

PD-1, TIM-3, and LAG-3 Expression in T Cells in a Patient with Recurrent Ossifying Fibroma: A Case Report

Alejandro García-Muñoz¹, Nayeli Goreti Nieto-Velázquez², Gabriela Damian-Morales², Carlos Liceaga-Escalera³, Luis Alberto Montoya-Perez³, Madeleine Cruz-Vélez³, Pabel Antonio Gómez-Hernández¹, Cynthia Trejo-Iriarte¹, Rodolfo Pastelin-Palacios⁴, Mario Adán Moreno-Eutimio^{4*}

1. *Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México (FES-Iztacala UNAM), Estado de México, México.*

2. *Research División, Hospital Juárez de México, Mexico City, Mexico.*

3. *Maxillofacial Surgery Service, Hospital Juárez de México, Mexico City, Mexico.*

4. *Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad de México, México.*

Submitted 2 August 2020; Accepted 15 November 2020; Published 27 January 2021

Central ossifying fibroma is a benign, slow-growing tumor of mesenchymal origin with a predilection for the mandibular premolar and molar areas. The immunophenotype of T cells involved in the antitumor response against this benign tumor is unknown. In this case report, we described a case of a 48-year-old woman presenting with a very large recurrent ossifying fibroma in the mandible, which was successfully treated with hemimaxillectomy. In addition, we evaluated the expression of programmed cell death 1 receptor (PD-1), lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and mucin-domain containing-3 (TIM-3), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), CD69 (activation inducer molecule), and CD25 (α chain of the high-affinity IL-2 receptor) in T cell populations from the tumor and peripheral blood of this uncommon lesion. The patient presented recurrent ossifying fibroma, and the tumor-infiltrating and peripheral blood T cells showed expression of PD-1, LAG-3, and TIM-3, suggesting an exhausted T cell response.

Key words: Immune checkpoint proteins, ossifying fibroma, programmed cell death 1 receptor, T-lymphocytes

According to the 2017 WHO classification, there are three variants of ossifying fibromas: cemento-ossifying fibroma, juvenile trabecular ossifying fibroma, and juvenile psammomatoid ossifying fibroma (1). In general, ossifying fibroma (OF) is a well-demarcated lesion composed of

fibrous connective tissue with varying degrees of cellularity and calcification areas. The calcified structures consist of rounded or lobulated basophilic masses (cementum-like), trabeculae of osteoid or bone, or combinations of the two. Due to the presence of this cementum-like material, OF has

*Corresponding author: Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad de México, México.
E-mail: marioadan@inmunoquimica.com

This work is published as an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by-nc/4>). Non-commercial uses of the work are permitted, provided the original work is properly cited.

also been called cementifying fibroma or cemento-OF (2). Clinically, OF is a slowly progressing tumor of craniofacial bones, with a tendency towards locally aggressive behavior and recurrence. This tumor usually shows bone expansion, is painless, shows no cortical perforation or tooth root resorption, and has a mixed unilocular radiodensity appearance and well-defined boundaries on radiological examination (3).

The adaptive antitumor immune response begins with professional antigen-presenting cells that capture tumor antigens and process and present them on the cell surface in complexes with major histocompatibility complex (MHC) class I and II molecules to naive T cells. This interaction results in the maturation and activation of tumor-specific cytotoxic T (Tc) lymphocytes capable of migrating to tumor sites, identifying tumor cells, and destroying them. Additionally, the interaction triggers a response by T helper (Th) cells, which stimulate the T and B cell arms of the antitumor immune response (4). Studies about infiltrating immune cells in OF or odontogenic tumors are limited.

Tumor cells evade the immune response and progress through different mechanisms (5), including activation of immune checkpoint pathways that suppress antitumor immune responses. Cancer immunotherapy with immune checkpoint blockade has been proven to be effective in the treatment of many tumors, such as melanoma and non-small-cell lung carcinoma (6). The immune checkpoint targets include molecules on T lymphocytes, such as programmed cell death protein 1 (PD-1) (7), lymphocyte activation gene-3 (LAG-3) (8), T cell immunoglobulin and mucin-domain containing-3 (TIM-3) (9), and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) (10), among others. Thus far, there are no studies that have explored the expression of these molecules in OF; immunotherapy by immune checkpoint blockade may be useful in OF. The present study

followed the medical and ethics protocols of the Declaration of Helsinki, and the institutional Committees of Research, Biosecurity, and Ethics approved it (Registration No. HJM 0623/19-1). Written informed consent for publication of the clinical details and clinical images was obtained from the patient.

