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Immunologiczne aspekty orbitopatii Graves'a

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

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Spis treści

WYKAZ STOSOWANYCH SKRÓTÓW

BCVA	najlepsza skorygowana ostrość wzroku	best-corrected visual acuity
CAS	wskaźnik klinicznej aktywności choroby	Clinical Activity Score
CD90	antygen różnicowania 90 (Thy-1)	cluster of differentiation 90 (Thy-1)
CLRs	receptory lektynowe typu C	C-type lectin receptors
DAMPs	wzorce molekularne związane z uszkodzeniami	damage-associated molecular patterns
DON	neuropatia nerwu wzrokowego	dysthyroid optic neuropathy
ERK1/2	kinaza regulowana zewnątrzkomórkowo 1/2	extracellular signal-regulated kinase 1/2
EUGOGO	Europejska Grupa do Spraw Orbitopatii Graves'a	European Group On Graves' Orbitopathy
GD	choroba Graves'a i Basedowa	Graves' disease
GO	orbitopatia Graves'a	Graves' orbitopathy
HIF-1α	czynnik indukowany hipoksją 1α	hypoxia-inducible factor 1α
HMG	grupa wysokiej mobilności	high mobility group
HMGB1	białko grupy wysokiej mobilności 1	high mobility group box 1
HSP	białka szoku cieplnego	heat shock proteins
iDCs	niedojrzałe komórki dendrytyczne	immature dendritic cells
IGF-1	insulinopodobny czynnik wzrostu 1	insulin-like growth factor-1
IGF-1R	receptor dla insulinopodobnego czynnika wzrostu	insulin-like growth factor-1 receptor
IκB	inhibitor kappa B	inhibitory protein kappa B
IL	interleukina	interleukin
IOP	ciśnienie śródgałkowe	intraocular pressure
LPS	lipopolisacharyd	lipopolysaccharide
NF-κB	czynnik jądrowy kappa B	nuclear factor kappa B
p38 MAPK	aktywowana mitogenami kinaza p38	p38 mitogen-activated protein kinase
PRRs	receptory rozpoznające wzorce	pattern recognition receptors
RAGE	receptor produktów zaawansowanej glikacji	receptor for advanced glycation end products
TGFβ	transformujący czynnik wzrostu β	transforming growth factor β
TLR	receptory Toll-podobne	Toll-like receptors
TNFα	czynnik martwicy nowotworów α	tumor necrosis factor α
TRAb	przeciwciała skierowane przeciwko TSHR	anti-TSHR antibodies
TSH	hormon tyreotropowy	thyroid-stimulating hormone
TSHR	receptor dla tyreotropiny	thyroid-stimulating hormone receptor

Streszczenie

Orbitopatia Graves'a (GO; ang. Graves' orbitopathy) jest jednym z pozatarczycowych objawów choroby Graves'a i Basedowa (GD; ang. Graves' disease), która charakteryzuje się nadczynnością tarczycy indukowaną przez autoprzeciwciała skierowane przeciwko TSHR (receptor dla tyreotropiny; ang. thyroid-stimulating hormone receptor). GO objawia się wytrzeszczem, podwójnym widzeniem, zaburzeniem widzenia barw i pogorszeniem ostrości widzenia. Dane literaturowe wskazują, że u podłoża GO leży przewlekły stan zapalny tkanek miękkich oczodołu, a naciek komórek zapalnych, produkcja glikozaminoglikanów oraz różnicowanie fibroblastów oczodołowych w adipocyty i miofibroblasty skutkuje obrzękiem mięśni gałki ocznej, rozplemem tkanki tłuszczowej i włóknieniem tkanek oczodołowych. Ta przebudowa tkanek powoduje wzrost ciśnienia w gałce ocznej i ucisk mięśni gałkoruchowych na nerw wzrokowy.

Przegląd piśmiennictwa na temat immunologicznych mechanizmów zaangażowanych w patogenezę GO posłużył do przygotowania pracy przeglądowej pt.: „Immunological Aspects of Graves' Ophthalmopathy". W publikacji zwrócono szczególną uwagę na fibroblasty oczodołowe i mechanizmy immunologiczne odgrywające kluczową rolę w rozwoju stanu zapalnego, ekspansji, przebudowie i włóknieniu tkanek oczodołowych. W artykule omówiono również udział autoantygenów w patogenezie GO z uwzględnieniem potencjalnych celów terapeutycznych w leczeniu choroby.

Kontynuacja badań w zakresie mechanizmów immunologicznych zaangażowanych w patogenezę GO dotyczyła analizy ekspresji wybranych białek odporności wrodzonej w mikrośrodowisku tkanki tłuszczowej oczodołów. Badania dotyczyły wieloligandowego receptora produktów zaawansowanej glikacji (RAGE; ang. receptor for advanced glycation end products) i jego liganda – białka HMGB1 (ang. high mobility group box 1). RAGE należący wraz z innymi receptorami do rodziny receptorów rozpoznających wzorce molekularne (PRRs; ang. pattern recognition receptors) odgrywa kluczową rolę w aktywacji odporności wrodzonej. Głównym ligandem dla RAGE jest endogenne białko HMGB1, które uwalniane jest w czasie stresu komórkowego, uszkodzenia tkanek lub w martwicy. Białko HMGB1 wraz z innymi endogennymi cząstkami należy do grupy tzw. cząstek molekularnych związanych z uszkodzeniem (DAMPs; ang. damage associated molecular patterns). Stymulacja PRRs

przez DAMPs skutkuje aktywacją stanu zapalnego, a także uruchomieniem mechanizmów naprawczych tkanek.

Przeprowadzone badania posłużyły do przygotowania oryginalnej publikacji pt.: „RAGE and HMGB1 Expression in Orbital Tissue Microenvironment in Graves' Ophthalmopathy". W pracy postawiono hipotezę, że oś RAGE/HMGB1 uczestniczy w patogenezie GO, a nasilenie ekspresji tych białek koreluje ze stanem klinicznym pacjentów z GO. Wykazano większą ekspresję badanych białek w tkance tłuszczowej oczodołowej u pacjentów z GO w porównaniu z grupą kontrolną. Stwierdzono również istotnie statystyczną korelację między ekspresją RAGE, a występowaniem neuropatii nerwu wzrokowego (DON; ang. dysthyroid optic neuropathy) i podwyższonym poziomem TRAb (przeciwciała skierowane przeciwko TSHR; ang. anti-TSHR antibodies). Ponadto w pracy wykazano obecność komórek zapalnych RAGE- i HMGB1- dodatnich, zlokalizowanych blisko naczyń, wskazując na ich potencjalny udział w rozwoju procesu zapalnego. Opublikowana praca jako pierwsza wykazuje ekspresję RAGE i HMGB1 w tkance tłuszczowej oczodołu metodą immunohistochemiczną.

RAGE i HMGB1 mogą uczestniczyć w rozwoju stanu zapalnego w tkankach oczodołowych, pełniąc istotną rolę w patogenezie GO. Dogłębne zrozumienie udziału immunologicznych procesów w patomechanizmie GO stanowi drogę do tworzenia nowych, skutecznych i bezpiecznych metod leczenia, a także usprawnienia monitorowania aktywności choroby.

Title of dissertation: Immunological Aspects of Graves' Orbitopathy

Abstract

Graves' orbitopathy (GO) is one of the extrathyroidal symptoms of Graves' disease (GD) that is characterized by hyperthyroidism, induced by circulating autoantibodies directed against the thyrotropin receptor (TSHR). GO is manifested by proptosis, double vision, impaired colour vision and deterioration of visual acuity. Literature data indicate that chronic inflammation of the orbital soft tissues underlies GO; and infiltration of immune cells, production of glycosaminoglycans and differentiation of orbital fibroblasts into adipocytes and myofibroblasts result in extraocular muscle edema, adipose tissue expansion and orbital tissues fibrosis. The remodeling of tissues leads to increased intraocular pressure and extraocular muscles compression on the optic nerve.

A review of the literature on the immunological mechanisms involved in the pathogenesis of GO was used to prepare the review article "Immunological Aspects of Graves' Ophthalmopathy". The paper focuses on orbital fibroblasts and immune mechanisms that play a key role in the development of inflammation, expansion, remodeling and fibrosis of the orbital tissues. The article also discusses the role of autoantigens in the pathogenesis of GO, taking into account potential therapeutic targets in the treatment of the disease.

The continuation of the research in the field of immunological mechanisms, involved in the pathogenesis of GO, concerned the analysis of the expression of selected innate immunity proteins in the microenvironment of the orbital adipose tissue. The research involved the multi-ligand receptor for advanced glycation end products (RAGE) and its ligand – HMGB1 protein (high mobility group box 1). RAGE which belongs with other receptors to the family of pattern recognition receptors (PRRs) plays a key role in the activation of innate immunity. The major ligand for RAGE is the endogenous HMGB1 protein, released during cellular stress, tissue damage or necrosis. The HMGB1 protein, with other endogenous particles, belongs to the group of the damage associated molecular patterns (DAMPs). Stimulation of PRRs by DAMPs results in the activation of inflammation as well as the activation of tissue repair mechanisms.

The conducted studies were used to prepare the original research article „RAGE and HMGB1 Expression in Orbital Tissue Microenvironment in Graves' Ophthalmopathy". The studies hypothesized that the RAGE/HMGB1 axis participates in the pathogenesis of GO, and the intensity of these proteins expression correlates with the clinical condition of GO patients. Elevated expression levels of these proteins were shown in the orbital adipose tissue of GO patients compared to the normal control. Moreover, a statistically significant correlation between RAGE expression and the occurrence of dysthyroid optic neuropathy (DON) and elevated TRAb levels (anti-TSHR antibodies) were demonstrated. Furthermore, in the research paper the presence of RAGE- and HMGB1- positive inflammatory cells closely located to the vessels was shown, indicating their potential participation in driving the inflammatory process. This is the first published paper showing RAGE and HMGB1 expression in the orbital adipose tissue using immunohistochemistry.

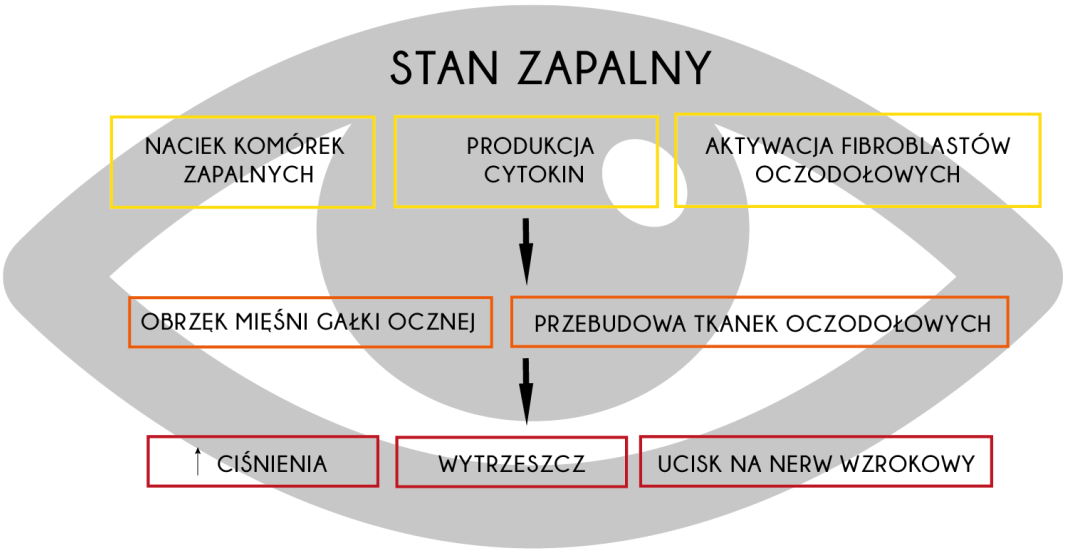
RAGE and HMGB1 may participate in the development of inflammation in the orbital tissues, playing a crucial role in the pathogenesis of GO. A thorough understanding of the participation of immune processes in GO pathomechanism is a way to create new, effective and safe methods of treatment as well as to improve monitoring of the disease activity.

