Author/s:
Marshall, A;Celentano, A;Cirillo, N;McCullough, M;Porter, S

Title:
Oral keratinocytes synthesize CTACK: A new insight into the pathophysiology of the oral mucosa

Date:
2018-02-01

Citation:
Marshall, A., Celentano, A., Cirillo, N., McCullough, M. & Porter, S. (2018). Oral keratinocytes synthesize CTACK: A new insight into the pathophysiology of the oral mucosa. Experimental Dermatology, 27 (2), pp.207-210. https://doi.org/10.1111/exd.13471.

DR. ANTONIO CELENTANO (Orcid ID : 0000-0003-4293-2511)

Article type : Letter to the Editors

Title: Oral keratinocytes synthesize CTACK: a new insight into the pathophysiology of the oral mucosa.

Short title: CTACK in oral epithelium and inflammation.

Authors: Alison Marshall,[a] Antonio Celentano,[b#] Nicola Cirillo,[bc] Michael McCullough,[b] Stephen Porter,[a]

[a] University College London, UCL Eastman Dental Institute, London, UK;

[b] Melbourne Dental School and Oral Health CRC, The University of Melbourne, Victoria, Australia.

[c] Centre for Innovation, Research, Education and Health (IRIS), Italy.

#**CORRESPONDING AUTHOR**: Melbourne Dental School, Oral Health CRC, The University of Melbourne, 720 Swanston street, 3053, Melbourne, Victoria, Australia. antony.celentano@gmail.com, antonio.celentano@unimelb.edu.au

Conflict of Interest

The authors have no conflict of interest to declare.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/exd.13471

Abstract

The skin-associated chemokine CTACK plays a key role in many inflammatory conditions and could be instrumental in the pathophysiology of tissue-specific immunological diseases such as oral lichen planus (OLP). In the present study, we investigated by RT-PCR, ELISA, chemotaxis assays, and fluorescence-activated cell sorting (FACS) the production of CTACK in oral keratinocytes, its expression in tissues from normal and OLP patients, and its role in T cell recruitment**.** CTACK was produced by the oral epithelium and it affects chemotaxis of memory CLA+ cells to the oral epithelium. CTACK mRNA was expressed constitutively in primary oral epithelium and was increased during pro-inflammatory IFN-γ treatment. We found a constitutive production of CTACK at a protein level in oral primary cells that increased after IFN-γ treatment. Moreover, we confirmed that CTACK attracts memory T cells and those T cells that express CLA above the level of basal migration.

Keywords: chemokine, CTACK, CCL27, oral inflammation, lichen planus

Background

Lichen planus (LP) is a chronic T-cell-mediated mucocutaneous inflammatory disease that targets the stratified epithelia. There is increasing evidence that epithelial-derived cytokines, immune receptors and co-stimulatory molecules promote and sustain the disease[1-4]. Oral lichen planus, (OLP) is known to be associated with production of IFN-γ by infiltrating T lymphocytes.

Cutaneous T-cell attractant chemokine CTACK, also known as CCL27, is known for its properties to attract memory T-cells. CTACK is highly expressed in the skin, both normal and inflamed, specifically by keratinocytes in the epidermis[5].

The oral cavity has many immunological features in common with the skin, however the role of CTACK in the oral cavity immunity has yet to be investigated. Further, anti-CCL27 was found to be more potent than tacrolimus at reducing inflammation in skin patients, highlighting a potential therapeutic role [6-11].

Questions addressed

Aim of this study was to investigate the production of CTACK in oral keratinocytes, its expression in tissues from normal and OLP patients, and its role as a chemotactic molecule mediating T cell recruitment..

Exp. design

Three primary normal oral keratinocyte strains (NHOK1, NHOK2, NHOK3), the UP cell line[12], and the H357[13] cell line were used in this study.

The cells were tested with TNF-α, IL-1β, and IFN-γ treatment or no treatment (for 3, 6 or 24 hours). RT-PCR was used to assess the expression of CTACK mRNA in epithelial cell lines. The supernatants were also tested for the presence of the CTACK protein using ELISA. Peripheral blood mononuclear cells (PBMC) were prepared from fresh blood obtained from healthy patients. Cell supernatants derived from the cytokine treatment assays were incubated with no antibody, monoclonal anti-human CTACK antibody, or mouse anti-human IgG_{2a} isotype control, for 30 minutes before placing in transwell.

Antibodies utilised to label the migrated PBL were monoclonal mouse anti-human CD45RO-R-Phycoerythrin, monoclonal rat anti-human CLA-FITC and monoclonal mouse anti-human CD3 Cy-chrome. The migrated cell populations were then analysed using a florescence activated cell sorter (FACS) machine.

