doi: 10.1111/j.1600-0714.2007.00519.x

J Oral Pathol Med (2007) 36: 215–22
© 2007 The Authors. Journal compilation © 2007 Blackwell Munksgaard · All rights reserved
www.blackwellmunksgaard.com/jopm

Survival signalling in keratinocytes of erythematous oral lichen planus

Andreas Karatsaidis[1,2], Katsuhiko Hayashi[1,3], Olav Schreurs[1], Kristen Helgeland[1], Karl Schenck[1]

[1]*Department of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway;* [2]*Department of Oral Surgery and Oral Medicine, University of Bergen, Bergen, Norway;* [3]*Department of Dentistry, Jikei University School of Medicine, Tokyo, Japan*

BACKGROUND: Keratinocytes in oral lichen (OL) planus have been shown to be exposed to potentially cell death-inducing factors such as tumour necrosis factor-α (TNF-α) and FasL, produced by the cells of the inflammatory infiltrate and by the keratinocytes themselves. Mostly, however, the lesions do not show ulceration, the clinical manifestation of substantial keratinocyte death. The aim of this study was to find support for the contention that there is activation of protecting anti-apoptotic mechanisms in keratinocytes in a form of chronic OL (erythematous OL; ERY OL), simultaneously with the pathological cell death signals.

METHODS: Biopsies from patients with normal oral mucosa (NOM) or with ERY OL were compared by immunohistological staining.

RESULTS: In ERY OL keratinocytes, both the pro-apoptotic FADD and the anti-apoptotic molecules p-IKK, NF-κB/p50, $FLIP_L$, cIAP-1 and cIAP-2 were strongly up-regulated when compared with NOM. There were no significant differences in the staining patterns for active caspase-3 and caspase-8 with only few positive cells for both enzymes.

CONCLUSIONS: The presently observed marked increase in expression of anti-apoptotic molecules in ERY OL epithelium may counteract the pro-apoptotic assault and rescue the epithelium from rampant cell death and thereby clinical ulceration.

J Oral Pathol Med (2007) **36**: 215–22

Keywords: apoptosis; keratinocytes; oral lichen planus; survival

Introduction

Oral lichen (OL), including oral lichenoid reactions and OL planus, is a common inflammatory mucosal disease that can affect all areas of the oral cavity. OL typically exhibits a chronic course with lesions that may persist for several months or years. Clinical features and accompanying symptoms of OL lesions vary, ranging from asymptomatic purely reticular forms to lesions that in addition display erythematous (ERY) and ulcerative areas associated with pain or discomfort (1, 2).

The histopathology of OL is typified by heavy infiltration by inflammatory cells in the connective tissue and basal cell degeneration, acanthosis, hyperkeratosis and thinning of the epithelium (3, 4). Aberrant cell death of keratinocytes has been proposed to be part of the pathological features of the OL epithelium (5–7). The inflammatory cells and the keratinocytes in OL express an array of cytokines and display membrane receptors that together induce a signalling network which is taken to be crucial in the pathological processes (8, 9). OL keratinocytes are exposed to potent extrinsic factors such as tumour necrosis factor-α (TNF-α) and FasL (8, 10, 11), which have pro-apoptotic (FasL) or both pro-apoptotic and anti-apoptotic (TNF-α) effects.

If the actions of death signalling cytokines were to be unrestricted in OL epithelium, this would lead to severe destruction of the basal epithelial cell layer and ulceration. Mostly, however, patients with OL do not suffer from ulceration. This suggests that anti-apoptotic factors may interfere and modulate epithelial cell death in the more quiescent phases of OL. Activation of NF-κB is particularly important for the induction of anti-apoptotic gene products, including the Inhibitor of Apoptosis Proteins 1 and 2 (cIAP-1 and cIAP-2), and the FLICE-Inhibitory Protein (FLIP; 12, 13).

The aim of the present study was to find support for the contention that pathological cell death signals are given to the keratinocytes of ERY OL, but that there is simultaneous activation of protecting anti-apoptotic mechanisms. To this purpose, we examined by immunohistology the protein expression in epithelium of ERY OL and normal oral mucosa (NOM) of the death-promoting Fas-associated death domain protein (FADD), active caspase-3 and caspase-8 and several molecules participating in cell survival: phosphorylated IκB kinase (p-IKK), NF-κB/p50, $FLIP_L$, cIAP-1 and cIAP-2. The results indicate that keratinocytes in OL are subjected to extrinsic death signals, but that the expres-

Correspondence: Karl Schenck, Department of Oral Biology, PO Box 1052, Blindern, N-0316 Oslo, Norway. Tel: +47 22 84 0360, Fax: +47 22 84 0302, E-mail: karl.schenck@odont.uio.no
Accepted for publication December 12, 2006

sion of anti-apoptotic molecules is significantly increased and that this may rescue the keratinocytes from extensive cell death.

