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Dendritic cell chimerism in oral mucosa of transplanted patients affected by graft-versus-host disease

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OBJECTIVE: Graft-versus-host disease (GVHD) is one of the main complications after haematopoietic stem cell transplantation. Clinical features of GVHD include either an acute (aGVHD) or a chronic (cGVHD) condition that affects locations such as the oral mucosa. While the involvement of the host's dendritic cells (DCs) has been demonstrated in aGVHD, the origin (donor/host) and mechanisms underlying oral cGVHD have not been completely elucidated. In this study, we intend to determine the origin of DCs present in mucosal tissue biopsies from the oral cavity of transplanted patients affected by cGVHD.

METHODS: We purified DCs, from oral biopsies of three patients with cGVHD, through immunobeads and subsequently performed DNA extraction. The origin of the obtained DCs was determined by PCR amplification of 13 informative short tandem repeat (STR) alleles. We also characterised the DCs phenotype and the inflammatory infiltrate from biopsies of two patients by immunohistochemistry.

RESULTS: Clinical and histological features of the biopsies were concordant with oral cGVHD. We identified CD11c-, CD207- and CD1a-positive cells in the epithelium and beneath the basal layer. Purification of DCs from the mucosa of patients affected by post-transplantation cGVHD was >95%. PCR-STR data analysis of DCs DNA showed that 100% of analysed cells were of donor origin in all of the evaluated patients.

CONCLUSION: Our results demonstrate that resident DCs isolated from the oral tissue of allotransplanted patients affected by cGVHD are originated from the donor. Further research will clarify the role of DCs in the development and/or severity of oral cGVHD.

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Keywords: chimerism; dendritic cells; graft-versus-host disease; oral mucosa

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Introduction

Allogeneic haematopoietic stem cell transplantation (AHSCT) consists of replacing the patient's defective haematopoietic system by an human leucocyte antigen (HLA)-compatible and functional haematopoietic system from a matched donor (1). This replacement is progressive, which means that cells from the recipient's haematopoietic system and those from the donor coexist during a given period of time. This cellular dualism in a patient is called *chimerism*, which can be complete (haematopoiesis is 100% from donor) or partial (mixed haematopoiesis from both recipient and donor) (2, 3). The chimeric status in allotransplanted patients has a prognostic significance and is strongly associated with immunological events such as graft-versus-host disease (GVHD), graft-versus-tumour effect (GVT) and graft loss (4).

During GVHD development, immunocompetent cells that are present in the graft generate an immune response directed against the host's healthy tissues. This response is generated despite the HLA compatibility and the use of immunosuppressive therapy (5). Clinically, GVHD may occur in two main forms: an acute manifestation of the disease (aGVHD) and a chronic one (cGVHD). Currently, there is no longer a time-based split of these GVHD manifestations, where the onset of both forms can appear before or after 100 days after AHSCT,

and the classification is mainly based on their specific clinical features (6). Although both GVHD types cause injury to the host's healthy tissues, the immunological processes involved in their development appear to be different (7).

A recent study supports the notion that three different diseases can constitute oral cGVHD manifestations: oral mucosal alterations, salivary dysfunction and limited mouth opening (8). One of the most frequently affected locations in patients with GVHD is the oral mucosa, resulting in poor quality of life and a serious challenge for dental clinicians. The growing number of AHSCT-treated patients with oral GVHD is increasing the number of these conditions encountered during regular dental practice. Oral mucosa affected by cGVHD is characterised by areas with whitish, hyperkeratotic and reticulated regions mixed with red, erosive plaques and ulcerations. These manifestations can range from very limited extension to more extensive disease with painful ulcerations. GVHD oral lesions usually mimic autoimmune diseases of natural occurrence such as lichen planus and systemic lupus erythematosus. Although these lichenoid inflammations can involve all intraoral sites, they particularly affect the tongue and buccal mucosa (9–11). In some cases, the lips can also be affected. Additionally, GVHD oral lesions can become infected with oral candidiasis and have also been associated with pre-cancerous and malignant oral lesions (12, 13).

It has been reported that donor T cells are responsible for tissue injury during GVHD (14). Furthermore, dendritic cells (DCs) have been shown to play an important role in the activation and maintenance of this important AHSCT complication (15, 16). Thus, the identification of the origin of the cell population implicated in the alloimmune responses in the oral mucosa (as either from donor or recipient) would improve our understanding of the immune mechanisms involved in this pathological condition. The latter determination would enable the prevention of onset and/or control the severity of GVHD (17).