Materials and methods are described under supplementary material 1 (S1).

Results

CTACK mRNA was expressed in primary unstimulated oral keratinocytes, and levels were increased when treated with IFN-γ (Fig. 1a, fig. 1b).

The ELISA test showed a increased production of CTACK in NHOK2, and a similar trend in NHOK1 and 3 (Fig. 1c). However the biological triplicate showed a statistically significant difference ($p=0.023638313$) between the IFN-γ treated and

non-treated cell lines. The average concentration produced in the IFN-γ treated cells was 557.74 pg/ml and 302.85 pg/ml in the unstimulated cells.

CTACK was released from the oral carcinoma cell line, H357 in similar concentrations to the oral primary cell line (Fig. 1d), suggesting that H357 are an adequate study model for CTACK over time. CTACK was produced by untreated H357 cells but the concentration produced decreases over time in culture. TNF-α and IL-1β treated cells released lower concentrations of CTACK at 3 hours compared to the untreated cells, but the amount increased over each time point and was significantly increased to the untreated cells after 6 and 24hours. IFN-γ treatment was also able to significantly increase the concentration of CTACK in this cell type after 24 hours (Fig. 1d).

In the UP cell line, CTACK was detected in untreated cells at all time points. IFN-γ treatment slightly increased CTACK production at 3 and 24 hours compared to control cells, but not to a significant level. Similarly, TNF-α and IL-1β treatment increased the production of CTACK at 24 hours (Fig. 1d).

The cell populations were analysed after migration with the peripheral blood lymphocyte population and gated upon the level of fluorescence to CD3 and CD45RO or CD3 and CLA into CD3+CD45RO+ cells and CD3+CD45RO+CLA+ cells (Fig. 2a, 2b).

Recombinant human CTACK attracted memory T cell population, significantly at 100 and 150 ng. It can also be shown that CTACK increased the migration of memory T cells expressing CLA from peripheral blood above the level of basal migration, to a significant degree at 100ng (Fig. S1).

Both H357 and UP cell supernatants induced the migration of memory and CLA positive T cells above the level of basal migration in a CTACK-dependent manner (Fig. 2c, 2d). TNF-α and IL-1 treatment of UP and H357 cells significantly increased the migration of memory T cells to the extracted supernatant compared to the control. H357 cell supernatant was able to induce a higher migration % of memory T cells than the UP cells regardless of cell treatment. Pre-incubation of oral and cutaneous keratinocyte cell line supernatants with anti-CTACK antibody reduced the migration of memory T cells and CLA+ T cells compared to those untreated, and to a significant extent in TNF-α /IL-1β-treated cells. Interestingly, by blocking CTACK activity there was a significant increase in the migration of naïve T cells to both cutaneous and oral supernatants compared to those not pre-treated (Fig. 2c-2f).

CTACK mRNA was expressed in the oral inflammatory disorder OLP and in NOM, however, to low levels and to differing degrees in different samples (Fig. S2).

Conclusions

CTACK is constitutively produced by oral keratinocytes, and increases during pro-inflammatory, IFN-γ, cytokine treatment. Further, it actively effects chemotaxis of memory CLA+ cells to the oral epithelium. This is the first report of a tissue-specific chemokine being produced by cells in the oral cavity. It suggests that CTACK may play a role in early recruitment of T cells that could be instrumental in the pathophysiology of tissue-specific immunological diseases such as oral lichen planus (OLP).

Surprisingly, in OLP and NOM tissue, low-level CTACK mRNA was expressed in most of the samples tested; however, CTACK may still play a role in progressing OLP lesions and not the chronic lesions tested in this study.

Author contribution

AM, AC, MMC and SP designed the study. AM and AC performed the experiments, analysed the data and wrote the manuscript. NC, MMC and SP critically reviewed the study proposal and discussed the data. All authors have read and approved the final version of the manuscript.

Acknowledgement

The authors have no acknowledgements to report.

Conflict of interest

The authors have declared no conflicting interests.

References

1) E.J. Kunkel, E.C. Butcher, Chemokines and the tissue-specific migration of lymphocytes, Immunity. 16 (2002) 1-4.

2) R. Lu, J. Zhang, W. Sun, G. Du, G. Zhou, Inflammation-related cytokines in oral lichen planus: an overview. J. Oral Pathol. Med. 44 (2015) 1-14.

