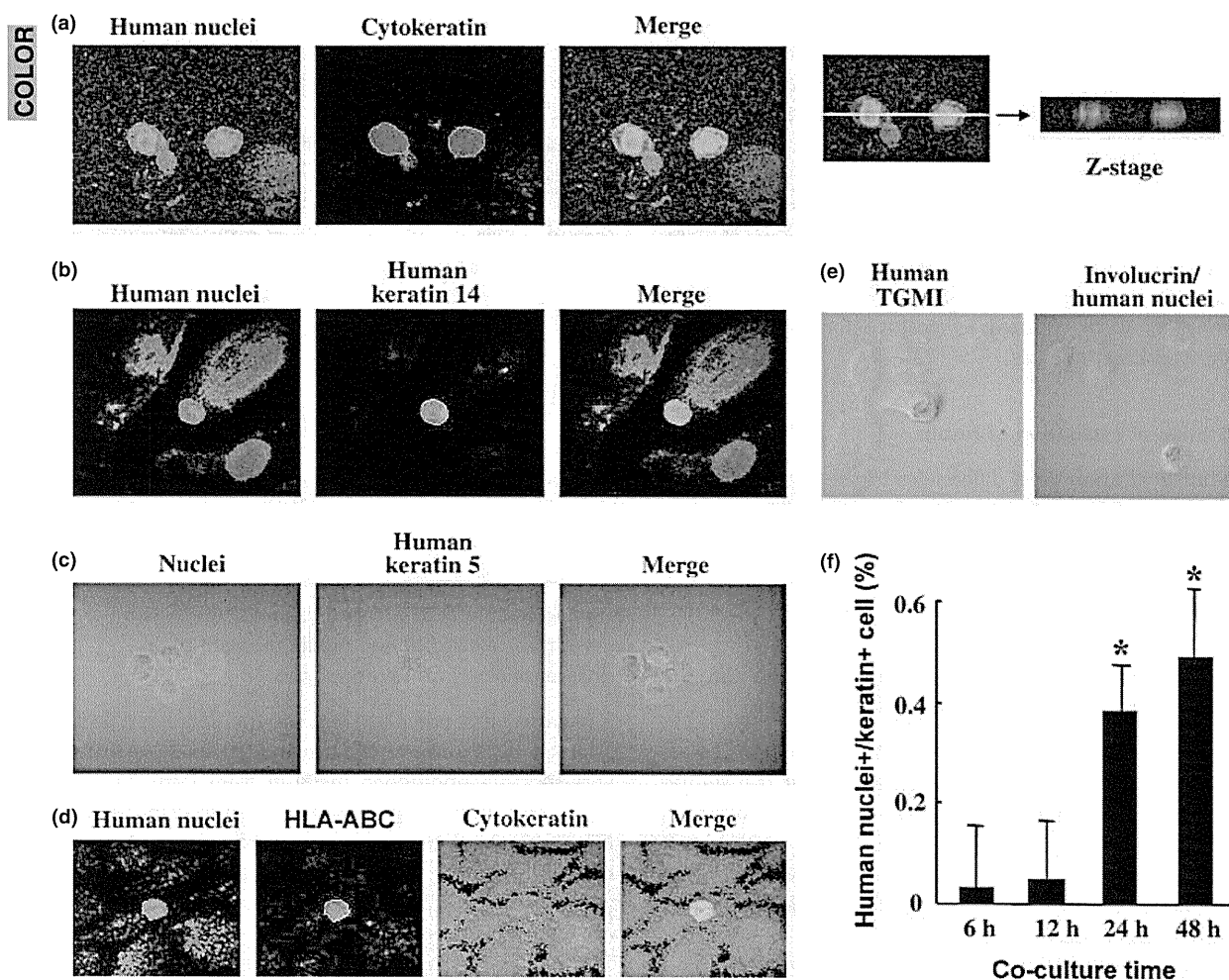
To determine whether the hNCs were generated through cell fusion or true differentiation, we examined

whether multinucleate cells could result from cell fusion between HSCs and keratinocytes.<sup>14</sup> Most hNCs were microscopically uninucleate, and only 0.1% of hNCs were binucleate, with human and nonhuman (presumed mouse) nuclei (Fig. 2a). To exclude the possibility of cell fusion at the initial hHSC to keratinocyte transition, hHSCs were cultured with 0.5% paraformaldehyde-fixed Pam 212 cells or NHEKs. It was predicted that the fixed cells would stabilize the cellular components, rendering live hHSCs resistant to fusion. This method has been shown to prevent fusion of live cells with fixed cells, while not disrupting receptor-mediated recognition and association of these cell types.<sup>15</sup> This procedure failed to convert hHSCs to hNCs (Fig. 2b), suggesting that hHSCs seldom fuse with keratinocytes and that the cell-surface molecules of keratinocytes do not induce hHSC differentiation into keratinocytes.