Dendritic cells are professional antigen-presenting cells (APCs) that can efficiently uptake both self and exogenous antigens, process them into peptides and present them to T cells in the context of the major histocompatibility complex (MHC) class I and class II (18, 19). DCs can migrate from peripheral tissues and activate naïve T cells in secondary lymphoid organs, thereby triggering an adaptive immune response (20, 21). Current evidence shows that the host's DCs are sufficient to trigger GVHD in its acute form. However, the origin and role played by both donor and host DCs in cGVHD are not completely understood, particularly in peripheral tissues such as the oral mucosa (22). Previous studies have shown that allotransplanted patients achieve complete DCs chimerism in peripheral blood within 2 weeks after the transplantation procedure (23), whereas in uninjured skin, the DC turnover occurs within the first 100 days after AHSCT (24). Orti-Raduan et al. (25) described a higher recruitment of Langerhans' cells in the oral mucosa of patients transplanted with cGVHD than in patients without cGVHD and normal samples. Moreover, Hasseus et al. (26) showed that cGVHD and oral lichen planus (OLP) have marked differences at the cellular level despite their similar clinical appearance, especially regarding the concentration of Langerhans cells in the affected tissues. However, a recent study showed no differences in infiltration of $CD1a^+$ cells between oral cGVHD and OLP (27). Additionally, the donor/host origin of DCs from oral cGVHD remains poorly studied. In this context, the study of DCs populations and particularly their chimerism at different sites affected by cGVHD, such as the oral mucosa, might provide important insights into the pathogenesis of this condition.

Here, we evaluated the origin of DCs infiltrating the oral mucosa of patients affected by cGVHD. We purified DCs derived from the oral mucosa of three transplanted patients affected by cGVHD with non-ablative conditioning regimen (reduced-intensity conditioning). The origin of the DCs was determined trough DNA isolation and amplification by PCR of short tandem repeats (STRs). In addition, histopathological analysis of the patients' mucosa samples showed an inflammatory infiltrate correlated with a clinical manifestation of cGVHD. Importantly, our results demonstrated that in all of the studied patients, 100% of the DCs isolated from biopsies of oral mucosa affected by cGVHD originated from the donor. Further studies are required to establish how donor DCs may contribute to the development, maintenance and severity of oral cGVHD.

Patients and methods

Patient selection

Five adult patients (three males and two females), diagnosed with moderate-to-severe oral mucosal cGVHD after non-ablative AHSCT treatment, were recruited from the Cell Therapy Unit at the University of Chile Clinical Hospital. The inclusion criteria were as follows: the engraftment and haematological recovery within the first 15 days after the transplantation procedure in the selected individuals, and clinical manifestations of oral mucosal cGVHD such as erythema, lichenoid changes, ulcerations, mucoceles, hyperkeratosis and pseudomembrane among others (6, 11, 28). Severity of oral mucosal cGVHD manifestations was established following the NIH criteria for organ scoring of cGVHD, where score 0, no symptoms; score 1, mild symptoms with disease signs but not limiting oral intake significantly; score 2, moderate symptoms with disease signs with partial limitation of oral intake; and score 3, severe symptoms with disease signs on examination with major limitation of oral intake (6). The histological criteria for the diagnosis of oral mucosal cGVHD were as follows: localised or generalised epithelial changes (lichenoid interface inflammation, exocytosis and apoptosis) (6, 29). The Bioethical Committee of the University of Chile Clinical Hospital approved the protocol used in this study, and all the patients signed an informed consent before enrolment.

Oral mucosa biopsies and DC purification

Patients were locally anaesthetised, and one excisional biopsy from the oral mucosa affected by cGVHD was obtained from each of the three patients (approximately $0.5 \times 0.5 \times 1.5$ cm in size). Biopsies were taken from the internal surface of the cheek (two biopsies) and dorsal tongue (one biopsy). Subsequently, specimens were incubated for 24 h in RPMI-1640 medium supplemented with 3% FBS and collagenase (2 mg/ml). Then, the cell suspensions were separated by density gradient centrifugation (Ficoll–Paque® PLUS, Stem

Cell Technologies Vancouver, BC, Canada). The target cell layer was collected by pipetting and resuspended in PBS. Finally, DC purification was performed using a CD1a micro beads kit with two rounds of purification according to the manufacturer's recommendations (MACS, Miltenyi Biotec, Teterow, Germany).