Materials and methods

Specimens

The Ethical Committee of Health, Oslo, Norway, approved the conduction of the study. After informed consent, buccal biopsies were taken from volunteers with ERY OL ($n = 10$), or from sex- and age-matched subjects with NOM ($n = 10$). None of the patients had *Candida* infection as tested by using Dentocult® dip slides (Orion Diagnostica, Espoo, Finland). The clinical and histopathological criteria used were those previously described in Ref. (4). The biopsies were taken from sites that typified the clinical diagnosis. Biopsies were divided into two pieces: one sample was snap-frozen on dry ice (−70°C) and one sample was fixed in 4% buffered formaldehyde for 10 h. The frozen biopsies were oriented and embedded in OCT compound (Sakura Finetek Co., Tokyo, Japan) and stored at −80°C. About 4-μm-thick sections were cut at −20°C in a cryostat and slides were stored at −20°C until used. The formaldehyde-fixed biopsies were processed through graded alcohol, orientated, embedded in paraffin and cut in 4-μm-thick sections on slides. Pathological diagnosis was confirmed on HE sections.

Immunohistology

Primary antibodies and concentrations used were: NF-κB/p50 (rabbit IgG, 2.0 μg/ml; NeoMarkers, Lab Vision Corp., Fremont, CA, USA); cIAP-1 (rabbit IgG, 2.0 μg/ml), cIAP-2 (rabbit IgG, 2.0 μg/ml), $FLIP_L$ (rabbit IgG, 0.7 μg/ml), FADD (rabbit IgG, 2.0 μg/ml) all from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA; cleaved caspase-3 (rabbit IgG, 0.2 μg/ml), p-IKK (rabbit IgG, 1.0 μg/ml) and cleaved caspase-8 (rabbit IgG, 10 μg/ml) all from Cell Signaling Technology, Inc., Beverly, MA, USA. All primary antibodies were used in stainings on frozen sections, except for the FADD antibodies, which were used on paraffin sections. Frozen sections were post-fixed in 4% paraformaldehyde for 20 min, except for those used in staining for $FLIP_L$, p-IKK, caspase-3 and NF-κB/p50, which were fixed in ice-cold acetone for 10 min. Paraffin sections were dewaxed and rehydrated according to standard procedures. Slides were washed in 2 × 5 min PBS and incubated with 0.3% H_2O_2 for 30 min after fixation and dewaxing/rehydration of frozen and paraffin sections, respectively. After rewashing, slides were incubated with 5% normal serum (species equivalent to the species of secondary antibody used, see below) for 30 min. Primary antibodies were diluted in PBS with 1% bovine serum albumin and incubated overnight at 4°C. Sections were washed in PBS and incubated 30 min with the appropriate secondary biotinylated antibody (horse anti-mouse IgG or goat anti-rabbit IgG; Vector Laboratories Inc., Burlingame, CA, USA). After washing in PBS, sections were incubated with avidin–biotin complex conjugated with horseradish peroxidase (ABC-HRP; Vector) for 30 min, washed and finally developed with 3,3′-diaminobenzidine as substrate (Sigma-Aldrich Corp, St Louis, MO, USA).

As negative controls, isotype-matched antibodies were used with the same concentrations as the primary antibodies, of which none showed staining with the immunohistological detection techniques used.

The length of the basal cell layer in tissue sections stained for active caspase-3 was estimated by monitoring images obtained by means of a digital camera connected to the microscope. An optical magnification of 20× was used, and measurements of basal cell layer lengths were made using a software program (Soft Imaging System GmbH, Münster, Germany).

Statistics

Student's *t*-test was used to compare countings for active caspase-3. Differences were considered to be statistically significant at $P < 0.05$.

Results

In NOM, moderate cytoplasmic and occasional nuclear staining for FADD was observed in keratinocytes of the basal and parabasal layers of the epithelium (Fig. 1a). Scattered cytoplasmic staining in upper spinous and granular layers was also seen (Fig. 1a). In ERY OL, very strong cytoplasmic staining for FADD was observed in basal and spinous layers (Fig. 1b). Significant nuclear staining was also observed in this area.

Active caspase-3 was occasionally observed in basal keratinocytes in both NOM and ERY OL. Typically, clusters of two or more basal keratinocytes showed nuclear and cytoplasmic staining for activated caspase-3 (Fig. 1c,d). The mean numbers of active caspase-3-positive keratinocyte clusters per mm basal cell layer in NOM and ERY OL epithelium were 4.95 ± 2.44 and 4.05 ± 1.62, respectively (mean ± SD). The difference was not statistically significant ($P > 0.05$). However, in ERY OL, scattered active caspase-3-positive cells with distinct round morphology were observed to infiltrate the keratinocytes in basal and spinous layers (Fig. 1d).

Weak cytoplasmic staining for active caspase-8 was seen in scattered cells in basal, spinous, granular and superficial epithelial layers in NOM and ERY OL (Fig. 1e,f). There was no significant difference in the staining pattern between ERY OL and NOM epithelium.

In NOM epithelium, cytoplasmic p-IKK staining was seen in basal keratinocytes and this was weaker in spinous, granular and superficial cell layers (Fig. 1g). In contrast, strong cytoplasmic staining for p-IKK was seen in all epithelial cell layers in ERY OL (Fig. 1h).

