Histol Histopathol (2011) 26: 997-1006

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Myeloid CD11c$^+$ S100$^+$ dendritic cells express indoleamine 2,3-dioxygenase at the inflammatory border to invasive lower lip squamous cell carcinoma

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Summary. The prevalence of squamous cell carcinoma of the lower lip (SCC-LL) is increasing worlwide. The expression of the enzyme indoleamine 2,3-dioxygenase (IDO) by antigen-presenting cells and/or tumor cells leads to tumor escape by inhibiting T cell-mediated rejection responses. The aim of this study was to determine the expression of IDO in SCC-LL. IDO-expression was analyzed in 47 SCC-LL, together with the expression of markers of T-cells (CD3), myeloid DCs (S100, CD11c), macrophages (CD68, CD11c), Langerhans cells (CD1a, Langerin (CD207)), plasmacytoid DCs (CD123), and regulatory T cells (Foxp3) by immunohistochemistry and immunofluorescence analysis. Twelve specimens out of 47 LL-SCCs contained cells that expressed IDO. IDO-positivity was strongly associated with the intensity of the cancer-associated infiltrate (P=0.0007). IDO-positive cells are located right along the border between the developing tumor and the inflammatory infiltrate. Immunofluorescence stainings showed that CD11c$^+$S100$^+$CD68$^-$ dendritic cells (DCs) express IDO in SCC-LL. IDO expression in LL-SCC may aid immune escape and chronic inflammation to promote cancer progression. Inhibition of IDO might be a therapeutic strategy to increase the anti-tumor immune response in SCC-LL.

Key words: Indoleamine 2,3-dioxigenase, Squamous cell carcinoma, Cancer immunology

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Introduction

The role of the adaptive immune response in controlling the invasion and growth of tumor cells has been controversial. Experiments with immunodeficient mice have provided data supporting the role of adaptive immunity in cancer immunosurveillance (Gallimore and Simon, 2008; Ljunggren, 2008; Kohlmeyer et al., 2009). However, immune escape is a crucial feature of cancer progression, the mechanisms of which are extremely divers and still obscure (Zou, 2005). One mechanism that has recently been suggested to add to tumor escape is the tryptophan catabolism mediated by the enzyme indoleamine 2,3-dioxygenase (IDO) (Munn and Mellor, 2007). IDO is expressed mainly by antigen-presenting cells (APCs) such as dendritic cells (DCs), monocytes or macrophages, but also by endothelial cells, fibroblasts or tumor cells (Uyttenhove et al., 2003; Huang et al., 2010; Paveglio et al., 2011). IDO is induced by inflammatory cytokines and mediators such as interferon-γ (IFN-γ), tumor-necrosis-factor-α (TNF-α), interleukin-6 (IL-6), Toll-like-receptor ligands (TLRs) such as lipopolysaccharide (LPS), or by cross-linking of specific surface receptors e.g. the high affinity receptor for IgE, FcεRI by allergens (von Bubnoff et al., 2002; Jurgens et al., 2009; Wang et al., 2010). By depriving the microenvironment from trp, IDO blocks T cell proliferation, induces regulatory T cells and regulatory DCs, thus creating a suppressive micromilieu (Baban et al., 2009; Brenk et al., 2009). IDO-expression by immunogenic mouse tumor cells prevents the rejection of these tumor cells by preimmunized mice (Uyttenhove et al., 2003). In a model of mouse melanoma, tumor-

Abbreviations: IDO (indoleamine 2,3-dioxygenase), DCs (Dendritic cells), SCC (squamous cell carcinoma), LL (lower lip)

draining lymph nodes harbour a small population of IDO-expressing CD19$^+$ plasmacytoid DCs that is able to potently suppress a host anti-tumor T cell response (Sharma et al., 2007).

Squamous cell carcinoma (SCC) of the skin (located to over 90% in the face) and lip is a cancer of older people (mean age: 70 years), which has locally destructive growth but with a low risk of metastasis (approximately 5% of patients with SCC). In contrast, oral cancer, which is mainly a malignancy of the tongue, has a much worse prognosis (Vartanian et al., 2004; Morselli et al., 2007). In cancer of the lip, it is particularly the lower lip (LL) that is affected and this is largely related to the exposure to ultra violet irradiation from the sun (van Leeuwen et al., 2009). Prolonged and repeated sun exposure is a major risk factor for developing LL-SCC, and LL-SCC is particularly found in fishermen, farmers, skiers and aquatic sportsmen. In a pioneering piece of work by Uyttenhove et al it became clear that many, but not all, human tumor cell types constitutively express IDO *in vivo*, such as prostatic carcinomas, cervical carcinomas, pancreatic carcinomas and others (Uyttenhove et al., 2003). One study detected a 100% expression of IDO in esophageal SCC samples and also in normal mucosa by RT-PCR (Sakurai et al., 2004). Another study examining human carcinoma of the endometrium and uterine cervix found IDO expression not only in carcinoma cells, but also in cancer surrounding cells (Sedlmayr et al., 2003).

