when stimulated with IL-17 and tumor necrosis factor-α. Out of 160 genes, 53 ''synergistic'' genes were identified in at least one of the aforementioned published studies; our RNA-seq analysis identified 14 additional upregulated DEGs (Supplementary Table S2 online), further emphasizing the contribution of this immunological circuit in the pathogenesis of psoriasis. One of these genes was *SPRR2B*; staining for the protein product of this gene confirmed increased expression in psoriatic lesional skin (Figure 2c), corroborating differences at the protein level of a gene identified uniquely by RNA-seq that is potentially informative with regard to active molecular pathways in psoriasis.

The transcriptomic profiling of psoriasis has led to an increased understanding of disease pathogenesis. Although microarray technologies have been instrumental in this regard, it is clear that these tools detect an incomplete set of DEGs. RNA-seq can be used to supplement these prior technologies. Here, the use of RNA-seq methods substantially increased the number of psoriasis-related DEGs. Furthermore, DEGs that were uniquely identified by RNA-seq, but not in other published microarray studies, further supported the role of IL-17 and tumor necrosis factor-α synergy in psoriasis. Examination of one of these factors at the protein level confirmed that RNA-seq is a powerful tool that can be used to identify molecular factors present in psoriasis lesions, and may be useful in the identification of therapeutic targets that to our knowledge have not been reported previously. Further studies are in progress to determine the biological significance of DEGs uniquely discovered by RNA-seq.

CONFLICT OF INTEREST

JGK has received honoraria and served as a consultant for Amgen, Centocor, Janssen, Ortho Biotech, Merck, Pfizer, Biogen Idec, and GlaxoSmithKline. The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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High Levels of CCL26 in Blister Fluid and Sera of Patients with Bullous Pemphigoid

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TO THE EDITOR

Bullous pemphigoid (BP) is a subepidermal blistering skin disease characterized by an autoimmune response to BP180 and BP 230, both of which are hemidesmosomal proteins in the basement membrane zone. Pathogenic mechanisms of BP include complement activation, recruitment of mast cells, neutrophils, and eosinophils, liberation of proteolytic enzymes, and direct interference with the adhesion function of BP180 (Kasperkiewicz and Zillikens, 2007; Iwata *et al.*, 2009).

CC chemokine ligand (CCL)24 and CCL26 are ligands for CC chemokine receptor (CCR)3, which is expressed on eosinophils and mast cells (Romagnani *et al.*, 1999). We have previously reported that serum levels of CCL26, but not CCL24, significantly elevate in patients with atopic dermatitis, and that serum CCL26 levels are correlated with

Abbreviations: BP, bullous pemphigoid; CCL, CC chemokine ligand; CCR, CC chemokine receptor

