Clin Oral Invest (2013) 17:1365–1373
DOI 10.1007/s00784-012-0814-1

ORIGINAL ARTICLE

Inflammatory cells of immunosuppressive phenotypes in oral lichen planus have a proinflammatory pattern of expression and are associated with clinical parameters

Marilena Vered · Eran Fürth · Yifat Shalev · Dan Dayan

Received: 16 April 2012 / Accepted: 23 July 2012 / Published online: 15 August 2012

Abstract
Objectives We sought to investigate the expression of cells with immunosuppressive/protumorigenic phenotypes in oral lichen planus (OLP), such as M2-tumor-associated macrophages (TAM2), myeloid-derived suppressive cells (MDSCs), and regulatory T cells (Tregs) in association with clinical parameters.
Materials and methods Cases of hyperkeratotic (HK)-OLP (n=23) and erosive (E)-OLP (n=26) were immunohistochemically stained to determine the percentages of CD163-TAM2, CD80-MDSCs, and FOXP3-Tregs of proinflammatory CD121a-Th17, CD4 and CD8 lymphocytes, and of cells positive for nuclear factor kappa B (NF-κB) and transforming growth factor beta. Clinical parameters included symptoms, treatment approach, treatment response, and others.
Results The inflammatory infiltrate in HK-OLP and E-OLP contained immunosuppressive cells; however, their pattern of expression was compatible with a proinflammatory response [membranous CD163-TAM2 staining (not extracellular), CD80+ lymphocytes (not macrophages), and a few Tregs]. The presence of CD4+, CD8+, and CD121a+ T lymphocytes was extensive. TAM2 were more frequent in E-OLP than in HK-OLP (P=0.017). A higher frequency of CD80+ lymphocytes was associated with partial to no response to treatment (P=0.028). Nuclear expression of NF-κB in the inflammatory cells was absent.
Conclusions The pattern of expression of the immunosuppressive cells, together with numerous CD4+, CD8+, and Th17-CD121a+ lymphocytes, suggest an extensive proinflammatory response rather than an immunosuppressive/protumorigenic response.
Clinical relevance The frequency of selective types of inflammatory cells calls for individual profile analyses of inflammatory infiltrates and individually adjusted treatment.

Keywords Oral lichen planus · Tumor-associated macrophages · Myeloid-derived suppressive cells · Regulatory T cells · Immune-suppressive/protumorigenic cells · Novel treatment approach

Introduction

Lichen planus (LP), a chronic inflammatory mucocutaneous disease of unknown etiology, involves the skin, oral and genital mucosal surfaces, scalp, and nails. Oral LP (OLP) can be the only manifestation of this disease, affecting 1–2 % of the population over 40 years of age. OLP has a slight female predominance [1, 2]. Clinically, the lesions can be classified into six variants, of which the hyperkeratotic/reticular and the erosive/atrophic are the most common [3]. In the long-standing, erosive/atrophic form, OLP is usually symptomatic, and treatment is aimed at relieving pain for which anti-inflammatory and immune-modulating agents, especially corticosteroids, topical cyclosporin or tacrolimus,

M. Vered (✉) · Y. Shalev · D. Dayan
Department of Oral Pathology and Oral Medicine,
School of Dental Medicine, Tel Aviv University,
Tel Aviv 69978, Israel
e-mail: lmy@netvision.net.il

M. Vered
Institute of Pathology, The Chaim Sheba Medical Center,
Tel Hashomer, Israel

E. Fürth
Oral and Maxillofacial Surgery Unit, Tel Aviv Sourasky
Medical Center,
Tel Aviv, Israel

and topical and systemic retinoids, are prescribed [4]. The 2005 WHO classification of head and neck tumors recognizes OLP as a premalignant condition [5].

All clinical variants of OLP share the same microscopic features that consist of a dense subepithelial lymphohistiocytic infiltrate, which has been extensively investigated, with emphasis on the predominant T cell lymphocytes. Specifically, cytotoxic T lymphocytes (CD8 phenotype) are prevalent within and adjacent to the epithelium, while noncytotoxic T helper 1 (Th1) lymphocytes (CD4 phenotype) are found mainly within the lamina propria [2]. In addition to these well-recognized cell types, another type of lymphocytic T cells has been identified in OLP, namely, the regulatory T cells (Tregs) [6] that are CD4-derived and are induced either by transforming growth factor beta (TGF-β) or by a subclass of myeloid-derived suppressor cells (MDSCs) through the CD80/B7-1 cell surface interactions [7]. Tregs have an immunosuppressive activity [8]. In addition to being a source for Tregs, CD4 can also be induced by TGF-β to develop into Th17 cells that represent a subclass of proinflammatory cells [9]. The presence of Th17 cells in OLP has not yet been investigated.

