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Short Communication

Evidence of COX-1 and COX-2 expression in Kaposi's sarcoma tissues

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ABSTRACT

Cyclooxygenases (COXs) are enzymes catalysing prostaglandin synthesis and are implicated in the carcinogenesis of some cancer types. In addition, an important role of these enzymes in herpesvirus infections was demonstrated and it has recently been proposed that COX-2 may participate in herpesvirus-induced neoplasia such as Kaposi's sarcoma (KS). To date no immunohistochemical study has been performed to determine the identification of COX-1 and COX-2 in KS. We have investigated 35 cases of classic KS and 27 cases of epidemic KS form in order to study the distribution and localisation of COXs. We have examined by immunohistochemistry the expression of COX-1 and COX-2 in classic and epidemic forms of KS also in relationship to the characteristic morphological phases (patch, plaque and nodular stage) of KS and cell localisation by double immunostaining. Moreover, we have obtained COX-1 and COX-2 expression by Western blot analysis. Our results establish that (a) COX-1 and COX-2 are overexpressed significantly in classic and epidemic KS compared with control skin tissues ($P < 0.01$ and $P > 0.03$, respectively, for COX-1; $P < 0.01$ and $P > 0.03$, respectively, for COX-2); (b) the extent and intensity staining for both COXs were higher in classic than in epidemic form of KS. Our data support the hypothesis that both COXs may be involved in the pathogenesis of KS.

1. Introduction

Kaposi's sarcoma (KS) still remains an enigmatic disease although important advances have recently been made. Herpesvirus type 8 (HHV-8), designed also as KS-associated herpesvirus (KSHV), is involved in the pathogenesis of KS and other lymphoproliferative disorders.[1–6] However, HHV-8 must be considered necessary but not sufficient for the development of KS, which is now considered as a multistep process which includes HHV-8 infection, genetic cofactors, production of several inflammatory cytokines and angiogenic factors.[1–8] From an initial reactive and inflammatory process (macular and plaque lesions of KS), some selective pressure and/or genetic alteration give rise to nodular lesion of KS,

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consisting of neoplastic monoclonal proliferation of spindle cells.[4]

In spite of some clinical and epidemiological differences, the four forms of KS (classic, epidemic, endemic and post-transplant) show a similar histopathologic picture, characterised by proliferation of spindle cells associated with neoangiogenesis.[4,7,8]

The spindle cells have always been regarded as KS tumour cells, but their histogenesis has been a matter of debate for many years. Actually, several immunohistochemical, ultrastructural, molecular and genetic data indicate that these cells are most likely derived from lymphatic endothelial cells.[4,9–12]

HHV-8 displays an essential role in the pathogenesis of KS because it induces several effects in tissues including upregulation of various cellular genes, suppression of expression of other genes, producing inhibition of apoptosis, increased proliferative activity and transcriptional reprogramming of fibroblastic and lymphatic endothelial cells.[2–6,9–11,13]

Moreover, HHV-8 associated tumours and infections either induce or functionally depend on cyclooxygenases (COXs) expression, which regulate angiogenesis, cell survival and proliferative activity in KS and other neoplasms.[14–19] Indeed, some studies have demonstrated overexpression of COX in KSHV-infected dermal endothelial cells.[19]

COXs catalyse the conversion of arachidonic acid, which is derived from membrane phospholipids by phospholipase, into prostaglandins.[20–24] There are two main COX isoforms: COX-1 and COX-2, that share the same substrates, generate similar products and catalyse reactions using identical mechanisms. Nevertheless, COX-2 is significantly more efficient than COX-1 as regards the enzymatic activity. A new splice variant of COX has been described, termed COX-3, which is prevalently expressed in cerebral cortex and heart in humans.[25,26]

Until recently, COX-2 has been regarded as the only inducible form of COX, whereas COX-1 has been assumed as the constitutive form of COX, which is expressed at a relatively constant level in normal tissues.[20–24]

Some investigations have displayed that COX-1 is evenly distributed through normal epidermis in all epithelial cells, but COX-2 is lacking or rarely identifiable in epithelial cells of superficial stratum of epidermis.[27,28]

Therefore, most studies[20–24,29–31] have focused only on COX-2 expression in neoplastic tissues, including premalignant lesions and cancer such as carcinoma of the breast, prostate, skin colorectum, lung, bladder and stomach, whereas a paucity of data[32,33] has reported about the role of COX-1 in carcinogenesis .

These studies are consistent with the fact that COX-2 expression may be the driving force for the development from inflammation to cancer or, more likely, a pathogenic factor in enhancing cancer development in the scenario of chronic inflammation.[32] Moreover, experimental studies[14–18] have shown that an increased expression of COX-2 occurs in herpesvirus associated tumours and infections. As regards KS, COX-2 appears to be an essential factor involved in some pathogenetic mechanisms, including neoangiogenesis, proliferative activity, regulation of expression of integrins, matrix metalloproteinases, p53 and bcl-2.[14–18]

Nevertheless, the simplistic notion that COX-2 expression is the inducible form of COX, and COX-1 is the constitutive form, implicated only in the physiological functions, is probably an overexemeplification of a much more complex regulatory framework.[24]

Indeed, various recent studies[24,33,34] have indicated that also COX-1 can promote tumourigenesis in certain situations, whereas COX-2 may be involved in physiological production of PGs.[35] In addition, other investigations[29,36–39] have demonstrated an overexpression of COX-1 and the occurrence of interaction mechanisms between two COX isoforms in some types of tumours.

To our knowledge, no immunohistochemical data have been reported about the identification of COX-1 and COX-2 in tissues of KS, although a wealth of investigations are reported in the literature about the expression of COXs in several types of cancers. The goal of the study was to assess the distribution and localisation of COX-1 and COX-2 in

Table 1 – COX-1 and COX-2 in normal skin

	Epidermis		Dermis	
	COX-1	COX-2	COX-1	COX-2
+[a]	20	–	–	–
++	5	–	–	–
+++	0	–	–	–
0[b]	–	17	9	11
A	–	8	16	13
B	–	0	0	1
C	–	0	0	0

a Semiquantitative scale from+ (weak staining) to ++ (moderate staining) and +++ (intensive staining).
b Score for percentage of positive cells from 0 (absent staining) to A (from 1 to 10% of positive cells), B (11–30% of positive cells) and C(>31% of positive cells).