Since the prevalence of LL-SCC is increasing worldwide, and since no studies are to date available analysing cellular IDO protein expression in skin SCC, we investigated the expression of this enzyme in 47 biopsies of LL-SCC. By immunohistochemistry and by double-immunofluorescence analysis, we demonstrate that in 12 out of 47 investigated human LL-SCC samples IDO is expressed and confined to large myeloid CD11c$^+$S100$^+$ DCs, while CD1a$^+$ Langerin$^+$ (CD207$^+$) Langerhans cells (LCs)/DCs, CD68$^+$ macrophages and tumor cells did not express IDO. These IDO$^+$ DCs are located along the pioneer front of the invading tumor.

Material and methods

Donors

Lesional skin biopsies from patients (n=47) with LL-SCC were taken at the dermatology department of the University of Bonn from 2001-2008 and were analyzed. Control biopsies were taken from unaffected lip. Informed consent was obtained from all donors. All patients were submitted to surgical treatment and none received radiotherapy, chemotherapy or other treatment prior to surgery. Clinical data (gender, age) and follow-up information in 2010 was obtained from medical follow-up care records. The study was performed according to local ethical guidelines and approved by the local regulatory committee.

Histology and tumor grading

Serial sections were prepared from formalin-fixed, paraffin-embedded skin biopsies. Standard H&E staining and periodic acid schiff reactions were performed for diagnostic purposes.

Histopathological grading of LL-SCC was evaluated according to the World Health Organization (WHO) classification of SCC Union Internationale Contre le Cancer (UICC 1997; TNM Classification of Malignant Tumors) (Sobin and Fleming, 1997; Wittekind et al., 2002): Grade I: well differentiated; Grade II: moderately differentiated; Grade III: poorly differentiated; Grade IV: not differentiated. As an additional histopathological parameter to classify the T class, the thickness of the tumors was analyzed in each specimen (Breuninger et al., 1990; Wittekind et al., 2002; Brantsch et al., 2008; Breuninger et al., 2008) and classified as: pT1-3a (tumor thickness ≤2mm and invasion level restricted to the dermis; pT1-3b (tumor thickness >2mm and ≤6mm and invasion level restricted to the dermis; pT1-3c (invasion to the subcutis and/or tumor thickness >6mm). N- and M- classifications of LL-SCC were evaluated according to WHO guidelines for carcinomas of the skin (Wittekind et al., 2002) and evaluated on the basis of clinical findings and imaging techniques during follow-up care records.

Scoring of the density of the inflammatory infiltrate

Cells of ten representative high-power-fields (HPF; x200), located at the invasive front of the tumor in each specimen were calculated. The density of the inflammatory infiltrate was scored as follows: None, if <25% of all cells were inflammatory cells in all HPFs; mild, if 25%-50% of all cells were inflammatory cells; moderate, if 50%-75% of all cells were inflammatory cells; and as intense, if nearly all (>75%) cells were of inflammatory origin and only few stromal cells were seen. All samples were evaluated independently by two investigators (MK and JW).

Immunohistochemistry (IHC)

Anti-IDO monoclonal antibody (mAb; clone 10.1, dilution 1:150, Millipore™, Billerica, USA) labeling was performed on paraffin embedded tissue sections (4 μm) after heat pre-treatment for 10 minutes in Target Retrieval Solution pH 9 (DAKO™, Hamburg, Germany). Appropriate isotype matched controls were included. Additional antibodies (Ab) for IHC included: anti-CD11c mAb (5D11, dilution 1:20, Novocastra™, Newcastle, UK), anti-S100 Ab (Z0311, dilution 1:2400, DAKO™), anti-CD68 mAb (PGM1, dilution 1:75, Novocastra™), anti-CD1a mAb (O10, dilution 1:10, Immunotech™, Marseille, France), anti-CD123 mAb (9F5, dilution 1:100, BD Bioscience™, San Jose, USA), anti-CD3 mAb (F7.2.38, dilution 1:1, DAKO™), anti-

CD207 mAb (306G9-01-HD24, dilution 1:75, antibodies-online™, Aachen, Germany), and anti-Foxp3 mAb (eBio7979, dilution 1:250, eBioscience™, San Diego, USA).

Visualisation was performed using the REAL detection system (4th edition, DAKO™) with fast red as chromogen. Photographs of stainings (microscope: Olympus BH-2; camera: Olympus DF 70) were visualized with the program AnalySIS 5.0 (Olympus Soft Imaging System GmbH).