3) A. Marshall, A. Celentano, N. Cirillo , M. Mirams, M. McCullough, S. Porter, Immune receptors CD40 and CD86 in oral keratinocytes: implications for oral lichen planus. J Oral Sci. 59 (2017) 373-382.

4) A. Marshall, A. Celentano, N. Cirillo, M.D. Mignogna, M. McCullough, S. Porter, Antimicrobial activity and regulation of CXCL9 and CXCL10 in oral keratinocytes. Eur. J. Oral Sci. 124 (2016) 433-439.

5) J. Morales, B. Homey, A.P. Vicari, S. Hudak, E. Oldham, J. Hedrick, R. Orozco, N.G. Copeland, N.A. Jenkins, L.M. McEvoy, A. Zlotnik, CTACK, a skin-associated chemokine that preferentially attracts skin-homing memory T cells. Proc. Natl. Acad. Sci. U. S. A. 25 (1999) 14470-5.

6) B. Homey, H. Alenius, A. Müller, H. Soto, E.P. Bowman, W. Yuan, L. McEvoy, A.I. Lauerma, T. Assmann, E. Bünemann, M. Lehto, H. Wolff, D. Yen, H. Marxhausen, W. To, J. Sedgwick, T. Ruzicka, P. Lehmann, A. Zlotnik. CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nat Med. 8 (2002) 157-65.

7) Y. Reiss, A.E. Proudfoot, C.A. Power, J.J. Campbell, E. Butcher. CC chemokine receptor CCR4 and the CCR10 ligand cutaneous T cell attracting chemokine (CTACK) in lymphocyte trafficking to inflamed skin. J. Exp. Med. 194 (2001) 1541-1547.

8) D.W. Brown, J. Furness, P.M. Speight, G.J. Thomas, J. Li, M.H. Thornhill, P.M. Farthing. Mechanisms of binding of cutaneous lymphocyte-associated antigen-positive and aeb7-positive lymphocytes to oral and skin keratinocytes. Immunology 98 (1999) 9-12.

9) L.J. Walton, M.H. Thornhill, M.G. Macey, P.M. Farthing. Cutaneous lymphocyte associated antigen (CLA) and alpha e beta 7 integrins are expressed by mononuclear cells in skin and oral lichen planus. J. Oral Pathol. Med. 26 (1997) 402-7.

10) M. Karakawa, M. Komine, Y. Hanakawa, H. Tsuda, K. Sayama, K. Tamaki, M. Ohtsuki M. CCL27 is downregulated by interferon gamma via epidermal growth factor receptor in normal human epidermal keratinocytes. J. Cell. Physiol. 229 (2014) 1935-45.

11) T.W. Rozycki, R.S. Rogers, M.R. Pittelkow, M.T. McEvoy, R.A. el-Azhary, A.J. Bruce, J.P. Fiore, M.D. Davis. Topical tacrolimus in the treatment of symptomatic oral lichen planus: a series of 13 patients. J. Am. Acad. Dermatol. 46 (2002) 27-34.

12) X.F. Pei, P.A. Gorman, F.M. Watt. Two strains of human keratinocytes transfected with HPV16 DNA: comparison with the normal parental cells. Carcinogenesis. 12 (1991) 277-84.

13) S.S. Prime, S.V. Nixon, I.J. Crane, A. Stone, J.B. Matthews, N.J. Maitland, L. Remnant, S.K. Powell, S.M. Game, C. Scully. The behaviour of human oral squamous cell carcinoma in cell culture. J. Pathol. 160 (1990) 259-69.