Mature macrophages (CD68 phenotype) are a consistent finding within the inflammatory infiltrate in OLP [10]. More recent studies have revealed the existence of less mature macrophages in the context of the inflammatory infiltrate that accompanies epithelial malignancies [11, 12]. Diversity, plasticity, and interplay are characteristic of these macrophages, which are called tumor-associated macrophages (TAMs) [7, 13]. TAMs can be polarized to have a proinflammatory, antitumorigenic phenotype (TAM1) or, inversely, an immunosuppressive phenotype (TAM2) that is noted for its potential to contribute to tumor growth, tissue remodeling, promotion of angiogenesis, and suppression of adaptive immunity [14]. The TAM2 phenotype can be induced by signals from Tregs [14]. Studies also argue in favor of a link between chronic viral, bacterial, and noninfectious inflammation, and the initiation, promotion, and progression of tumors, especially in organs, such as the gastrointestinal tract, pancreas, lungs, and bladder. This link is based on the knowledge that signaling pathways involved in inflammation operate downstream of oncogenic mutations. Nevertheless, not all the components of the inflammatory infiltrate are equally protumorigenic, and furthermore, one cell type may have a cancer-promoting or a cancer-inhibiting phenotype under different circumstances [14].

Defining the direction of the inflammatory/immune response into either a proinflammatory ("good" inflammation) or an immunosuppressive ("bad" inflammation) route is controlled by a plethora of cytokines, chemokines, and transcriptor factors [15–17]. Of special significance are the nuclear transcriptor factor kappa B (NF-κB), which plays a crucial role in programming the TAM1 and TAM2 phenotypes and thus masters the switch between them and TGF-β, which is a key factor in the balance between formation of CD4-derived Tregs and Th17 cells [9, 11, 14].

It was the aim of our study to investigate the presence and the pattern of expression of immunosuppressive/protumorigenic inflammatory cells in the chronic infiltrate of this disease, including the CD4-derived Tregs, MDSCs of the CD80 phenotype, and CD163-TAM2 macrophages, in parallel to the presence of already proven proinflammatory cells, such as CD4 and CD8 T lymphocytes and CD4-derived Th17 cells, together with the presence and pattern of expression of NF-κB and TGF-β. We also investigated associations between these various types of inflammatory cells and clinical parameters. This information should contribute to a more effective clinical outcome for OLP by making it possible to tailor the therapeutic approach and adjust it to the individual composition of the inflammatory cell phenotypes and their architectural organization.

Materials and methods

Study group

The study group was comprised of patients who were clinically and histopathologically diagnosed with OLP in the Department of Oral Pathology and Oral Medicine, Tel Aviv University, between 1999 and 2008. They had not been given any local or systemic treatment prior to diagnosis and were followed up for ≥12 months. The study was approved by the Ethical Committee of Tel Aviv University. The following parameters were collected from the patients' files: age, gender, clinical form of the disease [hyperkeratotic (HK)-OLP and atrophic/erosive (E)-OLP], symptoms of soreness, discomfort and pain, and administered treatment [systemic (consisting of prednisone at initial doses between 20 and 40 mg followed by tapering down) or local (consisting of local application of either 0.05 % clobetasone cream or 0.05 % dexamethasone mouth rinses)]. In addition, we recorded the response to administered treatment: "good response" if the lesions were found clinically to have disappeared or remarkably decreased in size, and the patient was free of symptoms at least for 6 months; "partial-to-no response" if the signs and symptoms improved for less than 6 months or no improvement whatsoever; and "not relevant" if the patient was given no treatment. The concomitant presence of cutaneous lesions of LP was also considered.

Antibodies for immunohistochemistry

These (including corresponding positive and negative controls) are summarized in Table 1 according to cell type and immune function.

Table 1 Antibodies used to identify cell-specific markers of the various immune response cell types according to their function

Types of cells/immune regulating factors and function	Cell marker	Clone, concentration, time of exposure, and antigen retrieval	Positive controls	Negative controls	Manufacturer	Reference
Lymphocytes, T helper 1 (Th1)	CD4	Clone ZT-17 (mouse, isotype IgG1-κ), ready to use, overnight, EDTA pH9, pressure cooker	Tonsil	Mouse IgG1[1]	Invitrogen/Zymed, San Francisco, CA, USA	Gannot et al. [18]
Lymphocytes, cytotoxic T cells	CD8	Polyclonal, ready to use, overnight, EDTA pH9, pressure cooker	Tonsil	Removal of the primary antibody	Invitrogen/Zymed, San Francisco, CA, USA	Hirshberg et al. [19]
Th17 lymphocytes (CD4-derived), proinflammatory activity	CD121a	Polyclonal, 1:50, overnight, EDTA pH9, pressure cooker	Placenta	Removal of the primary antibody	Acris, Herford, Germany	Rogers et al. [20]
Macrophages (TAM2)	CD163	Clone k20-T (rabbit, isotype IgG), 1:200, overnight, citrate buffer pH 6, pressure cooker	Placenta	Normal rabbit Serum[1]	Acris, Herford, Germany	Massimo et al. [21]
Regulatory T cells (Tregs) (CD4-derived), immuno suppression, protumorigenic	FOXP3	Clone mAbcam 22510 (mouse, isotype IgG1), 1:50, overnight, EDTA pH 9, pressure cooker	Tonsil	Mouse IgG1[1]	Abcam, Cambridge, UK	Matsumoto et al. [22]
MDSC, immunosuppression, protumorigenic	CD80	Clone 1G10 (mouse, isotype IgG2a), 1:50, overnight, citrate buffer pH 6, pressure cooker	Tonsil	Mouse IgG2a[1]	Abcam, Cambridge, UK	Lamb et al. [23]
Inflammation regulatory factor	NF-κB	Polyclonal, NF-κB/p65, 1:500, overnight, citrate buffer pH 6, pressure cooker	B cell lymphoma	Removal of the primary antibody	Alexis Biochemical San Diego, CA, USA	Quaranta et al. [24]
Control on the CD4-derived Th17 and Tregs	TGF-β	Polyclonal, 1:25, overnight, citrate buffer pH 6, pressure cooker	Placenta	Removal of the primary antibody	Acris, Herford, Germany	Theilig et al. [25]