1. Wstęp

1.1. Orbitopatia Graves'a

Choroba Graves'a i Basedowa (GD; ang. Graves' disease) to schorzenie o podłożu autoimmunologicznym, będące przyczyną nadczynności tarczycy. Zachorowalność jest szacowana na poziomie 20-50 nowych przypadków na 100 000 ludzi rocznie [1]. W patogenezie choroby kluczową rolę odgrywają autoprzeciwciała skierowane przeciwko receptorowi dla tyreotropiny (TSHR; ang. thyroid-stimulating hormone receptor), które imitują działanie hormonu tyreotropowego (TSH; ang. thyroid-stimulating hormone) i aktywują komórki tarczycy, indukując nadprodukcję hormonów. Jednym z pozatarczycowych objawów GD jest orbitopatia Graves'a (GO; ang. Graves' orbitopathy), definiowana jako przewlekły stan zapalny tkanek miękkich oczodołu o podłożu autoimmunologicznym; objawiająca się wytrzeszczem, podwójnym widzeniem, zaburzeniem widzenia barw i pogorszeniem ostrości widzenia [2]. Chociaż GO zazwyczaj towarzyszy nadczynności tarczycy, pacjenci mogą charakteryzować się eutyrozą lub niedoczynnością gruczołu. Według EUGOGO (Europejskiej Grupy do Spraw Orbitopatii; ang. the European Group On Graves' Orbitopathy) rozróżnia się 3 stopnie ciężkości GO: łagodna, umiarkowana/ciężka i zagrażająca utracie wzroku [3]. Mimo coraz lepszego poznania patomechanizmu, GO nadal stanowi poważne wyzwanie kliniczne i terapeutyczne.

Dane literaturowe wskazują, że u podstaw chorób autoimmunologicznych, w tym GO, leżą zaburzenia tolerancji immunologicznej, prowadzące do utraty kontroli nad stanem zapalnym. Naciek komórek zapalnych i wzmożona produkcja cytokin inicjuje zapalenie tkanek oczodołowych [2]. Obrzęk mięśni gałki ocznej oraz rozplem tkanki tłuszczowej oczodołowej prowadzą do wzrostu ciśnienia w oczodole, co z kolei może skutkować uciskiem na nerw wzrokowy i utratą wzroku. Zmiany obrzękowo-naciekowe obejmujące tkanki oczodołowe występują u 25-50% pacjentów ze zdiagnozowaną GD [4] (Rycina 1).



Rycina 1. *Udział procesu zapalnego w patogenezie GO.*

1.2. Udział układu immunologicznego w orbitopatii Graves'a

Fibroblasty oczodołowe, wśród których wyróżnia się dwie subpopulacje różniące się funkcjonalnie i fenotypowo: Thy-1(CD90)-dodatnie, wykazujące wysoką zdolność do różnicowania się w miofibroblasty, i Thy-1-ujemne, różnicujące się w adipocyty, odgrywają kluczową rolę w rozwoju GO [5, 6]. Naciek komórek immunologicznych, przeciwciał, produkowanych cytokin, chemokin i czynników wzrostu aktywuje fibroblasty, które stymulują produkcję macierzy pozakomórkowej (glikozaminoglikanów) oraz uczestniczą w ekspansji i przebudowie tkanek oczodołowych oraz w procesach włóknienia. Badania wykazały, że na fibroblastach oczodołowych dochodzi do ekspresji TSHR oraz receptora dla insulinopodobnego czynnika wzrostu IGF-1 (IGF-1R, ang. insulin-like growth factor-1 receptor), przeciwko którym skierowane są swoiste autoprzeciwciała [7]. Identyfikacja wspólnych autoantygenów w obrębie gruczołu tarczowego i tkanek oczodołowych może wyjaśniać powiązanie GD z GO. Nadczynność tarczycy w GD jest związana z aktywnością autoprzeciwciał TRAb (ang. anti-TSHR antibodies) skierowanych przeciwko TSHR, które ulegają ekspresji na powierzchni tyreocytów. Literatura podaje, że wspomniany receptor jest obecny również w tkance tłuszczowej oczodołowej, a poziom TRAb koreluje z kliniczną aktywnością i ciężkością GO [8-10]. Również wskazuje się na IGF-1R jako wspólny autoantygen i cel dla przeciwciał stymulujących. Receptor ten wykazuje ekspresję na tyreocytach i w tkance oczodołowej u pacjentów z GD i GO, a poprzez działanie

autoprzeciwciał anty-IGF-1R, uczestniczy w różnicowaniu fibroblastów oczodołowych w adipocyty oraz w syntezie hialuronianu w obrębie tkanek oczodołowych [11, 12]. Badania sugerują występowanie kompleksu sygnalizacyjnego TSHR–IGF-1R uczestniczącego w patogenezie GO, ze względu na ich wspólną lokalizację i powiązanie funkcjonalne [13, 14]. Blokowanie IGF-1R hamuje również szlak sygnalizacyjny TSHR, natomiast stymulacja kompleksu TSHR – IGF-1R aktywuje fibroblasty oczodołowe.

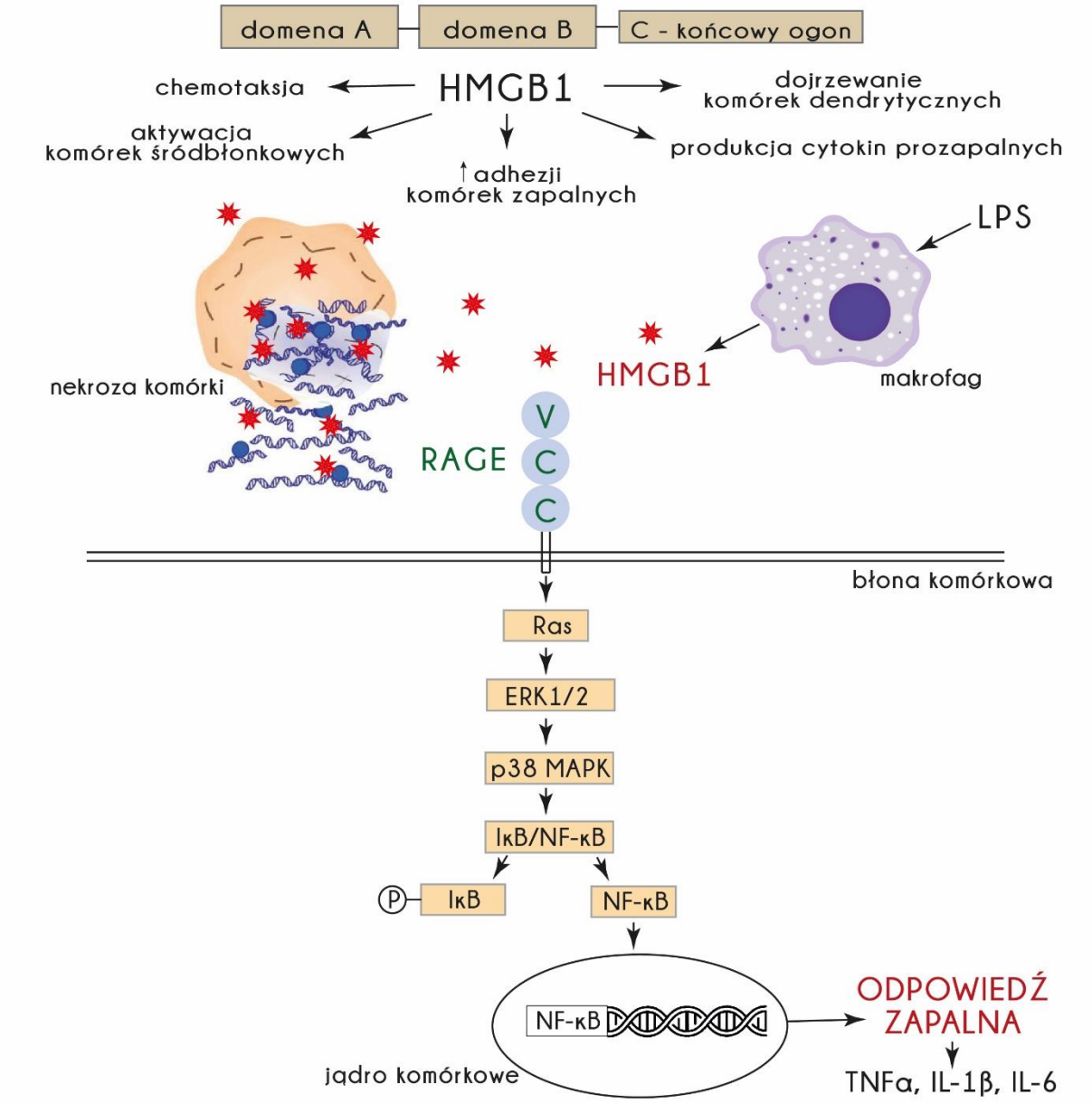
1.3. Odporność wrodzona, RAGE i HMGB1 w orbitopatii Graves'a

Odporność wrodzona/nieswoista (ang. innate immunity), obok odporności nabytej/swoistej (ang. adaptive immunity), jest drugim głównym ramieniem odpowiedzi immunologicznej. Stanowi ona bezpośrednią i natychmiastową linię obrony organizmu przed patogenami, ale także uruchamia mechanizmy zapalne i naprawcze w przypadku uszkodzenia własnych tkanek organizmu, stresu komórkowego lub śmierci komórek w przebiegu martwicy. W trakcie tych procesów dochodzi do uwalniania endogennych cząstek określanych jako struktury molekularne związane z uszkodzeniem tkanek (DAMPs; ang. damage associated molecular patterns) [15]. DAMPs obejmują m.in. produkty rozpadu macierzy zewnątrzkomórkowej, takie jak kwas hialuronowy, białka szoku cieplnego (HSP; ang. heat shock proteins), białko S100, kwas moczowy, β-amyloid, cytokiny (IL-1α, IL-33) oraz białko grupy wysokiej mobilności 1 (HMGB1; ang. high mobility group box 1) [16]. DAMPs wiążą się z receptorami określanymi jako tzw. receptory rozpoznające wzorce molekularne (PRRs; ang. pattern recognition receptors) [17]. Do PRRs należą m.in. wieloligandowy receptor produktów zaawansowanej glikacji (RAGE; ang. receptor for advanced glycation end products), receptory Toll-podobne (TLR; ang. Toll-like receptors), receptory lektynowe (CLRs; ang. C-type lectin receptors). Stymulacja PRRs przez DAMPs skutkuje sygnalizacją wewnątrzkomórkową i aktywacją układu odporności wrodzonej oraz nasileniem stanu zapalnego [18].

RAGE jest członkiem nadrodziny immunoglobulin i jako białko powierzchniowe wykazuje ekspresję na powierzchni limfocytów, makrofagów, komórek śródbłonka, mięśni gładkich, neuronów i innych [19]. Struktura RAGE składa się z krótkiego ogona cytoplazmatycznego, domeny transbłonowej oraz regionu zewnątrzkomórkowego obejmującego 1 domenę typu V (zmienna; ang. variable) i 2 domeny typu C (stałe; ang. constant), stabilizowane przez mostki disiarczkowe między resztami cysteiny.

Działając jako wspólny receptor dla różnych ligandów, RAGE aktywuje wiele szlaków sygnałowych w zależności od dostępności liganda, typu komórki i środowiska. Interakcja ligand-RAGE uruchamia dodatnie sprzężenie zwrotne i wzmacnia ekspresję receptora, przez co dochodzi do pobudzenia kolejnej fazy aktywacji komórki. Wskazuje się RAGE jako kluczową cząsteczkę w inicjacji i rozwoju zapalenia, miażdżycy naczyń krwionośnych, nadciśnienia, chorób neurodegeneracyjnych i wielu innych chorób przewlekłych, a także nowotworów [20-23]. Dane literaturowe podkreślają również związek między poziomem RAGE w surowicy, a aktywnością chorób o podłożu autoimmunologicznym [24].