Immunofluorescence staining

Immunofluorescence double staining was carried out using formalin fixed, paraffin embedded sections after heat antigen retrieval following standard protocols. The following primary Abs were used: anti-IDO polyclonal Ab (AHP833, dilution 1:200, Serotec™, Düsseldorf, Germany), anti-CD11c mAb (5D11, dilution 1:10, Novocastra™), anti-S100 Ab (Z 0311, dilution 1:1200, DAKO™), anti-CD68 mAb (PGM1, dilution 1:50, Novocastra™), anti-CD1a mAb (O10, dilution 1:1, Immunotech™), anti-CD123 mAb (9F5, dilution 1:50, BD Biosciences™), anti-CD3 mAb (PS1, dilution 1:50, Novocastra™) and anti-CD207 mAb (306G9-01-HD24, dilution 1:100, antibodies-online™).

As secondary Ab we used a FITC-conjugated donkey-anti-sheep-IgG (dilution 1:100, SerotecTM, Düsseldorf, Germany) to detect the IDO Ab. To detect the binding of S100, we used a CY3-conjugated goat-anti-rabbit-IgG (dilution 1:50, Invitrogen™, Darmstadt, Germany). For the other primary mAbs we used a CY3-conjugated goat-anti-mouse-IgG (dilution 1:200, Invitrogen™). Controls included staining with isotype-matched antibodies. Analysis was performed using a microscope (Leica DM LB KY-F754) interfaced with a 12V 100W halogen lamp (Osram, München, Germany). Photographs (camera JVC KY-F75U) were visualized with the program Diskus 4.60.1171 (Hilgers, Königswinter, Germany).

Statistical methods

Analyses were performed using Microsoft Excel 2007 and SAS software (version 9.2, Cary, USA). The correlation of IDO-expression with the density of the perineoplastic inflammatory infiltrate was performed with the Trend Test. *P* values lower than 0.05 were considered statistically significant.

Results

Patient characteristics

To avoid confounding external factors such as pretreatment or mode of sampling, 47 caucasian patients with LL-SCC were qualified and had their primary biopsies taken at the Department of Dermatology and Allergology at the University of Bonn, Germany. Surprisingly, IDO expression was detected in only 12 specimens out of 47 specimens with LL-SCC by immunohistochemistry (IHC). The mean age in the IDO^+ and IDO^- LL-SCC group was nearly identical, with 72 years and 70 years, respectively. During the follow-up care phase (2-9 years; median follow-up time: 6.3 years), only one patient, who belonged to the IDO-negative group, died of unknown causes unrelated to LL-SCC (after a 5 year follow-up). One patient of the IDO-negative group presented with submandibular lymph node metastasis (N-stage: N1). Another patient of the IDO-negative group had a distant metastasis retroauricularly on the contralateral side (M-stage: M1); both patients with metastases are alive (3 year follow-up). Patient characteristics are summarized in Table 1.

The rate of IDO-positive samples in LL-SCC increases with the density of the inflammatory perineoplastic infiltrate

Only 9% of IDO^- specimens showed an intense

Table 1. Patient characteristics, clinical and histological data.

Clinical features	IDO^+	IDO^-
Patients, n (%)		
Number of cases	12 (100)	35 (100)
Age		
≤70 years, n (%)	4 (33)	14 (40)
≥70 years, n (%)	8 (67)	21 (60)
Mean (± SD)	72.33 (±12.47)	70.46 (±9.93)
Min./Max.	51/94	52/96
Gender, n (%)		
Male	9 (75)	25 (71)
Female	3 (25)	10 (29)
Tumor thickness, histological (mm)		
Mean (± SD)	2.2 (±0.49)	2.3 (±1.07)
Histological grading (WHO grade), n (%)		
Grade I	9 (75)	33 (94)
Grade II	3 (25)	2 (6)
T stage, n (%)		
pT1-3a	7 (58)	17 (49)
pT1-3b	5 (42)	18 (51)
pT1-3c	0 (0)	0 (0)
N stage, n (%)		
N0	12 (100)	34 (97)
N1	0 (0)	1 (3)
M stage, n (%)		
M0	12 (100)	34 (97)
M1	0 (0)	1 (3)
Intensity of inflammatory infiltrate, n (%)		
None	0 (0)	4 (11)
Mild	0 (0)	14 (40)
Moderate	7 (58)	14 (40)
Intense	5 (42)	3 (9)
Clinical outcome, n (%)		
Dead	0 (0)	1 (3)
Alive	12 (100)	34 (97)

perineoplastic infiltrate, 40% of IDO$^-$ lesions disclosed a moderate and another 40% a mild infiltrate. No infiltrate was seen in 11% of IDO$^-$ LL-SCC samples. In contrast, a perineoplastic infiltrate was seen in all IDO$^+$ specimens. 42% of IDO$^+$ specimens showed an intense infiltrate and 58% of IDO$^+$ samples displayed a moderate cancer-associated infiltrate. IDO-expression in LL-SCC was never seen with a mild or no infiltrate. The correlation between the density of the cancer-associated inflammatory infiltrate and the cellular expression of IDO was statistically significant (P=0.0007; Fig. 1). The T stage or thickness of the tumors were not significantly different between IDO$^+$ and IDO$^-$ cancer lesions. The maximum thickness of the tumor in the IDO$^+$ specimens was 3 mm (mean 2.2±0.49 mm SD) and in the IDO$^-$ specimens 5 mm (mean 2.3±1.07 mm SD).