[1] R&D Systems, Minneapolis, MN, USA

Immunohistochemical evaluation, light microscopy at ×200

T lymphocytes of the CD4 phenotype

Immunostaining revealed that the apparently uniform lymphohistiocytic band seen on the hematoxylin and eosin-stained slides was occasionally composed of areas that contained high density aggregates of positively stained CD4 cells. Therefore, in each case, these cells were assessed in two ways: first as a general percentage of the entire lymphohistiocytic band ($CD4_{gen}$) and then as a percentage of the aggregates ($CD4_{agg}$). The positively stained mononuclear cells were assessed along the entire length of the epithelial–inflammatory interface. For $CD4_{gen}$, approximately 100 inflammatory cells were counted in each consecutive field in order to determine the mean percentage of positive cells from all the mononuclear cells per sample [26]. For $CD4_{agg}$, the sample was examined again, concentrating on the high-density areas of inflammation, and the percentage of stained cells out of 100 cells (or mean percentage if more than one focal aggregation was present) was calculated. $CD4_{agg}$ was not expected to be found in all cases.

T lymphocytes of CD8 phenotype

For cytotoxic T cells of the CD8 phenotype, the assessment was performed in a similar manner as for CD4-positive lymphocytes for both $CD8_{gen}$ and $CD8_{agg}$.

T lymphocytes, CD4-derived Th17 cells, identified by cell membrane CD121a

The CD121a-stained cells were assessed as the percentage from the total amount of lymphohistiocytic inflammatory cells in a similar way as for $CD4_{gen}$ and $CD8_{gen}$.

T lymphocytes and CD4-derived regulatory T cells of the FOXP3 phenotype

Since the number of positively stained cells was too small to be expressed as a percentage from the total number of

inflammatory cells, we used an alternative histomorphometric method of assessment that included photographing three representative fields (×100) from each case by means of a digital camera (DP 70, Tokyo, Japan) mounted on a light microscope (BX-50, Tokyo, Japan). Each of the photomicrographs was saved as a .jpeg file, which was then copied to a full-size Power Point slide with a 10×10 square graticule (also adjusted for the full size of the slide) superimposed on top of it. The number of FOXP-3-positive cells on the odd columns (i.e., 1, 3, 5, 7, and 9) of the graticule, starting from the square and overlapping the interface between the epithelium and the lymphohistiocytic infiltrate for a distance of three vertical squares that covered the width of the infiltrate, were counted in each of the three representative photomicrographs belonging to each case. The individual counts were summed up and then divided by 3 to obtain the mean number of FOXP-3 stained cells per field per case.

TAM2 of the CD163 phenotype

These are polarized, nonmature macrophages with an immunosuppressive/protumorigenic activity. The frequency of CD163-stained cells was assessed as the percentage from the total amount of lymphohistiocytic inflammatory cells, similarly to $CD4_{gen}$ and $CD8_{gen}$.

MDSCs of the CD80 phenotype

Assessment of the CD80-positive cells within the inflammatory cells was performed as described above for $CD4_{gen}$ and $CD8_{gen}$.

NF-κB

The activation of this transcription factor depends on its nuclear localization. Assessment of nuclear NF-κB expression was performed in both the inflammatory and epithelial cells, and the result was reported as the percentage of positively stained cells from the total number of cells in each compartment as described above for $CD4_{gen}$ and $CD8_{gen}$.

TGF-β

Cytoplasmic expression was assessed in the inflammatory cells as a percentage of the total of these cells as described above for $CD4_{gen}$ and $CD8_{gen}$.

Statistical analysis

Associations between the frequency of the various inflammatory cells and OLP variants (HK-OLP and E-OLP), presence or absence of symptoms, response to treatment, and skin involvement were examined by the *t* test. Associations between these cells and response to treatment were analyzed by one-way analysis of variance. Associations between OLP variants and the clinical parameters were analyzed by crosstabs. Data were analyzed by the SPSS software, version 16 (Chicago, IL, USA), and statistical significance was set at $P<0.05$.