Table 2 – COX-1 and COX-2 in KS tissues, classic form

	Epidermis							KS tissue					
	COX-1				COX-2			COX-1			COX-2		
	N	+[a]	++	+++	A[b]	B	C	A	B	C	A	B	C
Patch	5	0	2	3	3	2	0	0	3	2	2	3	0
Plaque	14	0	6	8	7	7	0	0	5	9	6	8	0
Nodular	16	3	7	6	10	3	3	0	7	9	7	9	0

a Semiquantitative scale from+ (weak staining) to ++ (moderate staining) and +++ (intensive staining).
b Score for percentage of positive cells from 0 (absent staining) to A (from 1 to 10% of positive cells), B (11–30% of positive cells) and C(>31% of positive cells).

Table 3 – COX-1 and COX-2 in KS tissues, epidemic form

	Epidermis							KS tissue					
	COX-1				COX-2			COX-1			COX-2		
	N	+[a]	++	+++	A[b]	B	C	A	B	C	A	B	C
Patch	17	1	9	7	9	7	1	3	12	2	14	3	0
Plaque	6	1	2	3	4	2	0	2	3	1	3	3	0
Nodular	4	1	2	1	2	1	1	1	2	1	2	2	0

a Semiquantitative scale from+ (weak staining) to ++ (moderate staining) and +++ (intensive staining).
b Score for percentage of positive cells from 0 (absent staining) to A (from 1% to 10% of positive cells), B (11–30% of positive cells) and C(>31% of positive cells).

classic and epidemic KS tissues in relationship with the stage of the disease.

2. Results

In normal skin, COX-1 immunostaining appeared uniformly distributed throughout the epidermis, showing faint or moderate degree of positivity, while COX-2 was absent or focally expressed in epithelial cells of upper layers (Table 1). In the dermis, a variable percentage of endothelial cells and fibroblasts showed COX-1 staining, whereas COX-2 positive sections were mainly detected in few macrophages, lymphocytes and fibroblasts. With regard to the cases of KS, immunostaining for both COXs in both classical and epidemic

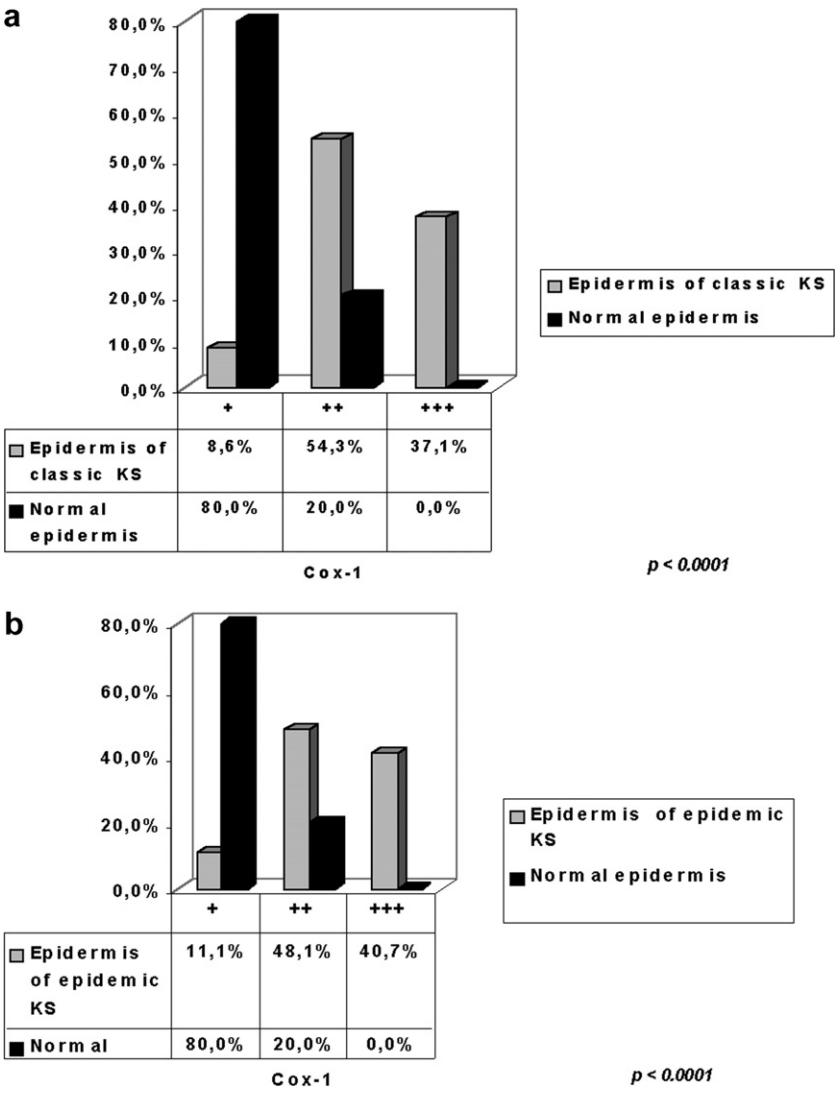

Fig. 1 – Comparison between COX-1 expression in epidermis of normal skin and in epidermis overlying classic KS tissues (a) and epidermis overlying epidemic KS tissues (b).

