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Systemic leukocyte activation in patients with central giant cell lesions

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BACKGROUND: Central giant cell lesion (CGCL) is a reactive lesion of the jaws with an associated inflammatory infiltrate. Since cell circulation allows for intense communication between different compartments in the body, we investigated whether the CGCL would lead to phenotypic and/or functional changes in circulating leukocytes.

METHODS: We obtained lymphocytes and monocytes from CGCL patients and control subjects, to evaluate cytokine and adhesion molecule expression using flow cytometry.

RESULTS: Our results revealed that $CD4^+$ T cells and $CD14^+$ monocytes from CGCL express elevated levels of CD11a and CD11b, respectively, when compared with controls. The frequencies of $CD4^+$ T cells expressing interferon (IFN)-γ and tumor necrosis factor (TNF)-α and the frequencies of $CD4^+$ and $CD14^+$ cells expressing interleukin (IL)-10 were increased in CGCL group, when compared with controls.

CONCLUSIONS: Our data indicate that, although CGCL is a localized lesion, the patients show systemic functional alterations in circulating leukocytes, suggesting their role in the inflammatory pathogenesis of CGCL.

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Introduction

Central giant cell lesion (CGCL) is a reactive bone lesion that occurs mainly in the mandible, characterized by the presence of multinucleated osteoclast-like giant cells in a background of oval- to spindle-shaped mononuclear cells positive for the macrophage marker CD68 (1, 2). An inflammatory infiltrate containing lymphocytes, plasma cells, macrophages, and neutrophils is often associated with CGCL and may play a role in tissue destruction and repair.

Leukocyte recruitment to inflammatory sites involves interactions between circulating leukocytes and vascular endothelium mediated by adhesion molecules. L-selectin (CD62L), leukocyte function-associated antigen 1 (LFA-1; CD11a/CD18), and macrophage antigen 1 (Mac-1; CD11b/CD18) are examples of adhesion molecules critical for controlling cell recruitment (3). Interactions between these molecules and their endothelial ligands are critical for leukocyte homing to different body compartments.

The fate of an immune response is under the control of cytokines, since these molecules coordinate most immunologic processes such as differentiation, antigen presentation, cellular recruitment and activation, and adhesion molecule expression. Tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and interferon (IFN)-γ are classical mediators of inflammation, controlling leukocyte recruitment and/or eliciting cellular immune responses (4). As opposed to inflammatory cytokines, IL-10 induces a decrease in major histocompatibility complex (MHC)-II and adhesion molecules expression, controlling inflammatory reactions (5). Differentiation of $CD4^+$ T cells into Th1 or Th2 is also an important regulatory mechanism of inflammatory reactions since Th1-derived cytokines, such as IFN-γ, induce inflammation, whereas Th2 cells can participate in its control by inhibiting Th1 cells and secretion of anti-inflammatory cytokines (6). The complex interactions established between cytokines and adhesion molecules are critical in

determining the establishment, as well the development of inflammatory lesions.

Communication between different compartments in the organism through physiologic cell circulation may lead to alterations in peripheral blood cells because of localized inflammatory lesions (7). Thus, it is important to determine the nature of the overall systemic immune response in individuals with CGCL pathologies to increase our understanding of the dynamics of disease progression and its relation with the overall immune system.

Materials and methods

Patients

This study was approved by the Ethical Committee of the Universidade Federal de Minas Gerais. A total of eight individuals, four patients with CGCL, previously diagnosed by histopathologic analysis of incisional biopsies, and four healthy donors (control group) were evaluated. The age ranges for the CGCL and control groups were statistically equivalent (mean ± SD: 26.75 ± 19.46 and 35.5 ± 11.21 years, respectively). In both groups, there were two females and two male individuals. All cases of CGCL were located in mandible and treated by curettage. Patients presented with good general health and did not report any previous episodes of CGCL. All patients were submitted to detailed hematologic and biochemical evaluation, including parathyroid hormone level, alkaline phosphatase activity, serum calcium and phosphorus concentrations. These parameters were within the reference ranges, excluding the occurrence of other diseases, which could compromise the final diagnosis of CGCL. Venous blood collections were performed a few minutes before complete tumor curettage in order to avoid any influences of the surgery in the circulating profile and allowing for the collection of material while active lesion is still present.

