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Orbital lymphocyte populations in three states of thyroid eye disease

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ABSTRACT

Purpose: We report histopathologic orbital tissue analysis from three patients with thyroid eye disease (TED) – active, chronic, and post-teprotumumab to better characterize orbital cellular populations in these varying states of TED.

Observations: Orbital tissues in TED demonstrate minimal lymphocytic infiltration in fat and Mueller's muscle. Following teprotumumab treatment, the tissues were devoid of lymphocytes with only perivascular cuffs of T-lymphocytes remaining in orbital fat.

Conclusions and importance: In active TED, post-teprotumumab treatment, and in quiescent TED, orbital fat may not show significant inflammatory infiltration. More work is warranted to characterize specific cellular effects of teprotumumab and other biologics.

1. Introduction

In thyroid eye disease (TED), orbital tissues undergo structural changes mediated by dysregulated fibroblasts and lymphocytes.[1] Antibodies binding the thyrotropin receptor (TSH-R) and insulin-like growth factor 1 receptor (IGF-1R) found on orbital fibroblasts as well as enhanced cytokine signaling cause increased production of hyalurons and glycosaminoglycans leading to infiltration and expansion of orbital connective tissues.[2–5] Clinically, these changes may lead to orbital swelling, eyelid retraction, strabismus, proptosis, and even optic neuropathy.[1,6,7] Teprotumumab, an IGF-1R monoclonal human antibody, is used to treat TED.[4] Other monoclonal antibodies used in TED, including rituximab and tocilizumab, have been associated with a depletion of inflammatory cells in orbital tissues.[8–11] We report cellular analysis of orbital tissues in patients with TED, including following treatment with teprotumumab.

2. Methods and Results

Three patients with a history of TED and one normal control were recruited for this study. Participants underwent surgical procedures as the usual component of their care including orbital decompression, levator recession, blepharoplasty, and Muller's muscle resection. The donation of tissues, which would otherwise have been discarded post-operatively, was discussed with each participant prior to surgery. Participants provided consent for donation and participation in the study. Orbital tissues included for histopathological analysis included nasal orbital post-septal fat as well as Mueller muscle. Tissues were subsequently sent to Contra Costa Pathology Associates for analysis. Study oversight and approval was obtained from the IRB of John Muir Hospital. This study adhered to the principles of the Declaration of Helsinki and all research activities were HIPAA compliant.

2.1. Active TED

A 60-year-old African American man with a history of Graves' disease and TED presented for evaluation. He reported increased visual blurriness and tearing. Medications included preservative-free artificial tears, methimazole 10 mg daily, and propranolol 10 mg three times daily. Best corrected visual acuity (BCVA) had declined from 20/20 OU to 20/70 + 2 in the right eye (OD) and 20/80 in the left (OS). Intraocular pressure was fifteen in each eye and pupils were equal. Color vision testing was impaired in each eye with Ishihara 1/7 plates being read. Hertel measurements at base 103 were 29 OD and 30 OS, with marked bilateral proptosis. Thyroid stimulating hormone (TSH) was low at <0.10 mIU/L, free T4 was normal at 0.95 ng/dL, and TSI was elevated at 396 IU/L. Computed tomography of the orbits with IV contrast revealed diffuse enlargement of all extra-ocular muscles with bilateral proptosis. With pain with eye movements, orbital swelling and injection, recent decrease in visual acuity and ocular motility, and increase in proptosis,

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the patient's clinical activity score (CAS) was seven. The patient was treated with intravenous solumedrol 1 mg/kg daily for 3 days and scheduled for surgical orbital decompression of the right and left orbit. Following completion of course of the intravenous course of steroids, CAS remained above 4 and orbital decompression was performed. Inflammation was significant at the time of surgical decompression, requiring temporary tarsorrhaphy for treatment of chemosis and swelling.

2.2. Quiescent TED

A 39-year-old Caucasian woman with an 11-year-history of Graves' disease and TED presented for evaluation. She reported significant dry eye symptoms refractory to aggressive ocular lubrication for years; other medications included levothyroxine 150 mcg daily. Her T3, T4, TSH, TSI levels had been within a stable normalized range without therapy for 2 years following surgical thyroidectomy. BCVA was 20/20 OD and 20/20 OS. Pupils, intraocular pressures, extraocular movements, and confrontational visual fields were normal. Hertel measurements at base 105 were 21 in each eye (OU), MRD1 was 4 OD and 5 OS, MRD2 was 6 OU with mild eyelid retraction. The patient expressed interest in orbital decompression. Surgery was performed bilaterally.