Results

A total of 49 OLP patients, 23 with HK-OLP (female/male ratio 13:10, mean age 49±11.8 years), and 26 with E-OLP (female/male ratio 21:5, mean age 60±9.4 years) were included in the study. Among the HK-OLP patients, 12 (53 %) were symptomatic, and 9 (39 %) received treatment (two systemic and seven local). Three of the treated patients reported a good response to treatment, and six reported that the treatment had partial-to-no response. Skin involvement was present in 4 of these 23 patients. Twenty-three of the E-OLP patients (90 %) were symptomatic, and 16 received treatment (3 systemic and 13 local), to which four had a good response, and 12 reported partial-to-no effect. Skin involvement was found in three of these patients.

Analysis of the associations between the clinical forms of OLP and clinical parameters revealed that a lack of symptoms was more frequently associated with HK-OLP cases (10/23, 43.5 %) than with E-OLP cases (4/26, 15.4 %) ($P=0.03$). One case of E-OLP developed carcinoma in situ at a previous site of OLP over a follow-up period of 4 years.

The mean percentages of the various types of inflammatory cells and immune-modulating factors NF-κB and TGF-β, in association with the clinical form of OLP, symptoms, treatment, response to treatment, and skin involvement are summarized in Table 2. An example of each of the immune cells immunostained by its selected marker is illustrated in Fig. 1.

T lymphocytes of the CD4 type

The frequency of $CD4_{agg}$ was higher than that of the $CD4_{gen}$ for all the investigated clinical parameters. A higher frequency of both $CD4_{agg}$ and $CD4_{gen}$ was associated with partial-to-no effect compared to a good response to treatment ($P=0.048$ and $P=0.034$, respectively).

T lymphocytes of the CD8 type

In general, the frequency of CD8 was usually lower than that of CD4 for both the aggregate and general patterns. The frequency of $CD8_{agg}$ was usually higher than that of $CD8_{gen}$ in a similar way as was observed with CD4. However, unlike CD4, there were no significant associations between

Table 2 The mean percentages (±SD) of the various types of inflammatory cells and of the epithelial cells stained for NF-κB and CD80, as a factor of the clinical form of OLP, HK-OLP, and E-OLP Data for FOXP3 are presented as mean number (±SD) cells per field

Types of cells/immune-modulating factors and function	Marker	Clinical form of OLP		Symptoms		Treatment			Response to treatment		Skin involvement	
		HK-OLP	E-OLP	Symptomatic	Asymptomatic	Systemic	Local	No treatment	Good	Partial-to-no effect	Yes	No
Lymphocytes, T helper 1 (Th1)	$CD4_{agg}$	77±20	68±24	74±22	67±23	87±6	66±24	76±20	54±29*	80±18*	72±23	72±22
	$CD4_{gen}$	39±23	45±27	45±25	34±23	67±15	35±23	46±25	28±18**	53±25**	35±15	43±26
Lymphocytes, cytotoxic T cells	$CD8_{agg}$	57±28	46±24	49±26	56±26	25±18	48±23	58±27	43±19	49±30	41±39	52±24
	$CD8_{gen}$	28±19	38±28	35±24	30±25	37±25	32±23	35±26	27±17	41±27	38±34	33±23
Th17 lymphocytes (CD4-derived), proinflammatory activity	CD121a	81±20	85±8	83±16	82±13	88±13	79±19	85±11	68±29	85±12	81±27	83±12
Regulatory T cells (Tregs) (CD4-derived), immune suppression, protumorigenic	FOXP3	2.9±2.8	2.5±2.3	2.6±2.3	2.9±3	5±3.3	2±1.4	2.9±2.9	1.2±1	3.3±2.6	2.9±1.5	2.7±2.7
Macrophages, TAM2	CD163	31±19***	48±25***	42±23	33±22	58±22****	46±24	30±19****	39±30	54±20	49±22	38±23
MDSC, immunosuppression, protumorigenic	CD80	66±19	72±20	72±17	61±23	70±14	67±21	71±19	57±23*****	77±13*****	70±19	69±20
Inflammation regulatory factor	NF-κB-E[a]	49±40	43±36	46±37	48±42	36±44	47±34	48±42	36±35	47±38	23±40	50±37
	NF-κB-I[b]	70±21	67±22	67±23	74±17	80±27	63±22	72±21	69±13	69±26	69±27	69±21
Control on the CD4-derived Th17 and Tregs	TGF-β	63±25******	30±20******	40±24	58±32	37±10*******	28±24********	55±27********, ********	20±5	50±17	55±21	44±28