Monoclonal antibodies

The following antihuman monoclonal antibodies were used for flow cytometric analysis: fluorescein isothiocyanate (FITC)-labeled CD4 (Pharmingen, San Diego, CA, USA), CD8, CD14, and CD68 (Caltag, Burlingame, CA, USA); phycoerythrin (PE)-labeled LFA-1/CD11a and L-selectin/CD62L (Pharmingen), ICAM-1/CD54, IL-4, IL-10, IL-12, IFN-γ, and TNF-α (Caltag); biotin CD11b/Mac-1 (Pharmingen); CyChrome-labeled CD4 (Caltag); streptavidin RPE-conjugated (Dako, Fort Collins, CO, USA), and appropriately labeled irrelevant isotype-matched control antibodies from the same suppliers.

Immunofluorescence

Approximately 20 ml of blood from each individual were drawn by venipuncture into heparinized tubes. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque gradient (Pharmingen). *Ex vivo* analysis were performed to evaluate the expression of adhesion molecules by lymphocytes and monocytes using double-labeling immunofluorescence followed by flow cytometric analysis, as previously performed by us (8). Briefly, 2×10^5 PBMC were plated in 96-well culture plates, stained for surface markers (CD4, CD8, CD14 or CD68, and CD11a, CD11b, CD54 or CD62L) for 15 min, washed with phosphate-buffered saline (PBS)/bovine serum albumin (BSA) 1%, and fixed using 2% formaldehyde.

To access cytokine expression, we performed intracellular staining (8). PBMC (2×10^5 cells) were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin and 100 g/ml gentamicin in 96-well culture plates. Antihuman CD3 and CD28 monoclonal antibodies were added at a final concentration of 0.5 g/ml each, followed by overnight incubation at 37°C with 5% CO_2. During the last 4 h of culture, brefeldin A (1 μg/ml), which impairs protein secretion by the Golgi complex, was added to the cultures. The cells were then harvested, stained for surface markers and fixed as described above. The fixed cells were permeabilized for 10 min with a 0.5% saponin solution and stained, for 30 min at room temperature, using anticytokine monoclonal antibodies directly conjugated with PE (IFN-γ, TNF-α, IL-4, IL-10, or IL-12). At least 30 000-gated events were acquired in each staining. Acquisition and analysis was performed using a FACSVantage (Becton Dickinson, San Jose, CA, USA) containing the CELL QUEST program. The frequency of positive cells was analyzed in lymphocyte (R1) or monocyte (R2) regions, determined based on size vs. granularity profiles (Fig. 1a) and markers were set based on negative populations and isotype controls (Fig. 1a). Comparisons were performed using the Tukey-Kramer all-pairs comparison test from JMP (SAS). Statistical significance was considered when $P < 0.05$.

Results

Increased frequency of $CD4^+CD11a^+$ lymphocytes and $CD14^+CD11b^+$ monocytes in the peripheral blood of patients with CGCL

Expression of CD11a, CD54, and CD62L was evaluated in $CD4^+$ and $CD8^+$ T-lymphocytes and CD11b in $CD14^+$ monocytes. Statistical analysis did not show any significant difference in the expression of all adhesion molecules by $CD8^+$ T cells between CGCL group and control group (Table 1). However, the frequency of $CD4^+CD11a^+$ lymphocytes was significantly higher in CGCL than in the control group (Table 1). No significant differences were detected for the intensity of expression of CD11a, CD54, and CD62L in both subsets of T-lymphocytes (CD4 and CD8) between CGCL and the control group (data not shown).