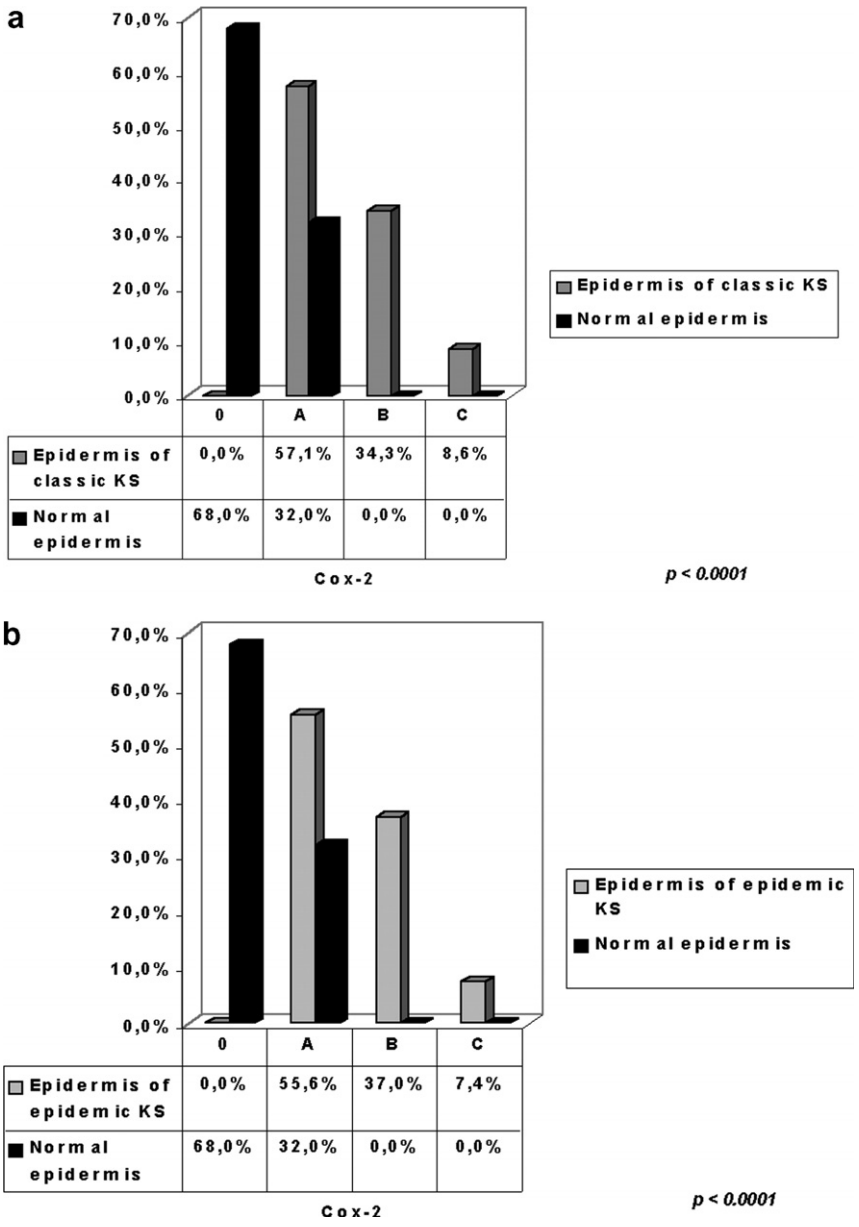

Fig. 2 – Comparison between COX-2 expression in epidermis of normal skin and in epidermis overlying classic KS tissues (a) and epidermis overlying epidemic KS tissues (b).

forms was more strong and diffuse in the epidermis overlying KS tissues as compared to normal skin control sections. COX-1 positivity was uniformly distributed throughout the epidermis, whereas COX-2 immunostaining was mostly detected in the upper layer of the epidermis overlying KS tissue. The staining was prevalently cytoplasmatic, occasionally nuclear and COX-1 generally showed higher intensity than COX-2. Highest expression of COX-2 was detected in the epithelium adjacent to the necrotic areas. Immunoreactivity for COXs in endothelial spindle cells of classic and epidemic KS tissues as well as in epidermis overlying KS lesions is reported in Tables 2 and 3, respectively.

The comparison between COXs expression in epidermis of normal skin and in epidermis overlying KS tissues is reported in Figs. 1 and 2, as percentage of positive cells. The differences in COXs expression between normal epidermis and epidermis overlying KS tissues both in classic form and in epidemic form of KS reached significant levels for COX-1 and for COX-2. Furthermore, no significant differences were found in epidermal expression of both COXs between epidemic form and classic form of KS. In KS tissues, both COXs were expressed in spindle cells as well as in endothelial cells of normal appearing vessels and in the irregular vascular spaces, such as clefts and slits. Nevertheless, the percentage of these positive cells was mostly higher for COX-1 than for COX-2 (Tables 2 and 3).

In addition, COX-2 staining was also frequently detected in lymphocytes and macrophages identifiable in KS tissues and

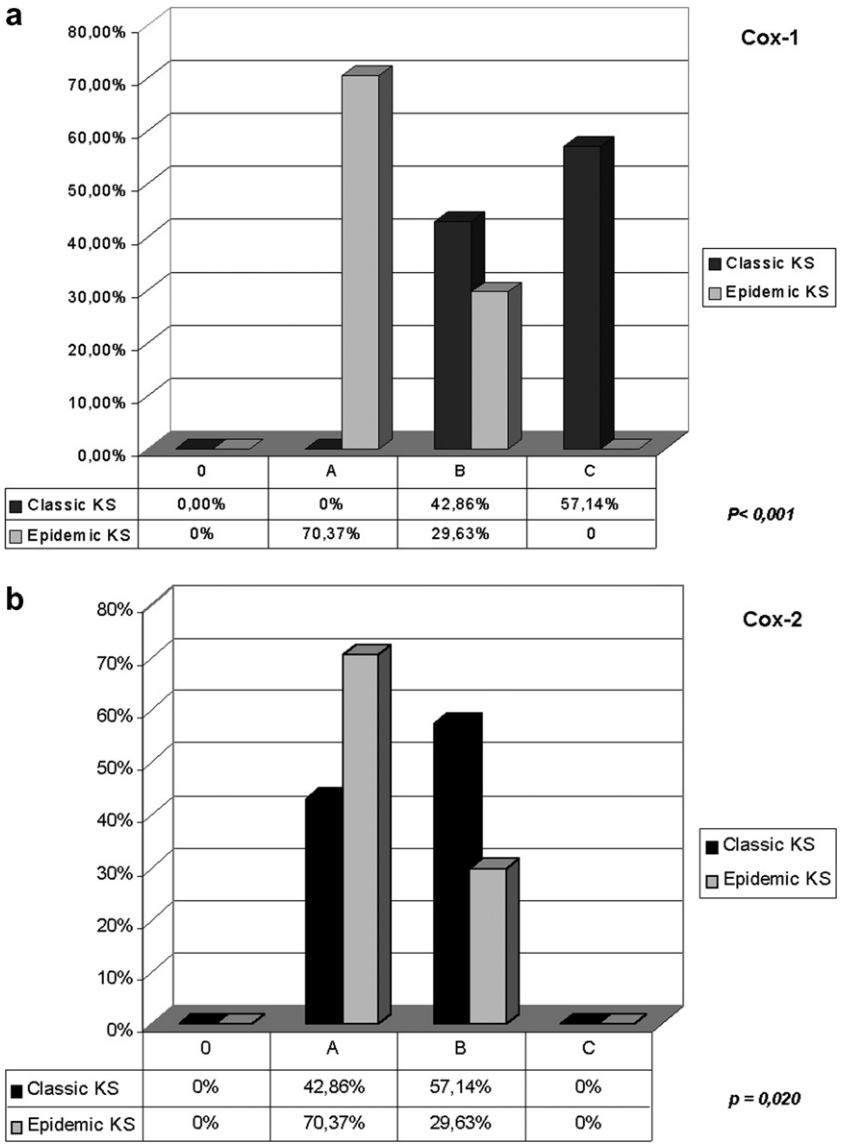

Fig. 3 – Comparison between classic form and epidemic form of KS in relation to expression of COX-1 (a) and COX-2 (b) in endothelial/spindle shaped cells of KS.

in the dermis. Spindle cells showed immunostaining for both COXs, but positivity for COX-1 was more strong and diffuse as compared to that of COX-2.