E epithelial lining, *I* inflammatory infiltrate

[a] Nuclear and cytoplasmic staining

[b] Cytoplasmic staining

*P=0.048; **P=0.034; ***P=0.017; ****P=0.026; *****P=0.028; ******P=0.005; *******P=0.054; ********P=0.056

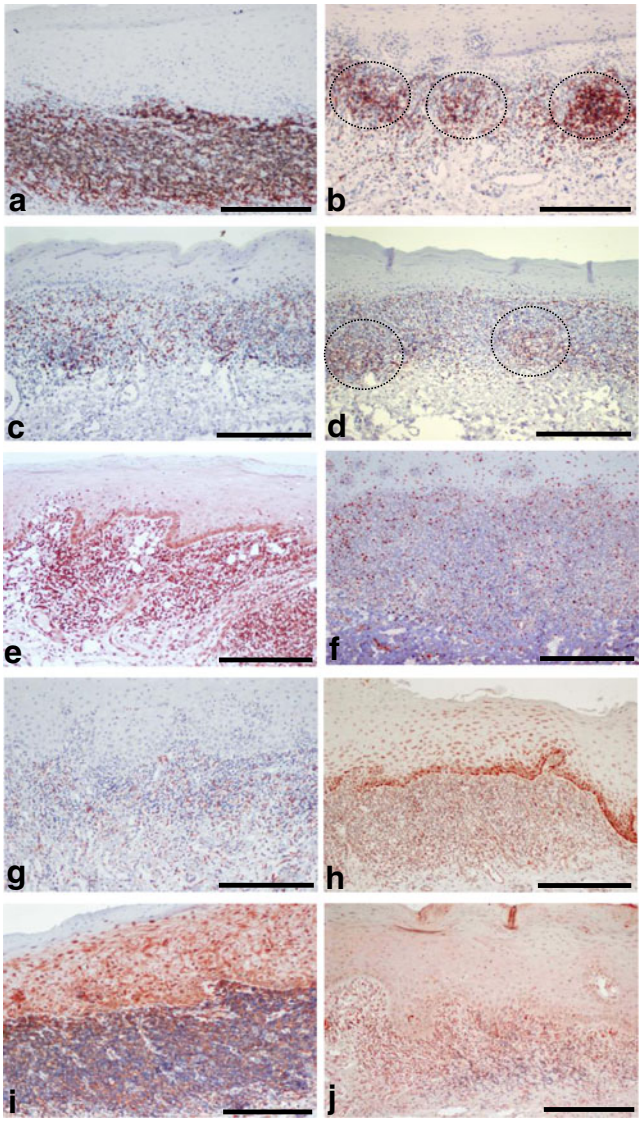

Fig 1 Oral lichen planus. The inflammatory cells and their different phenotypes and patterns of organization and expression patterns of NF-κB and TGF-β. CD4 lymphocytes appear with a general (**a**) and aggregated (**b**) patterns (aggregates are *encircled*); CD8 lymphocytes appear in a general (**c**) and aggregated (**d**) patterns (aggregates are *encircled*); CD4-derived Th17 (CD121a staining) lymphocytes (**e**); CD4-derived T regulatory cells (FOXP3) (**f**); TAM2 macrophages (CD163 staining) (**g**); CD80-positive lymphocytes (**h**); NF-κB (**i**) and TGF-β (**j**). Scale bar 200 μ

the frequency of either $CD8_{agg}$ or $CD8_{gen}$ and any of the selected clinical parameters.

Th17 lymphocytes (CD121a positive)

This is the first report on the expression and presence of this T cell phenotype in OLP. The staining pattern was membranous cytoplasmic, and it was abundant within lymphocytes in all cases, with a similar frequency for HK-OLP (81±20 %) and E-OLP (85±8 %).

Tregs (FOXP3 positive)

The staining pattern was nuclear, and only a few cells were identified, irrespective of the clinical form of OLP. There were no additional associations with the selected clinical parameters.

TAM2 cells (CD163 positive)

This is the first report on the expression and presence of this macrophage phenotype in OLP. Positively stained inflammatory cells had a spindle/dendritic morphology, and staining was membranous cytoplasmic. The relative frequency of these cells in HK-OLP (31±19 %) was the lowest compared to the other inflammatory cell types in this variant, and it was significantly lower than that in E-OLP (48±25 %) (P=0.017).

CD80-positive cells

Interestingly, the expression of these cells in the inflammatory infiltrate was almost exclusively found in those with a lymphocytic morphology. In addition to their expression within the inflammatory cells, the basal cells (if present) and/or the 2–3 suprabasal layers of the epithelium were usually positive as well. CD80-positive lymphocytes were more frequent in cases in which patients reported partial-to-no effect compared to patients with a good response to treatment (P=0.028).

NF-κB

Nuclear expression of NF-κB was found only in the lining of the epithelial cells, while the inflammatory cells exhibited solely a cytoplasmic expression. Staining within the lining revealed that keratinocytes had a similar frequency in cases of HK-OLP (49±40 %) and in E-OLP (43±36 %). Although the staining in the inflammatory infiltrate was more prominent (70±21 % in HK-OLP and 67±22 % in E-OLP), it is important to emphasize that it was almost entirely cytoplasmic and not nuclear.

TGF-β

A large proportion of inflammatory cells was TGF-β-positive in the cytoplasm, with a higher frequency in cases of HK-OLP (63±25 %) than in cases of E-OLP (30±20 %) (P=0.005).