The overall *ex vivo* frequency of $CD11b^+$ cells in the monocyte gate was greater in the CGCL group when compared with the control group (Fig. 1b). This difference was maintained following *in vitro* stimulation (Fig. 1c). Moreover, CGCL group expressed a striking increase, *ex vivo*, in the intensity of CD11b expression by $CD14^+$ and $CD14^-$ cells, when compared with control cells (Fig. 1b). Finally, following polyclonal stimulation, the increased intensity of expression of

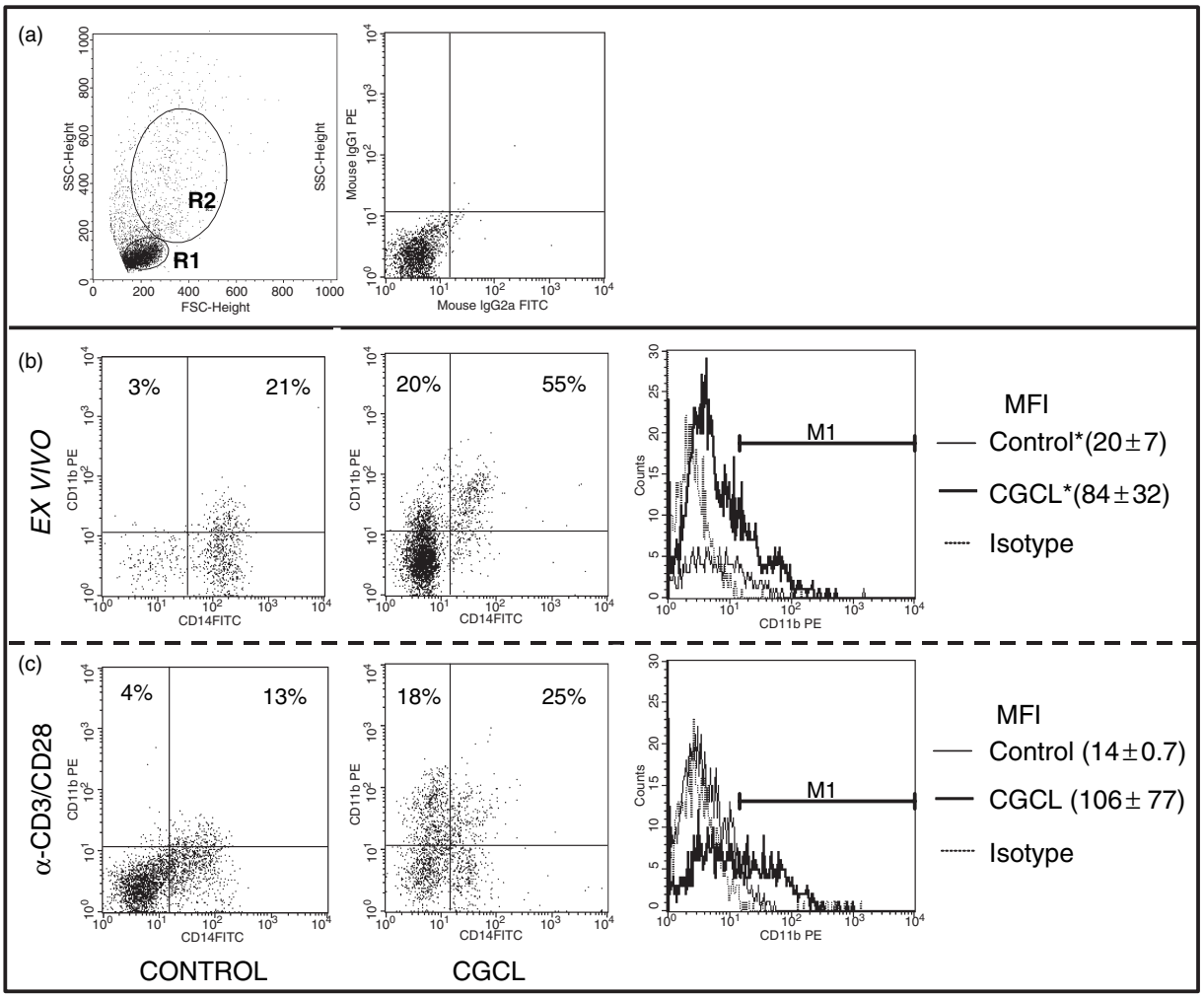

Figure 1 Representative profile of cytometric analysis of peripheral blood mononuclear cells (PBMC). (a) Selection of lymphocyte (R1) and monocyte (R2) regions for analysis, based on size vs. granularity distribution. Representative dot-plots of CD14 and CD11b expression by monocytes from central giant cell lesion (CGCL) and control individuals after incubation with media only (b) or with anti-CD3/-CD28 (c). Overlaid histograms show mean fluorescence intensity (MFI) of CD11b expression. The values in the quadrants represent the mean percentage of cells in each quadrant for each group. $*P < 0.05$ using Tukey-Kramer analysis.