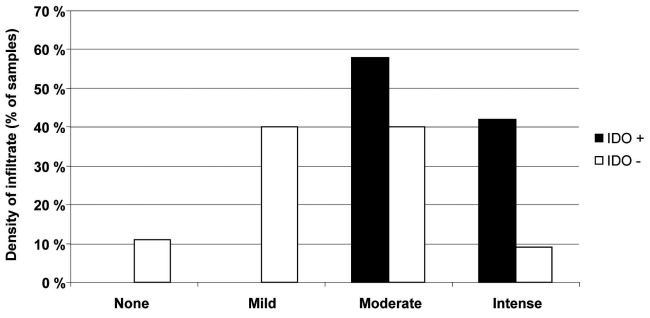


Fig. 1. Percentages of IDO$^+$ and IDO$^-$ LL-SCC samples with no, mild, moderate and intense perineoplastic infiltrate. The rate of IDO+ LL-SCC samples increases with the density of the perineoplastic infiltrate. This correlation was statistically significant (*P*=0.0007).

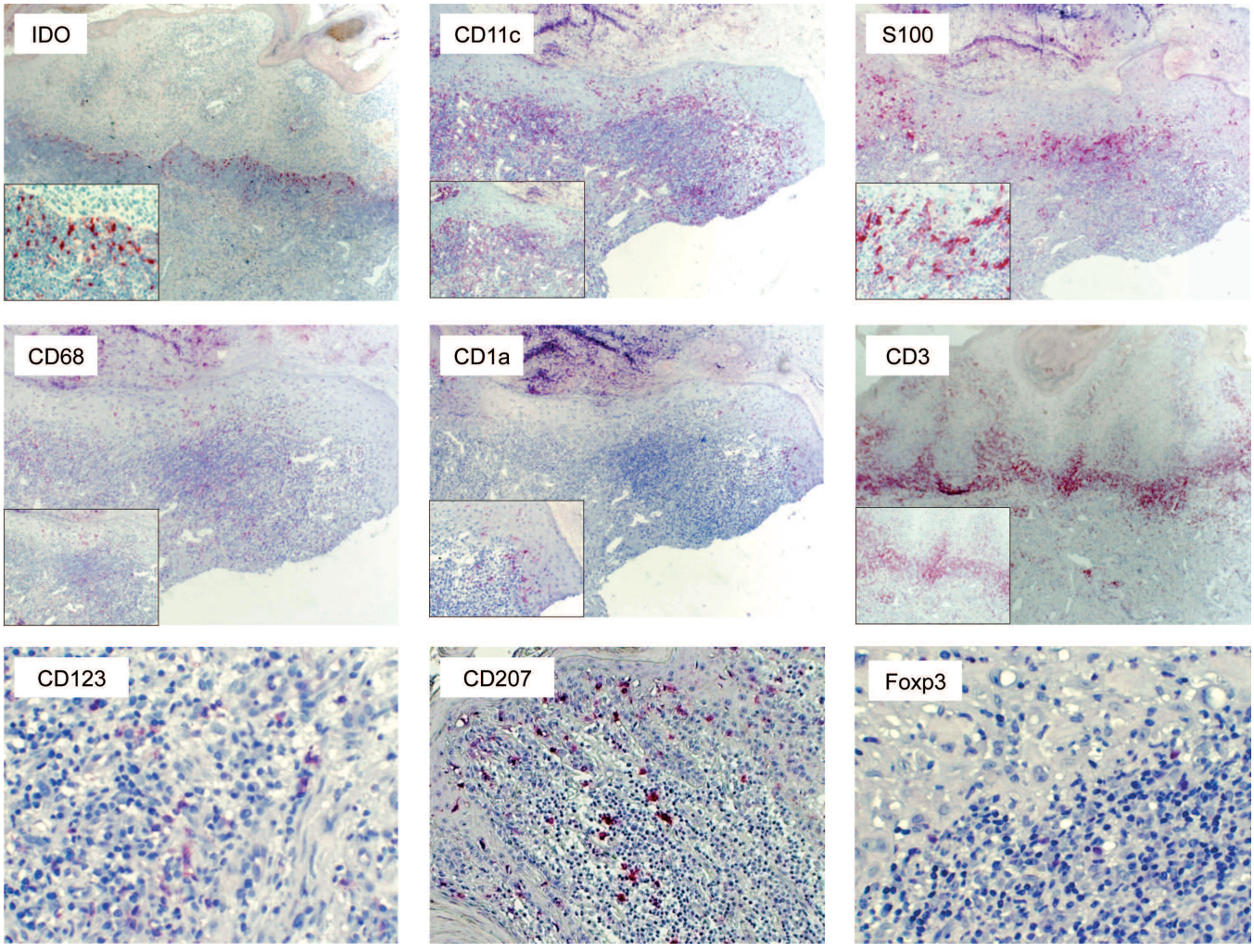


Fig. 2. Immunohistochemical staining for IDO, CD11c, S100, CD68, CD1a, CD3, CD123, CD207 (Langerin), and Foxp3 in lower lip (LL) SCC. Representative immunohistological pictures of one IDO$^+$ sample of LL-SCC. Large photographs are mainly at low power (x40) except CD123 (x400), CD207 (x200) and Foxp3 (x400). Small pictures are at higher power: IDO (x400); CD11c, S100, CD68, CD3 (x100); CD1a (x200). Note the IDO+ large dendritic cells just along the border of invasive SCC.

Indoleamine 2,3-dioxygenase (IDO) expression in LL-SCC is found in pioneer cells that align along the border of invasive tumor cells

In IDO$^+$ LL-SCC specimens the cells that expressed IDO were aligned just between the invasive front of the tumor cells and the inflammatory dermal infiltrate (Fig. 2). IDO$^+$ cells were relatively large and displayed typical dendrites. IDO$^+$ cells were virtually absent in deeper dermal sections. To better allocate IDO expression to other immune cells in the inflammatory infiltrate of LL-SCC samples the specimens were also stained with markers for CD11c (myeloid marker), S100 (dendritic cells; DCs), CD1a and CD207 (Langerhans cells), CD68 (macrophages), CD3 (T cells), CD123 (plasmacytoid DCs), and Foxp3 (regulatory T cells). Strikingly, the expression of the myeloid markers CD11c and S100 (myeloid DCs) showed the same arrangement as IDO$^+$ cells, i.e. in pioneering cells at the front side where tumor cells face the inflammatory dermal infiltrate. In addition to CD11c and S100, the marker CD3 (T cells) showed a strong expression in the perineoplastic infiltrate. Regulatory T cells (Foxp3) were moderately present within the perineoplastic inflammatory microenvironment in the T cell area (CD3). Apart from these markers, the expression of CD1a (Langerhans cells) was only seen clustered in some areas where the epithelial structure was still maintained and CD1a+ cells presumably dropped down into the adjacent inflammation. The expression of CD207 (Langerin) was

