AMERICAN ACADEMY OF OPHTHALMOLOGY®

Exploring the Molecular Intersection of Posterior Ocular Tuberculosis: *Mycobacterium tuberculosis* Proteins, Ocular Autoimmunity, and Immune Receptor Interactions

Işıl Kutlutürk Karagöz, MD, PhD,[1] *Mücahit Kaya, PhD,*[2] *Ulviye Kıvrak, PhD,*[1] *Marion R. Munk, MD, PhD*[3,4,5]

Purpose: The presentation of posterior ocular tuberculosis (TB) varies greatly along with the need for immunomodulatory therapy to control inflammation. In this study, we explore the potential mechanisms and pathways for autoimmune-related inflammation in ocular TB using molecular mimicry-based mathematical modeling.

Design: Computational protein analysis.

Methods: Twenty-three TB-related proteins, including ESAT-6 subgroup proteins, and 23 retinal ganglion cells, photoreceptor, and retinal pigment epithelium (RPE) cellular proteins were included in this study. The 3-dimensional structure and sequence of the TB AG proteins were compared to the above-mentioned retinal, photoreceptor, and RPE cellular proteins. All retinal proteins were obtained from the UniProt database. The sequence and 3-dimensional structure of TB-related proteins and retinal proteins were compared with the TM-align server. The interactions of proteins showing significant similarity (template modeling score above 0.5, root mean square deviation [RMSD] value below 5A°) with cytokines (interleukin [IL]6, IL10, IL12A, IL12B, TLR2, TLR3, and TLR4) were analyzed. Autoimmune and autoinflammation-related protein—receptor interaction of similar proteins was assessed using the CABS-dock web server.

Main Outcome Measures: Template modeling score, structural alignment accuracy using RMSD value, protein—cytokine interaction.

Results: We detected a high level of structural similarity between ESAT-6 (EsxA, EsxB) proteins and rhodopsin, HSPA1A, RPE-related BEST-1, ABCC-1, ABCC-4, ABCC-5, SLC47A1, SLC1A5, SLC38A7, SLC6A6, SLC5A6, LAT-1, and SLC16A1 proteins. When we evaluated the likelihood/potential to stimulate an immune response via a cytokine release, TLR-2 (most common), TLR-3, and TLR-4, which are highly susceptible to *Mycobacterium tuberculosis* ESAT-6 (ESXA and ESXB) proteins, showed a potential receptor—protein interaction with retinal proteins. Moreover, some eye-related proteins had the capacity to trigger the T-cell response by binding to cytokines such as IL-12, IL-10, and IL-6, which are all highly overexpressed in TB infections.

Conclusions: Our study demonstrates that TB proteins may have significant structural similarities with many eye-related proteins. These eye-related proteins are therefore immunological target sites and may be secondarily affected by any immune response toward TB.

Financial Disclosure(s): Proprietary or commercial disclosure may be found in the Footnotes and Disclosures at the end of this article. *Ophthalmology Science 2025;5:100698 © 2024 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Tuberculosis (TB) is a global health problem, and according to the World Health Organization, TB is one of the 10 most common causes of death worldwide.[1] One-third of the world's population is infected with *Mycobacterium tuberculosis* (MTB), and the microorganism persists latent among approximately 70% to 80% of immunocompetent infected people.[1] Tuberculosis can affect various organs in the body. A rare form is ocular TB, which can lead to severe vision loss. The prevalence of ocular TB ranges between 10% and 26% in high endemic regions such as India and Saudi Arabia, whereas it is estimated to be found in 1% to 4% in low endemic regions such as the United States, Europe, and Japan.[2]

Ocular TB can affect any tissue in the eye. It usually presents as TB uveitis (TBU). The prevalence of TBU varies from 0.2% to 10.5%.[2,3] Tuberculosis-related posterior uveitis and choroiditis are the most common forms of TBU. It has been suggested that MTB directly infects the eye tissues or may indirectly lead to intraocular inflammation through the presence of MTB in the vitreous, aqueous fluid, retinal pigment epithelium (RPE), or choroid in Tuberculous Serpiginous-like Choroiditis.[4–7] Tuberculosis uveitis

https://doi.org/10.1016/j.xops.2024.100698
ISSN 2666-9145/25

phenotypes vary widely, each potentially triggered by different mechanisms. Choroidal tuberculoma, for example, occurs as the immune system attempts to contain the infection by forming granulomas, which are clusters of immune cells that surround and isolate the bacteria. Although granulomas are meant to prevent the spread of MTB, they also create a localized area of intense inflammation. This immune response appears to be directly caused by microbial invasion from MTB, and it typically responds effectively to antitubercular therapy. Conversely, tubercular serpiginous–like choroiditis is primarily driven by indirect immune responses, requiring extensive immunomodulatory treatment to control the inflammation (Fig 1).[8] This condition is characterized by multifocal chorioretinal lesions mimicking idiopathic serpiginous choroiditis.[4–6] The direct immune response is quite well understood. It involves initially macrophages, dendritic cells, and neutrophils, which recognize MTB through pattern recognition receptors. The following response of the adaptive immunity involves particularly $CD4^+$ T cells, which release cytokines such as interferon gamma. However, the knowledge on the involvement of autoimmunity and crossreactivity, which seem to be very relevant in phenotypes such as serpiginous-like choroidopathy, is very limited.