2.3. Post-teprotumumab TED

A 42-year-old Asian woman was referred by her comprehensive ophthalmologist for management of TED. She underwent surgical thyroidectomy at age 16 with a subsequent 26-year history of persistent severe eye disease. TSH was low at 0.75 mIU/L and her medications were levothyroxine 112 mcg daily as well as losartan 100 mg daily. At her first appointment, she demonstrated diplopia, eyelid retraction and erythema, chemosis, conjunctival erythema, and swelling of the caruncle as well as proptosis (Hertel 25 OD and 27 OS) with impaired ductions. Teprotumumab (Tepezza®) treatment was initiated. Following an 8-infusion course of IV treatment (initial dose of 10 mg/kg followed by seven doses of 20 mg/kg) she demonstrated stable BCVA 20/30–2 OD and 20/20–5 OS, with diminished proptosis (Hertel 22 OD and 23 OS) however, she reported persistent diplopia in up gaze and uncomfortable severe bilateral eyelid retraction. The patient elected bilateral levator muscle recession with limited orbital fat removal.

3. Discussion

Teprotumumab is a monoclonal human antibody which targets aberrant signaling of the IGF-1R/TSH-R complex, likely regulating connective tissue remodeling by orbital fibroblasts.[12,13] For patients with TED, teprotumumab infusions may significantly reduce proptosis and diplopia by decreasing orbital fat and extraocular muscle volume.[14] Although teprotumumab offers promising benefits to patients with TED, the myriad effects of systemically targeting IGF-1R are yet to be fully elucidated.[15] IGF-1R and IGF-1 are expressed not only in the orbit, but throughout the human body so teprotumumab has the potential to impact many cellular processes and tissues.[15]

In the present study, we analyzed nasal orbital post-septal fat from participants with active TED, quiescent TED, and post-teprotumumab treatment (Table 1). All tissue samples demonstrated positive CD3 and CD34 staining, consistent with a small population of T-lymphocytes, fibroblasts, and the presence of benign blood vessels (Fig. 1). The quiescent TED patient had undergone surgical thyroidectomy 2 years prior to orbital decompression. All markers of Graves had normalized following thyroidectomy, yet she persisted with chronic inactive TED. Orbital fat from this quiescent TED state was devoid of inflammation (Fig. 1). The staining pattern seen in orbital fat from the post-teprotumumab TED was most similar to that seen in active TED however, in active TED, scant lymphocytes including B cells, were seen throughout the sections examined where teprotumumab-exposed fat was relatively depleted of lymphocytes, with perivascular cuffs of mature lymphocytes predominantly consisting of T cells (Fig. 2). Pre-treatment with steroids may impact the inflammatory infiltration in the orbital tissues of the patient with active TED. However, in other inflammatory orbitopathies chronic steroid use has not demonstrated consistent histopathologic effects.[16] Additionally, the active TED patient's CAS score remained markedly elevated at the time of surgery, despite intravenous steroid therapy. His tissues showed noteworthy evidence of inflammation. Mueller muscle tissue in post-teprotumumab TED was without signs of inflammation similar to the control muscle (Table 1). Previous work demonstrating that Mueller's muscle tissue in the quiescent phase of TED shows little sign of inflammation, suggests that the inflammatory state of teprotumumab-treated muscle tissue may most-closely resemble that of quiescent TED.[17]

In this case series, comparative histopathologic analysis of orbital and Mueller's muscle was made possible by clinical circumstance. Due to surgical approaches employed, we also limited our analysis to post-septal nasal fat as there may be variations within orbital fat depending on location.[18,19] Through differential cell surface expression,[19] lymphocyte populations theoretically could vary by orbital fat pad location, although the contents of orbital fat with regards to structure, fibroblast, and adipocyte populations appears to be homogeneous.[20] Within the orbit, the primary site of inflammation in TED is not only the orbital fat but also the extraocular muscles, where lymphocytes mediate elaboration of connective tissues by fibroblasts.[6,7] Although orbital fat often lacks lymphocytic infiltrate[6,7] and TED may be primarily driven by muscle expansion,[21] markers of inflammation including T-cell-derived cytokines found there may be key drivers of TED.[3,22] Our finding that all three samples of orbital fat in various states of TED were devoid of lymphocytic infiltration excluding perivascular cuffs is, therefore, consistent with previous histopathological analysis. Furthermore, inflammation in TED may be driven by mast cells, macrophages, and IgE which were not specifically studied.[23,24] Nevertheless, our comparison of lymphocyte populations in orbital fat may serve as a foundation for further investigation. Utilizing extraocular muscle tissue from strabismus surgery, especially the inferior and medial rectus, would be an informative next step.