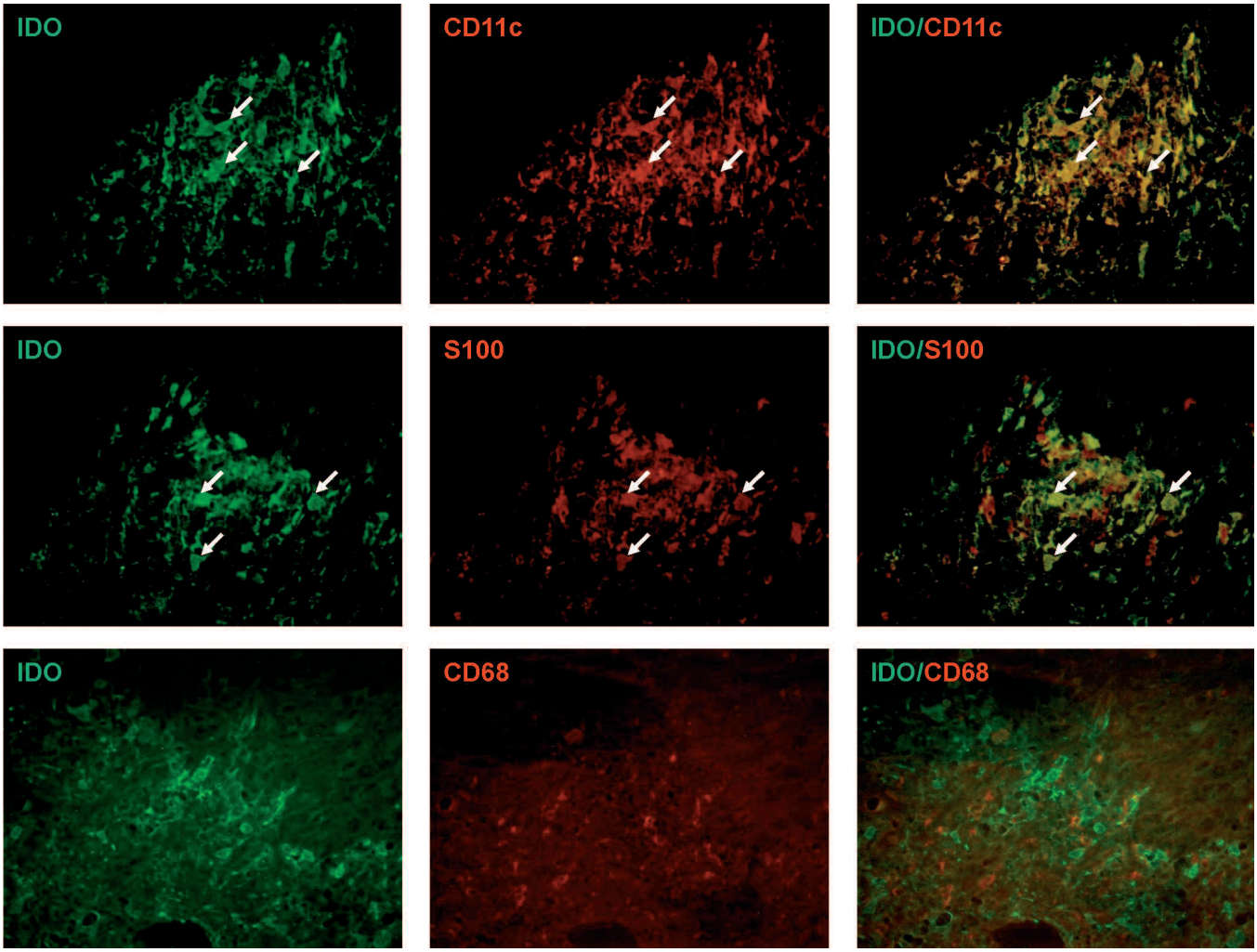