Discussion

We designed and conducted this study in order to identify inflammatory cells with an immunosuppressive/

protumorigenic function (i.e., CD163-positive TAM2, CD80-positive MDSC, and Tregs) since attempts have been made to link the inflammatory infiltrate to the possible malignant transformation of the lesional epithelium [27]. Our main result was that, in addition to the extensive presence of CD4, CD8, and CD121a lymphocytes, there is a remarkable positivity of the immunosuppressive/protumorigenic inflammatory cells, CD163-TAM2, CD80-MDSC, and Tregs. However, the patterns of expression of those inflammatory cells, including the membranous expression of CD163, the expression of CD80 by lymphocytes, and the small number of Tregs, together with the lack of nuclear NF-κB in the inflammatory cells, suggest an extensive proinflammatory immune response and not an immunosuppressive/protumorigenic response in OLP. This is the first report to provide evidence showing a link between the profile of the inflammatory infiltrate, including the cells with an immunosuppressive phenotype and the clinical findings and treatment outcomes.

The membranous expression of CD163-TAM2 was found to function in a net proinflammatory direction in psoriasis and allergic and antihelminthic responses [28, 29], unlike in the context of tumor microenvironment, where their immunosuppressive/protumorigenic activity is activated by a fraction from the extracellular domain of CD163 that sheds into the extracellular matrix ("secreted CD163") [30]. Moreover, the protumorigenic activity of CD163-TAM2 was shown to be dependent on the presence of nuclear NF-κB [7]. Our current study results showed an almost complete lack of nuclear expression of NF-κB within the inflammatory cells, indicating a proinflammatory rather than a protumorigenic direction of the CD163 macrophages in OLP [13]. CD80 is usually expressed on the membrane of antigen-presenting cells, such as macrophages, dendritic cells, and other hematopoietic cells (e.g., MDSCs). Our findings demonstrated that the expression of CD80 within the inflammatory infiltrate was predominantly within cells of a lymphocytic morphology, reflecting a state of activation of these T lymphocytes [31]. Furthermore, the lack of expression of CD80 on MDSCs/macrophages attenuates the emergence of Tregs from their CD4 precursors, thus explaining the small number of Tregs seen in the inflammatory infiltrate in our study. In addition, we found an extensive presence of CD4-derived Th17 lymphocytes, CD121a-positive cells, as well as CD4 and CD8 T cells. Collectively, the CD163 membranous expression of TAM2; the lymphocytic expression of CD80; the almost lack of Tregs in parallel with extensive expression of CD4, CD8, CD121a, and TGF-β; and the lack of nuclear NF-κB in the inflammatory cells are highly suggestive that the lymphohistiocytic infiltration of OLP is not immunosuppressive/protumorigenic, but proinflammatory.

The interplay between immune cell phenotypes can be described at many levels, where activated epithelial NF-κB (at the epithelial–inflammatory interface) has the potential to induce the emergence of both CD4-derived Tregs [32] and CD80-positive cells [33]. CD80-expressing cells, in turn, can also contribute to the emergence of Tregs [7, 33, 34]. Under the control of TGF-β, Tregs are in a dynamic phenotypical interplay with CD4-derived Th17 cells [35]. In addition, Tregs are able to induce the development of CD163 macrophages and vice versa [36]. This interplay between the various inflammatory cells may be controlled by the key inflammatory related factors, NF-κB, and TGF-β, which were also extensively expressed in our study. In addition, these interrelations, although presented in a very simplified version without considering the plethora of chemokines and cytokines associated with inflammatory/immune cells, reveal that proinflammatory cells can induce cells with immunosuppressive function and vice versa. Local and systemic therapeutic agents used in OLP can serve as factors that may modify the pro- versus immunosuppressive immune response.

In terms of the selected clinical parameters, the CD163 macrophages were significantly more frequent in the E-OLP than in the HK-OLP type. Considering that the E-OLP type is more painful and symptomatic, affected patients are usually treated with a corticosteroid-based agent [37]. However, this type of therapy has only a limited rate of success according to the conclusions of a recent meta-analysis on the effectiveness of steroids in OLP [38]. This finding can be explained by the fact that the induction of CD163 macrophages has been shown to be upregulated by glucocorticoids with a potential effect of enhancing the proinflammatory response [30] and, assumedly, of slowing down the resolution of the clinical OLP lesions and the improvement of symptoms. Furthermore, Sugerman et al. [39] raised the possibility that long-term constant use of steroids (and assumedly other immune-suppressing agents, like tacrolimus) may massively suppress T cell activation, thereby contributing to the possible malignant transformation in OLP. We can now explain that this assumption by the emergence of an immunosuppressive microenvironment, which following a shift to a protumorigenic inflammatory response [12], might increase the risk of malignant transformation.