The extent and intensity of immunostaining for both COXs in endothelial/spindle cells displayed by classic form of KS were higher than those observed in epidemic form of KS, as disclosed by the comparison between Tables 2 and 3 and Fig. 3. Particularly, these differences were statistically significant for COX-1 ($P < 0.001$) and for COX-2 ($P = 0.020$). In contrast, the differences in COXs expression between various stages of KS did not reach significant levels in both classic form and epidemic form of KS. Nevertheless, in patch stage of KS, the dissection of collagen bundles by irregular, ramifying thin-wall vessels was enhanced because of high immunoreactivity for COX-1 expressed by endothelial cells. Figs. 4 and 5 show the typical immunohistochemistry patterns seen in the present study.

In order to address the localisation of COX-1 and COX-2 in the context of other cells, double immunostaining experiments were also performed (Fig. 6). Finally, we performed Western blot analysis in some KS tissues from both classic and epidemic forms (Fig. 7). Data were consistent with overexpression of COX-1 and COX-2 seen in the immunohistochemical analysis.

3. Methods

3.1. *Study population*

Our study includes skin tissue specimens as follows:

(1) 35 cases of classic KS form (5 patch, 14 plaque and 16 nodular stage) obtained from the Department of Pathology 'L. Armanni', Second University of Naples.

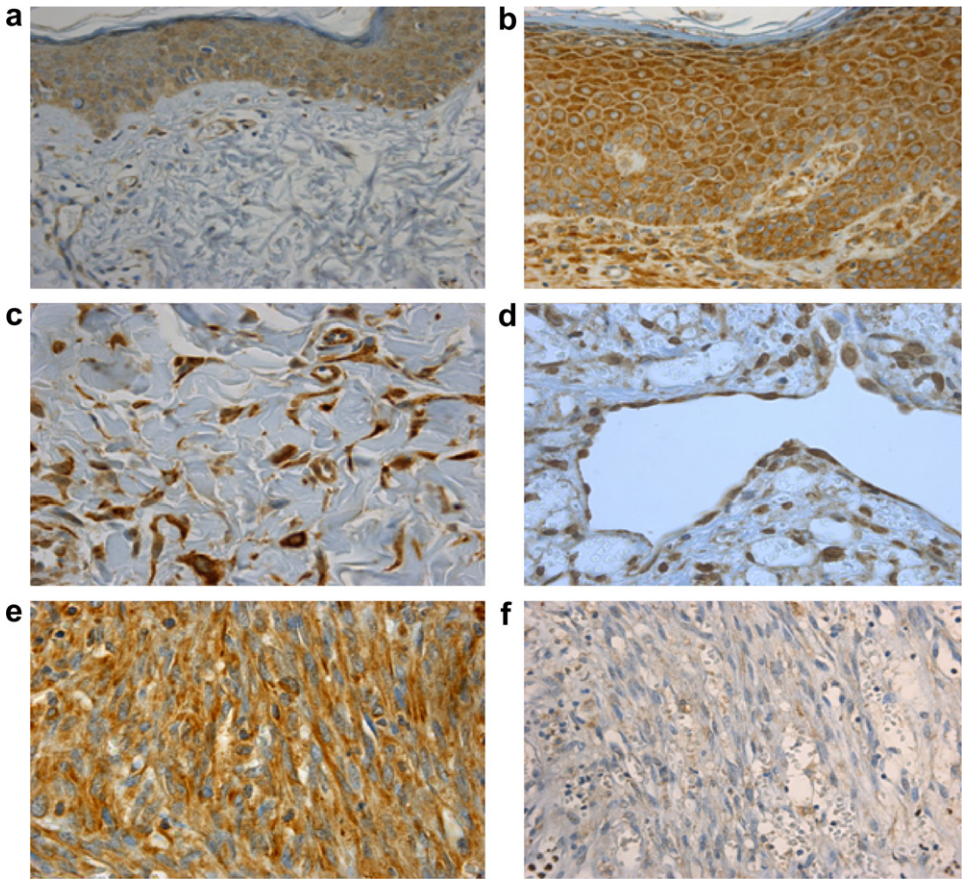

Fig. 4 – COX-1: (a) Epidermis of normal skin showing moderate staining in epithelial cells of all layer (100×). (b) Epidermis overlying tissues of classis KS, patch stage, displaying intensive and extent staining (250×). (c) Dissection of collagen bundles by irregular, ramifying thin-wall vessels enhanced by high immunoreactivity for COX-1 expressed by endothelial cells (250×). (d) 'Promontory sign' in early classic KS characterised by intense staining of endothelial cells (400×). (e) Intense and diffuse positivities in spindle cells in nodular stage of classic KS (250×). (f) Faint and focal staining of spindle cells in nodular stage of epidemic KS (250×).

(2) 27 cases of epidemic KS form (16 patch, 6 plaque and 4 nodular stage) obtained from Hospital 'D. Cotugno'.
(3) 25 cases of normal skin (from Plastic Surgery Units, ASL Napoli 1) used as controls.

All tissue specimens were formalin fixed and embedded in paraffin using routine histological procedure. 5 μm thick sections were cut from each specimen. Slides were deparaffinised and then rehydrated in graded alcohols according to the usual protocol.

3.2. *Immunohistochemistry and Western blot analysis*

Immunostaining and double-labelling technique were performed as described previously.[40,41] The following primary antibodies were used for immunohistochemistry: monoclonal mouse antibodies for CD-34 and CD-31 (Immunomarkers-Diapath, Martinengo, Italy), monoclonal mouse antibodies for COX-1 and COX-2 (Novocastra, Newcastle, United Kingdom). The sections were immunostained with streptavidin–biotin system using automated slide stainer (Benchmark, Ventana Medical System, Tucson, USA). Omission and inversion of primary antisera were also employed as negative controls. For each section, the staining pattern of COX-1 and COX-2 was scored in a blinded way for percentage of positive cells (A = <10% of positive cells ; B = from 11% to 30%; C = >31% of cells). Only for the COX-1 staining in the epidermis overlying KS tissue was evaluated the intensity of staining pattern, because COX-1 is generally expressed in almost all epithelial cells of all skin specimens. For this purpose, a semiquantitative scale was used: + = weak intensity; ++ = moderate intensity; +++ strong staining. γ-Tubulin normalised Western blot analysis was performed by using monoclonal antibodies for COX-1 and COX-2 (Santa Cruz, CA) as previously described in detail.[40]