Table 1 Analysis of adhesion molecule expression by $CD4^+$ and $CD8^+$ T cells

	Percentage of $CD4^+$ cells		*Percentage of $CD8^+$ cells*	
Adhesion molecules	*Control*	*CGCL*	*Control*	*CGCL*
CD11a	47.36 ± 4.02	58.29 ± 7.05*	11.72 ± 4.55	11.19 ± 4.55
CD54	7.97 ± 2.70	8.21 ± 0.14	2.81 ± 1.14	1.51 ± 1.08
CD62L	41.00 ± 4.90	48.28 ± 4.99	7.75 ± 2.83	7.79 ± 2.08

Data expressed as mean ± SD of the frequency of double positive cells.
CGCL, central giant cell lesion.
*Statistically significant when compared with control group ($P < 0.05$) by Tukey-Kramer test.

CD11b in CGCL when compared with the control group was maintained, although not statistically significant (Fig. 1c).

Cytokine expression in lymphocytes and monocytes from patients with CGCL

We evaluated the expression of IFN-γ, TNF-α, IL-12, IL-4, and IL-10 by $CD4^+$ and $CD8^+$ lymphocytes as well as $CD14^+$ or $CD68^+$ monocytes from patients with CGCL after short-term polyclonal stimulation using flow cytometry. Statistical analysis revealed that the percentages of total IFN-γ^+ cells and $CD4^+$IFN-γ^+ cells within the lymphocyte population from CGCL were increased when compared with control group (Table 2 and Fig. 2a). Moreover, considering a total frequency of IFN-γ^+ cells of approximately 1% in CGCL (Table 2), we observed that $CD4^+$ T cells are responsible for approximately 60% of the IFN-γ^+

Table 2 Analysis of cytokine expression by lymphocytes and monocytes

	Percentage of lymphocytes		*Percentage of monocytes*	
Cytokine	*Control*	*CGCL*	*Control*	*CGCL*
IFN-γ	0.76 ± 0.11	1.04 ± 0.07*	ND	ND
IL-10	0.32 ± 0.10	0.80 ± 0.20*	9.01 ± 1.87	29.96 ± 11.32*
TNF-α	0.29 ± 0.05	0.44 ± 0.14	15.41 ± 4.19	17.93 ± 4.59
IL-4	0.73 ± 0.16	0.80 ± 0.20	ND	ND
IL-12	ND	ND	18.32 ± 5.22	29.01 ± 20.33

Data expressed as mean ± SD of frequencies of cells expressing cytokines in each cell population.
CGCL, central giant cell lesion; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.
*Statistically significant when compared with control group ($P < 0.05$) by Tukey-Kramer test.

production in CGCL, being the main source of this cytokine (Fig. 2a). The frequency of total IL-10$^+$ lymphocytes was higher in CGCL when compared with control group (Table 2). Double-labeling analysis showed that CD4$^+$ T cells are responsible for approximately 50% of IL-10 production by CGCL (data not shown). Moreover, a higher frequency of CD4$^+$IL-10$^+$ T cells was observed in CGCL than in controls (Fig. 2b). Interestingly, IL-10 expression in the monocyte gate was significantly higher than in the lymphocyte gate for both groups analyzed, showing that monocytes are the major cells producing IL-10 (Table 2). Moreover, the percentage of monocytes expressing IL-10 was significantly higher in CGCL, when compared with control individuals (Table 2). We determined that 50% of the CD14$^+$ monocytes are