Białko HMGB1. Niehistonowe białko wiążące chromatynę – HMGB1 – należące do rodziny białek HMG (ang. high mobility group) jest zlokalizowane w jądrze, umożliwiając tworzenie kompleksów nukleoproteinowych, ale również ma zdolność przemieszczania się do cytoplazmy [25]. Uwalniane jest biernie poprzez uszkodzone lub martwicze komórki; lub aktywnie wydzielane z komórek śródbłonka, komórek odpornościowych, płytek krwi, neuronów i komórek nowotworowych podczas stresu komórkowego. Będąc wewnątrzkomórkowym regulatorem transkrypcji, białko HMGB1 odgrywa istotną rolę w utrzymaniu funkcji DNA. Strukturalnie, składa się z domeny A (ang. box A), domeny B (ang. box B) oraz C-końcowego ogona o charakterze kwaśnym. HMGB1 działa jako silny mediator zapalenia, a wiąże się nie tylko z RAGE, ale również m.in. z receptorami TLR2, TLR4, TLR9 [26, 27]. Szlak sygnałowy HMGB1/RAGE aktywuje w końcowym efekcie prozapalny czynnik transkrypcyjny NF-κB (czynnik jądrowy kappa B; ang. nuclear factor kappa B) [25]. Interakcja HMGB1 z RAGE uczestniczy w inicjacji odporności wrodzonej i nabytej, pobudza dojrzewanie i migrację komórek układu odpornościowego, a także nasila produkcję cytokin (TNFα, IL-1β, IL-6), sugerując udział zewnątrzkomórkowego HMGB1 w procesie zapalnym [28]. HMGB1 indukuje dojrzewanie niedojrzałych komórek dendrytycznych (iDCs; ang. immature dendritic cells), które przełączają swoją funkcję ze zbierania antygenów na ich prezentację, stając się wrażliwsze na chemokiny w węzłach chłonnych [29] (Rycina 2).



Rycina 2. *Budowa oraz zaangażowanie białka HMGB1 i receptora RAGE w proces zapalny.*

Białko HMGB1 strukturalnie składa się z domeny A, domeny B oraz C-końcowego ogona. Pełni istotną rolę w aktywacji komórek odpornościowych, ich chemotaksji, adhezji, dojrzewaniu i różnicowaniu. HMGB1 uwalniane jest do przestrzeni zewnątrzkomórkowej biernie z komórek nekrotycznych lub aktywnie z monocytów/makrofagów podlegających stymulacji np. poprzez LPS (lipopolisacharyd; ang. lipopolysaccharide). Uwolnione HMGB1 działa jak cytokina prozapalna. Receptorem dla HMGB1 jest m.in. RAGE, które jako białko powierzchniowe składa się z 3 domen zewnątrzkomórkowych (V-variable; C-constant), fragmentu transbłonowego i krótkiego ogona cytoplazmatycznego. Oś HMGB1/RAGE angażuje szlak sygnalizacyjny [Ras → ERK1/2 (kinaza regulowana zewnątrzkomórkowo 1/2; ang. extracellular signal-regulated kinase 1/2) → p38 MAPK (aktywowana mitogenami kinaza p38; ang. p38 mitogen activated protein kinase)], który prowadzi do aktywacji NFκB, związanego w cytoplazmie z inhibitorem IκB (inhibitor κB; ang. inhibitory protein κB). IκB podlega fosforylacji (na rycinie oznaczone jako P), co powoduje dysocjację kompleksu IκB/NFκB. Uwolniony NFκB aktywuje w jądrze komórkowym ekspresję genów prozapalnych.

RAGE i HMGB1 w orbitopatii Graves'a. Podwyższony poziom ligandów dla RAGE w chorobach przewlekłych sugeruje zaangażowanie RAGE w patogenezę chorób o podłożu zapalnym [28]. Dane literaturowe wykazują udział białka HMGB1 w patogenezie autoimmunologicznego zapalenia tarczycy i innych chorób przewlekłych na skutek indukowania procesów zapalnych [21, 30]. Wykazano, że blokowanie HMGB1, RAGE i TLR przyczynia się do zmniejszenia produkcji cytokin prozapalnych w modelu autoimmunologicznego zapalenia tarczycy, co wskazuje na ich udział w zapaleniu towarzyszącym GO [30]. Ostatnie doniesienia piśmiennictwa przedstawiają korelację między poziomem osoczowego HMGB1, a TRAb, a także w stosunku do klinicznej aktywności choroby (CAS; ang. Clinical Activity Score), czyniąc go wartościowym biomarkerem choroby [31]. HMGB1 i jego receptory uczestniczą w mechanizmach zapalnych GO, a blokowanie szlaku HMGB1 może mieć potencjalne zastosowanie w leczeniu pacjentów z GO.

2. Założenia i cel pracy

Na podstawie danych literaturowych wskazujących na kluczową rolę układu odpornościowego w przewlekłych chorobach o podłożu zapalnym i autoimmunologicznym, postawiono hipotezę, że oś RAGE/HMGB1 uczestniczy w patogenezie GO, a nasilenie ekspresji tych białek koreluje ze stanem klinicznym pacjentów z GO.

Celem pracy było:

1. Ocena roli układu immunologicznego w etiologii i patogenezie GO.
2. Określenie znaczenia klinicznego ekspresji RAGE i HMGB1 w mikrośrodowisku tkanki tłuszczowej oczodołów w GO.

3. Kopie opublikowanych prac

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Review Article

Immunological Aspects of Graves' Ophthalmopathy

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The body's autoimmune process is involved in the development of Graves' disease (GD), which is manifested by an overactive thyroid gland. In some patients, autoreactive inflammatory reactions contribute to the development of symptoms such as thyroid ophthalmopathy, and the subsequent signs and symptoms are derived from the expansion of orbital adipose tissue and edema of extraocular muscles within the orbit. The autoimmune process, production of antibodies against self-antigens such as TSH receptor (TSHR) and IGF-1 receptor (IGF-1R), inflammatory infiltration, and accumulation of glycosaminoglycans (GAG) lead to edematous-infiltrative changes in periocular tissues. As a consequence, edema exophthalmos develops. Orbital fibroblasts seem to play a crucial role in orbital inflammation, tissue expansion, remodeling, and fibrosis because of their proliferative activity as well as their capacity to differentiate into adipocytes and myofibroblasts and production of GAG. In this paper, based on the available medical literature, the immunological mechanism of GO pathogenesis has been summarized. Particular attention was paid to the role of orbital fibroblasts and putative autoantigens. A deeper understanding of the pathomechanism of the disease and the involvement of immunological processes may give rise to the introduction of new, effective, and safe methods of treatment or monitoring of the disease activity.

1. Introduction

Graves' disease (GD) is the most common underlying cause of hyperthyroidism, and the incidence of new cases is estimated at 20 to 50 per 100,000 people per year [1]. It is a multifactorial disease, influenced by genetic, environmental, and endogenous factors. The peak in the disease occurrence is between the ages of 30 and 50 years, but it can occur at any age and affects women more often than men [2]. The cause of hyperthyroidism in GD is circulating autoantibodies directed against the thyrotropin receptor (TSHR), which mimic the action of TSH and excessively activate thyroid follicular cells and consequently stimulate the secretion of thyroid hormones (triiodothyronine and thyroxine), thereby inducing thyroid growth and its vascularization [3]. These processes trigger the development of hyperthyroidism symptoms such as anxiety, fatigue, nervousness, weight loss, moist skin, hair loss, muscle weakness, and palpitations. The extrathyroidal symptoms include localized dermopathy, acropachy, and ophthalmopathy, edematous-infiltrative changes involving orbital soft tissues described as thyroid-associated orbitopathy (TAO), and thyroid eye disease or Graves' ophthalmopathy (GO) since more than 90% are due to GD [4]. GO, defined as an autoimmune inflammatory disorder involving the orbit, is observed in about 2 subjects per 10,000 a year and in 25–50% of patients with GD [5, 6].

Although these patients are predominantly hyperthyroid (90%), patients with GO may also be euthyroid (5%) or hypothyroid (5%) [7]. It is observed that the pathological autoimmune reaction is directed against cross-reactive autoantigens in the thyroid and retrobulbar tissues [6, 8]. Significant involvement of cytokines and immunological mechanisms in the pathogenesis of GO is suggested. Tissue infiltration by cytokine-producing inflammatory cells and extensive remodeling of the eye soft tissues results in a phenotypic picture of the disease (Figure 1). Clinical signs and symptoms include double vision, retracting eyelids, edema, proptosis, and erythema of the conjunctival and periorbital tissues [6]. According to the recommendations of the European Group on Graves' Orbitopathy (EUGOGO), GO is distinguished into three levels of severity: mild, moderate to severe, and sight-threatening [9]. Treatment depends on the GO severity and includes immunosuppressive therapy, orbital irradiation, and surgery (endoscopic orbital decompression). Understanding the role of the immune system in GO may enable the introduction of new therapeutic options in the future.

2. Pathogenesis

Similarly to GD, at the base of GO is the autoimmune response in which the sensitive T cells, as well as autoantibodies against a common autoantigen of the thyroid and retrobulbar tissues, play an important role [10]. This common antigen may be the TSH receptor, as it has been also expressed on fibroblasts and orbital preadipocytes [11]. A correlation between the degree of ocular changes and the level of stimulatory antibodies directed against TSHR (TRAb) has been reported [12]. It has been suggested that another autoantigen may be the insulin-like growth factor-1 receptor (IGF-1R), as immunoglobulins of GD patients may activate the IGF-1R [13, 14]. Autoantibodies directed against this receptor contribute to the activation of orbital fibroblasts in GO, and the increased expression of the IGF-1R has been shown in patients with GD in both the thyroid tissue and the orbital tissues. Varewijck et al. demonstrated a diminished stimulating activity of IGF-1R through the depletion of immunoglobulins of GD patients [15]. Although these antibodies against IGF-1R are potentially implicated in GO development, there are some discrepancies regarding this speculation. Minich et al. have obtained data that do not confirm that the circulation of stimulating antibodies (against IGF-1R) in the patient's blood aggravates GD, nor their usefulness as a diagnostic parameter of the disease [16].

The main processes involved in the pathogenesis of thyroid-associated orbitopathy are cytokine production and inflammation, hyaluronan synthesis, adipogenesis, and myofibrillogenesis. The main sites of ongoing inflammation are the orbital adipose tissue and fibrous tissue of extraocular muscles [17]. The orbital tissues are infiltrated by activated mononuclear cells, such as T cells, and to a lesser extent by plasmocytes, macrophages, and mast cells. Cytokines produced by leukocytes, such as IFN-γ, IL-1α (IL-5), and leukoregulin (lymphokine, produced by activated lymphocytes), lead to the synthesis of glycosaminoglycans (GAG) [18]. The

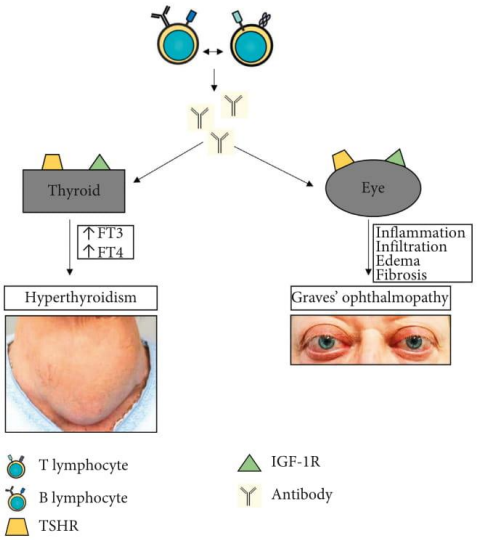

Figure 1: Pathogenesis of Graves' disease (GD) and Graves' ophthalmopathy (GO). GD is an autoimmune disease in which antibodies stimulate the thyroid to produce thyroid hormones leading to hyperthyroidism. One of the most common signs and symptoms is enlargement of the thyroid gland (goiter) while GO is the most frequent extrathyroidal involvement of GD. Inflammation and infiltration extraocular tissues result in edema and fibrosis of these tissues.

accumulation of GAG leads to extraocular muscle edema [19]. By means of inflammatory mediators (cytokines) or direct cellular interaction, orbital fibroblasts are activated, which exhibit different morphological and functional features as compared to fibroblasts in other localizations. Moreover, the activation of orbital fibroblasts by TRAb indicates the link between GD and GO [20, 21]. Activated orbital fibroblasts proliferate, differentiate into adipocytes and myofibroblasts, and play a key role in the production of the extracellular matrix. Excessive orbital fibroblast activity contributes to expansion, remodeling, and fibrosis of the orbital tissues. In the active phase of orbital changes, as a result of inflammatory cell infiltration and edema, the volume of tissues surrounding the eyes augments, in turn leading to an increase in the intraocular pressure [18]. As a consequence, the eyeball moves beyond the bony edges of the orbit. Moreover, optic nerve compression resulting in optic neuropathy, as well as impaired venous and lymphatic outflow from the orbit, can occur [22]. The final stage (inactive phase) of exophthalmos involves the fibrosis of the eye muscles (Figure 2).