Case presentation

At the maxillofacial service at Hospital Juárez de México, a 48-year-old female who had undergone surgery three years prior presented with a benign fibro-osseous lesion. The first treatment was enucleation, and the second treatment was remodeling. The final pathology results reported OF. Afterward, the patient presented with a mass that increased the volume of the left side of the nasogenian region and raised the nasal ala but was asymptomatic, without changes in color but with a firm consistency. Intraorally, the patient presented an increased volume in the vestibular furrow and palatal region from the upper lateral left incisor to the molar area on the same side. The mass was the same color as the adjacent mucosa, did not result in tooth mobility, and was also hard and firm.

Results

The imaging studies showed an osteolytic lesion covering the entire left maxillary and zygomatic parts of the same side (Figure 1A). Biopsies showed hypodensity of the lesion, with some areas of higher density from the orbital rim to the midline jaw.

To date, there is limited information about the interaction of odontogenic tumors and immune cells. The aim of this work was to analyze the immunophenotype of the T cell subset in the tumor and peripheral blood to analyze its activation function.

The pathological diagnosis was OF, and given that it was a recurrent tumor, left maxillectomy with the Weber-Ferguson approach was performed later.

A containment plate 2.0 system was used to prevent

depression of soft tissue, and tissue conditioner was

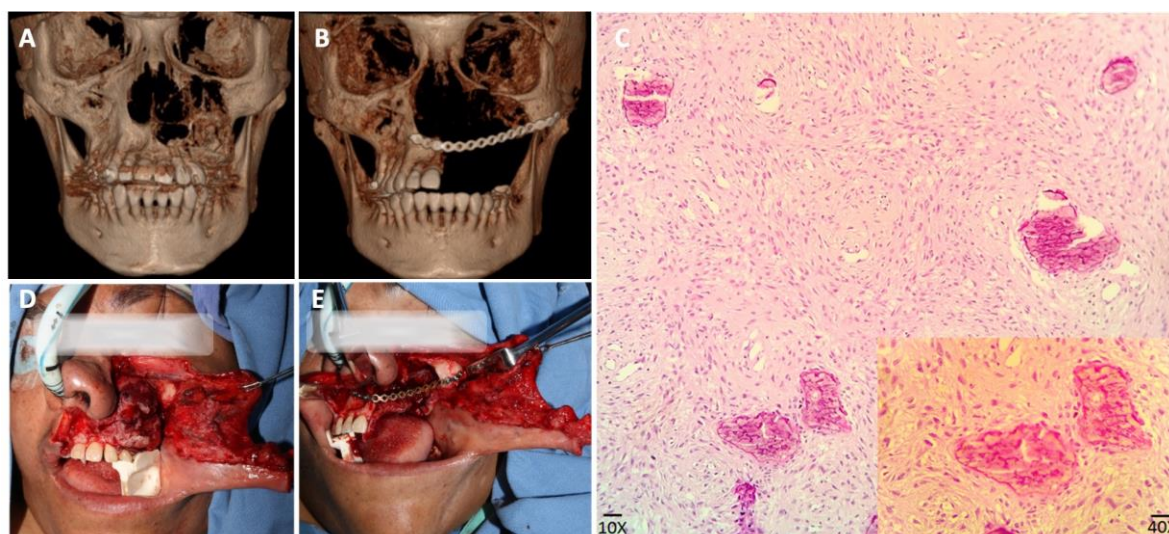


Fig. 1. Clinical appearance and surgical approach of the patient. A) On computed tomography, there was tissue destruction at the level of the nasogenian region, which extended to the area surrounding the malar bone and, at the level of the oral cavity, from the region of the lateral incisor to the molar region; B) Postsurgery computed tomography; C) The histopathologic appearance of the ossifying fibroma showing highly cellular fibroblastic stroma with small calcifying spherules (hematoxylin-eosin, 10x and 40x); D) Image of the tumor before surgery; E: mage of the area postsurgery, and the placement of the titanium plate used for facial reconstruction.

applied immediately to encourage healing of any tissue that was damaged during surgery (Figure 1 D and E). Finally, an injury-free control was imaged (Figure 1B).

Although the final pathology report was OF, the histopathology analysis showed proliferation of benign spindle-shaped fibroblastic cells with embedded mineralized structures, which presented as round to ovoid collections of bone with an osteoid rim. Additionally, there were particles with radiating collagen fibers, whereas there were multiple ossicles with a thick irregular collagenous rim (Figure 1C).