#### Human haematopoietic stem cells cultured with keratinocyte-conditioned media mediate their differentiation into keratinocytes

To clarify the potential role of the secretory factors released by keratinocytes, we treated hHSCs with the keratinocyte-conditioned medium from Pam 212 cells or NHEKs; interestingly, both types of medium induced hHSC conversion into keratinocytes (Fig. 2c). The number of hNCs in  $10^4$  hHSCs after 48 h in culture was 35 (0.35%) in NHEK-conditioned medium and 9 (0.09%) in Pam-212-conditioned medium (Table 1). The greater number in NHEK-conditioned medium compared with Pam-212-conditioned medium implies an association with species-specific factors. However, there were no significant difference between cultures with NHEK-conditioned medium and those with Pam-212-conditioned medium. Furthermore, 40 (0.40%) hNCs were detected in culture with fixed NHEK in NHEK-conditioned medium, and 29 (0.29%) in culture with fixed Pam 212 cells in Pam-212-conditioned medium. Fixed keratinocytes seemed to accelerate the conversion of hHSCs with keratinocyte-conditioned medium, but the difference was not significant. In addition, as control of cell type for the conditioned media, fibroblast-conditioned media never induced hHSCs to convert into keratinocytes.

To explore any additional effects of keratinocyte surface molecules on differentiation, we added blocking antibodies against keratinocyte surface molecules during culture. We chose human alpha 6-integrin and human N-cadherin as the surface molecules, as these molecules express on various stem cells, including



**Figure 1** Coculture of human haematopoietic stem cells (hHSCs) and a mouse keratinocyte cell line, Pam212. (a) Expression of human nuclei (green) and cytokeratin (red) after 2 days in coculture, and cross-section analysis of the same cells (Z-axis). (b) Expression of human nuclei (green) and human cytokeratin 14 (red) after 2 days in coculture. (c) Expression of human cytokeratin 5 (green) and nuclei (propidium iodide staining, red) after 2 days in coculture. (d) Expression of human nuclei (green), human leucocyte antibody-ABC (red) and cytokeratin (blue). (e) Left: expression of human transglutaminase I (green) with transmission after 2 days in coculture; right: expression of involucrin (green) and human nuclei (red) with transmission after 2 days in coculture. (f) Percentages of hHSCs expressing keratin after 6, 12, 24 and 48 h in culture. \* $P < 0.05$  vs. 6 h.

epidermal stem cells, playing an important role in differentiation.<sup>16,17</sup> Blocking antibodies during coculture of hHSCs and fixed NHEK with NHEK-conditioned medium did not influence the keratinocyte conversion (data not shown).

It is possible that the humoral induction of keratinocyte differentiation is mediated by a specific growth factor such as KGF and IGF.<sup>18</sup> However, we did not observe hNCs with exposure of hHSCs to KGF or IGF, which are secreted exclusively from keratinocytes (data not shown). These findings suggest that soluble factors

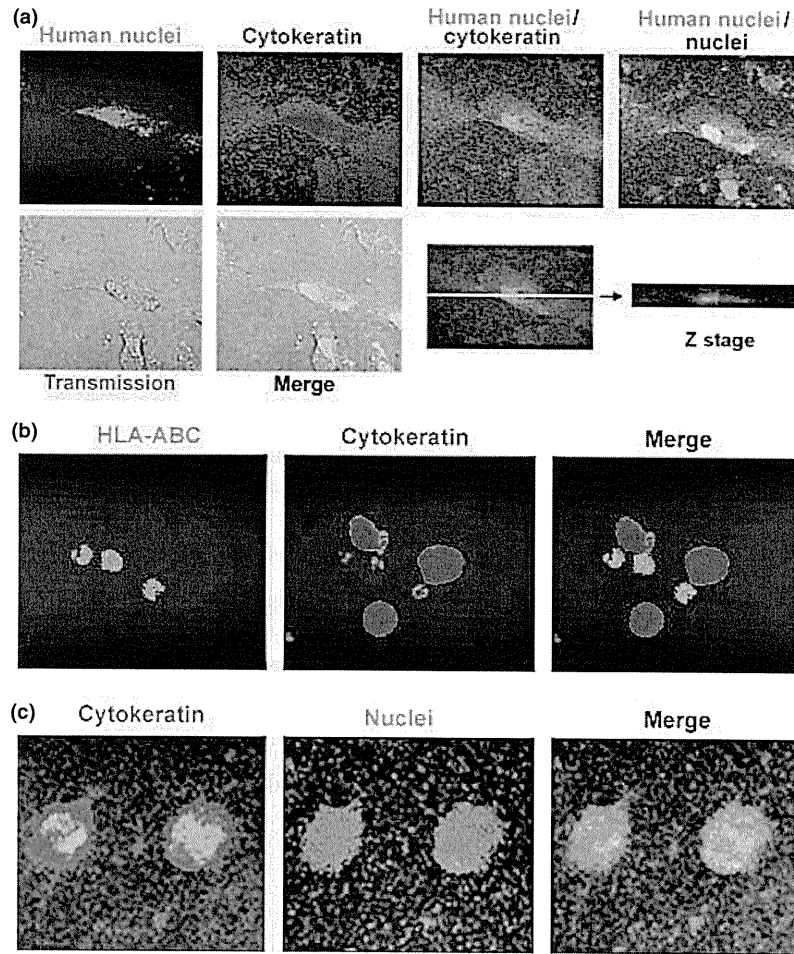
other than KGF and IGF in keratinocyte supernatant may mediate HSCs differentiation.