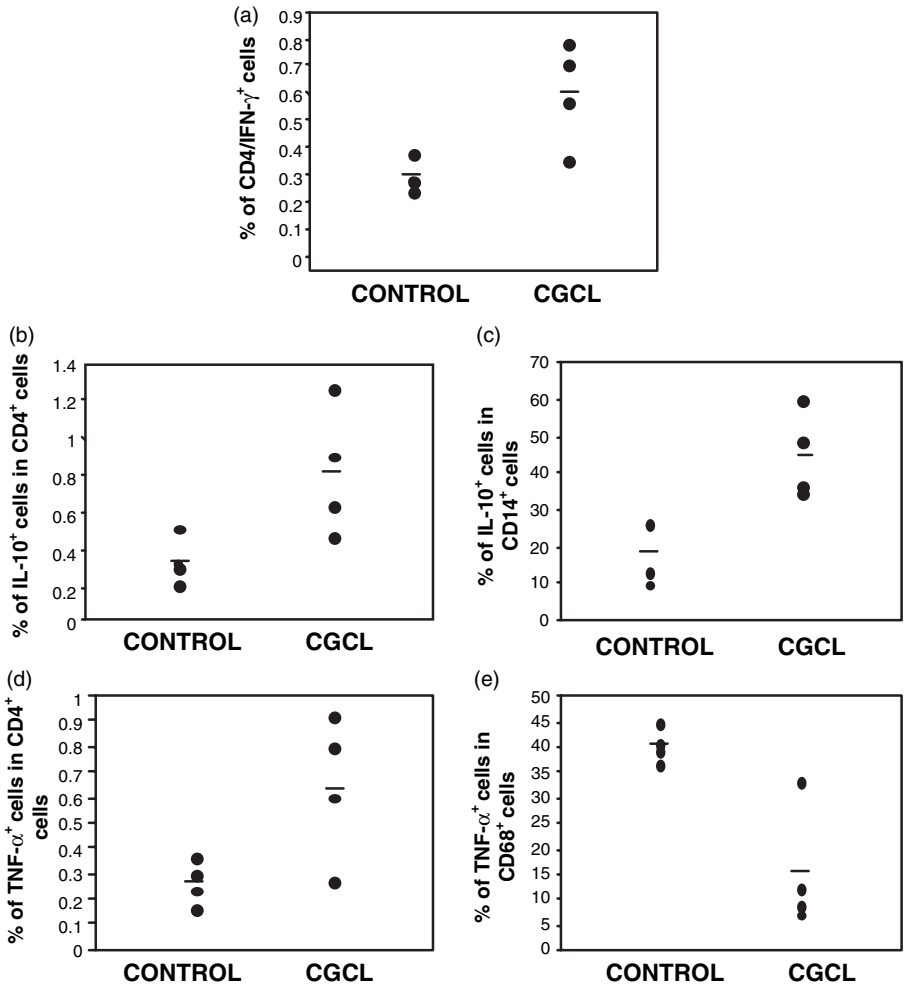

Figure 2 Frequency of cytokine expression by lymphocytes and monocytes from central giant cell lesion (CGCL) and the control group double-stained with fluorescein isothiocyanate (FITC)-labeled anti-CD4 or anti-CD14 and phycoerythrin (PE)-labeled anti-interferon (IFN)-γ (a), interleukin (IL)-10 (b–c), or tumor necrosis factor (TNF)-α (d–e). $P < 0.05$ using Tukey-Kramer analysis.

involved with IL-10 production in CGCL, whereas only 20% of the $CD14^+$ cells expressed IL-10 in control group (Fig. 2c). Although no significant differences were observed in the frequency of total lymphocytes expressing TNF-α (Table 2), we observed a higher frequency of TNF-α expression by $CD4^+$ T cells from patients with CGCL when compared with controls (Fig. 2d). The commitment of the $CD68^+$ monocytes with TNF-α production was lower in CGCL when compared with controls (Fig. 2e), despite the same overall TNF-α expression by the total monocyte population (Table 2). The frequencies of the Th1- and Th2-inducing cytokines, IL-12 and IL-4, respectively, did not show any statistically significant differences between the groups (Table 2). No significant differences were observed in the expression of cytokines by $CD8^+$ T cells between the CGCL and the control group (data not shown).