Basal keratinocytes in NOM epithelium showed cytoplasmic staining for NF-κB/p50 (Fig. 1i). Weaker cytoplasmic staining was observed in all suprabasal layers (Fig. 1i). Nuclear staining for NF-κB/p50 was observed in scattered keratinocytes throughout the whole epithelium in NOM (Fig. 1i). In ERY OL, there was strong cytoplasmic staining for NF-κB/p50 in all

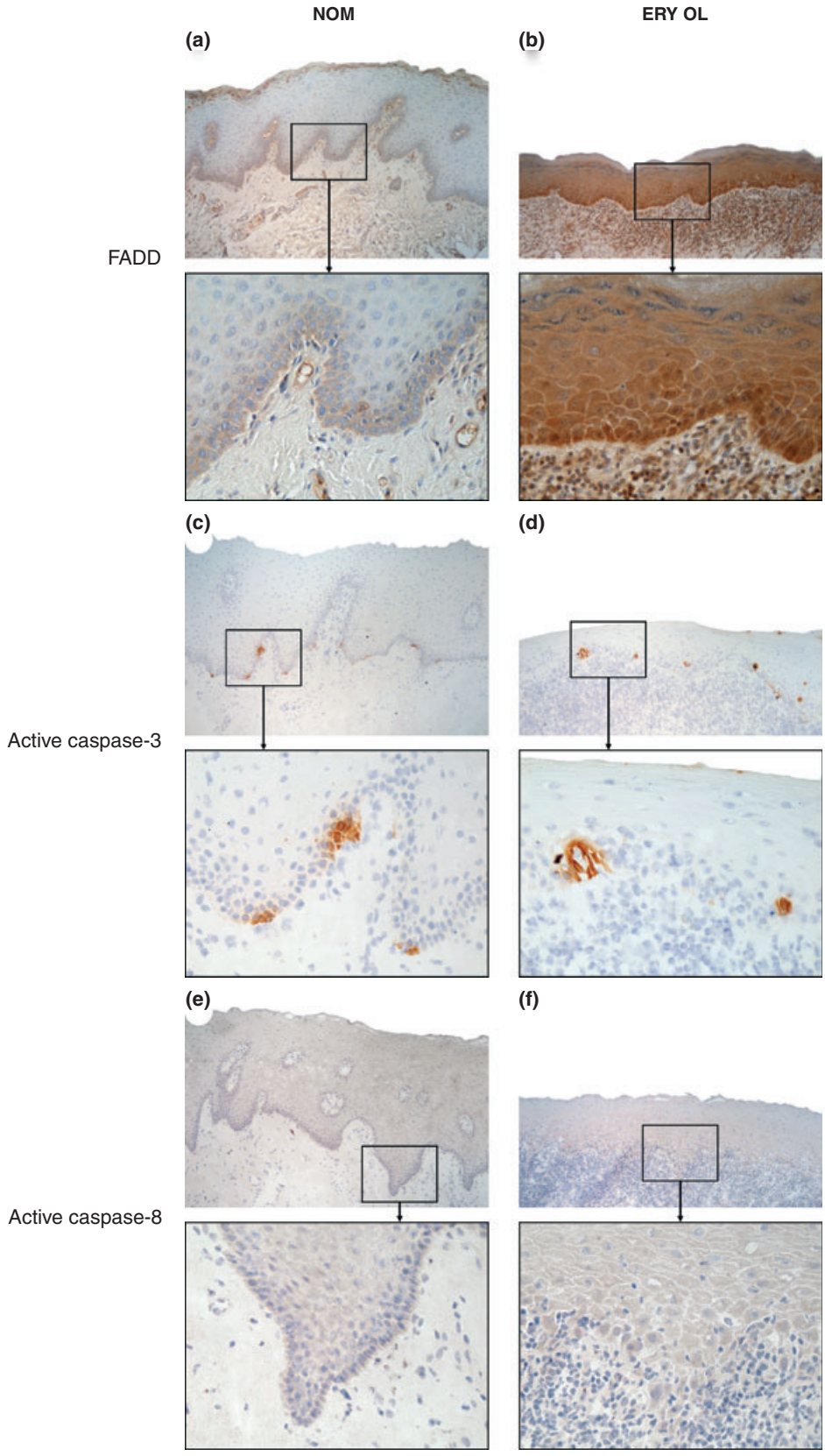

Figure 1 Micrographs of immunohistochemically stained sections from normal oral mucosa (left side: a, c, e, g, i, k, m, o, q) and ERY OL (right side: b, d, f, h, j, l, n, p, r). Labelled molecules are seen in brown and nuclei are counterstained with haematoxylin. Antibodies were used against (a and b) FADD; (c and d) active caspase-3; (e and f) active caspase-8; (g and h) p-IKK; (i and j) NF-κB/p50; (k and l) $FLIP_L$; (m and n) cIAP-1; (o and p) cIAP-2; (q and r) control rabbit IgG. Selected fields are shown at a higher magnification as indicated by frames and connecting arrows.