## Discussion

We have shown that hHSCs differentiate into keratinocytes in the presence of factors secreted from keratinocytes, without cell fusion. In this study, hHSCs converted into keratinocytes when cocultured with keratinocytes. By contrast, hHSCs cocultured with fixed keratinocytes were found never to convert into



COLOR



**Figure 2** Coculture of human haematopoietic stem cells (hHSCs) and Pam212 cells fixed with 0.5% paraformaldehyde, and culture of hHSCs in conditioned medium of Pam 212 cells. (a) Expression of human nuclei (green), mouse nuclei (blue) and cytoke- ratin (red) after 2 days in coculture of hHSCs and fixed Pam212 cells, and analysis of same cells by Z-axis or transmission. (b) Expression of human leucocyte antibody-ABC (green) and cytoke- ratin (red). (c) Expression of cytoke- ratin (blue) and human nuclei (green) in culture of hHSCs in the conditioned medium of Pam 212 cells.

**Table 1** Frequency of cytoke- ratin-positive cells derived from human haematopoietic stem cells (hHSCs).

Treatment	CK-positive cells, n*
Coculture with Pam 212 cells	49
Coculture with fixed Pam 212 cells	0
Coculture with mouse fibroblasts	0
Culture in Pam 212 CM	9
Culture with fixed Pam 212 in Pam-212 CM	29
Culture in NHEK CM	35
Culture with fixed NHEKs in NHEK CM	40

CK, cytoke- ratin; CM, conditioned medium; NHEK, normal human epidermal keratinocyte. \*In  $10^4$  hHSCs.

keratinocytes, and hHSCs cultured with keratinocyte- conditioned medium expressed keratinocyte-specific markers. These data support the existence of factors secreted from keratinocytes or the existence of relatively paraformaldehyde-sensitive cell surface molecules that induce hHSCs to differentiate into keratinocytes.

We did not observe differentiation after exposure of hHSCs to the growth factors KGF or IGF, which suggests that other soluble factors might mediate HSC differentiation. Indeed, a previous report on hepatocyte differentiation showed that the specific growth factors hepatocyte growth factor and fibroblast growth factor

4 failed to mediate such conversion.<sup>10</sup> Further investigation is required to identify specific soluble factors that affect differentiation of HSCs to keratinocytes.

Recently Mortier *et al.*<sup>19</sup> succeeded in generating a skin equivalent model from human cord blood cells, which contains heterogeneous cells including hHSCs and MSCs. Although the origin of the induced keratinocytes was not investigated, we propose that most of these cells are mesenchyme-derived, as our observation showed that purified hHSCs seldom convert to keratinocytes.

Murine BMSCs can contribute to the regeneration of injured adult tissues of various organs, including brain, liver and heart tissue, after bone-marrow transplantation.<sup>1,3,20</sup> These unexpected events were initially attributed to BMSC transdifferentiation, supporting the emerging idea of extended plasticity of adult stem cells. The alternative hypothesis of spontaneous cell fusion has also been proposed as the primary cause of unexpected cell-fate switches of BMSCs into various cell lineages.<sup>21,22</sup>

We found that the number of fused multinucleate cells (which are unlikely to undergo further cell division) in the skin was very low. Conversely, Fujino *et al.*<sup>23</sup> reported the observation of fused functional hepatocytes after hHSC injection into immunodeficient mice. Taking these results into consideration, it is likely that both cell fusion and conversion from HSCs play some role in the repair of damaged tissue.