3.3. *Data analysis*

Three observers (RR, RL and PM) estimated separately staining pattern and percentages of positive cells of each section. The level of concordance, expressed as percentage of agreement between observers, was 89% (128 of 144 specimens). All specimens with discordant score were re-evaluated by investigators using a multiheaded microscope and the consensus was reached after collegial revision. Descriptive statistics were used to summarise morphologic evaluation of

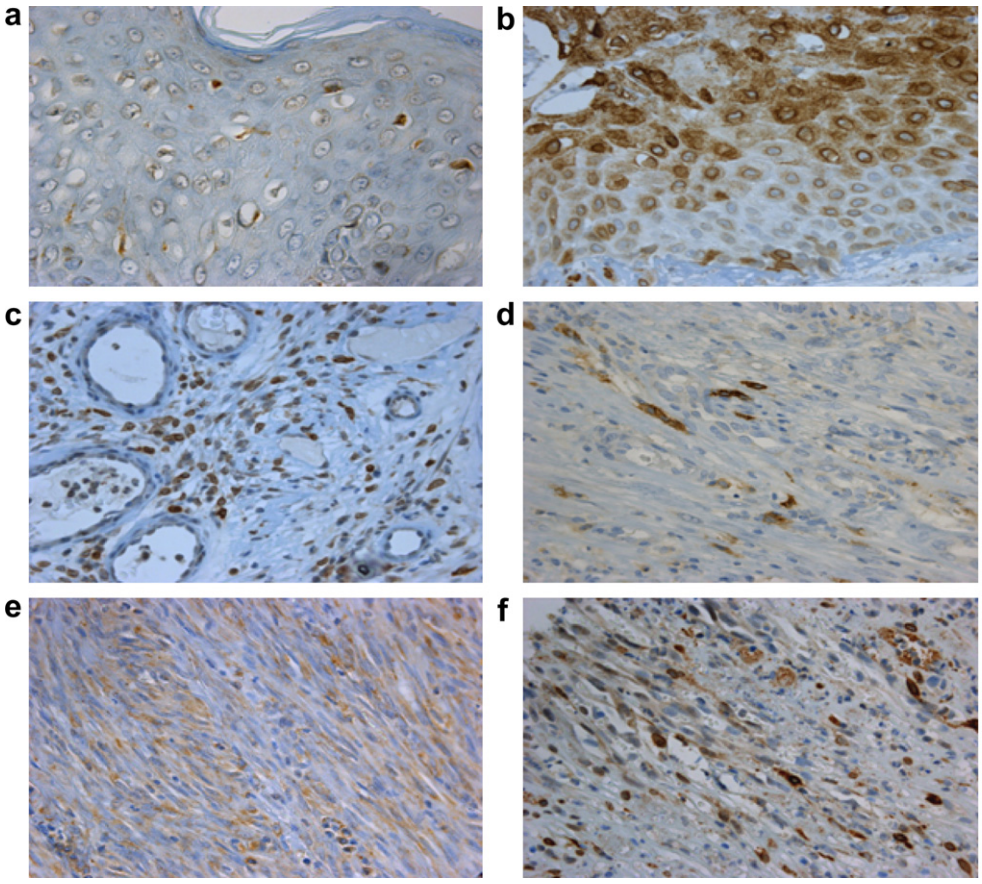

Fig. 5 – COX-2: (a) Few and weak positivities in some epithelial cells of superficial layer of epidermis of normal skin (100×). (b) Intensive staining mostly identifiable in epithelial cells of superficial layer of epidermis overlying early KS tissue (250×). (c) Positivities in macrophages and lymphocytes intermingled between lymphatic vessels in early classic KS (250×). (d) Focal positivities in spindle cells of epidemic KS (250×). (e) (400×) Moderate staining of spindle cells in nodular stage of classic KS (250×). (f) Moderate staining of spindle cells and lymphocytes in nodular stage of classic KS (100×).

immunohistochemical data. Comparisons between the groups were made by the Fisher exact test and Pearson χ^2 when appropriate, P value <0.05 was considered statistically significant.

4. Discussion

The major finding of the present study is that COX-2 expression is lacking or very low and restricted to only a few keratinocytes of the superficial layer of epidermis and dermal macrophages, lymphocytes and fibroblasts in normal skin. In parallel, COX-1 immunostaining may be detected in all cell layers of the epidermis as well as in fibroblasts and endothelial cells of dermal vessels. Our findings are in line with those reported by some investigators[27,28,31,42,43] that have found a similar distribution of COXs in normal skin tissue. Here, COX-1 and COX-2 immunostaining of the epidermis overlying KS tissues reveals higher intensity and extent than those displayed by normal skin control sections. This difference appears to be statistically significant for both COX-1 and COX-2. However, these measures might have a limited value because they are related to a comparison between two groups of non-homogeneous skin specimens. These findings allow to point out that epidermal cells also share in the development of KS lesions.

Interactions between epidermis and dermal tissues play an important role in regulating homeostasis , growth and differentiation of epithelial and mesenchymal tissues.[43] Other than COXs, keratinocytes synthesise and release a broad variety of factors, cytokines and chemokines modulating in a paracrine and in an autocrine manner the activity of other epithelial cells as well as of dermal fibroblasts, endothelial cells and macrophages.[43–46]

These data strongly suggest that the overlying epidermis is not just a protective barrier, but that epidermal cells interact closely with underlying dermis. In view of these data, the overexpression of both COXs at epidermal level might be related to the involvement of keratinocytes in the development of dermal KS lesions. In KS tissues, positivities for both COXs are identifiable in spindle cells and endothelial cells , but COX-2 immunostaining is also detected in macrophages and lymphocytes . These findings are consistent with other studies[47–49] that shows expression of COX-2 in lymphocytes, macrophages and dendritic cells.