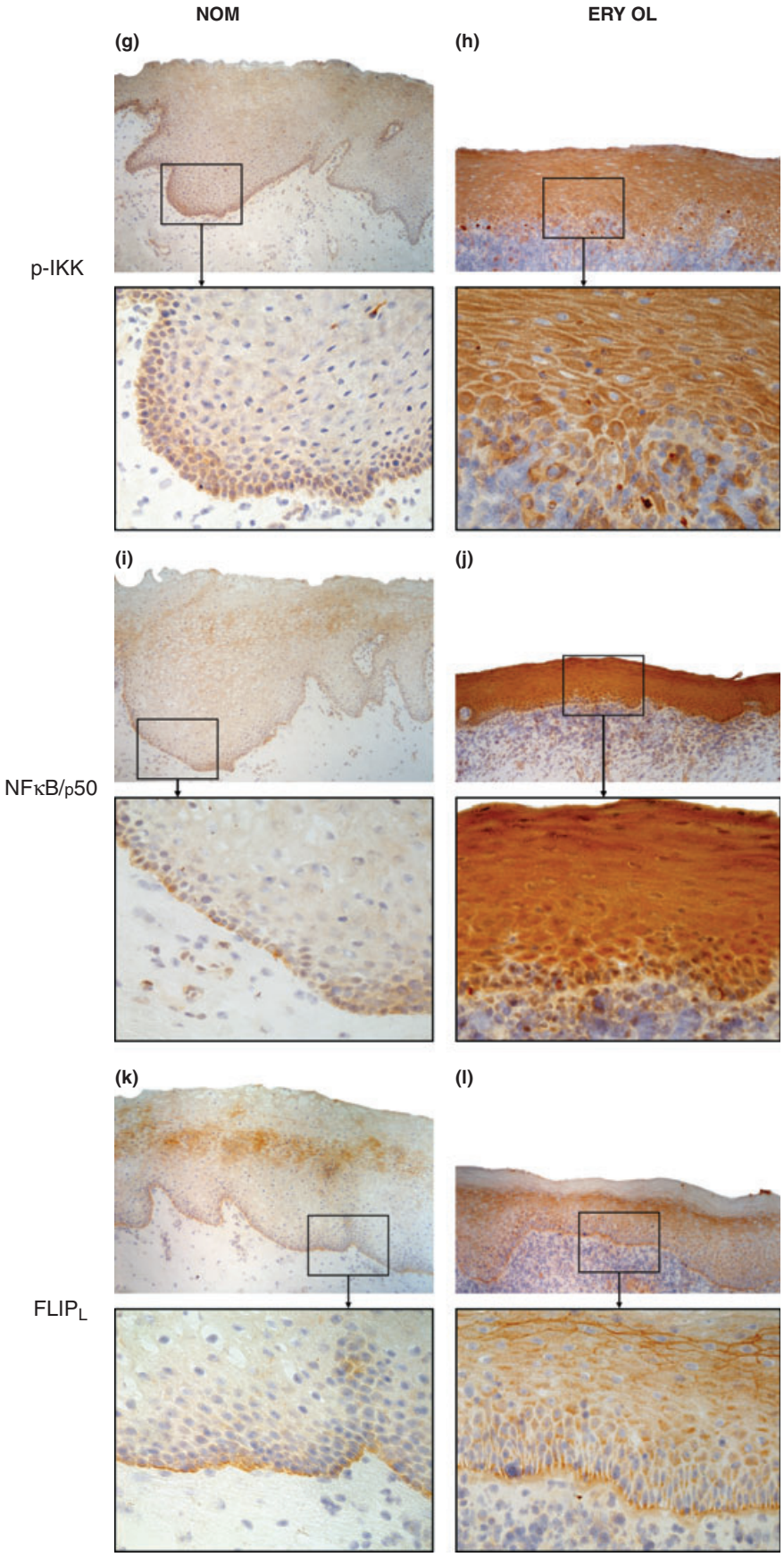

Figure 1 Continued

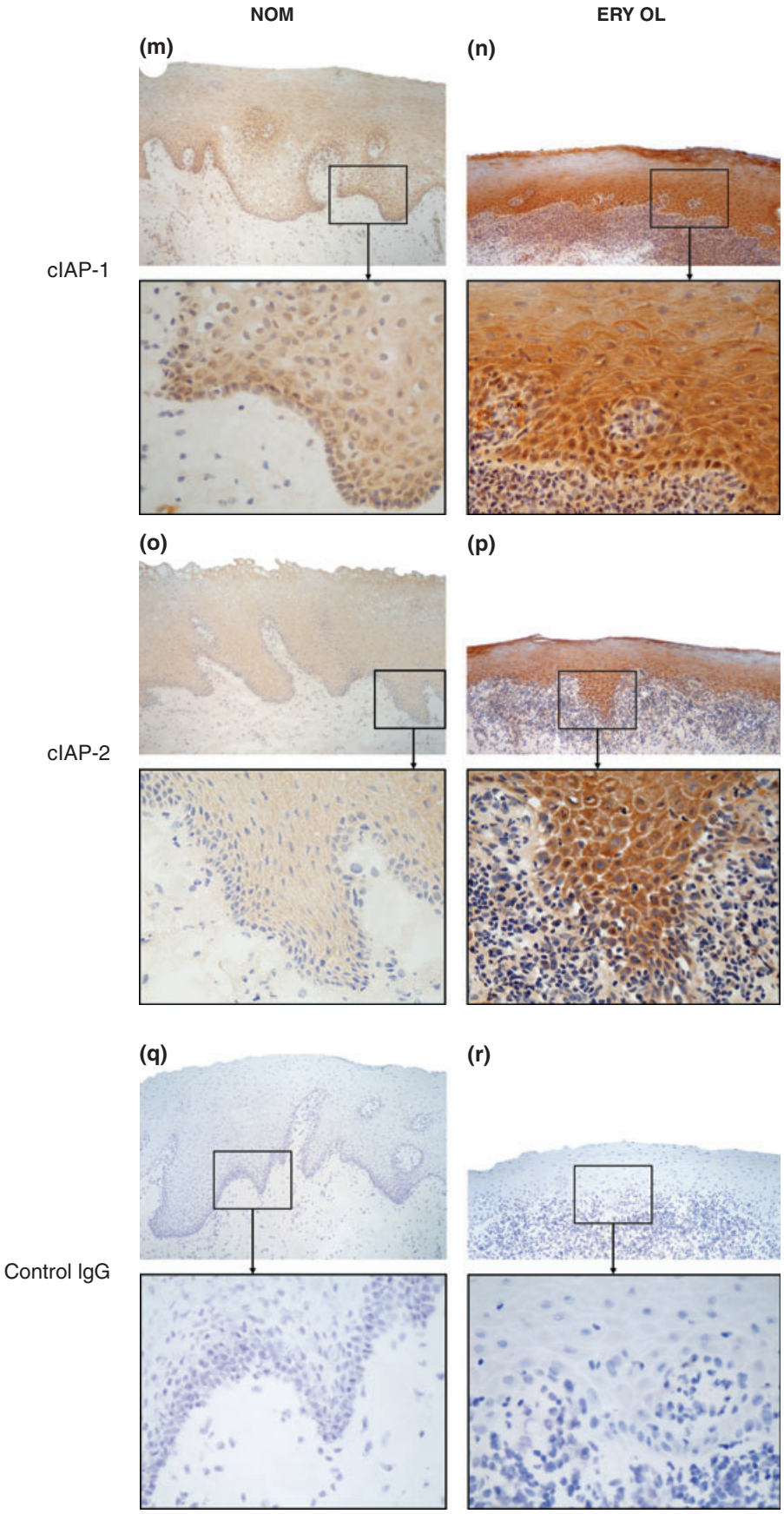

Figure 1 Continued

epithelial layers (Fig. 1j). Nuclear staining was also observed in all keratinocyte layers in ERY OL (Fig. 1j).

Basal keratinocytes of NOM showed cytoplasmic staining for $FLIP_L$ which was typically polarized towards the basement membrane (Fig. 1k). Cytoplasmic staining was occasionally also seen in keratinocytes in upper spinous and granular layers in NOM (Fig. 1k). ERY OL basal keratinocytes showed distinct and strong cytoplasmic staining which was also polarized towards the basement membrane (Fig. 1l). In addition, all suprabasal keratinocytes showed cytoplasmic staining for $FLIP_L$, which in the spinous and granular layers mainly was localized adjacent to the cell membrane (Fig. 1l).

In NOM, cIAP-1 showed weak to moderate cytoplasmic staining in basal and spinous cell layers (Fig. 1m). cIAP-2 also showed weak to moderate cytoplasmic staining, but this staining was seen throughout the whole NOM epithelium (Fig. 1o). Scattered basal and spinous keratinocytes in NOM displayed nuclear staining for cIAP-1 but not for cIAP-2 (Fig. 1m,o). In ERY OL, strong cytoplasmic staining, predominantly in basal and spinous layers, was seen both for cIAP-1 and cIAP-2 (Fig. 1n,p). Nuclear staining for cIAP-1, but not for cIAP-2, was seen in many basal and spinous keratinocytes in ERY OL (Fig. 1n,p).

Discussion

In skin and mucosal diseases, the balance between normal cell death and survival of keratinocytes can be disturbed, resulting in changed epithelial morphology and function (14). We have previously shown that chromatin condensation – a marker for normal cell death – is significantly reduced in ERY OL keratinocytes, indicating an aberrant form of cell death in ERY OL epithelium (15). We have also showed that TUNEL-positive cells observed in ERY OL epithelium are mainly infiltrating apoptotic lymphocytes and not keratinocytes (16). In the present study, we have investigated the *in situ* distribution of cell survival and death-inducing factors in ERY OL epithelium, when compared with NOM. Due to the dense infiltrate of inflammatory cells, the epithelium of ERY OL is exposed to a variety of cytokines including agents such as TNF-α and FasL (8, 10, 11). It has been suggested that these factors might propel keratinocytes in OL tissue into pathological cell death (8, 10, 11). However, agents such as TNF-α can have a two-sided effect being either pro-apoptotic or anti-apoptotic, depending on temporal or spatial factors (17). We hypothesized that disease-related extrinsic cell death-inducing signals are given to ERY OL keratinocytes, but that induction of counteracting survival pathways limit their effect.