Figure legends

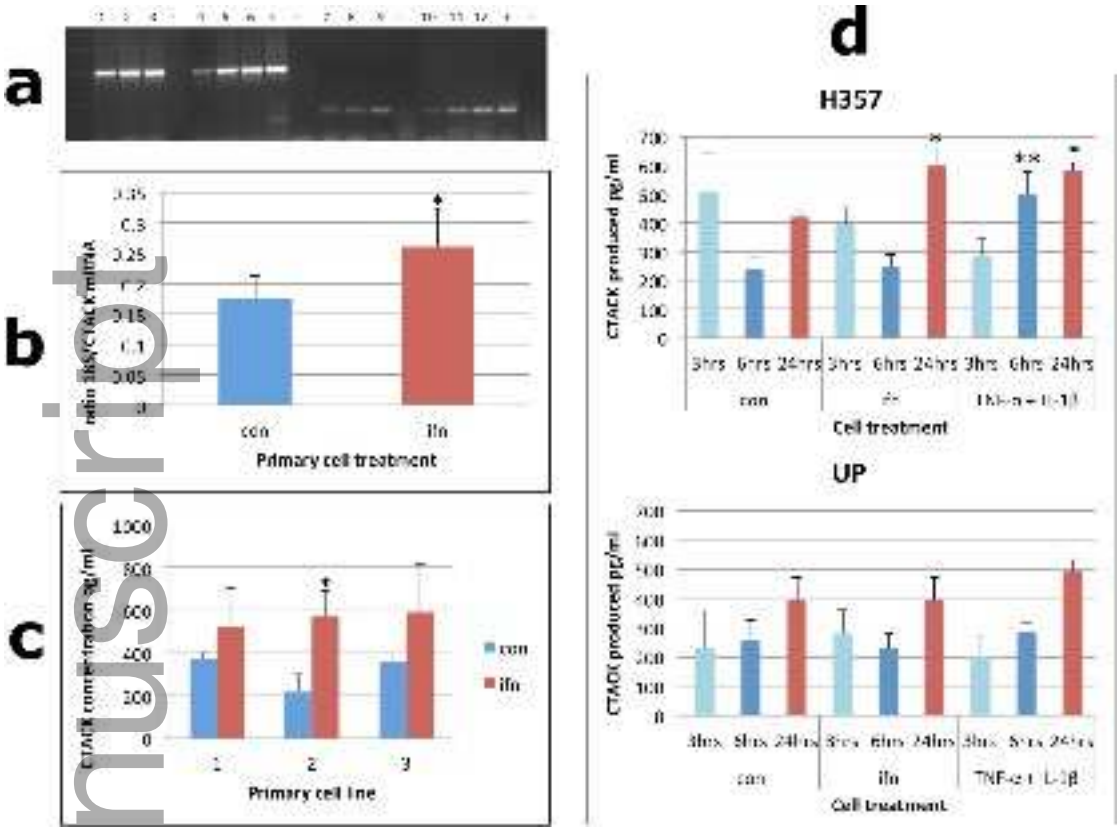
Figure 1: Human keratinocytes express the chemokine CTACK in vitro. **a)** Expression of 18S in the 3 epithelial cell lines non-stimulated (1-3), and treated with IFN-γ for 48hours (4-6), and CTACK mRNA expression in these cell types are represented in lanes 7-9 and 10-12 respectively. + represents the positive control for 18S and CTACK expression, skin cDNA. Lanes marked – are the negative controls for these samples. **b)** Densitometric analysis of the average CTACK mRNA expression (normalised to 18S expression) in the 3 different primary oral keratinocytes treated with IFN-γ (ifn) or non-treated (con). **c)** The mean concentration of CTACK produced in primary oral epithelial cell lines (n=3) stimulated with 1000U IFN-γ (ifn) or left untreated for 24 hours. **d)** The concentration of CTACK produced (pg/ml) in the supernatants of H357 or UP cells treated either with IFN-γ (ifn) or TNF-α and IL-1β (TNF-α +IL-1β) for 3, 6 or 24 hours as determined by ELISA. Each result is the mean of triplicate samples ±SD. *, ** represents a significant difference $p<0.05$ or $p<0.01$ respectively between the treated and control cells at the same time point.

Figure 2: 3 colour-FACS profile of T cells exposed to oral and cutaneous cell lines supernatants. The cytometry plots for the PBL population labelled with **a)** anti-CD3 and CD45RO with populations divided into CD3+CD45ROlo (R2) and CD3+CD45ROhi (R3) and **b)** anti-CD3 and CLA with gating corresponding to CD3+CLA+ (R4) or CD3+CLA- (R5). The normalised migration of T cell subsets to supernatants of non-treated (con) or TNF-α and IL-1 treated (tnf+il-1) keratinocyte