Our investigation also displays that overexpression of both COXs is detected in all stages of KS: from early inflammatory/angiogenic lesion to nodular stage, characterised by neoplastic monoclonal proliferation of spindle cells. The comparison between various stages of KS shows no significant differ-

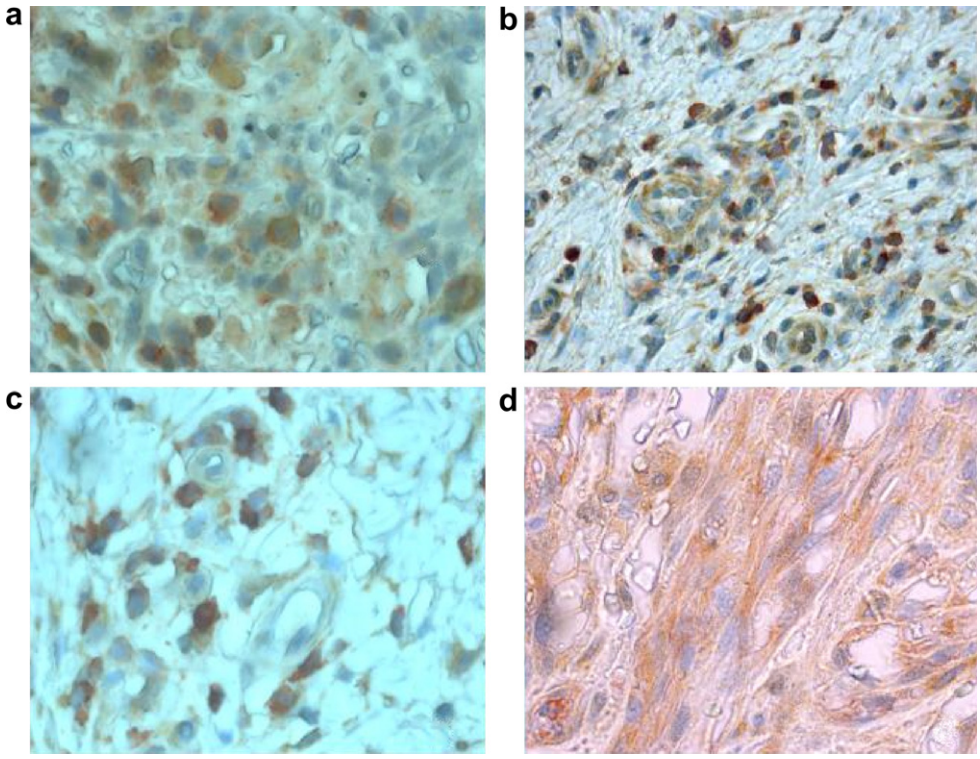

Fig. 6 – Double immunostaining using antibodies against CD 68, CD 45 (leucocyte common antigen) or CD 31 (chromogen AEC, red) and against COXs (chromogen DAB). (a) Double immunostaining for CD 68 (red) and COX-2 (brown) in macrophages (400×). (b) Double immunostaining for CD 45 (red) and COX-2 (brown) in leucocytes (250×). (c) Higher magnification of the previous panel b showing co-expression of red and brown chromogens in lymphocytes (400×). (d) Double immunostaining for CD 31 (AEC) and COX-1 (DAB) in spindle shaped cells (630×).

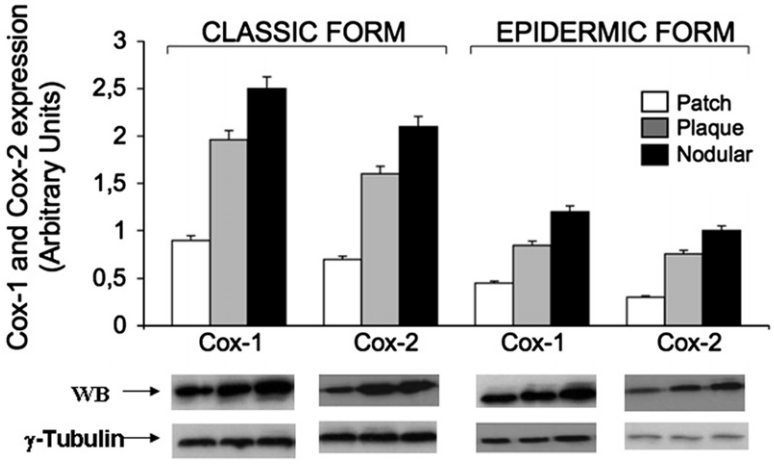

Fig. 7 – COX-1 and COX-2 expression in KS classic and epidemic forms detected by Western blots (data are the mean ± SE of densitometric analysis of samples, in Arbitrary Units). Below are representative experiments normalised for γ-tubulin.

ences. This overexpression suggests a possible role for both COX s in the pathogenesis of KS from onset to tumoural progression. Our findings show also a remarkable overexpression of COX-1 higher than that of COX-2 both in KS tissues and in the overlying epidermis . These results oppose a simplified view that COX-1 is constitutively expressed in normal tissues, whereas COX-2 is the inducible isoform, expressed only in pathological condition . COX-2 is responsible for physiological prostaglandins production in some human normal tissues[24] and COX-1 has recently been involved in the carcinogenesis of various types of tumours. Indeed, several studies[30,33,36–38] show that overexpression of COX-1 may occur independently or in combination with up-regulation of COX-2 in some type of tumour such as carcinoma of the lung, bladder, oesophagus, and cervix. Experimental investigations[29,34,39] have demonstrated upregulated and inducible expression of COX-1 in different biological models. Therefore, Hull[24] has stated recently that it is an 'old dogma' that COX-1 is involved

in physiological activities, whereas COX-2 is induced in pathological conditions.

Particularly, overexpression of COX-1 seems to enhance synthesis of prostanoids that may thus act in autocrine–paracrine manner to upregulate the expression of COX-2 and enhance the expression of vascular endothelial growth factor and fibroblast growth factor.[38] This mechanism led to recruitment of new vessels and to increase vascular sprouting and branching. Other investigations[33,50] have demonstrated that upregulation of both COXs isoforms is linked to overexpression of VEGF-C, an important modulator of lymphoangiogenesis. It is well known that several data[4,14,51] lead to support a lymphatic origin for KS tumour cells.

These data may support the hypothesis that both COXs are involved in the pathogenesis of KS. With regard to the comparison between two forms of KS in relation to the expression of COXs, we found a significant difference because COX-1 and COX-2 staining appeared less intensive and extended in the epidemic form as compared to the classic form in KS tissues ($P < 0.001$ for COX-1 and $P = 0.020$ for COX-2). In contrast, the comparison between classic and epidemic forms does not reach a statistically a significant level as regards the expression of both COXs in epidermis overlying KS tissues of both forms.

This difference in immunoreactivity in endothelial–spindle cells may be due in part to the use of antiretroviral therapy in HIV seropositive individuals that has dramatically changed the natural history of HIV/KS ,because it exhibits a less aggressive clinical pattern.[52] This therapeutic treatment induces several effects including antiangiogenic action, decrease in the concentration of some viral proteins, inhibition of HHV-8 DNA production and a decrease in peripheral blood of HHV-8 viral load.[53] These mechanisms might explain the reduction of COXs expression in KS tissues of epidemic form, because it is also well known that HHV-8 induces an increase in the expression of COXs to enhance and to establish efficient infections.[14]

Moreover, COXs expression may be induced or increased by a wide range of stimuli such as cytokines, chemokines and various factors produced by T-lymphocytes.[20,32,46,54] In addition, dendritic cells and activated T-lymphocytes may express COX-2.[48,54] Depletion of T-helper cells and dendritic cells occurring in AIDS patients might also inhibit the production of COXs. Further studies are needed to unravel the implications of both COXs in the pathogenic mechanisms related to the development of KS as well as the possible role of inhibitors of COXs in the treatment and prevention of KS.