The tumor-infiltrating immune cells were assessed in tumor cell samples and by multiparameter flow cytometry analysis. The analysis showed that 13% of cells were immune cells (CD45⁺ cells, Figure 2A), of which 29.8% were T lymphocytes (CD3⁺ cells, figure 2B). Within the T cell subset, 41.1% of the cells were Th (CD3⁺ CD4⁺), and 48.3% were Tc (CD3⁺, CD8⁺) (Figure 2C).

The relative expression of the CD69, CD25, CTLA-4, PD-1, LAG-3, and TIM-3 molecules in T lymphocyte populations was compared by normalized mean fluorescence intensity (nMFI), both in tumor cells and in peripheral blood cells, as observed in the histograms of Figure 2 D-E. High expression of the activating molecule CD69 by Th and Tc lymphocytes in the tumor and peripheral blood was observed; however, high expression of PD-1, which is an inhibitory molecule, was also found in Th and Tc lymphocytes infiltrating the tumor and in peripheral blood. In the tumor and peripheral blood samples, the expression of LAG-3 and TIM-3 was restricted to the population of Th lymphocytes and was mostly expressed by Tc lymphocytes. CD25 and CTLA4 expression by T cells was not observed in the tumor or peripheral blood. (Figure 2 D-E).

The high expression of PD-1 by the T lymphocyte populations in the tumor was also seen for lymphocytes from peripheral blood. These data suggest that the cells received strong inhibitory signals, which cause T cells to not respond to a

specific antigen. For this reason, we decided to carry out an *ex vivo* assay with PBMCs stimulated with a polyclonal activator (PMA/Ion) and to evaluate the expression of these molecules by T

lymphocytes 24 hours poststimulation (Figure 3 A-B). After activation, the cells showed higher expression of CD69, and maintained increased

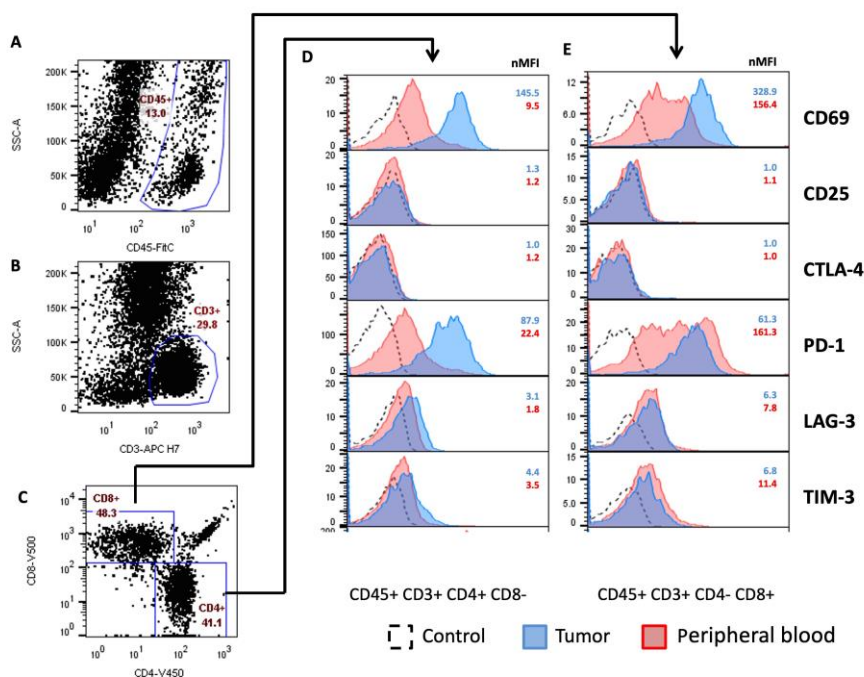


Fig. 2. The phenotype of T cells from the tumor and peripheral blood as analyzed by flow cytometry. A) Dot plot of the tumor-infiltrating immune cells (CD45⁺); B) T lymphocytes (CD3⁺, CD45⁺); C) T helper cells (CD45⁺, CD3⁺, CD4⁺, CD8⁻) and cytotoxic T cells (CD45⁺, CD3⁺, CD4⁻, CD8⁺); D-E: CD69, CD25, CTLA-4, PD-1, LAG-3, and TIM-3 expression in T helper cells (right) and cytotoxic T cells (left) analyzed from peripheral blood (PB) and tumor samples. The auto-fluorescence control defined the threshold between negative and positive cells. Data is shown as normalized mean fluorescence intensity (nMFI) by dividing the MFI of the stained sample to the MFI of fluorescence minus one (FMO) control.