3. Cytokine Production and Inflammation

The inflammatory process in orbital tissues leads to migration and infiltration of immune cells, which resembles the process occurring within the thyroid gland. T cells enter the

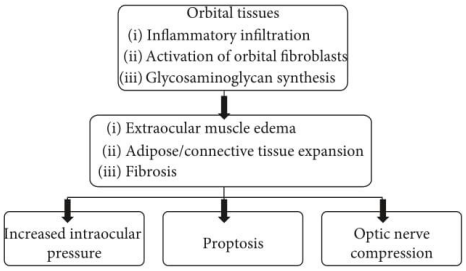

Figure 2: Pathogenesis of edematous-infiltrative changes. Inflammatory infiltration in periocular tissues and activity of orbital fibroblasts lead to expansion and remodeling of tissues. Increased intraocular pressure within the inflexible bony orbit results in proptosis and can contribute to developing optic nerve compression.

soft orbital tissue and release cytokines that contribute to reactivity and tissue remodeling [23]. The initial phase of GO is characterized by increased activity of Th1 lymphocytes, facilitating cell-mediated immunity and producing IL-1β, IL-2, TNF-α, and IFN-γ [24]. These proinflammatory cytokines enhance fibroblast proliferation and hydrophilic GAG production. Furthermore, the inflammatory process leads to the activation of Th2 lymphocytes, which release cytokines, such as IL-4, IL-5, IL-10, and IL-13, activating humoral reactions and the production of IgG [25]. The late phase of GO is characterized by tissue remodeling and fibrosis [26] (Figure 3).

Produced cytokines, chemokines, and growth factors have a huge impact on cells in orbital tissues. IFN-γ induces the production of CXCL9, CXCL10, and CXCL11 by fibroblasts, whereby the migration of lymphocytes to the orbital tissues is promoted [27]. In addition, IFN-γ stimulates the secretion of IL-1β and both (synergistically) stimulate the synthesis of GAG by orbital fibroblasts [28]. However, in contrast to IL-1β, IFN-γ inhibits adipogenesis of fibroblasts [29]. IL-1β has been shown to stimulate the orbital fibroblasts to produce IL-6, IL-8, CCL2, CCL5, and IL-16, which are chemoattractants for T and B cells, monocytes, and neutrophils [30, 31] (Figure 4).

Besides lymphocytes, macrophages, and thyrocytes, orbital fibroblasts also express the costimulatory protein CD40 [32]. The interaction between CD40 ligand (CD154) localized on T cells and the CD40 molecule on the orbital fibroblast surface stimulates the production of various inflammatory mediators (such as IL-1α, IL-6, IL-8, CCL2, and PGE2) by orbital fibroblasts as well as the activity and proliferation of these cells [32]. Prostaglandin E2 (PGE2) participates in B-cell maturation, stimulates the production of IL-6 by orbital fibroblasts, and activates mast cells [33, 34]. The production of PGE2 by orbital fibroblasts is also promoted by leukoregulin, IL-1β (released by macrophages and fibroblasts), and IFN-γ (secreted by activated T cells) [28, 35]. The process of recruitment of autoreactive T lymphocytes is supported by locally produced or circulating adhesion molecules, and the expression of these

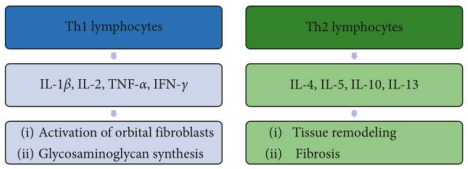

Figure 3: The proportion of T lymphocytes in the pathogenesis of Graves' ophthalmopathy. The initial phase of GO is characterized by increased activity of Th1 lymphocyte-producing cytokines that enhance fibroblast proliferation and GAG production. Th2 lymphocytes involved in the late phase participate in remodeling and fibrosis of periorbital tissues.

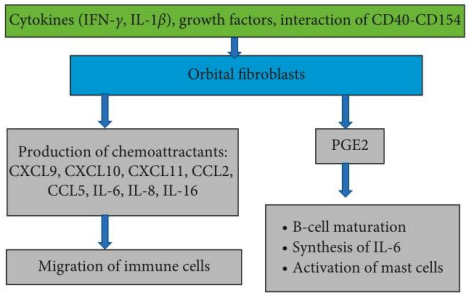

Figure 4: The participation of orbital fibroblasts in orbital inflammation. Cytokines, growth factors, and T cells stimulate orbital fibroblasts to produce chemokines and cytokines. PGE2 produced by orbital fibroblasts activates mast cells and B-cell maturation as well as stimulates the production of IL-6 by orbital fibroblasts.

molecules is induced by cytokines [36]. IL-1α, IL-1β, TNF-α, IFN-γ, and also CD40-CD154 interaction enhance the expression of intercellular adhesion molecule (ICAM-1) on orbital fibroblasts [30, 37, 38]. Adhesive molecules activate T cells and enhance their recruitment, resulting in an increased cell response and development of the active phase of ophthalmopathy. Elevated levels of L-selectin and ICAM-1 have been reported in patients in the active phase of the disease [39].

It is suggested that the cause of the development of GO is a lack of regulatory T lymphocytes (Tregs) control over the inflammatory reaction directed against self-tissues (antigens) [40]. Tregs are responsible for suppressing the immune response by the release of IL-10 and TGF-β [41]. Under physiological conditions, Tregs destroy autoreactive T lymphocytes, directed against thyroid follicular cell antigens [42, 43]. Glick et al. demonstrated an impaired suppressor function of Treg lymphocytes in patients with autoimmune thyroid disease (GD or Hashimoto's disease), who did not receive glucocorticosteroids for a minimum of six months [44]. Klatka et al. reported that patients with GD were characterized by a lower number of Tregs and a higher Th17 lymphocyte count compared to healthy subjects [45]. The significant contribution of Th17 lymphocytes to

inflammatory infiltration is also suggested as their role in autoimmune diseases has been demonstrated [46, 47]. The elevated concentration of Th17 lymphocytes in the peripheral blood of GO patients was reported, but there are no data on the presence of Th17 lymphocytes in the inflammatory infiltration of orbital fat.

4. Hyaluronan Synthesis

An important feature of the processes occurring in retroocular connective tissue, which affects the clinical picture of ophthalmopathy, is the synthesis of large amounts of GAG by orbital fibroblasts [48]. In particular, the accumulation of hyaluronan acid and collagen contributes to the retrobulbar tissue edema. *In vitro* culture of orbital fibroblasts treated with IFN-γ was characterized by higher production of GAG compared to the dermal fibroblasts culture [49]. Similar results were obtained using leukoregulin as a stimulant [50]. The effect of inflammatory mediators, such as IL-1, TNF-α, IFNγ, TGF-β, IGF-1, PDGF (platelet-derived growth factor), and prostaglandins, on the stimulation of orbital fibroblasts for the production of hyaluronan is also indicated [30, 48, 51–53]. Han et al. reported that IL-4 and IFNγ enhance the effect of IL-1β on GAG production by orbital fibroblasts as they augment the induction of hyaluronan synthase-2 (HAS2) expression by IL-1β [28]. Hyaluronan synthases (HASs) expressed on the cell membrane are responsible for the regulation of hyaluronan synthesis [54]. In GO, the major isoform of HAS involved in the synthesis of hyaluronan is HAS2. The balance between synthesis and degradation reflects hyaluronan accumulation. Zhang et al. reported the production of hyaluronidase by orbital fibroblasts [55].

5. Adipogenesis and Myofibrillogenesis

A portion of the orbital fibroblasts is called preadipocytes since they possess the capability to differentiate into mature adipocytes, which distinguishes them from fibroblasts from other locations in the body. This may be due to the high expression of the peroxisome proliferator-activated receptors (PPARγ) [56]. PPARγ belongs to the nuclear receptors of adipocytes, which act as transcription factors and regulate homeostasis of lipids and glucose. Adipogenesis in orbital fibroblasts is enhanced by the activation of PPARγ with rosiglitazone [57]. PPARγ agonists stimulate not only adipogenesis but also the expression of TSHR in cultured orbital preadipocytes. Moreover, they inhibit orbital inflammation and the production of hyaluronan [58]. Microarray studies have shown an upregulation of adipocyte-related genes (genes encoding PPARγ, IL-6, adiponectin, and leptin) in the orbit in GO. The activity of cyclooxygenase-2 (COX2) in activated T cells results in the production of proadipogenic prostaglandins (PPARγ ligands) [59]. COX2 is upregulated in the orbit in patients with GO, and as a result, prostaglandins provoke the process of adipogenesis in orbital fibroblasts [60].

Fibroblast subpopulations Thy1(CD90)+ and Thy1− can be distinguished based on the presence or absence of CD90 glycoprotein expression [61]. Thy1− fibroblasts have a strong ability to differentiate into adipocytes. Studies indicate that IL-1β, IL-6, and PGD2 stimulate fibroblasts towards adipogenesis [30, 52, 62]. It has been shown that this process is inhibited by TNF-α and IFNγ, but not by IL-4. These results agree with the claim that cytokines associated with Th1 lymphocytes are more involved in the early phase of ophthalmopathy rather than in the late phase associated with tissue remodeling and fibrosis. Thy1+ fibroblasts have the potential to differentiate into myofibroblasts, as demonstrated by fibroblasts stimulated by TGF-β, i.e., by a cytokine associated with Th2 lymphocytes [63, 64]. Myofibroblasts play a key role in muscle contraction and the accumulation of collagen in fibrotic tissue. Lehmann et al. have reported that adipocytic differentiation of Thy1− orbital fibroblasts can be inhibited by culture media from Thy1+ orbital fibroblasts, which produce antiadipogenic factors [63]. The involvement of adipose tissue or extraocular muscles in GO patients results from the proportion of Thy1+ and Thy1− orbital fibroblast populations and exposure to TGF-β or another stimulus [6] (Figure 5).