Previously, we reported that CTACK/CCL27 accelerates skin regeneration via accumulation of BMDCs.<sup>7</sup> Furthermore, bone-marrow transplantation improves type XVII collagen-knockout epidermolysis bullosa (EB) mice, in which the deficient type XVII collagen, a cutaneous structure protein produced by keratinocytes, was restored by BMSCs.<sup>8</sup> Because there have been ethical and safety concerns in using embryonic stem cells and induced pluripotent stem cells, therapies using HSCs are thought to be safer.<sup>24</sup> In the near future, stem cell therapies might be a candidate for the treatment of severe eEB, for which there is no effective treatment other than palliative care.<sup>25</sup>

## Conclusion

When exposed to skin tissue, hHSCs are capable of taking on many characteristics of the skin cell types, and this is mediated by the plasma environment rather than by direct cell–cell interactions, including the specific gene and/or protein expression and function of the cells.

## Learning points

- It is known that HSCs have the potential for conversion into keratinocytes.
- Several mechanisms, including direct cell–cell interaction between HSCs and damaged skin, and involvement of paracrine-regulated soluble factors from the organ have been speculated; however, there have been no reports identifying the precise mechanism involved.
- In this study, we found that the conversion of HSCs into keratinocytes is mediated by the plasma environment rather than by direct cell–cell interactions.

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 39.

# Macrophage Migration Inhibitory Factor Is Essential for Eosinophil Recruitment in Allergen-Induced Skin Inflammation

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Macrophage migration inhibitory factor (MIF) is a pluripotent cytokine that has an essential role in the pathophysiology of experimental allergic inflammation. Recent findings suggest that MIF is involved in several allergic disorders, including atopic dermatitis (AD). In this study, the role of MIF in allergic skin inflammation was examined using a murine model of AD elicited by epicutaneous sensitization with ovalbumin (OVA). We observed the number of skin-infiltrating eosinophils to significantly increase in OVA-sensitized MIF transgenic (Tg) mice compared with their wild-type (WT) littermates. On the other hand, eosinophils were virtually absent from the skin of MIF knockout (KO) mice and failed to infiltrate their skin after repeated epicutaneous sensitization with OVA. The mRNA expression levels of eotaxin and IL-5 were significantly increased in OVA-sensitized skin sites of MIF Tg mice, but were significantly decreased in MIF KO mice in comparison with the levels in WT littermates. Eotaxin expression was induced by IL-4 stimulation in fibroblasts in MIF Tg mice, but not in MIF KO mice. These findings indicate that MIF can induce eosinophil accumulation in the skin. Therefore, the targeted inhibition of MIF might be a promising new therapeutic strategy for allergic skin diseases.

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## INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing inflammatory disease of the skin with significant morbidity and an adverse impact on patient well-being (Morar *et al.*, 2006). AD is considered to result from a dysregulation of the normal interactions between the environment and genes, defects in skin barrier function, and systemic and local immunological responses (Leung *et al.*, 2004). The contribution of the immune response to the pathogenesis of AD has been attributed largely to abnormalities in adaptive immunity, with key roles being played by T-helper 1(Th1)/Th2 cell dysregulation, IgE production, dendritic cell signaling, and mast-cell hyperactivity, leading to the pruritic, inflammatory

dermatosis that characterizes AD (Leung *et al.*, 2004). In addition, accumulation of eosinophils is characteristic of the inflammation associated with AD (Honma *et al.*, 2000).

Macrophage migration inhibitory factor (MIF) was the first lymphokine reported to prevent random migration of macrophages (Bloom and Bennett, 1966). As the molecular cloning of MIF complementary DNA (Weiser *et al.*, 1989), MIF has been re-evaluated as a proinflammatory cytokine and pituitary-derived hormone that potentiates endotoxemia (Bernhagen *et al.*, 1993; Bucala, 1996). MIF has an important role in delayed-type hypersensitivity (Bernhagen *et al.*, 1998). Recently, it has been demonstrated that MIF also upregulates the expression of Toll-like receptor-4, which mediates lipopolysaccharide binding and activation of macrophages (Roger *et al.*, 2001). MIF is now recognized as a cytokine that exhibits a broad range of immune and inflammatory activities, including induction of inflammatory cytokines, and regulation of macrophage and lymphocyte proliferation. Furthermore, MIF induces the endothelial expression of E-selectin, ICAM-1, vascular cell adhesion molecule-1, IL-8, and monocyte chemoattractant protein-1, thus resulting in leukocyte recruitment (Gregory *et al.*, 2004, 2006; Cheng *et al.*, 2010). MIF originates from multiple cellular sources such as activated T lymphocytes, monocytes, eosinophils, and keratinocytes (Rossi *et al.*, 1998; Shimizu *et al.*, 1999; Yamaguchi *et al.*, 2000). MIF has also been shown to

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Abbreviations: AD, atopic dermatitis; KO, knockout; MIF, macrophage migration inhibitory factor; Tg, transgenic; WT, wild type

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