Histological and immunohistochemical analysis

A fragment of each biopsy specimen was fixed with 4% paraformaldehyde for 8 h, dehydrated with graded alcohols (75–100%) and paraffin-embedded following the standard procedure. Next, 4-μm-thick slices were cut from these blocks using a microtome and were mounted on a glass slide for histological analysis and immunohistochemistry. Tissue sections were stained with haematoxylin and eosin (H&E) to identify histologic manifestation of oral cGVHD such as acanthosis, spongiosis, lichenoid interface and mononuclear cells infiltrate. For inmunohistochemical analysis, sections were treated with proteinase K for antigen retrieval followed treatment with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity and thereafter washed twice with PBS. Finally, the sections were incubated with horse serum for 1 h and then with anti-hCD11c, anti-hCD207, anti-hCD1a and anti-hCD3 (Abcam, London, UK) for an additional hour at 37°C. Next, sections were washed twice with PBS before incubation with the secondary antibody for 20 min at 37°C. After further washing with PBS, the samples were incubated with ABC solution (Vectastain ABC Kit; Vector Laboratories, Burlingame, CA, USA) for 20 min at 37°C, washed twice with PBS and the reaction developed using DAB (Vector Laboratories, Burlingame, CA, USA). Samples were analysed under light microscopy at different magnifications (4×, 10×, 40× and 100×).

Flow cytometry

The purity of the isolated DCs was assessed by flow cytometry analysis. Briefly, cells were centrifuged at 400 ***g*** for 5 min at 4°C, washed with PBS and incubated on ice with an anti-CD1a-PE-conjugated monoclonal antibody (eBioscience, San Diego, CA, USA) for 30 min. After being washed twice with PBS, the specimens were centrifuged at 400 ***g*** for 5 min at 4°C and fixed in 100 μl of PBS 1% paraformaldehyde. Samples were acquired on a FACScan flow cytometer (BD Bioscience, San Jose, CA, USA) and analysed with the CellQuest software (BD Bioscience).

DNA extraction

The DNA of purified cells was isolated with the commercial kit 'JETQUICK' (GENOMED, Löhne, Germany). Briefly, cells were resuspended in 200 μl PBS, and 200 μl of cell lysis buffer containing 2 mg/ml proteinase K was added. Incubation proceeded at 70°C for 10 min, and 200 μl of absolute ethanol was added. The total volume was transferred to a silica column and centrifuged at 13 000 ***g*** for 2 min. The column was washed twice with ethanol, and the DNA was subsequently eluted from the column by adding 100 μl of water pre-heated at 70°C.

Measurement of chimerism

The isolated DNA was amplified through PCR using the following eight different STR alleles: D16S539, D7S820, D13S317, CSF1PO, F13A01, TPOX, TH01 and F13B (Table 2). The primers for each of the STR alleles were labelled with the FAM-3 cys fluorophore (GenePrint STR system kit; Promega Inc. Madison, WI, USA) that incorporates to the PCR product. The PCR program indicated by the manufacturer (Promega Inc.) was used. The informative alleles were determined for each patient and were used to establish DC chimerism. PCR fragments were analysed in a capillary electrophoresis device that detects the size and the fluorescence emitted by the amplified PCR product.

Results

Five patients recruited for this study underwent non-ablative transplantation and presented complete chimerism in

Table 1 Demographic and clinical characteristics of patients selected for oral DCs chimerism analysis

	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*	*Patient 5*
Age (years)	40	57	32	20	18
Gender (donor/receptor)	Female/female	Male/male	Male/male	Male/male	Male/female
Disease	NHL anaplastic	CLL	NHL skin B	AML	Anaplastic anaemia
Disease status (at biopsy time)	Complete remission	Complete remission	Complete remission	Cytogenetic remission	Complete remission
Graft type	PBSC	PBSC	PBSC	PBSC	PBSC
SCT conditioning protocol	Cy-Flu	Cy-Flu	Cy-TBI	Cy-Flu	Cy-Flu
Time of mucosal biopsy (days post-SCT)	728	542	181	438	310
Acute GVHD	−	−	−	−	+
Chronic GVHD	+	+	+	+	+
Oral cGVHD	+	+	+	+	+
Peripheral blood T-cell chimerism (>45 days post-SCT)	Complete	Complete	Complete	Complete	Complete
CD34 × 10^6/kg	5.8	3.1	5.6	3.5	2.0
CD3 × 10^8/kg	1.8	2.2	3.0	3.0	4.3

NHL, non-Hodgkin lymphoma; SCT, stem cell transplantation; CLL, chronic lymphocytic leukaemia; Cy-Flu, cyclophosphamide – fludarabine; PBSC, peripheral blood stem cells; TBI, total body irradiation; GVHD, graft-versus-host disease; AML, acute myeloid leukaemia; cGVHD, chronic graft-versus-host disease; DC, dendritic cells.

peripheral blood starting from 15 days after AHSCT treatment (Table 1). All of the patients were affected by oral mucosal cGVHD at the time of mucosal biopsy, when the peripheral blood chimerism status continued to originate from the donor. One patient, who also presented with GVHD affecting the lungs, was treated with cyclosporine to attain immunosuppression and suffered recurring infections as a side effect of this therapy. All of the patients were