Although ours was a medium-sized study sample, it introduced a novel notion regarding the necessity for an alternative line of therapeutic agents to effectively treat OLP by modulating the immune response while avoiding the emergence of a protumorigenic polarization of the inflammatory response. This could be feasible, for example, by attenuating the inductive effect of the epithelial NF-κB on the interface inflammatory response and regulating the action of TGF-β [40]. In line with this, curcumin, a natural agent with the potential to modulate various molecular targets and cellular functions, including NF-κB and TGF-

β and those of inflammatory cells [41], would be a suitable therapeutic candidate for treating OLP. The benefits of curcumin in OLP have been recently reported in an empirical, small-sized study that showed its being efficacious in controlling the signs and symptoms of the disease [42]. The new findings, added by our current study to the understanding of the immunological forces, their potential directions, and clinicopathological correlations, can serve as the platform for future treatment approaches that may combine immune-modulating nutraceutical agents (i.e., curcumin) with conventional pharmacological ones. This can be expected to achieve a more effective clinical outcome for OLP, particularly when the therapeutic approach will be case tailored and adjusted to the individual composition of the inflammatory cell phenotypes and their architectural organization.

Acknowledgments The authors would like to thank Mrs. Hana Vered for her technical assistance. Ms. Esther Eshkol is thanked for here editorial assistance. The study was supported by the Dave and Sarah Babish fund, School of Dental Medicine, Tel Aviv University.

Conflict of interest None.

References

1. Roopashree MR, Gondhalekar RV, Shashikanth MC, George J, Thippeswamy SH, Shukla A (2010) Pathogenesis of oral lichen planus—a review. J Oral Pathol Med 39:729–734
2. Lodi G, Scully C, Carrozzo M, Griffiths M, Sugerman PB, Thongprasom K (2005) Current controversies in oral lichen planus: report of an international consensus meeting. Part 1. Viral infections and etiopathogenesis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 100:40–51
3. Zhang J, Zhou G, Du GF, Xu XY, Zhou HM (2011) Biologics, an alternative therapeutic approach for oral lichen planus. J Oral Pathol Med 40:521–524
4. Crincoli V, Di Bisceglie MB, Scivetti M, Lucchese A, Tecco S, Festa F (2011) Oral lichen planus: update on etiopathogenesis, diagnosis and treatment. Immunopharmacol Immunotoxicol 33:11–20
5. El Naggar AK, Reichart PA (2005) Proliferative verrucous leukoplakia and precancerous condition. In: Barnes L, Eveson JW, Reichart P, Sidransky D (eds) World Organization Classification of tumours. Pathology and genetics. Head and neck tumours. IARC, Lyon, pp 180–181
6. Tao XA, Xia J, Chen XB, Wang H, Dai YH, Rhodus NL et al (2010) FOXP3 T regulatory cells in lesions of oral lichen planus correlated with disease activity. Oral Dis 16:76–82
7. Sica A, Bronte V (2007) Altered macrophage differentiation and immune dysfunction in tumor development. J Clin Invest 117:1155–1166
8. Wang HY, Wang RF (2007) Regulatory T cells and cancer. Curr Opin Immunol 19:217–223
9. Lee YK, Mukasa R, Hatton RD, Weaver CT (2009) Developmental plasticity of Th17 and Treg cells. Curr Opin Immunol 21:274–280
10. Sato M, Tokuda N, Fukumoto T, Mano T, Sato T, Ueyama Y (2006) Immunohistopathological study of the oral lichenoid lesions of chronic GVHD. J Oral Pathol Med 35:33–36
11. Karin M, Greten FR (2005) NF-kappa B: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 5:749–759
12. Phipps KD, Surette AP, O'Connell PA, Waisman DM (2011) Plasminogen receptor S100A10 is essential for the migration of tumor-promoting macrophages into tumor sites. Cancer Res 71:6676–6683
13. Hallam S, Escorcio-Correia M, Soper R, Schultheiss A, Hagemann T (2009) Activated macrophages in the tumour microenvironment-dancing to the tune of TLR and NF-kappaB. J Pathol 219:143–152
14. Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-related inflammation. Nature 454:436–444
15. Lin WW, Karin M (2007) A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest 117:1175–1183
16. Allavena P, Germano G, Marchesi F, Mantovani A (2011) Chemokines in cancer related inflammation. Exp Cell Res 317:664–673
17. Sethi G, Sung B, Aggarwal BB (2008) Nuclear factor-kappaB activation: from bench to bedside. Exp Biol Med (Maywood) 233:21–31
18. Gannot G, Gannot I, Vered H, Buchner A, Keisari Y (2006) Increase in immune cell infiltration with progression of oral epithelium from hyperkeratosis to dysplasia and carcinoma. Br J Cancer 86:1444–1448
19. Hirshberg A, Amariglio N, Akrish S, Yahalom R, Rosenbaum H, Okon E et al (2006) Traumatic ulcerative granuloma with stromal eosinophilia—a reactive lesion of the oral mucosa. Am J Clin Pathol 126:522–529
20. Rogers HW, Sheehan KC, Brunt LM, Dower SK, Unanue ER, Schreiber RD (1992) Interleukin 1 participates in the development of anti-*Listeria* responses in normal and SCID mice. Proc Natl Acad Sci USA 89:1011–1015
21. Massimo H, Ohguro N, Nomura S, Hashida N, Nakai K, Tano Y (2008) Neutrophil chemotaxis and local expression of interleukin-10 in the tolerance of endotoxin-induced uveitis. Invest Ophthalmol Vis Sci 49:5450–5457
22. Matsumoto Y, Horiike S, Ohshiro M, Yamamoto M, Sasaki N, Tsutsumi T et al (2010) Expression of master regulators of helper T-cell differentiation in peripheral T-cell lymphoma, not otherwise specified, by immunohistochemical analysis. Am J Clin Pathol 133:281–290
23. Lamb RJ, Capocasale RJ, Duffy KE, Sarisky RT, Mbow ML (2007) Identification and characterization of novel bone marrow myeloid DEC205+ Gr-1+ cell subsets that differentially express chemokine and TLRs. J Immunol 178:7833–7839
24. Quaranta MG, Mattioli B, Spadaro F, Straface E, Giordani L, Ramoni C et al (2003) HIV-1 Nef triggers Vav-mediated signaling pathway leading to functional and morphological differentiation of dendritic cells. FASEB 17:2025–2036
25. Theilig F, Enke AK, Scolari B, Polzin D, Bachmann S, Koesters R (2011) TGF-β tubular deficiency of von Hippel-Lindau attenuates renal disease progression in anti-GBM glomerulonephritis. Am J Pathol 179:2177–2188
26. Kastelan M, Prpić Massari L, Gruber F, Zamolo G, Zauhar G, Coklo M et al (2004) The role of perforin-mediated apoptosis in lichen planus lesions. Arch Dermatol Res 296:226–230
27. Mignogna MD, Fedele S, Lo Russo L, Lo Muzio L, Bucci E (2004) Immune activation and chronic inflammation as the cause of malignancy in oral lichen planus: is there any evidence? Oral Oncol 40:120–130
28. Timmermann M, Buck F, Sorg C, Hogger P (2004) Interaction of soluble CD163 with activated T lymphocytes involves its association with non-muscle myosin heavy chain type A. Immunol Cell Biol 82:479–487
29. Ben-Neriah Y, Karin M (2011) Inflammation meets cancer, with NF-κB as the matchmaker. Nat Immunol 12:715–723