6. Putative Autoantigens and Potential Treatment

6.1. TSH Receptors. Hyperthyroidism associated with GD results from the action of autoantibodies directed against TSHR expressed on the surface of thyrocytes (thyroid epithelium). Studies have demonstrated the presence of the receptor in orbital adipose tissue and also suggested that the shared autoantigen hypothesis can explain the pathogenesis of GO (a common autoantigen of the thyroid and orbital tissues). Orbital adipose tissue of patients with GO (including euthyroid patients) is characterized by greater expression of TSHR than control tissues from people without GD [65, 66]. An elevated level of TSHR has been also noticed in pretibial connective tissue from patients with thyroid-associated dermopathy [67]. Some studies have shown that the level of antibodies against TSHR (TRAb) correlates with the clinical activity and severity of GO [68, 69]. Active GO is associated with a higher expression of TRAb compared to inactive GO. It is suggested that the extrathyroidal and thyroidal TSHR exhibit similar properties [70]. The response of orbital fibroblasts to TRAb is augmented by PDGF-AB and PDGF-BB, whereas TGF-β reduces TSHR expression [51, 71]. TSH, TRAb, and GD-IgG activate orbital fibroblasts and initiate cAMP and PI3K (phosphoinositide 3-kinase) signaling and the production of hyaluronan, ICAM-1, and cytokines, e.g., IL-6, IL-8, CCL2, and CCL5 [72]. In addition, the activation of TSHR induces adipogenesis in orbital fibroblasts [73].

Studies indicate that enhanced *de novo* adipogenesis in the orbit of GO patients increases TSHR expression in this tissue. Cultured orbital fibroblasts under adipogenic conditions have shown higher TSHR expression in mature fat cells than in preadipocyte fibroblasts [74]. Furthermore, PPARγ agonist rosiglitazone and adipogenic conditions trigger the enhanced expression of TSHR and adipocyte-associated genes (adiponectin, leptin, and PPARγ) [57, 65]. Similar findings have been obtained in orbital adipose tissue. In addition, monoclonal TRAbs stimulate adipogenesis in

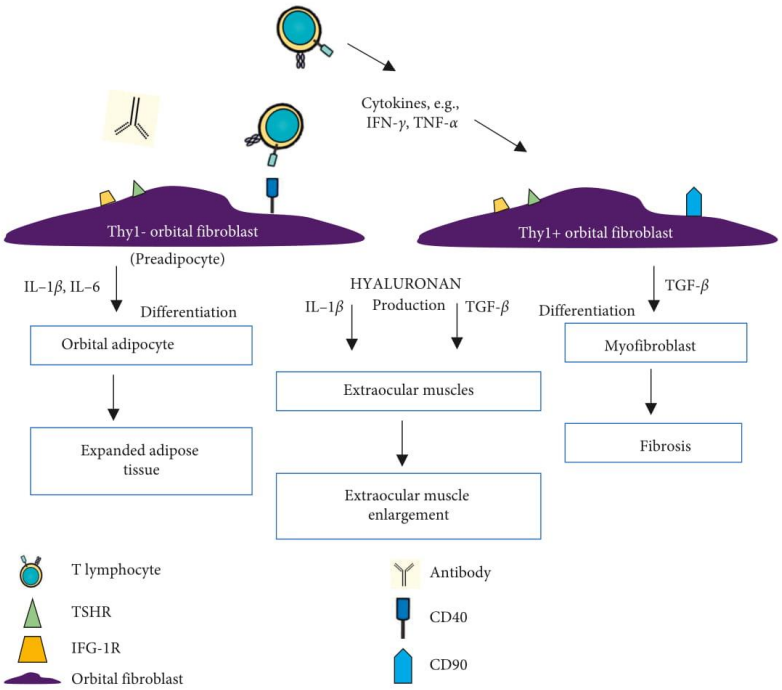

FIGURE 5: The participation of orbital fibroblasts in orbital tissue remodeling. Orbital fibroblasts express TSHR, IGF-1R, and CD40. Infiltrated immune cells, antibodies, secreted cytokines, chemokines, growth factors, and also CD40-CD154 interactions activate orbital fibroblasts. Inflammatory mediators (Il-1β and IL-6) that enhance adipogenesis activate Thy1− orbital fibroblasts to differentiate into adipocytes. And Thy1+ orbital fibroblasts (with CD90 expression), activated by TGF-β, differentiate into myofibroblasts. Proliferative activity of orbital fibroblasts, their differentiation, and capacity to synthesize extracellular matrix contribute to orbital tissue expansion, remodeling, and fibrosis.

orbital preadipocyte fibroblasts, which indicates the involvement of autoantibodies not only in the overproduction of thyroid hormones in GD but also in an orbital adipose tissue volume increase in GO.

Smith and Hoa have discovered that purified immunoglobulins from patients with GD (GD-IgG including TRAb and other IgGs) participate in the production of hyaluronan [75]. They found that GD-IgG enhances hyaluronan synthesis in GO orbital fibroblasts (through IGF-1R) whereas such properties have not been demonstrated for human recombinant TSH (hrTSH). In addition, only orbital fibroblasts that have undergone adipocyte differentiation are induced to hyaluronan production by GD-IgG, but not by hrTSH [72, 76]. On the other hand, Zhang et al. have shown that, in undifferentiated orbital fibroblasts (not in GO fibroblasts), bovine TSH and TRAb stimulate hyaluronan synthesis [54]. They also demonstrated that GO orbital fibroblasts containing the transfected TSHR-activating mutation increase hyaluronan production.

Due to the fact that the TSHR plays an important role in the pathogenesis of GD, it is believed that this receptor may be a therapeutic target for the treatment of GD [77]. Considering the orbital fibroblast activation through TSHR signaling, small-molecule TSHR antagonists can be used to block signal transduction [78]. These molecules have been found to inhibit cAMP production in human thyrocytes induced by TSH and GD-IgG [79]. TSHR-blocking monoclonal antibodies inhibit hyaluronan production and adipogenesis in cultured human orbital fibroblasts [80]. TRAb K1-70 has antagonist activity and can be useful in the inhibition of stimulating TRAb in GD patients [81]. ATX-GD-59 is an apitope that decreases the production of stimulating TRAb and demonstrates potential for the prevention and treatment of GO [82]. Apitopes—antigen processing independent epitopes—mimic naturally processed CD4+ T-cell epitopes. Regulatory-like T cells (type 1) with immunosuppressive features are induced after the administration of apitopes.

6.2. Insulin-Like Growth Factor-1 Receptor (IGF-1R). Another crucial autoantigen potentially involved in the pathogenesis of GO is IGF-1R. This receptor is expressed

Table 1: Potential therapeutic targets in GO [6, 103].

Target	Treatment	Potential benefit	
TSHR	TSHR-blocking antibody; TSHR antagonist	Inhibition of hyaluronan production and adipogenesis	[109]
IGF-1R	Teprotumumab—IGF-1R-blocking antibody	Inhibition of hyaluronan production and adipogenesis	[87, 88]
CD3	Teplizumab and otelixizumab—CD3 monoclonal antibodies	Induction of tolerance	[89]
CTLA4	Abatacept—CTLA4 analogue	Increased T-cell activation	[90]
CD20	Rituximab—CD20 monoclonal antibody	Increased TRAb production	[5, 94, 95]
TNF and TNF receptor	Adalimumab—TNF-blocking monoclonal antibody; Etanercept—soluble TNF receptor	Inhibition of hyaluronan production and inflammation	[99, 110]
TGF-β	TGF-β-blocking monoclonal antibody	Reduction in fibrosis	[111]
IL-6 receptor	Tocilizumab—IL-6 receptor monoclonal antibody	Inhibition of hyaluronan production and inflammation	[101, 112]
IL-1 receptor	Anakinra—IL-1 receptor antagonist	Inhibition of hyaluronan production and inflammation	[113]

in many tissues, particularly in the thyrocytes and orbital adipose tissue in patients with GD and GO. It belongs to the tyrosine kinase receptors and is involved in processes such as cellular metabolism, growth, apoptosis, and immunity [77]. It also plays a role in the activation of T and B cells. Studies show higher IGF-1R expression in GO orbital fibroblasts than in normal cells [83]. Increased expression of IGF-1R has been found not only in the retroorbital tissue of GO patients but also in the thyroid tissue of GD patients [14]. The stimulation of GO orbital fibroblasts by GD-IgG leading to the synthesis of T-cell chemoattractants, i.e., IL-16 and chemokine RANTES is attenuated by autoantibodies blocking IGF-1R or by transfecting fibroblasts with a dominant negative mutant IGF-IR. This draws attention to the vital role of signaling through IGF-1R in this process [84]. The chemoattractant effect contributes to the recruitment of inflammatory cells into the orbital tissues and promotes the autoimmune response. IGF-1R is found to participate in the differentiation of orbital fibroblasts into adipocytes and in the synthesis of hyaluronan through the action of autoantibodies directed against this receptor [70].

Research indicates that IGF-1 and TSH cooperate in the differentiation and metabolism of thyroid cells [85]. Their common location has been demonstrated in the membrane, in cytoplasmic and nuclear thyroid regions, and also in orbital fibroblasts. Tsui et al. have demonstrated that a monoclonal IGF-1R-blocking antibody inhibits kinase signaling induced by TSH. This antibody can also inhibit M22 (monoclonal TRAb) induced hyaluronan production by orbital fibroblasts. It can result from an association (physical and functional) between IGF-1R and TSHR [86]. Studies have shown that blocking IGF-1R through teprotumumab, a monoclonal antibody, inhibits IGF-1 and TSH action in fibrocytes and reduces the expression of IGF-1R and TSHR [87]. Teprotumumab infusions have great potential in reducing proptosis and the clinical activity score (CAS) in GO [88]. In 2016, the Food and Drug Administration described teprotumumab as a "breakthrough therapy." At present, it is being evaluated in phase III RCT.

6.3. Other Potential Targeted Treatments. Antibodies targeting T cells can be used as a potential therapy since the participation of these cells in the pathogenesis of GO is crucial. Antibodies against CD3 (teplizumab and otelixizumab) lead to the depletion of T cells as in the case of type 1 diabetes [89]. Studies have also found that abatacept, a CTLA4 analogue, diminishes the activation of T cells. This approach was reported to be useful in corticosteroid-resistant rheumatoid arthritis [90]. Furthermore, the application of synthetic peptides in the silencing of autoimmune responses and the induction of T-cell tolerance to autoantigens has been used in experimental autoimmune encephalomyelitis in an animal model of multiple sclerosis [91]. However, none of these approaches connected with inhibiting T cells were investigated in autoimmune thyroid disease [92]. Because CD40-CD154 pathway participates in GD pathogenesis, the anti-CD40 antibody may be a promising approach in the treatment of GD. Iscalimab is one such immunomodulating, human, blocking anti-CD40 monoclonal antibody which can successfully treat Graves' hyperthyroidism [93].

Rituximab, a monoclonal antibody directed against CD20 on B cells, is actively investigated as it expresses an immunosuppressive effect. This monoclonal antibody decreases the production of TRAb [94]. Salvi et al. have demonstrated an improvement in GO activity and severity after the application of rituximab [95]. Although studies conducted by these researchers have also shown favorable effects of treatment by rituximab compared with intravenous methylprednisolone, Stan et al. did not confirm this in the prospective trial [5, 96]. However, long disease duration before treatment initiation may have significantly impacted different results of the mentioned researchers. It seems that rituximab may be vital in the case of a poor response to corticosteroids in patients with GO.