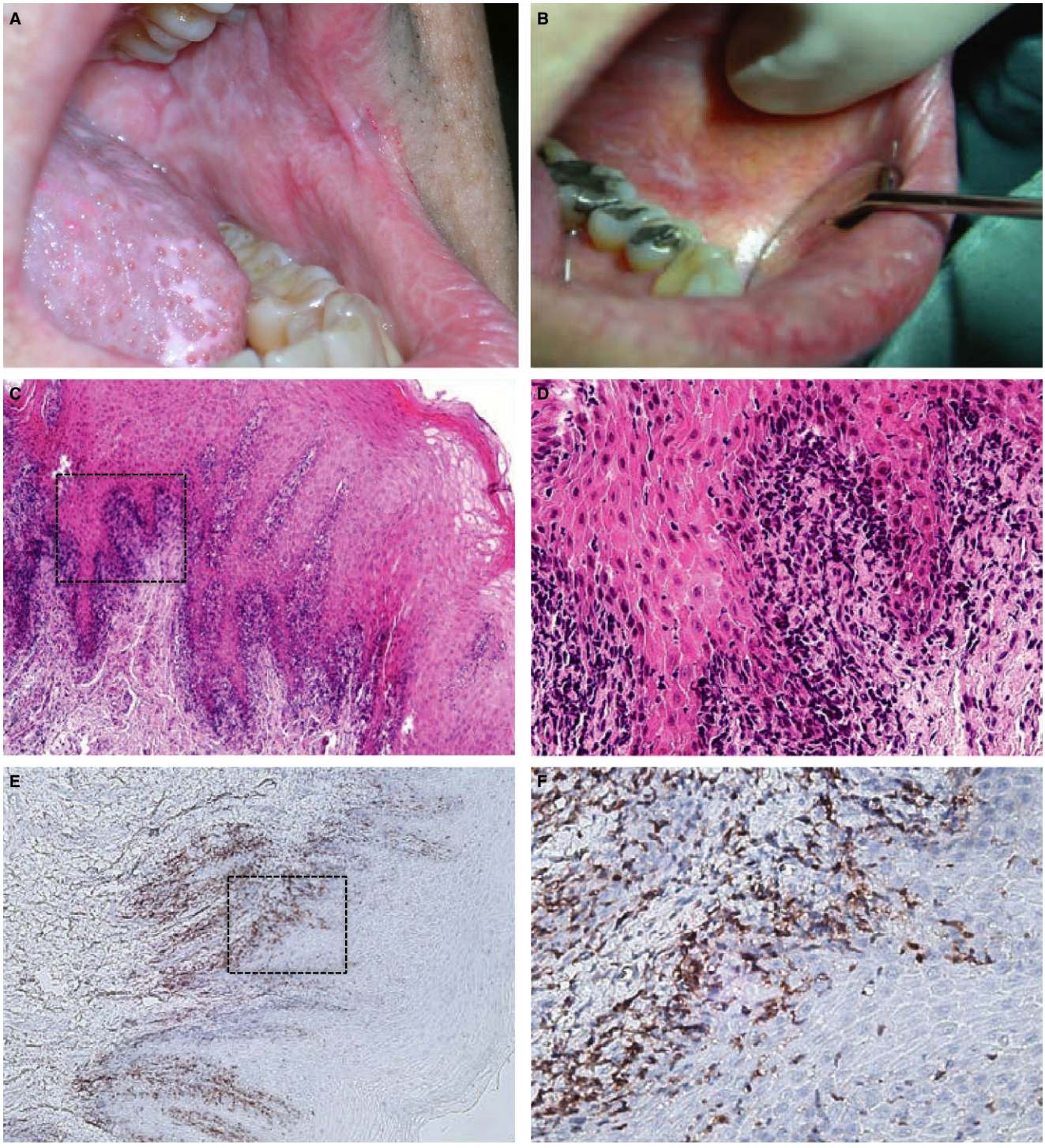

Figure 1 Clinical and histological characteristics of patients with oral manifestations of chronic graft-versus-host disease (cGVHD). (A and B) Clinical images from two of five analysed patients before the biopsy (case 1 and case 2, respectively). (C) Histological view of the oral mucosa showing inflammatory features and the altered tissue architecture. 4× magnification. (D) Magnification of the previous image. A subepithelial mononuclear lymphocytic infiltrate correlated with a multilayered squamous epithelium with acanthosis could be seen, which is consistent with the histological features of cGVHD. 40× magnification. (E and F) The lymphocytes presence is additionally demonstrated by CD3 positive immunostaining. 10× and 40× magnifications, respectively.