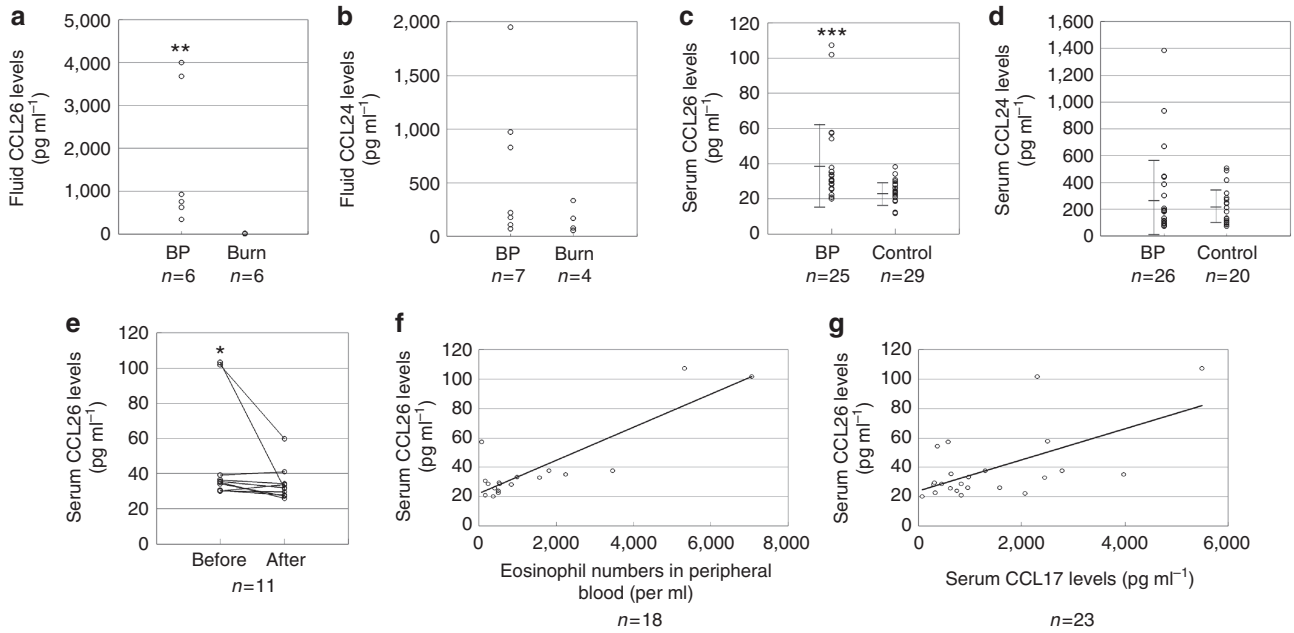

Figure 1. Blister fluid and serum CCL26 levels. Blister fluid CCL26 (**a**) and CCL24 (**b**) levels in bullous pemphigoid (BP) and burn patients. Serum CCL26 (**c**) and CCL24 (**d**) levels in patients with BP and control subjects. (**e**) Serum CCL26 levels in patients with BP, before and after oral corticosteroid treatment. Data are presented as mean ± SD. *$P<0.05$, **$P<0.005$, and ***$P<0.0001$. Correlation coefficient between serum CCL26 levels and eosinophil numbers in peripheral blood (**f**), or serum CCL17 levels (**g**) in BP patients.

eosinophil numbers in peripheral blood (Kagami *et al.*, 2003). We have also revealed that fibroblasts from lesional skin of cutaneous T-cell lymphoma express higher amounts of CCL26 mRNA (Miyagaki *et al.*, 2010). These patients often show eosinophilia. However, the relationship between CCL26 and BP remains unclear.

In this study, we quantified both blister fluid and serum CCL26 levels in BP patients. We also examined the expression of CCL26 in lesional skin from BP patients by immunohistochemistry.

After giving informed consent, 25 patients with BP (13 men and 12 women, mean ± SD age: 67.2 ± 20.2 years) and 29 healthy individuals (16 men and 13 women, 53.7 ± 20.3 years) were enrolled in this study. In addition, we collected blister fluid from six BP patients and six burn patients. The medical ethics committee of the University of Tokyo approved all aspects of this study according to the Declaration of Helsinki Principles. The diagnosis of BP was made based on clinical and histological features. All sera and blister fluid were collected and stored at −20 °C, until use.

We used CCL24 and CCL26 immunoassay kits (R&D systems, Minneapolis, MN). These levels were measured according to the manufacturer's instructions. Optical densities were measured at 450 nm with a Bio-Rad Model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA). Statistical analyses were performed using Mann–Whitney *U*-test for comparison between BP and controls. We used the Wilcoxon signed-ranks test in the comparison of serum CCL26 levels before and after treatment. Correlation coefficients were determined by using the Spearman rank correlation test. $P<0.05$ was considered significant.

Biopsy specimens obtained from lesional skin of patients with BP and control subjects were used for immunohistochemical staining. We used paraffin-embedded tissue sections and anti-human CCL26 goat polyclonal antibody (AF653, R&D systems, 7.5 μg ml^{-1}). Purified goat IgG (sc-2028, Santa Cruz Biotechnology, Santa Cruz, CA) was used as an isotype-matched control.