Another possible pathway in the treatment of GO is targeting TNF because of the impact of TNF on the production of MCP-1 by preadipocytes, which is crucial in attracting macrophages [97]. Adalimumab, a monoclonal antibody directed against TNF, was found to reduce

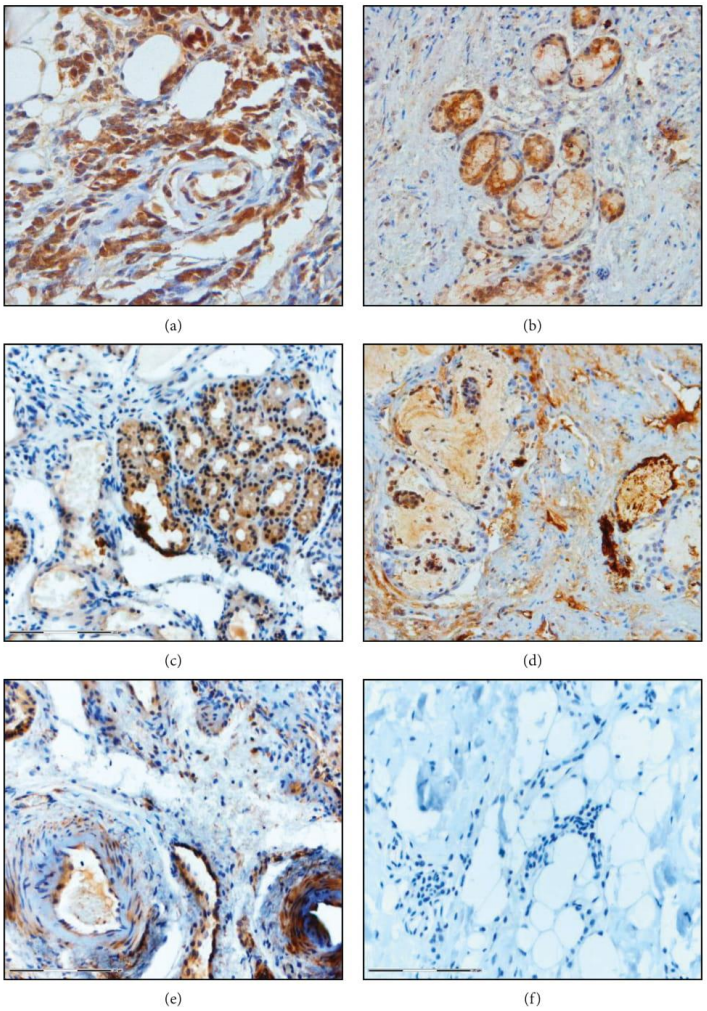

FIGURE 6: Immunohistochemistry on formalin-fixed paraffin-embedded tissue section of fat tissue of the eye socket obtained from patients who underwent endoscopic orbital decompression due to dysthyroid optic neuropathy: (a) TGF-β (×200); (b) TLR-4 (×100); (c) NF-kappa B (×100); (d) HIF-1α (×100); (e) IL-17 (×100); (f) isotype control (×100). The color reaction was visualized using DAB as a chromogen.

inflammation in active GO and etanercept (soluble TNF receptor) can improve soft tissue changes [98, 99]. As TGF-β demonstrates a profibrotic effect, especially in patients with inactive GO, neutralizing this effect can be beneficial.

Serum concentrations of soluble IL-6 receptor are elevated in patients with active GO and correlate with disease activity [100]. Treatment with an IL-6 monoclonal antibody (tocilizumab) leads to decreased proptosis and improvement in eye muscle motility as well as in severity and activity in corticosteroid-resistant GO [101]. IL-1 is also markedly involved in the pathogenesis of GO. Studies carried out on cultured human orbital fibroblasts have shown that an antagonist of the IL-1 receptor (anakinra) inhibits hyaluronan production and decreases inflammation [102]. Potential therapeutic targets in GO are summarized in Table 1 [6, 103].

7. Conclusions and Future Prospects

GD is an autoimmune disease underlying immune tolerance disorders and reactivity to thyroid autoantigens. One of the

nonthyroid symptoms is GO, in which the autoreactive inflammatory process in the orbital tissues plays the main role. Extraocular muscles and connective tissues are infiltrated by immune cells. This inflammatory infiltration and cytokine production result in the activation of orbital fibroblasts, differentiation, and synthesis of GAG. As a consequence, muscle swelling, adipose tissue expansion, and fibrosis develop. Orbital fibroblasts exhibit particular features as they are a target for TSHR and IGF-1R autoantibodies and also possess the ability to differentiate into adipocytes and myofibroblasts. Our preliminary study indicates that, in the orbital adipose tissue of patients with GO, TGF-β, Toll-like receptor 4 (TLR-4), hypoxia-inducible factor-1α (HIF-1α), nuclear factor kappa B (NF-kappa B), and IL-17 are expressed (unpublished data). It is well known that the expression of these proteins is associated with increased fibrosis, inflammation, hypoxia, and autoimmunity (Figure 6). Toll-like receptors (TLR) are classified as pattern recognition receptors and exhibit expression on monocytes, macrophages, dendritic cells, B cells, and T cells. The signaling pathway activates NF-kappa B, leading to cytokine production. TLRs participate in the development of autoimmune and inflammatory diseases [104]. Liao et al. have reported that TLR-9 gene polymorphisms were associated with an increased risk of GO in male GD patients [105]. HIF-1α is activated in response to cellular hypoxia, which results in tissue remodeling in GO through activation of HIF-1α-dependent pathways in orbital fibroblasts. HIF-1α levels in these cells correlate with the clinical activity score of GO patients [106]. Due to insufficient knowledge regarding the pathomechanism of GO, there is no effective and safe method of treating this disease. The current treatment with the use of methylprednisolone pulses is effective in active moderate to severe GO in about 50% of cases and it carries the risk of complications, including fatalities (thromboembolic complications, sudden cardiac deaths, and severe liver damage) [107, 108]. An in-depth understanding of the function of immune cells as well as fibroblasts, adipocytes, and cytokines in GD patients may, in the future, help to define new treatment modalities or improve monitoring of the disease activity.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

RAGE and HMGB1 Expression in Orbital Tissue Microenvironment in Graves' Ophthalmopathy

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Graves' ophthalmopathy (GO) is a chronic autoimmune inflammatory disorder involving orbital tissues. A receptor for advanced glycation end products (RAGE) and its ligand high mobility group box 1 (HMGB1) protein trigger inflammation and cell proliferation and are involved in the pathogenesis of various chronic inflammatory diseases. This study was aimed to evaluate RAGE and HMGB1 expression in GO to determine its potential clinical significance. To the best of our knowledge, this is the first study showing RAGE and HMGB1 expression in orbital tissue using immunohistochemistry. Sections of orbital adipose tissue obtained from patients diagnosed with GO (23 patients; 36 orbits) and normal controls (NC) (15 patients; 15 orbits) were analyzed by immunohistochemistry for RAGE and HMGB1 expression. Expression profiles were then correlated with clinical data of the study group. RAGE and HMGB1 expression were elevated in GO patients in comparison with NC ($p = 0.001$ and $p = 0.02$, respectively). We observed a correlation between RAGE expression and occurrence of dysthyroid optic neuropathy (DON) ($p = 0.05$) and levels of TSH Receptor Antibodies (TRAb) ($p = 0.01$). Overexpression of RAGE and HMGB1 might be associated with GO pathogenesis. In addition, RAGE and HMGB1 proteins may be considered as promising therapeutic targets, but this requires further research.

1. Introduction

Graves' disease (GD) represents an autoimmune process in which circulating autoantibodies directed against thyrotropin receptor (TSHR)—TRAb (TSHR antibodies)—activate the thyroid gland, causing hyperthyroidism [1]. One of the extrathyroidal symptoms of GD is Graves' ophthalmopathy (GO), defined as a chronic autoimmune inflammatory disorder involving orbital tissues [2]. Although patients with GO are mostly hyperthyroid, they can also be euthyroid or hypothyroid. Moreover, GO may be reported in Hashimoto's thyroiditis [3]. Cytokine production, inflammatory infiltration, and orbital fibroblast activity result in expansion and remodeling of extraocular tissues—mainly orbital adipose tissue and fibrous tissue of extraocular muscles. Edematous-infiltrative changes involving orbital soft tissues are observed

in 25–50% of patients with GD [4]. Clinical manifestations of GO include lid retraction, double vision, soft tissue swelling, and erythema of the conjunctival and periorbital tissues. Increased intraocular pressure within the inflexible bony orbital walls can contribute to the development of proptosis and optic nerve compression, including dysthyroid optic neuropathy (DON). According to the European Group on Graves' Orbitopathy (EUGOGO), severity of GO is rated as mild, moderate-to-severe, and sight threatening (including DON and/or severe keratitis) [5].

Multiligand receptor for advanced glycation end products (RAGE) is suggested to initiate and amplify immune and inflammatory responses [6]. Increased levels of RAGE ligands in chronic disorders indicate that RAGE is involved in the pathogenesis of various inflammatory diseases [7]. Cellular stress causes the generation of RAGE ligands such as high mobility group box 1 (HMGB1) protein, S100 proteins, and nucleic acids, while prolonged hyperglycemia and inflammation induce the release of the ligands AGE and amyloid [8]. HMGB1 is one of the most significant members of the DAMP (damage-associated molecular patterns) family. DAMPs involve molecules released by dying or necrotic cells and can induce inflammation, cell proliferation, and migration [9]. HMGB1-RAGE interaction affects inflammation via the activation of proinflammatory transcription factor NF-κB (nuclear factor kappa B) which furthermore regulates RAGE [10].

In this study, we test the hypothesis that RAGE and HMGB1 are overexpressed in orbit tissue in GO and that expression patterns correlate with disease severity. To the best of our knowledge, this is the first study showing RAGE and HMGB1 expression in orbital tissue by immunohistochemistry.

2. Materials and Methods

2.1. Patients and Tissue Collection. Archival tissue paraffin blocks were used for the studies. All orbital adipose tissue samples used in the study were collected during surgery for routine histopathological examination between 2016 and 2020. Tissues were obtained from 23 patients (18 females, 5 males, 36 orbits) diagnosed with GO who underwent transnasal endoscopic orbital decompression surgery in the Otolaryngology Department, Centre of Postgraduate Medical Education in Warsaw, Poland, or were derived from a tissue bank at the Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Poland, from previous studies. Inclusion criteria for the study were in accordance with the EUGOGO guidelines: (1) moderate-to-severe GO or (2) sight-threatening GO. Patients with any of the following conditions were excluded from the study: (1) age under 18 years, (2) a history of eye surgery, (3) a previous history of metabolic diseases or other diseases that may affect orbital connective tissues, except for thyroid disease, and (4) pregnancy or lactation. As controls, orbital adipose tissue samples were obtained from 15 patients (15 orbits) operated on the orbit due to trauma at the Department of Maxillofacial Surgery, Medical University of Warsaw, Poland. The study was approved by Local Ethics Committees at the Medical University of Warsaw in Poland (#AKBE/86/2018 to P.M.; KB/126/2016 to M.J.S) and at the Centre of Postgraduate Medical Education (#16/PB/2018 to I.K.). Tissues were fixed in 10% formaldehyde and embedded in paraffin. Sections were stained with hematoxylin-eosin (H+E) and evaluated by light microscopy before performing immunohistochemistry.

2.2. Clinical Characteristics of Graves' Ophthalmopathy. Ocular involvement, including GO severity and activity, was defined as absent, mild, moderate-to-severe, and sight-threatening—active or inactive, according to criteria reported in EUGOGO recommendations [5, 11]. Patients were classified as "active" or "inactive" based on a Clinical Activity Score (CAS). Dysthyroid optic neuropathy (DON) was defined by visual dysfunction secondary to GO when other causes for visual impairment had been excluded. The diagnosis of DON was based on at least two criteria from the following: (1) reduced visual acuity (<1.0), (2) relative afferent pupillary defect, (3) reduced color vision (more than two errors in Ishihara plates), (4) optic disc swelling in the affected eye, and (5) magnetic resonance imaging of orbit showing apical crowding or optic nerve stretching. Corneal involvement (keratitis) was defined as absent or punctuate keratopathy/ulcer. Best-corrected visual acuity (BCVA) was examined using Snellen charts and expressed as a decimal fraction. Exophthalmos was measured by a Hertel exophthalmometer. The intraocular pressure was measured in the primary position using an applanation tonometer. All the ophthalmology examinations for all patients were carried out by the same ophthalmologist. Clinicopathological characteristics of Graves' ophthalmopathy (GO) patients are presented in Table 1.