180 days post-AHSCT (Table 1). Clinically, patients' oral mucosa showed several whitish and reticulated areas mixed with red contoured ulcerations and mainly affecting the cheeks and the dorsal tongue (Fig. 1A,B). Histological analysis of the processed biopsies demonstrated a lichenoid reaction associated with the clinical manifestation of cGVHD. Haematoxylin–eosin staining of the oral mucosa sections shows the epithelium with signs of spongiosis and

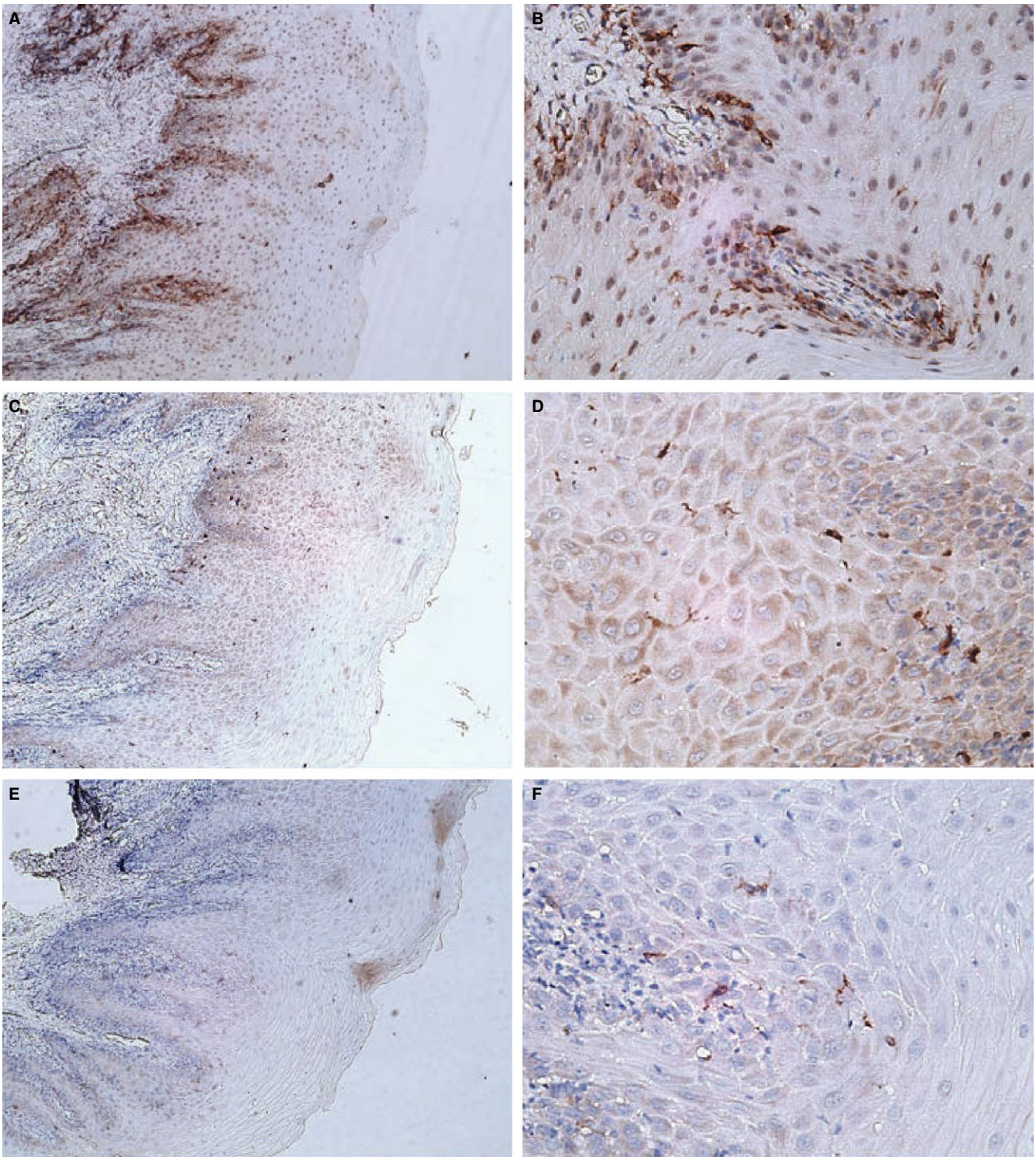

Figure 2 Dendritic cells (DCs) with different phenotypes are present into the oral mucosa affected by chronic graft-versus-host disease. (A and B) Immunohistochemical detection of CD11c-positive DCs was observed in the basal layer of the epithelium and into the connective tissue beneath the epithelium. (C and D) Positive cells for the Langerhans' cell marker CD207 (Langerin) were preferentially located into the basal and intermediate layers of the epithelium. (E and F) CD1a-positive DCs were detected in the basal layer of the epithelium. 4× and 40× magnifications, respectively