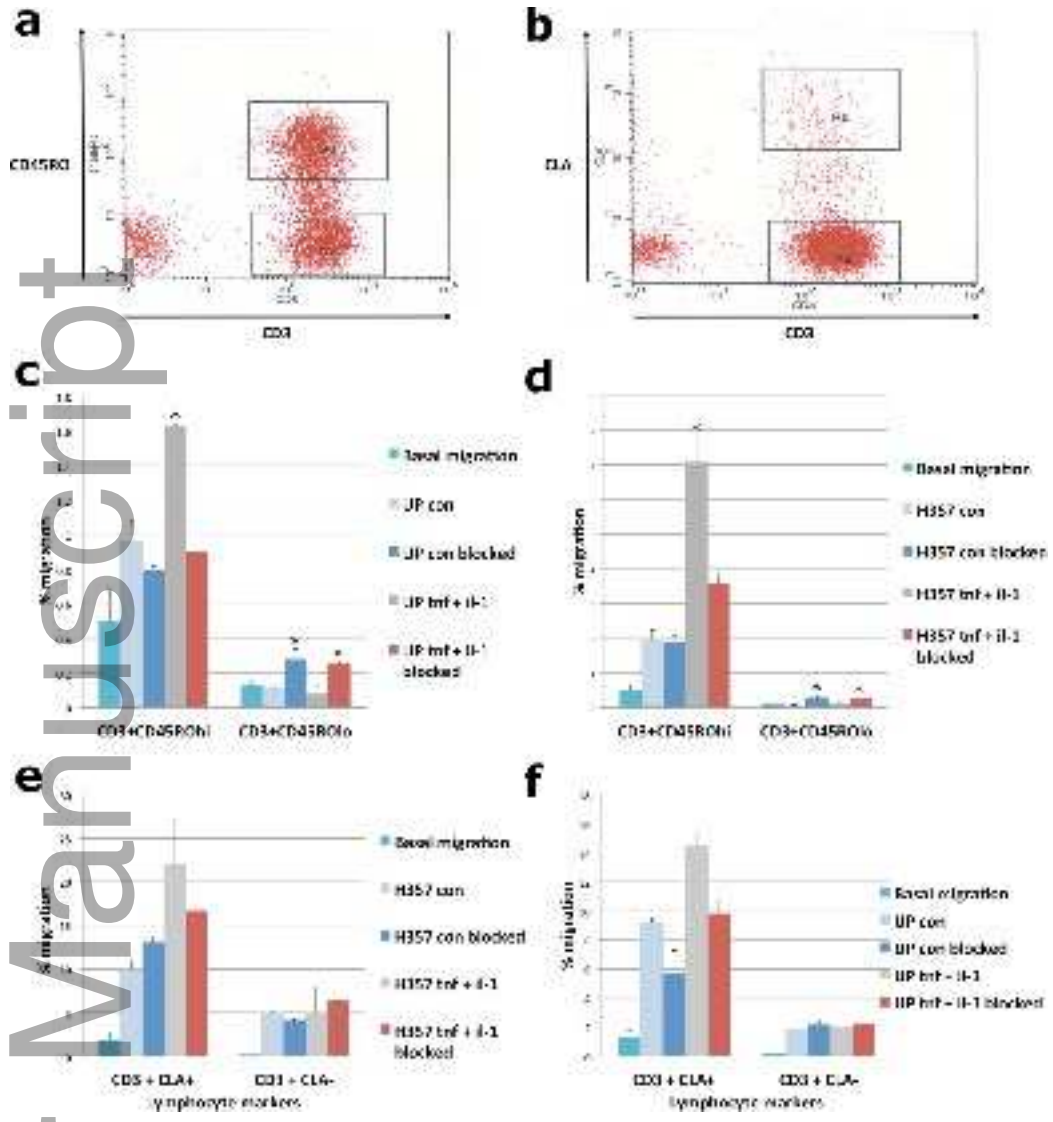
cell lines. The migration of CD3+CD45ROhi ('memory' T cells) or CD3+CD45ROlo ('naïve' T cells) to **c)** oral (H357) or **d)** cutaneous (UP) cell line supernatants, with or without pre-incubation with an anti-CTACK antibody (blocked). The migration of CLA+CD3+ or CLA-CD3+ cells to **e)** oral (H357) or **f)** cutaneous (UP) cell line supernatants, with or without pre-incubation with an anti-CTACK antibody (blocked). Basal migration is the migration of these cell types to cell culture medium alone. * represents a significant difference ($p<0.05$) in the migration induced by supernatants pre-treated with anti-CTACK or non-treated.

Supplementary figure 1: The chemotaxis of T cells to recombinant CTACK. PBL that had migrated were labelled with florescent antibodies to T cell marker CD3, memory cell marker CD45RO and 'skin-homing' marker CLA. % migration is the normalised chemotaxis of **a)** CD3+CD45RO+ cells or **b)** CD3+CD45RO+CLA+ compared to the number of specific input cells to 50, 100 or 150ng of CTACK (ctack 50, ctack 100 and ctack 150 respectively). Basal migration is the migration of cells to cell culture medium only. * represents a significant increase in the % migration ($p<0.05$) compared to basal migration.

Supplementary figure 2: Expression of 18S and CTACK mRNA in OLP samples (1-8), NOM tissue (1-6) and positive control (+), un-inflamed skin mRNA.



exd_13471_f1.tif



exd_13471_f2.tif