30. Fuentes-Duculan J, Suárez-Fariñas M, Zaba LC, Nograles KE, Pierson KC, Mitsui H et al (2010) A subpopulation of CD163-positive macrophages is classically activated in psoriasis. J Invest Dermatol 130:2412–2422
31. Yamazaki T, Akiba H, Koyanagi A, Azuma M, Yagita H, Okumura K (2005) Blockade of B7-H1 on macrophages suppresses CD4+ T cell proliferation by augmenting IFN-gamma-induced nitric oxide production. J Immunol 175:1586–1592
32. Long M, Park SG, Strickland I, Hayden MS, Ghosh S (2009) Nuclear factor-κB modulates regulatory T cell development by directly regulating expression of Foxp3 transcription factor. Immunity 31:921–931
33. Torihata H, Ishikawa F, Okada Y, Tanaka Y, Uchida T, Suguro T et al (2004) Irradiation up-regulates CD80 expression through two different mechanisms in spleen B cells, B lymphoma cells, and dendritic cells. Immunology 112:219–227
34. Yang R, Cai Z, Zhang Y, Yutzy WH, Roby KF, Roden RB (2006) CD80 in immune suppression by mouse ovarian carcinoma–associated Gr-1+CD11b+ myeloid cells. Cancer Res 66:6807–6815
35. Valmori D, Raffin C, Raimbaud I, Ayyoub M (2010) Human RORγt+ TH17 cells preferentially differentiate from naive FOXP3+Treg in the presence of lineage-specific polarizing factors. Proc Natl Acad Sci USA 107:19402–19407
36. Liu G, Ma H, Qiu L, Li L, Cao Y, Ma J et al (2011) Phenotypic and functional switch of macrophages induced by regulatory CD4+ CD25+ T cells in mice. Immunol Cell Biol 89:130–142
37. Lodi G, Scully C, Carrozzo M, Griffiths M, Sugerman PB, Thongprasom K (2005) Current controversies in oral lichen planus: report of an international consensus meeting. Part 2. Clinical management and malignant transformation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 100:164–178
38. Keenan AV, Ferraiolo D (2011) Insufficient evidence for effectiveness of any treatment for oral lichen planus. Evid Based Dent 12:85–86
39. Sugerman PB, Savage NW, Walsh LJ, Zhao ZZ, Zhou XJ, Khan A et al (2002) The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med 13:350–365
40. Mani H, Sidhu GS, Kumari R, Gaddipatil JP, Seth P, Maheshwari RK (2002) Curcumin differentially regulates TGF-β1, its receptors and nitric oxide synthase during impaired wound healing. Biofactors 16:29–43
41. Fu S, Kurzrock R (2010) Development of curcumin as an epigenetic agent. Cancer 116:4670–4676
42. Chainani-Wu N, Madden E, Lozada-Nur F, Silverman S (2011) High-dose curcuminoids are efficacious in the reduction in symptoms and signs of oral lichen planus. J Am Acad Dermatol. doi:10.1016/j.jaad.2011.04.022