Orbital lymphocyte populations may be altered by monoclonal antibody treatments including rituximab which targets CD20, a protein expressed on B-lymphocytes,[25] and tocilizumab which targets IL-6, a pro-inflammatory cytokine.[8] In Graves' disease, pathogenic immunoglobulins stimulate the thyrotropin receptor leading to hyperproduction of thyroid hormone. Binding of these immunoglobulins in the orbit leads to an inflammatory cascade mediated by lymphocytes and fibroblasts

Table 1
Summary of histopathological findings from subjects: Active TED, Quiescent TED, Post-teprotumumab TED, and Control subject. Tissues analyzed included nasal orbital post-septal fat (OF) and Mueller Muscle (MM). Stains for CD3, CD4, CD8, CD20, CD21, and CD34 were compared.

Subject	Age	Laterality	Tissue	Condition	CD3	CD4	CD8	CD20	CD21	CD34	Interpretation
1	60	OS	OF	Active TED	+	+	+	+	–	+	Minimal inflammation
2	39	OD	OF	Quiescent TED	+	–	–	–	–	+	Minimal inflammation
3	42	OD	OF	Post-Tepro	+	+	+	+/–	–	+	Focal perivascular T-cell lymphoid population
4	74	OD	OF	Control	+	–	–	–	–	+	Rare, scattered T-cells, from blood
3	42	OD	MM	Post-Tepro	+	+	+	–	–	+	Minimal T-cell population, No lymphocytic infiltration
4	74	OD	MM	Control	–	–	–	–	–	+	No inflammation

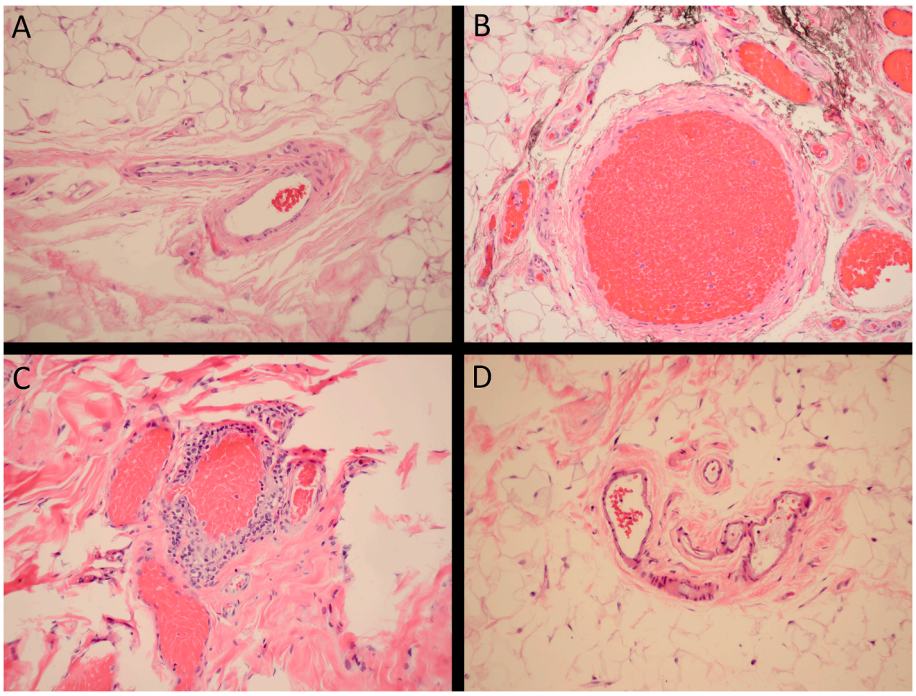

Fig. 1. Hematoxylin and Eosin stain of orbital fat taken at 200 x magnification. Active TED (A) and minimal inflammation present in the orbital fat. Quiescent TED (B) and minimal inflammation present. Post-teprotumumab (C) showing perivascular T-cell population. Control nasal orbital fat from patient who underwent blepharoplasty and mullerectomy with (D) showing scattered T-cells from blood.