The intracellular signalling events triggered by TNF-α and FasL binding to the receptors TNF-R1 and Fas, respectively, have been extensively studied in recent years, but not all regulatory details are known. Initially, upon TNF-α binding to its receptor TNF-R1, a complex I is formed on the receptor's cytoplasmic domain, consisting of the bridging protein TRADD, the protein kinase RIP1 and the signal transducer TRAF2 (18). This complex signals cell survival through IKK activation of the transcription factor nuclear factor-κB (NF-κB; 18–21). NF-κB belongs to a highly conserved Rel-related family, which includes Rel A (p65), Rel B, c-Rel, NF-κB 1 (p105/p50) and NF-κB 2 (p100/p52). The p50/p65 heterodimer, commonly called NF-κB, is the most abundant and ubiquitous (22). In this classical TNF-α-induced pathway, IKK phosphorylates and inactivates IκB, an NF-κB inhibitor, which allows NF-κB to translocate into the nucleus and induce the transcription of various anti-apoptotic genes such as FLIP, cIAP-1 and cIAP-2 (12, 13). At a later stage, complex I can be released from the receptor into the cytoplasm and associate with the adaptor protein FADD and pro-caspase 8 to form complex II, creating a Death-Inducing Signaling Complex (DISC; 18). During Fas activation, however, caspase-8 is recruited to the DISC complex on the cell inner surface without any prior formation of a complex I. Thus, FasL is a weak NF-κB activator but a strong death signalling activator. If there is a low NF-κB activity in the cell, pro-caspase-8 is autocleaved and activated in the DISC (18). The active form of caspase-8 is then able to cleave and activate other downstream caspases such as caspase-3, which will lead to apoptosis of the cell (18). The present study shows very strong expression of NF-κB in ERY OL epithelium with significantly increased nuclear staining and this may have an important role in suppressing caspase-8 autocleavage and thereby downstream activation of the apoptotic machinery (see below).

Complex II induces apoptosis only when the NF-κB survival signalling from complex I fails to induce the expression of FLICE-Inhibitory Protein ($FLIP_L$; 12, 18). FLIP is expressed in two splicing isoforms; the 55 kDa $FLIP_L$ and 26 kDa $FLIP_s$ variants. $FLIP_L$ is nearly identical to caspase-8, but lacks a protease domain which leaves it without any enzymatic activity (23, 24). $FLIP_L$ specifically interacts with pro-caspase-8 in the DISC, hindering the generation of active caspase-8 (18, 23), and it is therefore an efficient inhibitor of TNF-α and FasL-induced cell death (12). The increased expression of $FLIP_L$ in ERY OL epithelium, and the constitutive expression of $FLIP_L$ in basal keratinocytes in NOM, indicates that this protein is constantly available at levels which may prevent caspase-8 activation. In OL epithelium, $FLIP_L$ may therefore have an important role in deflecting extrinsic signals induced by death receptors towards cell survival through NF-κB. The sustained IKK and NF-κB activation found in ERY OL can be induced by additional signals. In recent studies on human epithelial cells, late NF-κB signalling depends on secondary release of IL-1α and IL-1β (25, 26). IL-1 cytokines, which are increasingly generated in oral lesions, serum and saliva of OL patients (9, 27), may therefore play an important role in the presently observed sustained IKK activation.

This study shows a significantly increased expression of FADD in ERY OL keratinocytes. This indicates that there is increased downstream signalling through the

Fas and TNF-R1 death receptors. In hepatocytes, FADD levels in the cytoplasm have been shown to increase in response to different types of cellular insult, such as treatment with actinomycin D (alone or in combination with TNF-α), cycloheximide, ultraviolet radiation and heat shock (28, 29). The mechanism for the upregulation is not yet fully established. In the hepatocytes, increase in FADD levels was accompanied by augmented cell death but changes in levels of FLIP and cIAP proteins were not observed. In analogy, FADD levels in ERY OL as seen in the present study may be upregulated in response to inflammatory signals, e.g. TNF-α itself. In contrast to what was seen in hepatocytes (29), however, FLIP and cIAP proteins are also upregulated in ERY OL keratinocytes. These molecules are factors that can outweigh the increase in FADD levels that otherwise might have induced keratinocyte cell death.

Until recently, FADD was mainly known as a death receptor adaptor protein, assumed to be primarily a cytoplasmic protein. Closer examination has shown that FADD also translocates to the nucleus and plays a role in genome surveillance (30). In our biopsies, FADD was seen both in the cytoplasm and in the nucleus and this suggests that FADD in ERY OL keratinocytes can have multiple functions.

