respectively) (Figure 2b and c). Other factors were not correlated with serum CTACK/CCL27 levels (data not shown).

In this study, we showed that serum CTACK/CCL27 levels in patients with CTCL strongly correlated with types of skin lesions, TBI, serum soluble IL-2 receptor, and thymus and activation-regulated chemokine/CCL17 levels, all of which are already reported to be good makers of disease activity (Wasik *et al.*, 1996; Schmid *et al.*, 1999; Kakinuma *et al.*, 2003b). In addition, serum CTACK/CCL27 levels in patients with CTCL decreased after treatment. This is the first report describing the relationship between CTACK/CCL27 and CTCL. CTACK/CCL27 is expressed at a small amount even in normal skin (Kakinuma *et al.*, 2003a) and that may be the reason of a large overlap of CTACK/CCL27 levels between healthy controls and an early stage of CTCL (Figure 1a). Serum CTACK/CCL27 levels in patients with tumor were higher than those in erythrodermic patients probably because three out of five patients with skin tumor had systemic involvement. In addition, erythrodermic CTCL is very rare and the number of cases may not be enough. Therefore, further study with larger number of patients would be useful to fully determine the potential usefulness of CTACK/CCL27 levels as a treatment- and diagnosis-related biomarker.

Previous reports showed that CCR10 is expressed in CTCL (Notohamiprodjo *et al.*, 2005) and that CCR10 engagement by CTACK/CCL27 allows melanoma cells to escape host immune antitumor killing mechanisms (Murakami *et al.*, 2003). The same pathway may be involved when CTCL cells escape host immunity. Our data suggest its possibility and that functional blocking of CTACK/CCL27 would be another target for treatment.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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The Receptor for *Cis*-Urocanic Acid Remains Elusive

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TO THE EDITOR

The number of human diseases in which the immunomodulatory effects of UV radiation play a role is expanding, and include not only the development of skin cancers but also autoimmune and infectious diseases. In addition, UV has long been recognized as an important therapeutic for the treatment of inflammatory skin conditions. Molecules in skin that absorb UV radiation include the DNA and membrane lipids of epidermal cells, as well as *trans*-urocanic acid (*trans*-UCA), a major molecule of the stratum corneum. On irradiation, it is converted to the more soluble *cis*-isomer. The action spectrum of UVB-induced systemic suppression of contact hypersensitivity responses in mice closely follows the absorption spectrum of UCA (De Fabo and Noonan, 1983).

Abbreviations: DOI, 2,5-dimethoxy-4-iodophenyl-2-aminopropane; 5-HT, 5-hydroxytryptamine; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor-alpha; UCA, urocanic acid

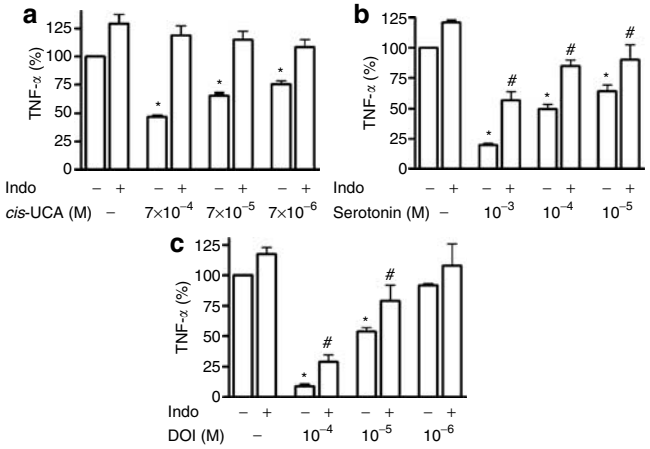

Figure 1. The effect of indomethacin (10^{-5} M) on the regulation of LPS-induced TNF-α production by (a) *cis*-UCA, (b) serotonin, and (c) DOI. PBMCs from three separate donors were incubated with LPS (500 ng/ml) for 24 h, ± the potential regulators. LPS-induced level has been normalized to 100%, mean±SEM, with * and # showing significant suppression compared with LPS-treated cells without and with Indo, respectively, $P<0.05$. Indo (10^{-5} M) significantly increased LPS-induced TNF-α production.

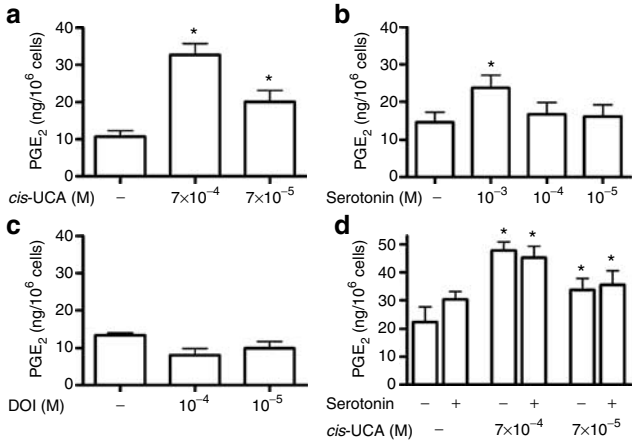

Figure 2. The effect of (a) *cis*-UCA, (b) serotonin, and (c) DOI on LPS-induced PGE_2 production. PGE_2 was measured in the same supernatants for which TNF-α levels are shown in Figure 1 (no PGE_2 was detected in indomethacin-treated cultures). In (**d**), PBMCs from two further donors were incubated with *cis*-UCA, together with a concentration of serotonin (10^{-4} M) that did not significantly induce PGE_2 levels. Mean±SEM; *significant induction compared with LPS-treated controls, $P<0.05$.