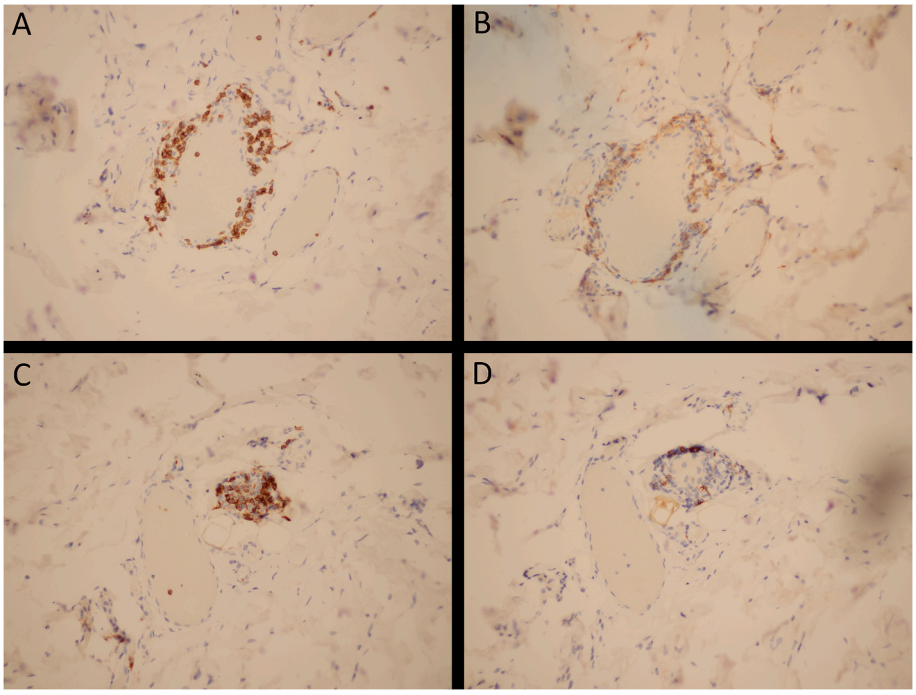

Fig. 2. Immunohistochemical stains of orbital fat taken at 200 x magnification, post-teprotumumab TED. (A) CD3 staining shows perivascular lymphocyte population. (B) CD4 staining shows perivascular lymphocyte population composed of T-cells. (C) CD8 staining shows an additional population of perivascular T-lymphocytes. (D) CD20 staining shows minimal perivascular B-cell population.

which in turn produce abnormal tissues.[1,13] These cells may aberrantly express IGF-R, the target of teprotumumab.[26] Therefore, teprotumumab has the mechanistic potential to affect the concentration of inflammatory cells within the orbit. Orbital fat from our post-teprotumumab patient showed only perivascular cuffs of lymphocytes (Fig. 2) and the post-treatment muscle tissue was devoid of B-lymphocytes. Comparing these staining patterns to those of active TED, we suggest that lymphocyte infiltration may change following teprotumumab treatment.

Although for some TED patients with proptosis and diplopia, teprotumumab offers significant benefits, other patients will be non-responders and responders may experience symptom flares following

initial treatment which may be less-amenable to teprotumumab treatment.[12] When 5 nonresponders and 8 patients with subsequent flare in the OPTIC study were offered re-treatment during the OPTIC-X study, only 2 nonresponders and 62.5% of flare patients had a second teprotumumab response.[12] As teprotumumab and other biologics are deployed in chronic TED,[27] the long-term effects of treatment will also become increasingly important. A better understanding of how these immunomodulatory therapies, especially monoclonal antibodies, interact with orbital lymphocytes and fibroblasts could augment clinical decision making in active, chronic, and refractory cases.

This series highlights some of the important questions which remain regarding the cellular effects of immunomodulatory systemic therapies for TED like teprotumumab. Our case series has significant limitations. Given the small number of cases, we cannot prove that teprotumumab treatment changes lymphocyte infiltration patterns within orbital tissues. To answer this question, future investigation could sample tissues from individual participants before and after teprotumumab treatment, providing an internal control to allow measurement of change in lymphocyte populations within each participants' tissue. Optimally, future investigation would also include analysis of extraocular muscle tissue. As IGF-1 and IGF-1R are ubiquitously expressed throughout the human body,[15] future investigations may also investigate how, following teprotumumab therapy, inflammatory markers are altered in blood, within the thyroid gland itself, as well as within off-target sites.

Patient consent

Written consent for participation was obtained from each study participant.

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Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

Declaration of competing interest

The following authors have no financial disclosures: SG, RZS.

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