In conclusion, our findings reported herein lead to hypothesise that COXs, particularly COX-1, are implicated in the pathogenesis of KS from onset to tumour progression.

REFERENCES

1. Wilkins K, Turner R, Doley JC, Le Bouit PE, Berger TG, Maurer TA. Cutaneous malignancy and human immunodeficiency virus disease. *J Am Acad Dermatol* 2006;**54**:189–206.
2. Schulz TF. The pleiotropic effects of Kaposi's sarcoma herpesvirus. *J Pathol* 2006;**208**:187–98.
3. Xie J, Pan H, Yoo S, Gao SJ. Kaposi's sarcoma associated herpesvirus induction of AP-1 and interleukin 6 during primary infection mediated by multiple mitogen-activated protein kinase pathways. *J Virol* 2005;**79**:15027–37.
4. Gessain A, Duprez R. Spindle cells and their role in Kaposi's sarcoma. *Intern J Biochem Cell Biol* 2005;**37**:2457–65.
5. An J, Lichtenstein AK, Brent G, Rettig MB. The Kaposi sarcoma-associated herpesvirus (KSHV) induces cellular interleukin 6 expression: role of the KSHV latency-associated nuclear antigen and AP1 response element. *Blood* 2002;**99**:649–54.
6. Wang HW, Boshoff C. Linking Kaposi virus to cancer associated cytokines. *Trends Mol Med* 2005;**11**:309–13.
7. Schwartz RA. Kaposi's sarcoma: an update. *J Surg Oncol* 2003;**87**:146–51.
8. Buonaguro FM, Tornesello ML, Buonaguro L, Ruocco E, Castello G, Ruocco V. Kaposi's sarcoma: aetiopathogenesis, histology and clinical features. *J Europ Acad Dermatol Venerol* 2003;**17**:138–54.
9. Weninger W, Partanen TA, Breiteneder-Geleff S, Mayer C, Kowalski H, et al. Expression of vascular endothelial growth factor receptor-3 and podoplanin suggests a lymphatic endothelial cell origin of Kaposi's sarcoma tumor. *Lab Invest* 1999;**79**:243–51.
10. Wang HW, Trotter MW, Lagos D, et al. Kaposi's sarcoma herpesvirus-induced cellular reprogramming contributes to the lymphatic endothelial gene expression in Kaposi's sarcoma. *Nat Genet* 2004;**36**:687–93.
11. Hong YK, Foreman K, Shin JW, et al. Lymphatic reprogramming of blood vascular endothelium by Kaposi's sarcoma-associated herpesvirus. *Nat Genet* 2004;**36**:683–5.
12. Carroll PA, Brazeau E, Lagunoff M. Kaposi's sarcoma-associated herpesvirus infection of blood endothelial cells induces lymphatic differentiation. *Virology* 2004;**328**:7–18.
13. An J, Lichtenstein AK, Brent G, Rettig MB. The Kaposi sarcoma-associated herpesvirus (KSHV) induces cellular interleukin 6 expression: role of the KSHV latency-associated nuclear antigen and AP1 response element. *Blood* 2002;**99**:649–54.
14. Shelby BD, Nelson A, Morris C. γ-herpesvirus neoplasia: a growing role COX-2. *Microsc Res Technol* 2005;**68**:120–9.
15. Steer SA, Corbett JA. The role and regulation of COX-2 during viral infection. *Viral Immunol* 2003;**16**:447–60.
16. Stine JT, Wood C, Hill M, et al. KSHV-encoded CC chemokine vMIP-III is a CCR4 agonist, stimulates angiogenesis, and selectively chemoattracts TH2 cells. *Blood* 2000;**95**:1151–7.
17. Poole LJ, Yu Y, Kim PS, Zheng QZ, Pevsner J, Hayward GS. Altered patterns of cellular gene expression in dermal microvasculature endothelial cells infected with Kaposi's sarcoma-associated herpesvirus. *J Virol* 2002;**76**:3395–420.
18. Janelle ME, Gravel A, Gosselin J, Tremblay MJ, Flamand L. Activation of cyclooxygenase-2 gene expression by human herpesvirus: role for cyclic AMP-responsive element-binding protein and activator-protein 1. *J Biol Chem* 2002;**277**:30665–74.
19. Naranatt PP, Krishnan HH, Svojanovsky SR, Bloomer C, Mathur S, Chandran B. Hose gene induction and transcriptional reprogramming in Kaposi's sarcoma associated herpervirus (KSHV/HHV-8) infected endothelial, fibroblast and B cells: insight into modulation events early during infection. *Cancer Res* 2004;**64**:72–84.
20. Rouzer CA, Marnett LJ. Structural and functional differences between cyclooxygenase: fatty acid oxygenase with a critical role in cell signalling. *Biochem Biophys Res Commun* 2005;**338**:34–44.
21. Warner TD, Mitchell JA. Cyclooxygenase: new forms, new inhibitors, and lessons from the clinic. *FASEB J* 2004;**18**:790–804.