2.3. Immunohistochemistry. Paraffin sections of GO patients and NC patients were immunostained using NovoLink Polymer Detection Systems (Novocastra Laboratories, Newcastle, UK) and the following primary antibodies diluted 1 : 100 in Antibody Diluent (Dako): rabbit polyclonal anti-human HMGB1 (LS-C2691, LifeSpan BioSciences, Inc., Seattle, WA, USA) and mouse monoclonal anti-human RAGE (LS-B6042, LifeSpan BioSciences, Inc., Seattle, WA, USA). Deparaffinated and rehydrated sections were stained according to the manufacturer's instructions, as previously described [12]. The activity of endogenous peroxidase was blocked by Peroxidase Block (NovoLink Polymer Detection System; Novocastra Laboratories). Sections were incubated with primary antibodies overnight and were subsequently incubated with Post Primary (rabbit anti-mouse IgG; NovoLink Polymer Detection System; Novocastra Laboratories) which detected mouse antibodies and with Polymer (NovoLink Polymer Detection System; Novocastra Laboratories) to recognize rabbit antibodies and detect Post Primary and finally with 3,3′-diaminobenzidine chromogen (NovoLink Polymer Detection System; Novocastra Laboratories). Nonspecific binding of the primary antibodies and the polymer was eliminated by application of Protein Block (NovoLink Polymer Detection System; Novocastra Laboratories) before adding the primary antibodies. Sections were counterstained with

Table 1: Clinical characterization of the Graves' ophthalmopathy (GO) patients included in this study at the day of surgery.

Characteristics	Patients ($n = 23$) 36 orbits	Normal controls ($n = 15$) 15 orbits
Sex (number of patients)		
Female	18	4
Male	5	11
Age (years)		
Range	35-73	36-56
Median	65	46
Disease status (number of orbits)		
Moderate-to-severe GO	14	
Severe GO		
With DON	12	
With severe keratitis	10	
Graves' disease (number of patients)	23	
Median CAS	2	
Thyroidectomy	7	
Median proptosis (mm)	23	
Median IOP (mmHg)	16	
Median BCVA	0.9	
Median fT4 (pmol/L)	19.4	
Median TRAb (U/L)	12.4	
Median aTPO (U/mL)	89.3	
Median aTG (U/mL)	144	

Abbreviations: DON: dysthyroid optic neuropathy; CAS: clinical activity score; IOP: intraocular pressure; BCVA: best-corrected visual acuity; fT4: free thyroxine; TRAb: anti-TSHR antibodies; aTPO: antithyroid peroxidase; aTG: antithyroglobulin.

hematoxylin (Dako), dehydrated, coverslipped, and evaluated by light microscopy ZEISS Observer Z1 (Axiovision 4.8 software; illumination system LUMEN 200; PRIOR) in high power field (magnification ×400). Results were scored by two independent investigators (M.J.S. and D.Ł.) as positive (++), heterogeneous (+), or negative (-), when the number of stained cells in each section was >75 cells, between 25 and 75 cells, and <25 cells, respectively, as previously described [13].

2.4. Statistical Analysis. Statistical analysis was performed by professional statistician using the Statistica 13 software package. The Fisher exact test was used for the binary variable. When comparing continuous variable, without normal distribution (checked with a Kolmogorov-Smirnov test), differences between groups were measured using Mann–Whitney's U test. The significance level was established at $p \leq 0.05$.

3. Results

3.1. RAGE and HMGB1 Expression in Tissue Sections. RAGE expression levels were elevated in GO tissues compared to those from NC ($p = 0.001$; Figure 1(a)). In GO tissues, RAGE was detected in the cytoplasm, and the expression was positive or heterogenous with staining intensities ranging from weak to strong. In contrast, RAGE expression was observed in only 25% of NC tissues and its staining intensity ranged from negative to weak (Figure 1(a)).

HMGB1 was detectable in the nuclei and cytoplasm in all tissues of GO patients and NC (Figure 1(b)). Intensity of HMGB1 staining was evaluated as moderate or strong (Figures 1(a) and 1(b)). Differences between GO and NC in terms of HMGB1 expression were statistically significant ($p = 0.02$; Figure 1(a)).

3.2. RAGE Expression Correlates with Disease Severity. In the GO cohort, we observed differences in RAGE positivity depending on occurrence of DON and TRAb levels (Figure 2). GO patients with DON had stronger expression of RAGE (positivity "++") than tissues from patients without DON ($p = 0.05$) (Figure 2(a)). Moreover, RAGE staining correlated with TRAb levels in GO patients. Positivity "++" was characteristic for high levels of TRAb ($p = 0.02$; Figure 2(b)).

The correlation of HMGB1 expression levels in the GO cohort with clinical data revealed no statistically significant differences ($p > 0.05$). However, we observed the tendency between increased levels of HMGB1 expression in patients with sight-threatening GO with DON (data not shown).

3.3. RAGE and HMGB1 Expression in Inflammatory Infiltrates. In GO tissues, we observed elevated numbers of inflammatory cells that expressed RAGE and HMGB1 proteins. Those polymorphonuclear and mononuclear cells were mainly localized in close proximity to the vessels (Figure 3).

4. Discussion

In our study, we demonstrated significant differences between GO and NC tissues for RAGE and HMGB1 expression. Enhanced expression of RAGE occurs in conditions of inflammatory mediators and ligands for RAGE accumulation [8]. Literature data indicate that the inflammatory process comprising orbital tissues underlies the pathogenesis of GO [14, 15]. Immune cell infiltration as well as cytokine production activates orbital fibroblasts and GAG synthesis resulting in adipose tissue expansion. Yoon et al. have demonstrated that mRNA and protein levels of the proinflammatory cytokines TNF-α and IL-1β are increased in GO tissues compared to healthy controls [16]. Furthermore, our preliminary study has shown that a variety of proteins and cytokines involved in inflammation, autoimmunity, hypoxia, and fibrosis are expressed in GO orbital adipose tissue microenvironment, including TGF-β, TLR-4 (Toll-like receptor 4), HIF-1α (hypoxia-inducible factor-1α), NF-κB (nuclear factor kappa B), and IL-17 [14].

Current evidences point out that HMGB1 protein is involved in numerous chronic inflammatory and autoimmune diseases including rheumatoid arthritis, atherosclerosis, and systemic lupus erythematosus (SLE) [17, 18]. Released HMGB1 interacts with its cell-surface receptor—RAGE, activating the main signaling pathways responsible for the pathogenesis of these diseases. HMGB1 is involved

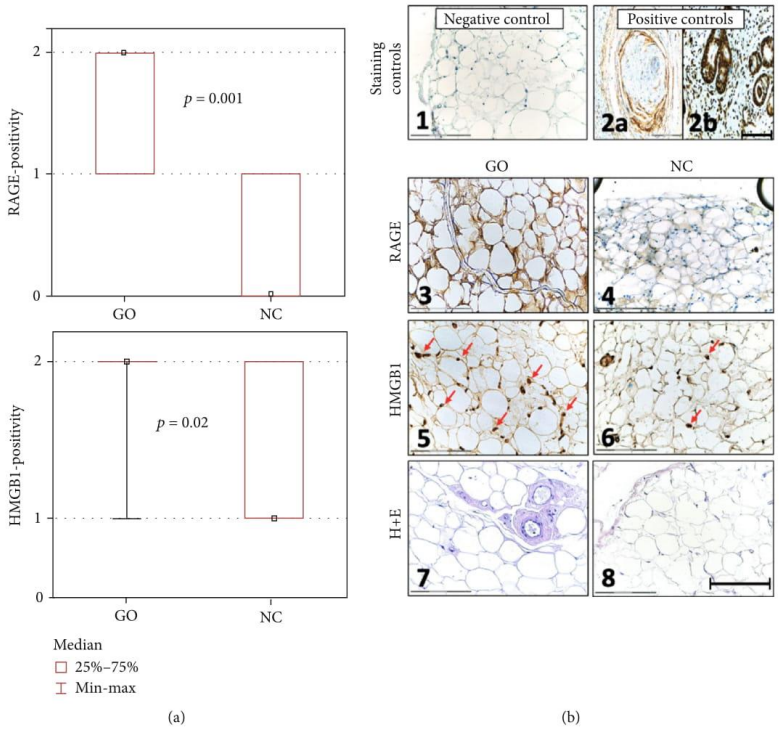

FIGURE 1: RAGE and HMGB1 expression in normal control (NC) and in Graves' ophthalmopathy (GO) tissues. (a) Statistical analysis of RAGE and HMGB1 expression in GO tissues compared to NC. The sections were scored as described in Material and Methods. (b) Representative images of immunohistochemistry staining. B1: isotype negative control of GO tissue. B2: positive control. (a) RAGE expression on head and neck squamous cell carcinoma tissue. (b) HMGB1 expression on nasal cavity mucosa. B3: RAGE expression in GO tissue. B4: RAGE expression in NC tissue. B5: HMGB1 expression in GO tissue (arrows). B6: HMGB1 expression in NC tissue (arrows). B7: hematoxylin and eosin (H+E) staining of GO tissue. B8: H+E staining of NC tissue. p value ≤ 0.05 was considered to be significant. Bar = 150 μm.

in the activation of innate and adaptive immunity, development of inflammation, and increased production of cytokines. In patients with rheumatoid arthritis, the production of HMGB1 and the number of cells secreting HMGB1 at specific inflammation areas are elevated [19, 20]. Experimental models of arthritis showed inhibitory effects of anti-HMGB1 antibodies on the development of synovial inflammation. HMGB1 is also found in SLE patients' plasma, and after being released from apoptotic cells, HMGB1 is bound to nucleosomes [21]. In turn, autoantibodies directed against nucleosomes and double-stranded DNA are characteristics for SLE. Our previous study demonstrated the expression of RAGE and HMGB1 in epithelial cells of sinonasal mucosa samples obtained from patients with chronic rhinosinusitis or in epithelial cells of middle ear cholesteatoma [12, 22]. We observed a strong correlation between the disease severity and RAGE expression.

Studies have shown that HMGB1 is involved in the pathogenesis of autoimmune thyroiditis and other chronic diseases by augmenting inflammation signaling and inflammatory infiltration [22–24]. Han et al. demonstrated that HMGB1 and its receptors are associated with the inflammatory mechanisms of GO and blocking of the HMGB1 pathway can be utilized to treat GO patients [25]. The authors demonstrated higher gene expression levels of RAGE and TLRs and higher mRNA and protein levels of HMGB1 in GO tissues compared to non-GO tissues. Moreover, blocking of HMGB1, RAGE, and TLR caused diminished production of proinflammatory cytokines confirming the participation of HMGB1 in GO inflammation. Plasma levels of HMGB1 were shown to correlate with the clinical activity score (CAS), indicating a valuable biomarker of disease activity. Being coherent with previous studies, we confirm the expression of RAGE and HMGB1 in GO tissues using immunohistochemistry. We observed a strong correlation between DON and RAGE expression in GO tissues. In addition, in GO tissues we demonstrated the presence of RAGE and HMGB1 positive inflammatory cells which were closely located to

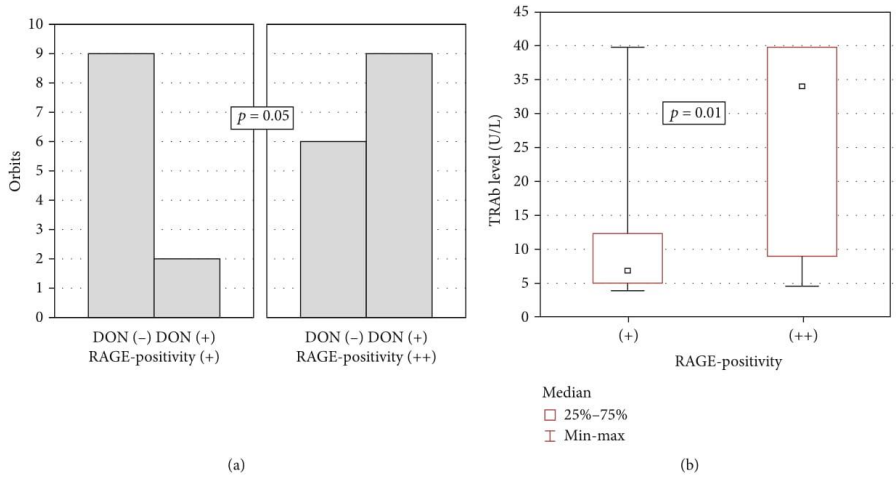

FIGURE 2: RAGE expression in correlation with clinical data. (a) RAGE positivity vs. occurrence of dysthyroid optic neuropathy (DON) ($p = 0.05$). (b) RAGE positivity vs. levels of TSHR antibodies (TRAb; $p = 0.01$). p value ≤ 0.05 was considered to be significant.