Discussion

In this work, we demonstrated the occurrence of phenotypic and functional systemic alterations in leukocyte populations of CGCL patients through the analysis of adhesion molecule and cytokine expression by circulating cells. Our results demonstrated that the frequency of $CD4^+$ T cells expressing CD11a was significantly increased in CGCL patients. The β2-integrin LFA-1 (CD11a/CD18) plays a critical role in recruitment of leukocytes to inflammatory sites (3) and acts as a co-stimulatory signal for T cells when the CD3 complex is engaged (9). Increased expression of LFA-1/CD11a by T cells is correlated to activation (10). Thus, the increased expression of this molecule in $CD4^+$ T cells from patients with CGCL may be indicative of cell activation and suggest that these cells are suitable for recruitment to CGCL sites. Previous studies demonstrated that cells in the CGCL express MHC-II molecules (11), which could explain the preferential activation of $CD4^+$ over $CD8^+$ T cells in CGCL. Future analysis of phenotypic and functional characteristics of T-lymphocytes present at CGCL sites would confirm this finding.

Monocytes leave the circulation and enter healthy and inflamed tissues by migration across the endothelium, using Mac-1 (CD11b/CD18) (12). We observed an increase in CD11b expression by monocytes in the CGCL group when compared with the control group, *ex vivo* and after *in vitro* stimulation (Fig. 1b,c). These findings are indicative of an activated state of circulating monocytes and can be explained by the altered cytokine environment (13, 14). The activity of cytokines and other mediators *in vivo* could lead to an increase in CD11b expression in monocytes passing through lesion sites, favoring their recruitment and contributing to the maintenance of the inflammatory infiltrate. In fact, we observed a higher frequency of IFN-γ^+ cells in CGCL when compared with the control, after short-term polyclonal stimulation (Table 2), suggesting the influence of this inflammatory cytokine in the expression of CD11b.

Despite the reactive nature of the CGCL, this lesion shows higher proliferative activity than the giant cell tumor, which shows neoplasic characteristics and a more aggressive behavior (15). Cytokines secreted by lesion cells or associated inflammatory cells, could up-regulate the activation and proliferation of lesion cells, contributing to the development of the lesion. Here, we showed that the percentages of total IFN-γ^+, $CD4^+$ IFN-γ^+, and $CD4^+$TNF-α^+ cells were increased in CGCL patients when compared with the control group (Table 2, Fig. 2). These results demonstrate the recall response of *in vivo* activated $CD4^+$ T cells that are able to produce proinflammatory cytokines after T-cell receptor (TCR) engagement and co-stimulatory stimuli. Moreover, the increased expression of these cytokines could explain previous data obtained by others (11) that showed high expression of MHC class II by CGCL cells. We also observed an increased expression of IL-10 by lymphocytes from CGCL, when compared with the control (Table 2). Previous studies have shown that circulating human memory T cells are able to produce IFN-γ and high levels of IL-10 after re-stimulation with anti-CD3 (16). Thus, we demonstrated that T cells from CGCL patients express inflammatory and anti-inflammatory cytokines.

Our results demonstrated a dramatic increase in expression of IL-10 by monocytes in CGCL patients (Table 2). There are some possible hypotheses that must be considered to explain this finding. First, the high production of this cytokine by cells of the innate arm of the immune response could reflect an attempt to control the fast developing inflammatory process associated with CGCL. On the contrary, it is possible that CGCL is associated with a deficiency of the individuals to respond to IL-10, as previously shown in other pathologic conditions (17). Analysis of IL-10 receptor expression would provide important information about the immune dynamics in CGCL. Moreover, since IL-10 inhibits IL-1, IL-6, and TNF-α production by activated macrophages (18), the higher levels of IL-10 expression could also explain the decreased frequency of TNF-α^+ cells in $CD68^+$ circulating monocytes observed in CGCL patients. Further studies are necessary to confirm this hypothesis.