There were only occasional cells staining for active caspase-8 and caspase-3 and there was no difference between ERY OL and NOM epithelium. This indicates and supports the hypothesis that apoptotic signals induced by complex II may be counteracted by the increased expression in ERY OL keratinocytes of the anti-apoptotic molecule $FLIP_L$, but also by cIAP-1 and cIAP-2. cIAPs exert their anti-apoptotic effects primarily by binding to and blocking the activity of caspase-3, caspase-7 and caspase-9 (31, 32). The unexpectedly low number of active caspase-3-positive keratinocytes in ERY OL epithelium observed in this study may therefore partly be due to blocking activity by the increased expression of cIAP-1 and cIAP-2.

Active caspase-8 can cleave the pro-apoptotic Bcl-2 family member Bid which induces the mitochondrial cytochrome *c* release into the cytosol. Cytosolic cytochrome *c* forms a complex with Apoptosis protease activating factor-1 (Apaf-1) which induces activation of caspase-9 (33–35). In ERY OL epithelium, we presently observed low activity of caspase-8 (probably due to the high levels of $FLIP_L$) and increased expression of cIAP-1 and cIAP-2 (which inhibits caspase-9 activity) and this therefore indicates that the mitochondrial apoptotic pathway may also be blocked in ERY OL.

Caspase-3 is one of the main effector caspases that is activated during the early stages of apoptosis (36). Double labeling with active caspase-3 and TUNEL performed at our laboratory, showed significant co-localized staining of scattered cells in ERY OL epithelium (data not presented). This, and in accordance with our previous report showing that most of the TUNEL-positive cells in ERY OL epithelium are infiltrating lymphocytes (16), indicates that the caspase-3-positive cells presently observed infiltrating the ERY epithelium in this study are lymphocytes. This means that in ERY OL, there are only a few epithelial cells that display active caspase-3, a pattern that agrees with a study where active caspase-3 staining in NOM was compared with oral squamous carcinoma; the authors also found only scanty staining for active caspase-3 in basal keratinocytes of NOM (37).

In conclusion, this study shows that both cell death and survival signalling are initiated in keratinocytes of ERY OL epithelium, but that upregulation of survival factors, solitarily or in combination, may limit or block rampant pathological cell death. This response is, however, probably not a part of the pathogenesis of OLP but rather a physiological response to cell damage. The action of survival factors can allow basal keratinocytes to progress to a certain extent into their differentiation and this may partly explain why there remains a covering (albeit atrophic) epithelium in ERY OL lesions.

References

1. Thorn JJ, Holmstrup P, Rindum J, Pindborg JJ. Course of various clinical forms of oral lichen planus. A prospective follow-up study of 611 patients. *J Oral Pathol* 1988; **17**: 213–8.
2. Silverman S Jr, Gorsky M, Lozada-Nur F, Giannotti K. A prospective study of findings and management in 214 patients with oral lichen planus. *Oral Surg Oral Med Oral Pathol* 1991; **72**: 665–70.
3. Hedberg N, Ng A, Hunter N. A semi-quantitative assessment of the histopathology of oral lichen planus. *J Oral Pathol* 1986; **15**: 268–72.
4. Schiodt M. Oral discoid lupus erythematosus: III. A histopathologic study of sixty-six patients. *Oral Surg Oral Med Oral Pathol* 1984; **57**: 281–93.
5. Kim SG, Chae CH, Cho BO, et al. Apoptosis of oral epithelial cells in oral lichen planus caused by upregulation of BMP-4. *J Oral Pathol Med* 2006; **35**: 37–45.
6. Santoro A, Majorana A, Bardellini E, et al. Cytotoxic molecule expression and epithelial cell apoptosis in oral and cutaneous lichen planus. *Am J Clin Pathol* 2004; **121**: 758–64.
7. Tobon-Arroyave SI, Villegas-Acosta FA, Ruiz-Restrepo SM, Vieco-Duran B, Restrepo-Misas M, Londono-Lopez ML. Expression of caspase-3 and structural changes associated with apoptotic cell death of keratinocytes in oral lichen planus. *Oral Dis* 2004; **10**: 173–8.
8. Khan A, Farah CS, Savage NW, Walsh LJ, Harbrow DJ, Sugerman PB. Th1 cytokines in oral lichen planus. *J Oral Pathol Med* 2003; **32**: 77–83.
9. Yamamoto T, Osaki T. Characteristic cytokines generated by keratinocytes and mononuclear infiltrates in oral lichen planus. *J Invest Dermatol* 1995; **104**: 784–8.
10. Neppelberg E, Johannessen AC, Jonsson R. Apoptosis in oral lichen planus. *Eur J Oral Sci* 2001; **109**: 361–4.
11. Sklavounou A, Chrysomali E, Scorilas A, Karameris A. TNF-alpha expression and apoptosis-regulating proteins in oral lichen planus: a comparative immunohistochemical evaluation. *J Oral Pathol Med* 2000; **29**: 370–5.
12. Micheau O, Lens S, Gaide O, Alevizopoulos K, Tschopp J. NF-kappaB signals induce the expression of c-FLIP. *Mol Cell Biol* 2001; **21**: 5299–305.

13. Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin AS Jr. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* 1998; **281**: 1680–3.
14. Lippens S, Denecker G, Ovaere P, Vandenabeele P, Declercq W. Death penalty for keratinocytes: apoptosis vs. cornification. *Cell Death Differ* 2005; **12** (Suppl. 2): 1497–508.
15. Karatsaidis A, Schreurs O, Helgeland K, Axell T, Schenck K. Erythematous and reticular forms of oral lichen planus and oral lichenoid reactions differ in pathological features related to disease activity. *J Oral Pathol Med* 2003; **32**: 275–81.
16. Karatsaidis A, Schreurs O, Axell T, Helgeland K, Schenck K. Identity of TUNEL-positive cells in the oral buccal epithelium of normal mucosa and lichen lesions. *J Oral Pathol Med* 2004; **33**: 264–8.
17. Muppidi JR, Tschopp J, Siegel RM. Life and death decisions: secondary complexes and lipid rafts in TNF receptor family signal transduction. *Immunity* 2004; **21**: 461–5.
18. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* 2003; **114**: 181–90.
19. Poyet JL, Srinivasula SM, Alnemri ES. vCLAP, a caspase-recruitment domain-containing protein of equine Herpesvirus-2, persistently activates the Ikappa B kinases through oligomerization of IKKgamma. *J Biol Chem* 2001; **276**: 3183–7.
20. Hsu H, Huang J, Shu HB, Baichwal V, Goeddel DV. TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. *Immunity* 1996; **4**: 387–96.
21. Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* 1996; **84**: 299–308.
22. Moynagh PN. The NF-kappaB pathway. *J Cell Sci* 2005; **118**: 4589–92.
23. Krueger A, Schmitz I, Baumann S, Krammer PH, Kirchhoff S. Cellular FLICE-inhibitory protein splice variants inhibit different steps of caspase-8 activation at the CD95 death-inducing signaling complex. *J Biol Chem* 2001; **276**: 20633–40.
24. Irmler M, Thome M, Hahne M, et al. Inhibition of death receptor signals by cellular FLIP. *Nature* 1997; **388**: 190–5.
25. Janes KA, Gaudet S, Albeck JG, Nielsen UB, Lauffenburger DA, Sorger PK. The response of human epithelial cells to TNF involves an inducible autocrine cascade. *Cell* 2006; **124**: 1225–39.
26. Wehkamp K, Schwichtenberg L, Schroder JM, Harder J. *Pseudomonas aeruginosa*- and IL-1beta-mediated induction of human beta-defensin-2 in keratinocytes is controlled by NF-kappaB and AP-1. *J Invest Dermatol* 2006; **126**: 121–7.
27. Rhodus NL, Cheng B, Myers S, Bowles W, Ho V, Ondrey F. A comparison of the pro-inflammatory, NF-kappaB-dependent cytokines: TNF-alpha, IL-1-alpha, IL-6, and IL-8 in different oral fluids from oral lichen planus patients. *Clin Immunol* 2005; **114**: 278–83.
28. Wang Y, Kim PK, Peng X, et al. Cyclic AMP and Cyclic GMP suppress TNFalpha-induced hepatocyte apoptosis by inhibiting FADD up-regulation via a protein kinase A-dependent pathway. *Apoptosis* 2006; **11**: 441–51.
29. Kim PK, Wang Y, Gambotto A, et al. Hepatocyte Fas-associating death domain protein/mediator of receptor-induced toxicity (FADD/MORT1) levels increase in response to pro-apoptotic stimuli. *J Biol Chem* 2002; **277**: 38855–62.
30. Screaton RA, Kiessling S, Sansom OJ, et al. Fas-associated death domain protein interacts with methyl-CpG binding domain protein 4: a potential link between genome surveillance and apoptosis. *Proc Natl Acad Sci U S A* 2003; **100**: 5211–6.
31. Roy N, Deveraux QL, Takahashi R, Salvesen GS, Reed JC. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO J* 1997; **16**: 6914–25.
32. Deveraux QL, Roy N, Stennicke HR, et al. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J* 1998; **17**: 2215–23.
33. Adrain C, Martin SJ. The mitochondrial apoptosome: a killer unleashed by the cytochrome seas. *Trends Biochem Sci* 2001; **26**: 390–7.
34. Zou H, Li Y, Liu X, Wang X. An APAF-1.cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. *J Biol Chem* 1999; **274**: 11549–56.
35. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* 1998; **94**: 481–90.
36. Leist M, Jaattela M. Four deaths and a funeral: from caspases to alternative mechanisms. *Nat Rev Mol Cell Biol* 2001; **2**: 589–98.
37. Hague A, Eveson JW, MacFarlane M, Huntley S, Janghra N, Thavaraj S. Caspase-3 expression is reduced, in the absence of cleavage, in terminally differentiated normal oral epithelium but is increased in oral squamous cell carcinomas and correlates with tumour stage. *J Pathol* 2004; **204**: 175–82.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.