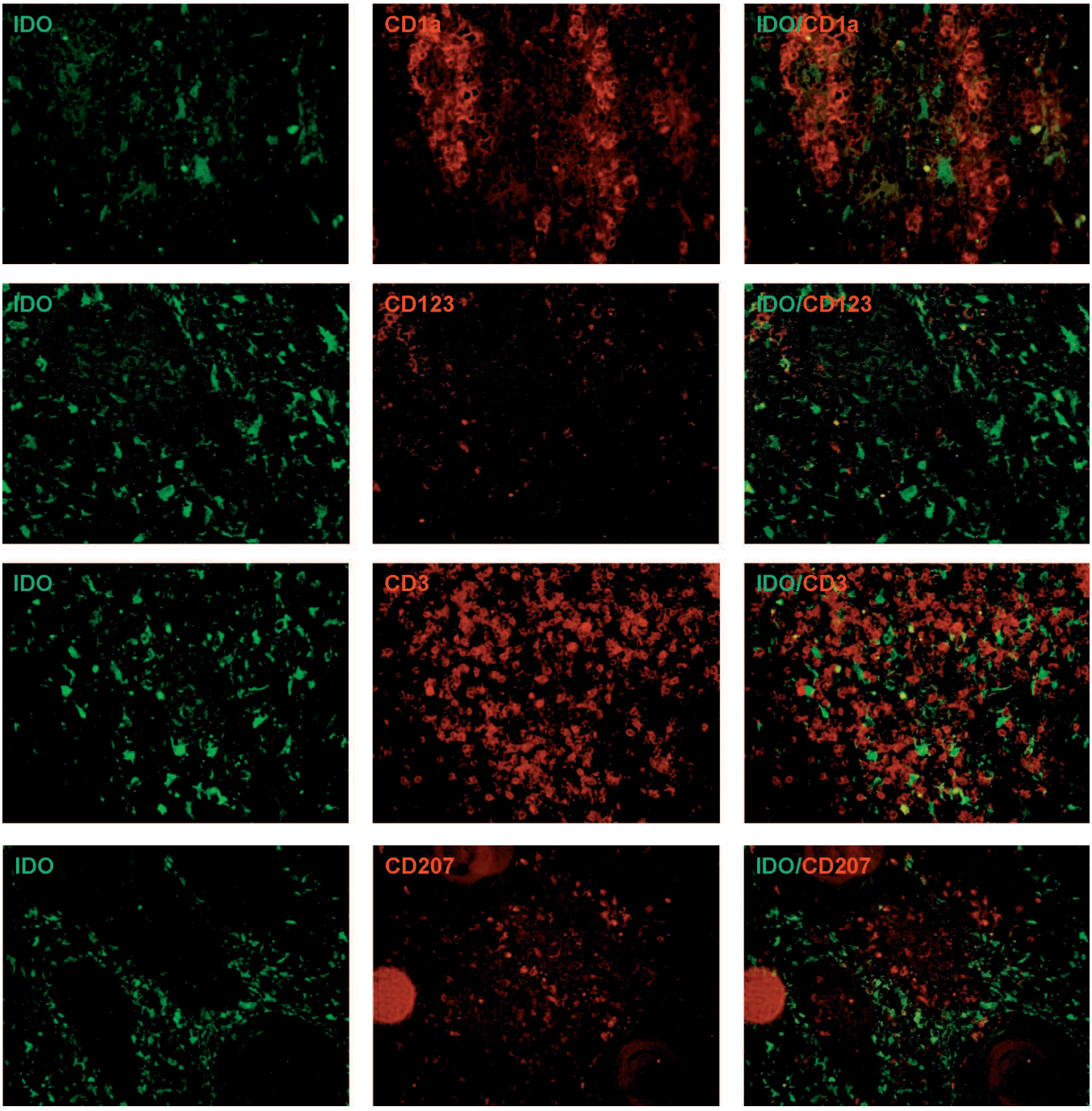


