

# Targeting fibrocytes in autoimmunity

Richard J. Bucala<sup>a,1</sup>

Thyroid-associated ophthalmopathy, also known as Graves' ophthalmopathy, is a proliferative disorder of the orbit of the eye with an autoimmune etiology. Disease arises from the enlargement of the extraocular muscles, adipose, and the associated connective tissue that, if untreated, leads to a compressive optic neuropathy and blindness. In Graves' disease, hyperthyroidism develops from autoantibodies directed against the thyroid-stimulating hormone receptor (TSHR) expressed on the follicular endothelial cells of the thyroid gland. Anti-TSHR autoantibodies stimulate the excessive production of thyroid-stimulating

hormone, which in turn leads to the clinical manifestations of hyperthyroidism: thyroid gland enlargement, weight loss, tremor, palpitations, dermatopathy, and, in up to 30% of subjects, autoimmune inflammation of the orbital structures (1). In PNAS, Fernando et al. provide evidence for a proximal and central role for the circulating fibrocyte in Graves' ophthalmopathy pathogenesis (2).

The autoimmune diseases comprise at least 100 nosologically distinct entities that afflict as many as 5% of the US population (3). Autoimmunity can be organ specific, as in the case of an autoantibody

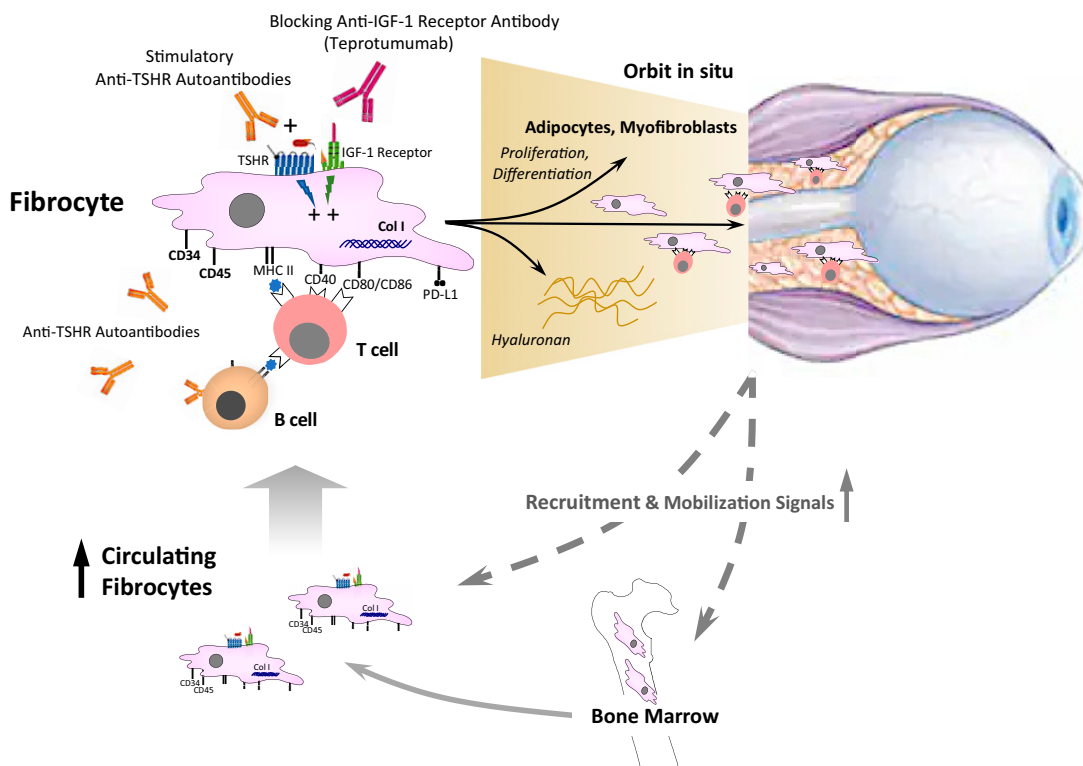


Fig. 1. Scheme for the role of the fibrocyte in Graves' ophthalmopathy.

<sup>a</sup>Section of Rheumatology, Allergy & Immunology, Department of Medicine, Yale School of Medicine, New Haven, CT 06520

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<sup>1</sup>Email: richard.bucala@yale.edu.

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attack against the neuromuscular junction in myasthenia gravis, or systemic in nature or manifestations, as in the immune complex pathology of systemic lupus erythematosus. The presence of anti-TSHR antibodies in nearly all patients with Graves' ophthalmopathy together with the correlation between their circulating levels and both the clinical features and prognosis of ophthalmopathy (and dermopathy) implicate them in immunopathogenesis (4). Notably, the hallmark lupus antinuclear autoantibody also occurs in Graves' disease, affirming a fundamental dysregulation in host immunity (5).