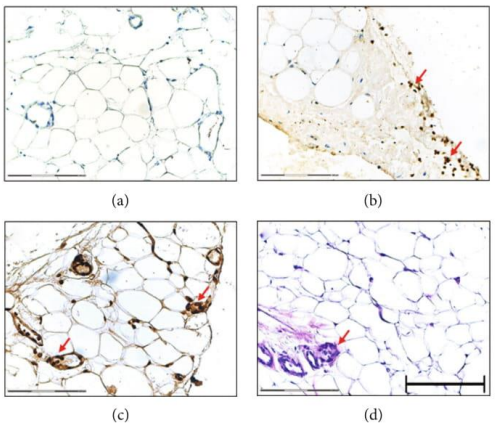

FIGURE 3: RAGE and HMGB1 expression on inflammatory infiltrates in GO tissues. (a) Isotype negative control staining of GO tissue. (b) RAGE expression on inflammatory infiltrates (arrows). (c) HMGB1 expression on inflammatory infiltrates (arrows). (d) H+E staining of GO tissue. Bar = 150 μm.

the vessels. We hypothesize that those cells may also play an important role in driving the inflammatory process. According to our knowledge, this is the first study that has shown the presence of RAGE and HMGB1 positive inflammatory cells in the GO adipose tissue microenvironment.

Peng et al. demonstrated that HMGB1 and RAGE expression on monocytes isolated from PBMCs was higher in patients with autoimmune thyroid diseases (AITD) compared to those isolated from healthy controls [26]. Their findings suggested a vital role of RAGE and HMGB1 in the pathogenesis of AITD. RAGE participates in inflammatory cell recruitment and chronic inflammation development, which are considered crucial aspects of GO pathogenesis [25, 27].

Pathogenesis of GO is closely associated with the presence of TRAb which involves autoantibodies directed against TSHR expressed not only on thyrocytes but also on orbital tissues. TRAb levels correlate with the clinical activity of GO [28]. Diana et al. reported a correlation between TRAb and the degree of ocular changes in GD [29]. However, the relationship between TRAb level and HMGB1 expression

was not observed by us. In turn, Han et al. demonstrated a strong correlation between plasma level of HMGB1 and TRAb as well as CAS. Literature data report a correlation between RAGE levels in the serum and disease activity of other autoimmune diseases [30]. Studies analyzing the involvement of RAGE and HMGB1 in the pathogenesis of various inflammatory diseases are accompanied by experimental studies showing anti-RAGE/HMGB1 antibodies that prevent chronic inflammation [31, 32].

In summary, we demonstrated for the first time by using immunohistochemistry that RAGE and HMGB1 are overexpressed in GO patients in comparison with NC. We also observed a correlation between RAGE expression and occurrence of dysthyroid optic neuropathy (DON) or elevated levels of TSH Receptor Antibodies (TRAb) in GO patients. Overexpression of RAGE and HMGB1 might be associated with GO pathogenesis.

Data Availability

The research and clinical data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

M.J.S. designed the study; D.Ł. and A.G. conducted the experiments; M.J.S., I.K., K.B.P., K.C., P.M., A.J.P., and Z.S. collected the clinical data; M.M.G. statistically analyzed the data; M.J.S., N.L., P.M., A.J.P., and J.B. interpreted the data; D.Ł., M.J.S., and N.L. prepared the manuscript; M.J.S., N.L., D.Ł., and J.B. analyzed the literature; D.Ł. and M.J.S. funded the study.

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4. Podsumowanie i wnioski

Przegląd piśmiennictwa na temat roli układu odpornościowego i mechanizmów immunologicznych zaangażowanych w etiologię i patogenezę GO posłużył do przygotowania pracy przeglądowej pt.: „Immunological Aspects of Graves' Ophthalmopathy". W artykule opisano jednostkę chorobową jaką jest GO. Zwrócono szczególną uwagę na fibroblasty oczodołowe oraz omówiono udział autoantygenów w patogenezie GO z uwzględnieniem potencjalnych celów terapeutycznych w leczeniu choroby. Wskazano również na zaangażowanie TGFβ (transformujący czynnik wzrostu β; ang. transforming growth factor β), TLR4, HIF-1α (czynnik indukowany hipoksją 1α; ang. hypoxia-inducible factor-1α), NF-κB i IL-17, których ekspresję wykazano we wstępnych oznaczeniach immunohistochemicznych wykonanych na tkankach oczodołowych, uzyskanych od pacjentów z GO (dane nieopublikowane). Ekspresja wymienionych białek jest związana z rozwojem stanu zapalnego, hipoksji i procesów włóknienia.

W swojej pracy badawczej zwróciłam uwagę na receptor RAGE i jego ligand HMGB1 ze względu na ich udział w patogenezie chorób o podłożu zapalnym. Wyniki oznaczeń immunohistochemicznych oceniających ekspresję RAGE i HMGB1 w tkance tłuszczowej oczodołowej pobranej od pacjentów z GO zostały opublikowane w artykule pt. „RAGE and HMGB1 Expression in Orbital Tissue Microenvironment in Graves' Ophthalmopathy". W pracy wykazano istotne statystycznie różnice w ekspresji zarówno RAGE, jak i HMGB1 między grupą badaną i grupą kontrolną. Kontrolę stanowiła tkanka oczodołowa uzyskana od pacjentów operowanych z powodu urazów w obrębie oczodołów. Następnie w grupie badanej oceniono korelację ekspresji RAGE i HMGB1 z wybranymi parametrami klinicznymi takimi jak: wskaźnik klinicznej aktywności choroby (CAS), neuropatia nerwu wzrokowego (DON; ang. dysthyroid optic neuropathy), zapalenie rogówki, najlepsza skorygowana ostrość wzroku (BCVA; ang. best-corrected visual acuity), wytrzeszcz i ciśnienie śródgałkowe (IOP). Zaobserwowano silną korelację pomiędzy ekspresją RAGE, a występowaniem DON, a także poziomem TRAb u pacjentów z GO. W grupie badanej nie wykazano natomiast istotnych statystycznie korelacji pomiędzy ekspresją HMGB1, a ocenianymi parametrami klinicznymi. Z kolei Han i in. wykazali, że poziom osoczowego białka HMGB1 jest istotnie wyższy u pacjentów z aktywną postacią GO i koreluje z poziomem TRAb oraz wskaźnikiem CAS [31]. Należy podkreślić, że przeprowadzone przeze mnie badania nie oceniały HMGB1

w surowicy pacjentów, a jedynie ekspresję miejscową w mikrośrodowisku tkanki tłuszczowej oczodołów.

Badania przeprowadzone w ramach pracy doktorskiej po raz pierwszy oceniają ekspresję RAGE i HMGB1 metodą immunohistochemiczną na unikalnym materiale biologicznym jakim jest tkanka tłuszczowa oczodołów. Ponadto w pracy po raz pierwszy wykazano obecność komórek nacieków zapalnych RAGE- i HMGB1- dodatnich, zlokalizowanych blisko naczyń, wskazując na ich potencjalny udział w rozwoju procesu zapalnego w GO. Wnioski płynące z przeprowadzonych badań wskazują na potencjalną rolę RAGE i HMGB1 w rozwoju stanu zapalnego w tkankach oczodołowych w patogenezie GO. Dogłębne zrozumienie udziału immunologicznych procesów w patomechanizmie GO stanowi drogę do tworzenia nowych skutecznych i bezpiecznych metod leczenia, a także usprawnienia monitorowania aktywności choroby.

Opinia Komisji Bioetycznej

CENTRUM MEDYCZNE KSZTAŁCENIA PODYPLOMOWEGO
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Uchwała Komisji Bioetycznej nr 16/PB/2018
z dnia 21 LUT. 2018

Komisja Bioetyczna przy Centrum Medycznym Kształcenia Podyplomowego powołana przez Dyrektora Centrum Medycznego Kształcenia Podyplomowego Zarządzeniem nr 39/2015 z dnia 25.03.2015r. zapoznała się z wnioskiem o wyrażenie opinii o projekcie eksperymentu medycznego/badania klinicznego:

pt: **„Ocena histologiczna tkanki tłuszczowej oczodołu u pacjentów z orbitopatią tarczycową i po urazach oczodołów"**

zgłoszonym przez głównego badacza:

dr hab. n. med. Ireneusza Kantora

Klinika Otolaryngologii CMKP
Mazowiecki Szpital Bródnowski w Warszawie Sp. z o.o.
Bródnowskie Centrum Kliniczne
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Po zapoznaniu się z całością dokumentacji Komisja Bioetyczna działając zgodnie z art. 29 ust. 2, 2a ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty (Dz. U z 2017r. poz. 125, z późn. zm.) i § 6 rozporządzenia Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 roku w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych (Dz. U. z 1999 r. Nr 47, poz. 480) oraz zasadami GCP:

- wyraziła pozytywną opinię* o rozpoczęciu eksperymentu medycznego/badania klinicznego zgodnie z przedstawionym wnioskiem, o którym stanowi Załącznik nr 1 do niniejszej uchwały.
- ~~odrzuciła wniosek* z powodu:~~

(* - niepotrzebne skreślić)

Data

14 MAR. 2018

PRZEWODNICZĄCA KOMISJI BIOETYCZNEJ
przy Centrum Medycznym Kształcenia Podyplomowego

prof. dr hab. n. med. Ewa Marcinowska-Suchowierska

1

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KB/..126/2016

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dotyczącym: wyrażenia opinii w sprawie badania pt. **„Ocena histologiczna tkanki tłuszczowej oczodołu u pacjentów z orbitopatią tarczycową i po urazach oczodołów"**

wyraża następującą
o p i n i ę

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- ~~stwierdza, że jest ono niedopuszczalne i niezgodne z zasadami naukowo-etycznymi.*~~

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Komisja działa zgodnie z zasadami GCP .
W załączeniu: skład komisji oraz lista obecności

Przewodniczący Komisji Bioetycznej

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po zapoznaniu się z wnioskiem /wymienić wnioskodawcę/ - **w dniu 15 marca 2011r.**
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dotyczącym: wyrażenia opinii w sprawie badania pt.: " Nowe markery biochemiczne i molekularne oceniające ciężkość i przebieg choroby Gravesa i Basedowa."

Uwagi Komisji-verte

wyraża następującą

o p i n i ę

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Pouczenie-w ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje Prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym.
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W załączeniu- skład Komisji oraz lista obecności.

Przewodniczący
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Oświadczenia wszystkich współautorów publikacji określające indywidualny wkład każdego z nich w ich powstanie

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Warszawa, 05.05.2021 r.

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dotyczył zebrania danych klinicznych i wyniósł 20 %.

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STATEMENT

I declare that my contribution to the preparation of the publication :

Łacheta Dominika , Poślednik Krzysztof , Czerwaty Katarzyna , Ludwig Nils , Molińska-Glura Marta , Kantor Ireneusz , Jabłońska-Pawlak Anna , Miśkiewicz Piotr Stefan, Głuszko Alicja , Stopa Zygmunt , Brzost Jacek , Szczepański Mirosław Jerzy.
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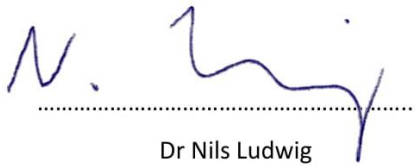
concerned assistance in data interpretation, literature analysis and manuscript preparation.
The contribution was 4 %.



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