Fig. 3. Large myeloid CD11c$^+$S100$^+$CD68$^-$ DCs, and not CD68$^+$ macrophages or CD1a$^+$CD207$^+$ Langerhans cells, are the IDO expressing cells in LL-SCC. Immunofluorescence double staining for IDO with CD11c, S100, CD68, CD1a, CD123, CD3, and CD207 was performed in IDO$^+$ lesional specimens of LL-SCC. Representative photographs of the single stainings are shown, as well as the overlay of IDO (green) with the individual markers (red) in order to investigate the origin of IDO$^+$ cells. Double positive cells are yellow. x 40

still seen in cells of proliferating tumor clusters and also within the inflammatory peritumoral infiltrate. $CD68^+$ cells (macrophages) and $CD123^+$ cells (plasmacytoid DCs) were predominantly located within the most prominent dermal inflammatory infiltrates and were also seen in the deeper dermis. Tumor stroma cells and tumor



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cells were absent of IDO expression.

Myeloid CD11c$^+$S100$^+$ DCs, but not CD68$^+$ macrophages or CD1a$^+$ CD207$^+$ antigen-presenting cells express IDO in LL-SCC

To further characterize the nature of IDO$^+$ cells at the invasive front of the tumor cells, we performed double-staining immunofluorescence (IF) using the IDO mAb in combination with multiple markers (Fig. 3). Co-staining with an antibody specific for CD11c revealed that virtually all IDO$^+$ cells were positive for this myeloid marker. Staining for IDO together with S100 and CD68 allowed further discrimination. Almost all S100$^+$ myeloid DCs stained positive for IDO whereas, unexpectedly, CD68-positive macrophages did not. Interestingly, CD1a$^+$ and CD207$^+$ APCs also failed to show IDO expression; these cells were located within the tumor front surrounded by the IDO-containing inflammatory infiltrate. As expected from previous studies, CD3$^+$ T cells were clearly negative for IDO, although present in high numbers peritumorally. Interestingly, here, the numerous small CD3$^+$ T cells were found clustered around and often adjacent to the IDO$^+$ cells. Plasmacytoid CD123$^+$ DCs did not express IDO and were loosely scattered within the IDO$^+$ infiltrate.

In summary, in LL-SCC myeloid CD11c$^+$S100$^+$ DCs, and not CD68$^+$ macrophages or CD1a$^+$CD207$^+$ APCs, seem to create a tolerogenic microenvironment right at the tumor front by the expression of IDO.

Discussion

In the present study we provide evidence that large veiled myeloid S100$^+$CD11c$^+$ DCs, located at the pioneer front of invasive human LL-SCC express IDO. IDO-positivity was not constitutive, but correlated highly with the density of the peritumoral infiltrate. In SCC, the density of the peritumoral infiltrate itself is associated with tumor progression as it increases from intraepithelial to invasive SCC (Muhleisen et al., 2009). IDO expression in DCs in the infiltrate is thus a feature linked to progression of LL-SCC.

Oncogenesis in SCC is initiated by mutations through UV irradiation or other mutagens. However, it is the tissue microenvironment that probably critically determines and modifies the progression of the tumor cells (Clark and Pavlis, 2009). Initially, a variety of immune cells might be attracted to the tumor microenvironment and increase in numbers, and powerfully contribute to tumor suppression (Koebel et al., 2007). Despite the presence of chronic inflammation, it is conceivable that the quality of the inflammatory infiltrate eventually changes in favor of the tumor (Dunn et al., 2004). The appearance of IDO$^+$ APCs could represent such a moment where the tumor and its infiltrate have evolved tactics, i.e. a change of specific cytokines or gene expression, that work to dampen the immune response and to reduce the possibility of over-activation. Recent studies suggest that one prominent mechanism is IDO-activity (Prendergast et al., 2010). In LL-SCC, the expression of IDO detected in this study will, as it does in many situations, very likely reduce the immune response and therefore facilitate tumor progression. IDO-expression is almost regularly found during an immune response, and it may therefore not be unexpected that it correlates with the density of the infiltrate in LL-SCC, as the density is an indicator of the intensity of the immune response. The more intense the response the greater the likelihood will be that counter-regulatory mechanisms like IDO are triggered (Muller et al., 2008). It is possible that IDO is a rather early mechanism in the initiation of tumor escape (Munn et al., 2004). In our study IDO-expression was not correlated to greater tumor thickness; in contrast, there was a tendency toward an association with smaller tumor thickness. This view is in accordance with the observation that the thickest tumors in our study did not show IDO expression, and IDO expression was not seen in our patients with the local and distant metastases. Once the tumor is past a certain stage IDO will cease to be of importance, as by then the immune system may have grown tolerant to the tumor and may not respond. Other factors instead may drive tumor growth at a later stage of tumor development. In one study involving human colon cancer IDO-expression was downregulated in lymph node metastases compared to tumor-draining lymph nodes without metastases, supporting the hypothesis of a rather early tumor escape mechanism by IDO-expression in human APCs (Gao et al., 2009).