acanthosis with deep papillary projections (Fig. 1C), all histopathological characteristics concordant with oral cGVHD (29). Lymphocytic infiltration is shown in the basal cell layer and beneath the epithelium as lichenoid features (Fig. 1D). The presence of an abundant amount of T cells along the basal layer of the oral mucosa was additionally demonstrated by CD3-positive immunostaining (Fig. 1E,F). Immunohistochemical detection of CD11c positive cells was observed in the basal layer of the epithelium and into the connective tissue beneath the epithelium (Fig. 2A,B). Langerin (CD207) immunostaining showed positive cells located only in the basal and intermediate layers of the epithelium (Fig. 2C,D). CD1a-positive cells were detected in the basal layer of the epithelium (Fig. 2E,F).

The purification of DCs from patients' biopsies using immunomagnetic beads enriched the $CD1a^+$ population from ~1% in the initial cell suspension to more than 95% of purity after two rounds of selection (Fig. 3A). We assumed that the same proportion of DCs from the host and donor was lost during the purification procedure. It is important to note that we cannot discard the presence of other APCs subsets, especially plasmacytoids DC that could be the 10–15% of resting cells. The number of isolated DCs (2–2.5×10^5 cells) was sufficient for DNA recovering and further PCR-STR analysis.

Figure 3B,C shown the results of patients' DNA amplification by capillarity electrophoresis and polyacrylamide gels electrophoresis revealed with silver staining, respectively. Each peak (Fig. 3B) or electrophoretic band (Fig. 3C) obtained after the PCR represents one allelic form of the studied STR gene. For example, in case 1, the patient carried alleles 6 and 8 of the TH01 STR, and the donor carried allele 6 only. Therefore, this gene TH01 allowed us to discriminate between donor and host genetic profiles for this specific patient. These allelic differences enabled us to compare the amplification profiles of the post-ACTH DCs samples with the respective patterns from the host/donor pairs analysed in this work. Finally, the analysis of obtained data showed that 100% of DCs present in the mucosa of patients affected by post-transplantation cGVHD were of donor origin in all evaluated patients (Fig. 3B,C and Table 2), indicating that the DC population in the oral mucosa affected by cGVHD has a complete chimerism.

Discussion

In the present study, we used PCR-STR to determine the origin and chimerism status of resident DCs from the oral mucosa of three patients who presented with cGVHD after undergoing allotransplantation. Our results clearly demonstrated that DCs in the samples analysed from oral mucosa affected by cGVHD were of donor origin in a complete chimerism for this cell type. Despite we studied a small sample of patients and cannot state this is a universal phenomena, we can establish that complete chimerism of resident DCs in oral mucosa affected by cGVHD as a phenomena that occurs in some patients affected by this condition. Although it is not possible to infer that these donor DCs are involved in the initiation of cGVHD, they might contribute to the development, maintenance and severity of the disease as described in the murine models (30).

In humans, it has been demonstrated that the population of $CD11c^+$ DCs from peripheral blood of patients with cGVHD is exclusively of donor origin, while the persistence of host DCs was observed in some patients without GVHD (31). Here, we found $CD11c^+$ cells at the basal layer of the epithelium and into the connective tissue of the affected oral mucosa. Additionally, CD207- and CD1a-positive cells were found into the epithelium and in the basal layer of the epithelium, respectively, establishing the presence of different DCs phenotype in these lesions. In a study conducted by Chan et al. (22), a correlation between persistence of the recipient's DCs and the development of acute and chronic GVHD was demonstrated, while Perruche et al. (32) reported that the recipient's DCs do not influence the onset of the lesions found in cGVHD.

The repopulation of the patient's tissues with donor DCs begins within 10 days of transplantation (33). However, the recipient's DCs may remain in uninjured tissues for over 1 year (34, 35). Under inflammatory conditions, host tissue-infiltrating DCs are rapidly replaced by the donor's DCs from the blood (36, 37). Here, the studied patients presented with complete chimerism in peripheral blood at 30 days after AHSCT and showed features of GVHD over 180 days after AHSCT. This outcome could explain why it is possible to find purely donor DCs in inflammatory conditions and to establish complete DCs chimerism as a common feature in oral mucosa affected by cGVHD. Moreover, the absence of