In conclusion, although CGCL is lesion localized in the jaws, it may cause significant systemic functional alterations in circulating leukocytes. These observed phenotypic and functional changes are compatible with recruitment of lymphocytes and macrophages to lesion sites and with involvement of these leukocyte populations in the inflammatory process. Whether CGCL causes peripheral leukocyte activation or this activation is caused by other factors and stimulates the formation of CGCL is a possibility that we cannot rule out solely based on the data presented here. Further studies were such changes would be followed by the establishment of CGCL could clarify this hypothesis. Functional changes in circulating monocytes and lymphocytes from CGCL patients can contribute to better comprehension about the immunopathologic process involved in the development and maintenance of CGCL.

References

1. Flanagan AM, Nui B, Tinkler SM, Horton MA, Williams DM, Chambers TJ. The multinucleated cells in giant cell

granulomas of the jaws are osteoclasts. *Cancer* 1988; **62**: 1139–45.
2. O'malley M, Pogrel MA, Stewart JC, Silva RG, Regezi JA. Central giant cell granulomas of the jaws: phenotype and proliferation-associated markers. *J Oral Pathol Med* 1997; **26**: 159–63.
3. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994; **76**: 301–14.
4. Romagnani S. Cytokines and chemoattractants in allergic inflammation. *Mol Immunol* 2002; **38**: 881–5.
5. Moore KW, O'garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. *Annu Rev Immunol* 1993; **11**: 165–90.
6. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989; **7**: 145–73.
7. Mackay CR. Migration pathways and immunologic memory among T lymphocytes. *Semin Immunol* 1992; **4**: 51–8.
8. Bottrel RA, Dutra WO, Martins FA, et al. Flow cytometric determination of cellular sources and frequencies of key cytokine-producing lymphocytes directed against recombinant LACK and soluble Leishmania antigen in human cutaneous leishmaniasis. *Infect Immun* 2001; **69**: 3232–9.
9. Van Seventer GA, Shimizu Y, Horgan KJ, Shaw S. The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. *J Immunol* 1990; **144**: 4579–86.
10. Wacholtz MC, Patel SS, Lipsky PE. Leukocyte function-associated antigen 1 is an activation molecule for human T cells. *J Exp Med* 1989; **170**: 431–48.
11. Regezi JA, Zarbo RJ, Lloyd RV. HLA-DR antigen detection in giant cell lesions. *J Oral Pathol* 1986; **15**: 434–8.
12. Meerschaert J, Furie MB. The adhesion molecules used by monocytes for migration across endothelium include CD11a/CD18, CD11b/CD18, and VLA-4 on monocytes and ICAM-1, VCAM-1, and other ligands on endothelium. *J Immunol* 1995; **154**: 4099–112.
13. Detmers PA, Lo SK, Olsen-Egbert E, Walz A, Baggiolini M, Cohn ZA. Neutrophil-activating protein 1/interleukin 8 stimulates the binding activity of the leukocyte adhesion receptor CD11b/CD18 on human neutrophils. *J Exp Med* 1990; **171**: 1155–62.
14. Vaddi K, Newton RC. Regulation of monocyte integrin expression by β-family chemokines. *J Immunol* 1994; **153**: 4721–32.
15. Souza PEA, Paim JFO, Carvalhais JN, Gomez RS. Immunohistochemical expression of p53, MDM2, Ki-67 and PCNA in central giant cell granuloma and giant cell tumor. *J Oral Pathol Med* 1999; **28**: 54–8.
16. Yssel H, de Waal Malefyt R, Roncarolo MG, et al. IL-10 is produced by subsets of human CD4+ T cell clones and peripheral blood T cells. *J Immunol* 1992; **149**: 2378–84.
17. Avdiushko R, Hongo D, Lake-bullock H, Kaplan A, Cohen D. IL-10 receptor dysfunction in macrophages during chronic inflammation. *J Leukoc Biol* 2001; **70**: 624–32.
18. Fiorentino DF, Zlotnik A, Mosmann TR, Howars M, O'garra A. IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991; **147**: 3815–22.

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