Fluid CCL26 levels, but not CCL24 levels, in BP patients were significantly higher than those in burn patients (1718.5 ± 676.9 vs. 16.1 ± 5.3 pg ml^{-1}, $P<0.005$, and 615.9 ± 691.7 vs. 155.9 ± 126.6 pg ml^{-1}, respectively; Figure 1a and b). We also measured serum CCL26 levels in BP patients and compared them with those of healthy individuals. BP patients showed higher serum CCL26 levels, but not CCL24 levels, than healthy individuals (39.9 ± 22.2 vs. 25.3 ± 6.0 pg ml^{-1}, $P<0.0001$, and 269.4 ± 305.4 vs. 234.5 ± 128.4 pg ml^{-1}, respectively; Figure 1c and d). Furthermore, serum CCL26 levels significantly decreased from 46.0 ± 28.0 to 33.7 ± 9.6 pg ml^{-1} after oral corticosteroid treatment ($n=11$, $P<0.05$; Figure 1e). In addition, we assayed serum CCL26 levels of pemphigus vulgaris and pemphigus foliaceus patients, but no significant difference was found (data not shown).

Next, we compared serum CCL26 levels in BP patients with other data that reflect the disease activity of BP (Kakinuma *et al.*, 2003). The serum CCL26 levels were significantly correlated with eosinophil numbers in

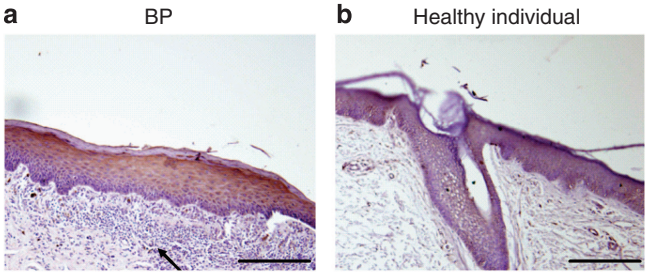

Figure 2. Immunohistochemical staining of CCL26 in BP. CCL26 expression in the lesional skin of bullous pemphigoid (BP) patients (**a**) and healthy individuals (**b**) were examined with immunohistochemical staining. An arrow indicates endothelial cells. Bar = 0.2 mm.

peripheral blood ($r = 0.59$, $P < 0.05$; Figure 1f) and serum CCL17 levels ($r = 0.45$, $P < 0.05$; Figure 1g).

CCL26 was expressed strongly in lesional and adjacent epidermal keratinocytes of patients with BP and weakly in vascular endothelial cells of the lesional skin. In particular, superficial keratinocytes showed higher CCL26 levels than basal keratinocytes (Figure 2a). In normal skin, CCL26 was expressed weakly in epidermal keratinocytes but no positive signal was found in the dermis (Figure 2b).

Increased expressions of CCR3 ligands, such as CCL11 and CCL13, in lesional skin of BP have been reported (Gounni Abdelilah *et al.*, 2006). We have previously demonstrated that serum levels of CCL28, another ligand of CCR3, are increased in patients with BP or atopic dermatitis (Kagami *et al.*, 2005a). In this context, CCR3 ligands seem to be important for BP. However, CCL24 production was not upregulated in BP patients. Other CCR3 ligands, but not CCL24, are produced by keratinocytes or fibroblasts, which may account for the difference between CCL24 and other CCR3 ligands.

Teraki *et al.* (2001) have shown that IL-4- and IL-13-producing skin-homing memory T cells are increased in peripheral blood and blister fluid of BP patients. In addition, IL-4 and IL-13 induce production of CCL26 by keratinocytes (Kagami *et al.*, 2005b). Therefore, we speculate a possible role of CCL26 in BP. BP patients have T cells that secrete IL-4 and IL-13 (Teraki *et al.*, 2001). These cytokines stimulate production of CCL26 by keratinocytes, which induces migration of mast cells into dermis (Romagnani *et al.*, 1999). Mast cells enhance migration of neutrophils and eosinophils, both of which cleave the extracellular, collagenous domain of 180-kDa hemidesmosomal protein of the basement membrane zone, which finally causes blister formation in BP (Kasperkiewicz and Zillikens, 2007).

In conclusion, we showed that CCL26, but not CCL24, levels were significantly increased in BP and decreased after treatment, and that CCL26 was strongly expressed in the lesional skin from patients with BP. These results suggest that serum CCL26 levels are useful for assessing the disease activity of BP, and that CCL26 has some role in the development of BP. Functional blocking of CCL26 may be another target for treatment.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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