Table 2 STR alleles used to discriminate between receptor and donor

	STR alleles							
Cases	*D16S539*	*D7S820*	*D13S317*	*CSF1PO*	*F13A01*	*TPOX*	*TH01*	*F13B*
Patient 1	11, 12	8, 10	11, 11	10, 11		8, 9	6, 8	
Donor 1	11, 12	11, 12	11, 11	10, 11	ND	8, 8	6, 6	ND
Post-DCs 1						8, 8	6, 6	
Patient 2	11, 12	11, 11	9, 13	11, 12		8, 8		
Donor 2	11, 12	11, 11	9, 13	9, 11	ND	8, 12	ND	ND
Post-DCs 2				9, 11		8, 12		
Patient 3	11, 12	8, 12	11, 12		6, 7	8, 10		6, 6
Donor 3	11, 12	8, 10	12, 13	ND	6, 6	8, 10	ND	6, 10
Post-DCs 3					6, 6			6, 10

ND, not determined; DC, dendritic cells; STR, short tandem repeat.

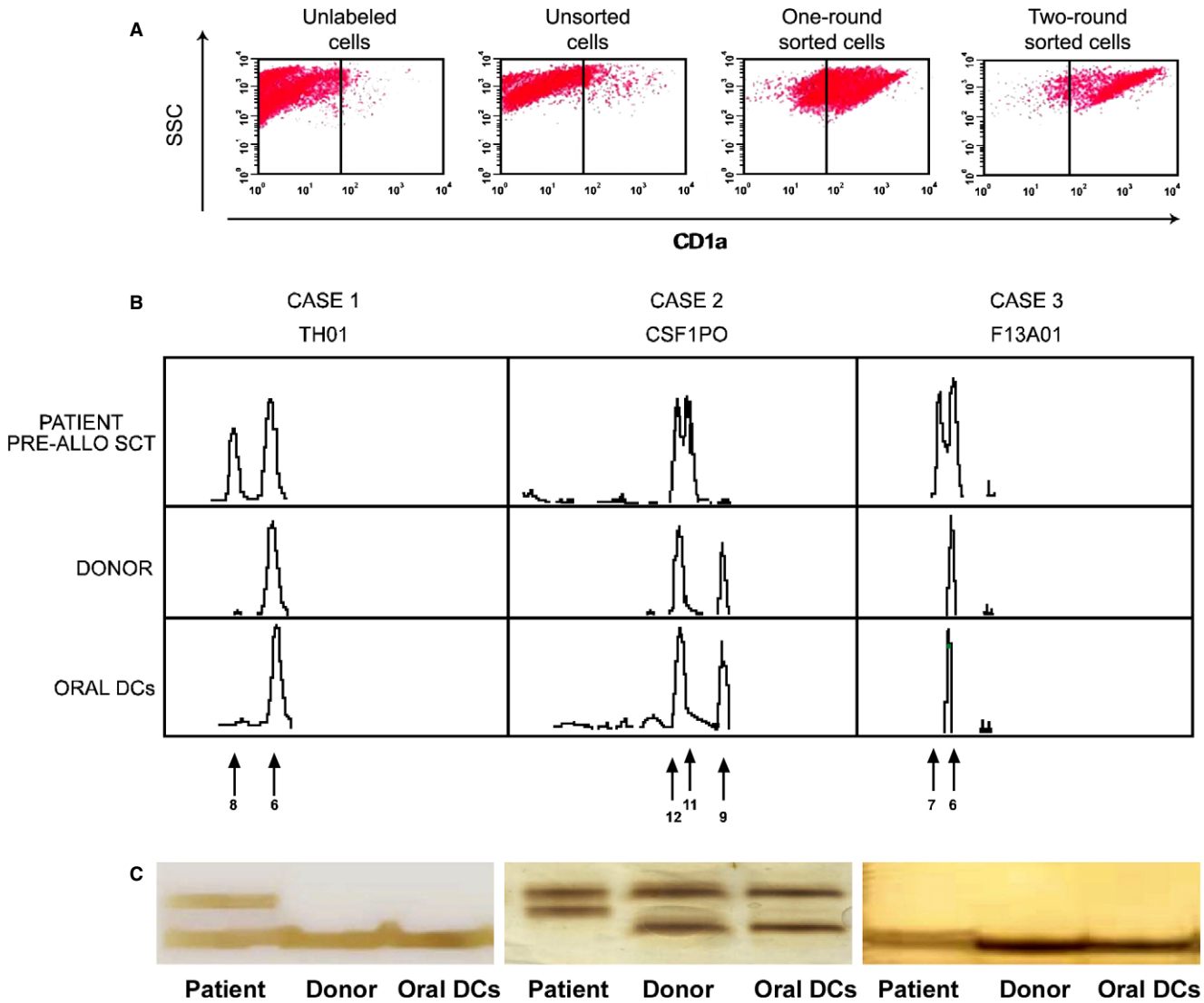

Figure 3 Dendritic cells (DCs) from oral mucosa affected by chronic graft-versus-host disease (cGVHD) are of donor origin. (A) Flow cytometry analysis of the purity of isolated DC populations from oral mucosa biopsies of allotransplanted patients affected by cGVHD. (B) DC chimerism was determined through the use of informative short tandem repeat (STR) alleles. A capillary electrophoresis analysis of PCR products from CSF1PO, TH01 and F13A01 STRs showed differences between receptors (patients) and donors, establishing the patient's chimeric state. The arrows shown the specific allele numbers identified as informative ones for each patient and STR. (C) Representative gels showing the informative STR alleles for each patient (CSF1PO, TH01 and F13A01).