The underlying inflammatory pathology of thyroid-associated ophthalmopathy appears to target the orbital fibroblast (6). There is evidence for functional synergy between the TSHR and the insulin-like growth factor-1 (IGF1) receptor, which is expressed in orbital fibroblasts, such that anti-TSHR autoantibodies stimulate IGF1 signaling intermediates, leading to an overproduction of hyaluronan, which increases in deposition in the soft tissues of the orbit, and prompting both the proliferation and the differentiation of fibroblasts into adipocytes. Inflammatory signaling by locally produced cytokines further potentiates these pathologic signals. Over time, the cellular alterations enlarge the volume of tissues within the bony retro-orbital space to produce the clinically distinctive proptosis or forward displacement of the eye that characterizes thyroid-associated ophthalmopathy. The pathologic role of the IGF1 receptor also underlies the basis for the successful blockade of the anti-IGF1 receptor by the monoclonal antibody teprotumumab in the treatment of Graves' ophthalmopathy (7).

An accumulation of recent data has implicated the fibrocyte, a fibroblast precursor derived from the bone marrow and distinguished by the expression of CD34, CD45, and collagen I, in the pathogenesis of Graves' ophthalmopathy (8–10). Arising from bone marrow precursors, fibrocytes circulate in elevated levels in different inflammatory and fibrosing disorders, including myelofibrosis, systemic sclerosis, rheumatoid arthritis, and interstitial lung disease (11–16). Fibrocytes are present within the orbital tissues of Graves' disease patients and their blood levels are increased fivefold, with those having the most severe orbital disease also exhibiting the highest circulating concentrations (8, 17). Both circulating and orbital CD34+ fibrocytes express high levels of the IGF1 receptor and the TSHR, which are responsive to stimulation by the expression of inflammatory cytokines, including tumor necrosis factor, interleukin (IL)-1 $\beta$ , and IL-12 (18, 19). As in the case of other organ pathologies where fibrocytes appear, it is unknown if the excess fibrocytes in Graves' disease orbital tissue arise from local proliferation or from the recruitment of blood fibrocytes, which may be mobilized from bone marrow by systemic inflammatory signals (20).

Prior work has shown that fibrocytes exhibit pleotropic features, including the ability to differentiate into myofibroblasts and adipocytes, which is notable in the context of the pathologic changes in Graves' ophthalmopathy (21, 22). Given their myeloid character, fibrocytes also were reported at the outset to be fully

capable of antigen presentation, expressing major histocompatibility complex class II (MHC II) and the necessary costimulatory molecules to prime naive T cells, and with a potency rivaling dendritic cells that prompted their testing in cancer immunotherapy (e.g., FibroVax) (23). Indeed, fibrocytes isolated from the circulation are often in close apposition with T cells, which modulate their differentiation (22, 24). The sum of these fibrocyte properties—localization and accumulation in the orbit, autoantigen (e.g., TSHR) expression, and antigen presentation and inflammatory activation—places these cells in a central immunopathologic position in Graves' ophthalmopathy (Fig. 1).

## In PNAS, Fernando et al. provide evidence for a proximal and central role for the circulating fibrocyte in Graves' ophthalmopathy pathogenesis.

Fernando et al. (2) investigate the state of fibrocyte activation in Graves' ophthalmopathy patients treated with teprotumumab. Besides clinical benefit, treated subjects showed marked reduction in the expression by circulating fibrocytes of MHC II and the costimulatory molecules CD80 and CD86. CD4+ T cell expression of IL-17A and interferon- $\gamma$ , which are two inflammatory cytokines produced by T cells, also were reduced in treated subjects and in cocultures of fibrocytes and T cells, supporting the role of fibrocytes in maintaining a T cell autoimmune inflammatory phenotype in Graves' patients. IGF1 signaling further is known to activate the mTor/FRAP/p70<sup>S6k</sup> pathway to favor inflammatory Th17 over regulatory T cell polarization. The authors' observed reduction in the fibrocyte expression of the programmed death receptor ligand-1 (PD-L1) also is noteworthy, as PD-L1/PD-1 signaling can mediate peripheral immune tolerance to antigen.

The authors had previously reported an important regulatory interaction in the orbit between mature (CD34–) fibroblasts and fibrocytes, specifically with respect to the ability of the former resident cells to secrete the protein Slit2, which down-regulates the inflammatory phenotype of fibrocytes (25). As the newly reported data were obtained from an analysis of peripheral blood cells, the question now posed is whether the therapeutic benefit of teprotumumab is from IGF1 blockade at the site of disease, e.g., the orbit, or from the blockade of systemic or circulating fibrocyte–T cell interactions. In either case, the insights made by Fernando et al. (2) about the ability of teprotumumab to attenuate a fundamental pathway for autoimmunity in Graves' ophthalmopathy augurs well for testing IGF1 blockade in other organ-directed or systemic fibrosing disorders that have immunologic etiologies.

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