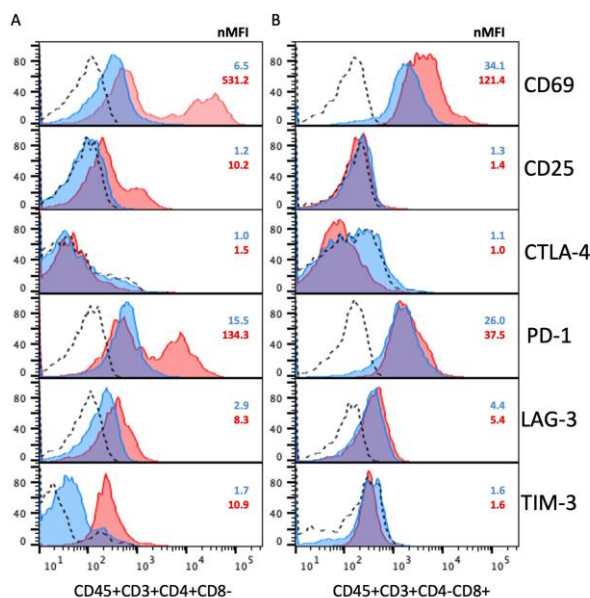


Fig. 3. Ex vivo T cells activation from peripheral blood. Peripheral blood mononuclear cells (PBMCs) from the patient were stimulated with a polyclonal activator (PMA/Ion). CD69, CD25, CTLA-4, PD-1, LAG-3, and TIM-3 post-stimulation expression by T cells were evaluated. A: CD4⁺ T cells; B: CD8⁺ T cells. Data is shown as normalized mean fluorescence intensity (nMFI) by dividing the MFI of the stained sample to the MFI of fluorescence minus one (FMO) control.

expression of PD-1; however, there was also expression of CTLA-4, LAG-3, and TIM-3, which are molecules that produce negative signals. This phenomenon was more marked in Tc lymphocytes than in Th lymphocytes. Interestingly, it was observed that the Th population showed expression of CD25, which is related to the proliferation of T cells, but the Tc population showed no expression of CD25.

Discussion

In this case report, we described for the first time the expression of inhibitory molecules (PD-1, LAG-3, and TIM-3) in the T cell subset that had not previously been described in OF and odontogenic tumors. We found high expression of the PD-1 molecule on the infiltrating T cells in OF and restricted expression of the LAG-3 and TIM-3 molecules.

These findings suggest that inhibitory signals predominated and probably kept the CD4⁺ and CD8⁺ T cells in an exhausted state, which perhaps prevented the immune response from inducing tumor remission.

The role of T cell exhaustion in oral OF is yet to be explored. These first findings and future studies could suggest immune checkpoint molecules as new therapeutic targets in the treatment of odontogenic tumors that could contribute to conventional therapy and management of these types of benign tumors.

Acknowledgement

This work was supported by the National Autonomous University of Mexico (UNAM: PAPIIT IN225820).

Conflict of interest

All authors declare that they do not have conflicts of interest.

References

1. El-Naggar AK, Chan JKC, Takata T, et al. The fourth edition of the head and neck World Health Organization blue book: editors' perspectives. *Hum Pathol* 2017;66:10-2.
2. Wright JM, Vered M. Update from the 4th Edition of the World Health Organization Classification of Head and Neck Tumours: Odontogenic and Maxillofacial Bone Tumors. *Head Neck Pathol* 2017;11:68-77.
3. Chrcanovic BR, Gomez RS. Juvenile ossifying fibroma of the jaws and paranasal sinuses: a systematic review of the cases reported in the literature. *Int J Oral Maxillofac Surg* 2020;49:28-37.
4. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* 2013;39:1-10.
5. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 2011;331:1565-70.
6. Zappasodi R, Merghoub T, Wolchok JD. Emerging Concepts for Immune Checkpoint Blockade-Based Combination Therapies. *Cancer Cell* 2018;34:690.
7. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2006;439:682-7.
8. Richter K, Agnellini P, Oxenius A. On the role of the inhibitory receptor LAG-3 in acute and chronic LCMV infection. *Int Immunol* 2010;22:13-23.
9. Ferris RL, Lu B, Kane LP. Too much of a good thing? Tim-3 and TCR signaling in T cell exhaustion. *J Immunol* 2014;193:1525-30.
10. Khaitan A, Unutmaz D. Revisiting immune exhaustion during HIV infection. *Curr HIV/AIDS Rep* 2011;8:4-11.