DCs from the recipient in injured tissues is due to the fact that these cells are also described as T-cell targets during GVHD pathogenesis (38). In addition, tissue injury persists due to the recruitment of new immune-inflammatory cells, including DCs, to peripheral tissues. Although the immune mechanisms responsible for the contribution of allogeneic DCs to the development of cGVHD are not clearly understood, they might be directly involved in the activation of T cells through alloantigen presentation as in acute GVHD or indirectly through inflammatory cytokines secretion to reactivate, maintain and expand memory T cells (39). Based on the present study, early T-cell activation cannot be discarded. Such early activation would generate memory T cells that mediate tissue injury regardless of the presence of new DCs arriving to the inflamed tissue. In fact, Teshima et al. (40) demonstrated that the presence of memory T cells and not the alloantigen presentation by APCs is sufficient to cause tissue damage. In addition, Matte et al. (30) emphasised the role of donor DCs in the extent of tissue injury.

Myeloid-derived DCs (mDC) have a high functional plasticity and depending on the stimulus could generate pro-inflammatory immune responses (41, 42). The number of inflammatory mDCs is higher in oral mucosa than in healthy tissues, which is similar to findings in other autoimmune pathologies (26). These cells express high levels of costimulatory molecules such as CD80 and CD86 (27), suggesting that the antigen presentation of host tissue-derived antigens by DCs is occurring at the tissue level. The expression of 'alloantigens' involves minor histocompatibility antigens (mHAgs) that differ between the donor and host. In addition to an emerging inflammatory process, this phenomenon might explain the development of GVHD in

individuals having reached complete DC chimerism within the tissues. Importantly, other donor cell populations found in the peripheral blood of transplanted individuals with cGVHD are plasmacytoid DCs (pDC) and $CD4^+$ $CD25^+$ regulatory T cells (43). These cell types have regulatory roles and induce tolerance by several mechanisms including the development of micro-chimerism and the induction of anergy and T-cell apoptosis (44). In fact, pDC may be used as an alternative therapy instead of immunosuppressant drugs in inflammatory or 'alloimmune' processes such as GVHD (45).

Currently, the use of immunosuppressive therapeutic schemes that prevent either graft rejection or the onset of acute GVHD favours the persistence of mixed chimeric statuses. The mechanisms underlying this condition include the inhibition of DC migration towards draining lymph nodes. This inhibition would affect donor/host DC turnover in peripheral tissues and eventually delay the onset of GVHD after immunosuppressive therapy. While none of the patients developed GVHD before 100 days post-AHSCT, their clinical events appeared subsequent to the withdrawal of immunosuppressive therapy.

It is interesting to assess the eradication of cells that are involved in the development of GVHD. The inflammatory process triggered during GVHD would cause mDC recruitment, antigen capture and T-cell stimulation by these cells. Additionally, loss of tolerance would trigger an increased inflammatory process and a generalised injury involving specific tissues or even an entire organ. Although donor mDCs may contribute to tissue damage in inflammatory conditions, they may be 're-educated' to generate a specific tolerogenic response and be able to control the onset and/or the severity of GVHD (46). Tolerance to tissue alloantigen, which is generated by DCs, is naturally attained in the absence of infectious or inflammatory stimuli (47). During this condition, DCs capture soluble antigens or antigens from apoptotic bodies and migrate to lymph nodes, thereby silencing reactive lymphocytes or generating new regulatory lymphocytes (48). Therefore, generation of a therapy against GVHD requires identification of specific antigens that are not expressed by cancer cells to avoid affecting GVT activity and to minimise recurrent infections caused by immunosuppression.

In conclusion, our results demonstrate that myeloid DCs present in oral tissues affected by cGVHD were of donor origin. Although no relationship was established between donor DCs and GVHD appearance, further research may address the specific role of DCs in the generation and development of GVHD in addition to possible therapeutic strategies aimed to control this disease and improve patients'quality of life.

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Conflict of interest

None of the authors have any potential financial conflict of interest related to this manuscript.