The main stimuli that induce IDO in APCs are microbial molecules on one hand (such as LPS and other TLR-ligands) and host inflammatory factors, especially interferons (IFNs), on the other hand (Gu et al., 2010). In the anti-tumor response both may be involved. Stressed or transformed cells may express endogenous TLR-ligands (Frenzel et al., 2006; Pries et al., 2008), and the presence of IFN-γ in the LL-SCC infiltrate has been described earlier (Wenzel et al., 2008; Huang et al., 2009; Wakita et al., 2009; Gu et al., 2010).

The immunosuppressive effect of IDO works through the localised reduction in trp-levels, which has been shown to change the function of both CD3$^+$ T cells and DCs. One aspect of IDO-activity is the induction and activation of regulatory T cells (Tregs) (Fallarino et al., 2006; Sharma et al., 2007). In this study, Foxp3$^+$ T cells were found along the tumor border and IDO$^+$ DCs were seen surrounded by numerous CD3$^+$ T cells. The appearance of Tregs thus may be the result of the observed IDO-expression (Prendergast et al., 2009). Through their inhibitory function, Tregs antagonise the immune response and will decrease the efficiency of the anti-tumor response in LL-SCC (Gasparoto et al., 2010). The other aspect of low trp levels is the induction of regulatory DCs (Brenk et al., 2009) and it was shown

that myeloid DCs from human SCC are poor stimulators of T-cell proliferation, a hallmark of regulatory DCs (Popov and Schultze, 2008; Bluth et al., 2009).

Perhaps more surprisingly is the fact that, apart from $S100^+CD11c^+$ myeloid DCs, other APCs within the peritumoral infiltrate in LL-SCC such as $CD68^+$ macrophages or $CD1a^+CD207^+$ LCs/ DCs and the tumor cells themselves were negative for IDO. This is remarkable since SCC involves the transitional epithelium of the lip where LCs are located. There might be several possible reasons for this. We have previously demonstrated that human keratinocytes do not respond with IDO protein activity upon stimulation with IFNs. In contrast, highly enriched human epidermal LCs were found to be able to respond with IDO protein activity upon stimulation with IFN-γ *in vitro* (von Bubnoff et al., 2004). However, although transcriptional profiling studies identified an upregulation of IFN-associated genes such as Mx1, CXCL9 or IRF1 in human skin SCC (Wenzel et al., 2008) it is possible that IFNs act to induce IDO in some APCs but not in the others, or the level of IFNs reached in the epidermal lesions are too low to induce IDO in LCs. In cancer, the activation of an efficient immune response is primarily dependent on presentation of tumor-derived peptides by professional DCs (Smits et al., 2009; Robson et al., 2010a,b). Inactivation of DCs through tumor-derived factors results in impairment of the antitumor immune response and to the development of regulatory DCs (Kuang et al., 2008). Emerging evidence suggests that the expression of IDO at key sites where the immune system encounters tumor antigens is of pivotal importance for tumor progression (Munn and Mellor, 2007).

In summary, this study clearly shows that IDO expression in human skin arises from a strong cancer-associated inflammatory infiltrate. The appearance of IDO in myeloid DCs in the peritumoral infiltrate of LL-SCC might implicate a subversion of immune surveillance in favor of the tumor. It is conceivable that the inhibition of IDO enhances the anti-tumor immune response in LL-SCC and therefore might be an option for therapeutic strategy (Liu et al., 2010).

Acknowledgements. We thank Sabine Zahn, Department of Dermatology, Friedrich-Wilhelms-University, Bonn 53105, Germany, for technical assistance. We are grateful to Dr. Rolf Fimmers, Institute for Medical Biometry, Informatics, and Epidemiology, University of Bonn, for statistical help. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 704/TP A15) and BONFOR. This work was supported by a grant (BONFOR) from the University of Bonn, Germany, and by the Deutsche Forschungsgemeinschaft (SFB 704, A15).

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Accepted February 7, 2011