22. Helliwell RJA, Adams FA, Mitchell MD. Prostaglandins synthase: recent development and a novel hypothesis. *Prostglandins Leucot Essent Fatty Acids* 2004;**70**:101–13.
23. Zha S, Yegnasubramanian V, Nelson WG, Isaacs W, De Marzo AM. Cyclooxygenase in cancer: progress and prospective. *Cancer Lett* 2004;**215**:1–20.
24. Hull MA. Cyclooxygenase-2: how good is it as a target for cancer chemoprevention? *Eur J Cancer* 2005;**41**:1854–63.
25. Chandrasekharam NV, Dai H, Roos KL, et al. COX-3 cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci USA* 2002;**99**:13926–31.
26. Dinchuk JE, Liu RQ, Trzaskos JM. COX-3: in the wrong frame in mind. *Immunol Lett* 2003;**86**:121.
27. Kabashima K, Miyachi Y. Prostanoid in the cutaneous immune response. *J Dermatol Sci* 2004;**34**:177–84.
28. Leong J, Hughes-Fulford M, Rakhlin N, Habib A, Maclouf J, Goldyne ME. Cyclooxygenase in human mouse skin and cultured human keratinocytes: association of COX-2 expression with human keratinocyte differentiation. *Exp Cell Res* 1996;**224**:78–97.
29. Bauer A, Dwyer-Nield L, Malkinson AM. High cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) contents in mouse lung tumors. *Carcinogenesis* 2000;**4**:543–50.
30. Mohammed SI, Dhawan D, Abraham S, Lu M, Stewart J, Knapp DK. Cyclooxygenase inhibitors in urinary bladder cancer: in vitro and in vivo effects. *Mol Cancer Ther* 2006;**5**:329–36.
31. Kagoura M, Toyoda M, Matsui C, Morohashi M. Immunohistochemical expression of cyclooxygenase-2 in skin cancer. *J Cutan Pathol* 2001;**28**:298–303.
32. Lu H, Ouyang W, Huang C. Inflammation, a key event in cancer development. *Mol Cancer Res* 2006;**4**:221–33.
33. Sales KJ, Katz AA, Howard B, Soeters RP, Millar RP, Jabbour HN. Cyclooxygenase 1 is up-regulated in cervical carcinomas: autocrine/paracrine regulation of cyclooxygenase-2, prostaglandin E receptors and angiogenic factors by Cyclooxygenase-1. *Cancer Res* 2002;**62**:424–32.
34. Kitamura T, Itoh M, Matsuura M, Wakabayashi K. Combined effects of cyclooxygenase-1 and cyclooxygenase-2 selective inhibitors on intestinal tumorigenesis in adenomatous polyposis coli gene knockout mice. *Int J Cancer* 2004;**109**:576–80.
35. Fitzgerald GA. COX-2 and beyond: approaches to prostaglandin inhibition in human disease. *Nav Rev Drug Discov* 2003;**2**:879–90.
36. Hasturk S, Kemp B, Kalapurakal SK, Kurie JM, Hong WK, Lee JS. Expression of cyclooxygenase-1 and cyclooxygenase-2 in bronchial epithelium and non small cell lung carcinoma. *Cancer* 2002;**94**:1023–31.
37. Mohammed SI, Dhawan D, Abraham S, Lu M, Stewart J, Knapp DK. Cyclooxygenase inhibitors in urinary bladder cancer: in vitro and in vivo effects. *Mol Cancer Ther* 2006;**5**:329–36.
38. Burkhard HA, von Rahden BHA, Stein H, et al. Coexpression of cyclooxygenases (COX-1, COX-2) and vascular endothelial growth factors (VEGF-A, VEGF-C) in esophageal adenocarcinoma. *Cancer Res* 2005;**65**:5038–44.
39. Chulada PC, Thompson MB, Doyle CM, et al. Genetic disruption of Ptgs-1, as well as of Ptgs-2, reduces intestinal tumorigenesis in Min mice. *Cancer Res* 2000;**60**:4705–8.
40. D'Armiento FP, Bianchi A, de Nigris F, et al. Age-related effects on atherogenesis and scavenger enzymes of intracranial and extracranial arteries in men without classic risk factors for atherosclerosis. *Stroke* 2001;**32**:2472–9.
41. Condorelli G, Aycock JK, Frati G, Napoli C. Mutated p21/WAF/CIP transgene overexpression reduces smoth muscle cell proliferation, macrophage deposition, oxidation-sensitive mechanisms, and restenosis in hypercholesterolemic apolipoprotein E knockout mice. *FASEB J* 2001;**15**:2162–70.
42. Akita Y, Kozaki K, Nakagawa A, et al. Cyclooxygenase-2 is a possible target of treatment approach in conjunction with photodynamic therapy for various disorders in skin and oral cavity. *Br J Dermatol* 2004;**151**:472–80.
43. Funayama E, Chodon T, Oyama A, Sugihara T. Keratinocytes promote proliferation and inhibit apoptosis of the underlying fibroblasts: an important role in the pathogenesis of keloid. *J Invest Dermatol* 2003;**121**:1326–31.
44. Bamba H, Ota S, Kato A, Itoyama S, Matsuzaki F. High expression of cyclooxygenase-2 in macrophages of human colonic adenoma. *Int J Cancer* 1999;**83**:470–5.
45. Bellemare J, Roberge CJ, Bergeron D, Lopez-Vallè CA, Roy M. Epidermis promotes dermal fibrosis: role in the pathogenesis of hypertrophic scars. *J Pathol* 2005;**206**:1–8.
46. Karrer S, Bossheroff AK, Weiderer P, Landthaler M. Keratinocyte-derived cytokines after photodynamic therapy and their paracrine induction of matrix metalloproteinases in fibroblasts. *Br J Dermatol* 2004;**151**:776–83.
47. Fogel-Petrovic M, Long JA, Knight DA, Thompson PJ, Upham JW. Activated dendritic cells express inducible cyclooxygenase and synthesize prostaglandin E2 but not prostaglandin D2. *J Cell Biol* 2004;**82**:47–54.
48. Iniguez MA, Punzon C, Fresno M. Induction of cyclooxygenase-2 on activated T-lymphocytes: regulation of T-cell activation by cyclooxygenase-2 inhibitors. *J Immunol* 1999;**163**:111–9.
49. Ryan EP, Pollack SJ, Murant TI, Bernstein SH, Felgar RE, Phipps RP. Activated human B-lymphocytes express cyclooxygenase-2 and cyclooxygenase inhibitors attenuate antibody production. *J Immunol* 2005;**174**:2619–26.
50. Su JL, Shih JY, Yen ML, Jeng YM, Chang CC, et al. Cyclooxygenase-2 induces EP1- and HER-2/Neu-dependent vascular endothelial growth factor C up-regulation : a novel mechanisms of lymphoangiogenis in lung adenocarcinoma. *Cancer Res* 2004;**64**:554–64.
51. Hong YK, Lacoste V, Briere J, et al. Lymphatic reprogramming of blood vascular endothelim by Kaposi's sarcoma. *Nat Genet* 2004;**36**:683–5.
52. Stebbing J, Sanitt A, Nelson M, Powles T, Gazzard B, Bower M. A prognostic index for AIDS-associated Kaposi.s sarcoma in the era of highly active retroviral therapy. *Lancet* 2006;**367**:1495–502.
53. Casper C. Defining a role for antiviral drugs in the treatment of persons with HHV-8 infection. *Herpes* 2006;**13**:42–7.
54. Fogel-Petrovic M, Long JA, Knight DA, Thompson PJ, Upham JW. Activated human dendritic cells express inducible cyclo-oxygenase and synthesize prostaglandin E2 but not prostaglandin D2. *Immunol Cell Biol* 2004;**82**:47–52.