The role of *cis*-UCA in UV-induced immunomodulation varies with the response examined and the experimental model adopted, but significant roles have been confirmed by neutralization studies with a *cis*-UCA monoclonal antibody (reviewed in Ullrich, 2005). *In vitro* effects of *cis*-UCA have been described on purified keratinocytes, Langerhans cells, fibroblasts, T lymphocytes, natural killer cells, monocytes, and recently on peripheral sensory nerves (reviewed in Khalil *et al.*, 2001). The receptor for *cis*-UCA, a potential target for therapeutic intervention, has however not been identified. Further, characterization of the receptor for *cis*-UCA may provide insights into the biological actions of *cis*-UCA. At first, it was hypothesized that *cis*-UCA bound to histamine receptors, but the different biological effects of histamine and *cis*-UCA (Hart *et al.*, 1993; Bouscarel *et al.*, 1997) as well as poor competitive binding to rat cortex membranes (Laihia *et al.*, 1998) showed that *cis*-UCA did not bind to histamine receptors. Instead, 10^{-3} M *cis*-UCA, but not *trans*-UCA, was able to compete off to approximately 50% the binding of radiolabeled γ-amino-butyric acid to rat cortical membranes (Laihia *et al.*, 1998). The finding that *cis*-UCA, but not γ-amino-butyric acid, alters microvascular blood flow at the base of a blister induced in the rat hind footpad (Khalil *et al.*, 2001) suggests that the functional *cis*-UCA receptor is not the γ-amino-butyric acid receptor.

More recently, it has been recognized that *cis*-UCA and serotonin share structural similarities. *Cis*-urocanic acid has a 5-membered ring with the inner nitrogen sharing a strong intramolecular hydrogen bond with the carboxylic acid moiety. In aqueous solution, this forms a stable six membered ring similar in structure to that of serotonin (Ash *et al.*, 1997). Further, it has been suggested that both *cis*-UCA and serotonin bind to the 5-hydroxytryptamine $(5\text{-HT})_{2A}$ receptor (Ngheim DX, Walterscheid JP, Nutt LK, McConkey DJ, Ullrich SE (2002) *Cis*-urocanic acid causes immunosuppression through 5-HT receptor signaling. *Proc Mutag Exp Path Soc Aust* (abstr. 14)), to which the agonist, 2,5-dimethoxy-4-iodophenyl-2-amino-propane (DOI) also binds (Johnson *et al.*, 1987).

To further analyze the receptor for *cis*-UCA and in experiments approved by the Princess Margaret Hospital Ethics Committee, human peripheral blood mononuclear cells (PBMC) were incubated with lipopolysaccharide (LPS) and regulation of tumor necrosis factor-alpha (TNFα) and prostaglandin E_2 (PGE_2) production examined. *Cis*-UCA (Hart *et al.*, 1993), serotonin, and DOI (Cloez-Tayarani *et al.*, 2003) can suppress TNF-α production by human PBMCs. However, in the absence of any cell death, the mechanism by which TNF-α production was suppressed varied and suggested use of different membrane receptors by *cis*-UCA, serotonin, and the 5-HT_{2A} receptor agonist. Suppression of LPS-induced TNF-α production by *cis*-UCA ($\geqslant 7\times10^{-6}$ M) was completely reversed by incubation with indomethacin (10^{-5} M) (Figure 1a). However, significant suppression by serotonin and DOI of LPS-induced TNF-α production was consistently detected in the presence of indomethacin (Figure 1b and c). Measurement of PGE_2 levels in the culture supernatants supported these results. *Cis*-UCA induced PGE_2 production in a dose-dependent manner (Figure 2a), whereas there was a consistent increase in response to serotonin only at the very highest (supra-physiological) concentration of 10^{-3} M (Figure 2b). DOI had no significant effect on LPS-induced PGE_2 production (Figure 2c). In a further experiment, we hypothesized that if serotonin and *cis*-UCA competitively bound to the same receptor, serotonin at 10^{-4} M

should affect PGE_2 production in response to *cis*-UCA. This did not happen (Figure 2d).

Cis-UCA and serotonin did not have the same biological effects on PBMCs, a result confirmed using monocytes obtained by elutriation (>85% enriched). Further, the biological effects of DOI, an agonist of the $5HT_{2A}$ receptor, on TNF-α and PGE_2 production by LPS-stimulated cells suggested that *cis*-UCA was not binding to this receptor. Studies with agonists of the 5-HT_3, 5-HT_4, and 5-HT_7 receptors also did not have the same functional outcomes as *cis*-UCA (data not shown). Further, serotonin (Cloez-Tayarani *et al.*, 2003; Durk *et al.*, 2005) but not *cis*-UCA (Hart *et al.*, 1993) can induce IL-1β production by LPS-stimulated monocytes.

We conclude that the effects of *cis*-UCA on PBMCs are not mediated via the receptor to which serotonin binds. These findings suggest that *cis*-UCA, and serotonin if it is involved, signal independently in pathways of UVB-induced immunomodulation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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