In this study, we explore the immune pathways, cytokines, receptors, and target proteins involved in TBU and explore the developmental mechanism of autoimmunity during MTB infection by utilizing mathematical models based on molecular mimicry.

Methods

Obtaining Protein Data

Twenty-three structural and infection-related MTB proteins, including ESAT-6 subgroup proteins (Esx A, B, H, L, N, O, Q, and R), and 23 ocular proteins, including retinal ganglion cell, photoreceptor (PR), and RPE proteins, were assessed in the study (Table 1). A 3-dimensional structure and sequence comparison between all the MTB-related and the ocular proteins was performed. Protein data of MTB and all ocular-related proteins and cytokines were obtained by the AlphaFold estimation method in the UniProt database (https://www.uniprot.org/) (https://www.nature.com/articles/s41586-021-03819-2). We used mTM-Align (efficient protein structure comparisons) and CABS-dock Server (flexible protein–peptide docking) to target the sequence and leverage the calculated structural similarities (Fig 2). This study is purely informatic, devoid of human or animal research, thereby negating the need for ethics approval, which was consequently not sought.

mTM-Align (Efficient Protein Structure Comparisons)

The TM-align web tool was employed to compare the sequence and 3-dimensional structure of ocular and MTB protein structures of unknown sequence-independent equivalence (https://yanglab.nankai.edu.cn/mTM-align/). Through this methodology, we achieved the optimal superposition and assessed the structural similarity between the 2 protein structures, quantified by the template modeling score (TM-score). Typically, TM scores range from 0 to 1, with a score of 1 indicating a perfect alignment between the 2 protein structures. According to previous literature, we established a cutoff value of $\geq$0.5 in our study as the threshold to consider the 2 proteins as structurally similar.[9]

CABS-Dock Server (Flexible Protein–Peptide Docking)

The CABS-dock web server offers a platform for modeling protein–peptide interactions through a sophisticated protocol that facilitates the flexible insertion of peptides into proteins (accessible at http://biocomp.chem.uw.edu.pl/CABSdock). Consequently, the docking procedure initiates with the generation of a random peptide structure, followed by its random positioning on the receptor site. All possible conformations were then determined using the Monte Carlo algorithm.[10,11] Root mean square deviation (RMSD) values resulting from docking that were below 5.5 to 6 Å were deemed significant, whereas values above this range were classified as insignificant. We analyzed the interactions of proteins that demonstrated significant similarity (TM-score above 0.5, RMSD value under 5 Å) with specific cytokines (interleukin [IL]6, IL10, IL12A, IL12B, TLR2, TLR3, and TLR4) that have been shown to play an important role in TBU using the CABS-dock web server (accessible at http://biocomp.chem.uw.edu.pl/CABSdock). Furthermore, we utilized the CABS-dock tool to investigate protein–receptor interactions related to autoimmunity and autoinflammation in proteins with similar structures.[12]

The databases of experimentally verified peptides, mimicDB and miPepBase, were queried as well.

The study adheres to the Declaration of Helsinki.

Results

A significant structural similarity and similar epitopic regions were found between RPE and PR proteins and both Esx-A (also known as the 6 kDa early secretory antigenic target, ESAT-6) and Esx-B of the MTB-related 23 proteins. There was no similarity between the assessed retinal ganglion cell proteins and MTB-related proteins. Apart from these 2 secreted MTB proteins, no similarity was found between other MTB-related proteins and ocular proteins (Tables 1 and 2). Esx-A (ESAT-6) and Esx-B (CFP-10) proteins are the most important proteins responsible for the virulence of a TB infection. The Esx-A protein demonstrated structural similarity and shared epitopic domains with 13 proteins associated with the RPE and PRs (TM-score >0.5; Table 2): Two PR-related proteins, rhodopsin (RHO) and heat shock 70 Da 1A (HSPA1A), had similar epitopic domains with ESX-A protein. The remaining 11 proteins were RPE-related and included bestrophin-1 (BEST-1), multidrug resistance–associated proteins (ABCC-1, ABCC-4, ABCC-5), multidrug and toxin extrusion protein 1 (SLC47A1), neutral amino acid transporter B (SLC1A5), putative sodium–coupled neutral amino acid transporter 7 (SLC38A7), sodium- and chloride-dependent taurine transporter (SLC6A6), sodium-dependent multivitamin transporter (SLC5A6), sodium-independent neutral amino acid (LAT-1), and monocarboxylate transporter 1 (SLC16A1) protein (Table 2, Fig 3). Ganglion cell-related proteins exhibited no similarity with Esx-A.

When the Esx-B structural protein similarity results were evaluated, similar epitopic areas were detected in 10 ocular-related proteins (TM-score >0.5; Table 2). This included

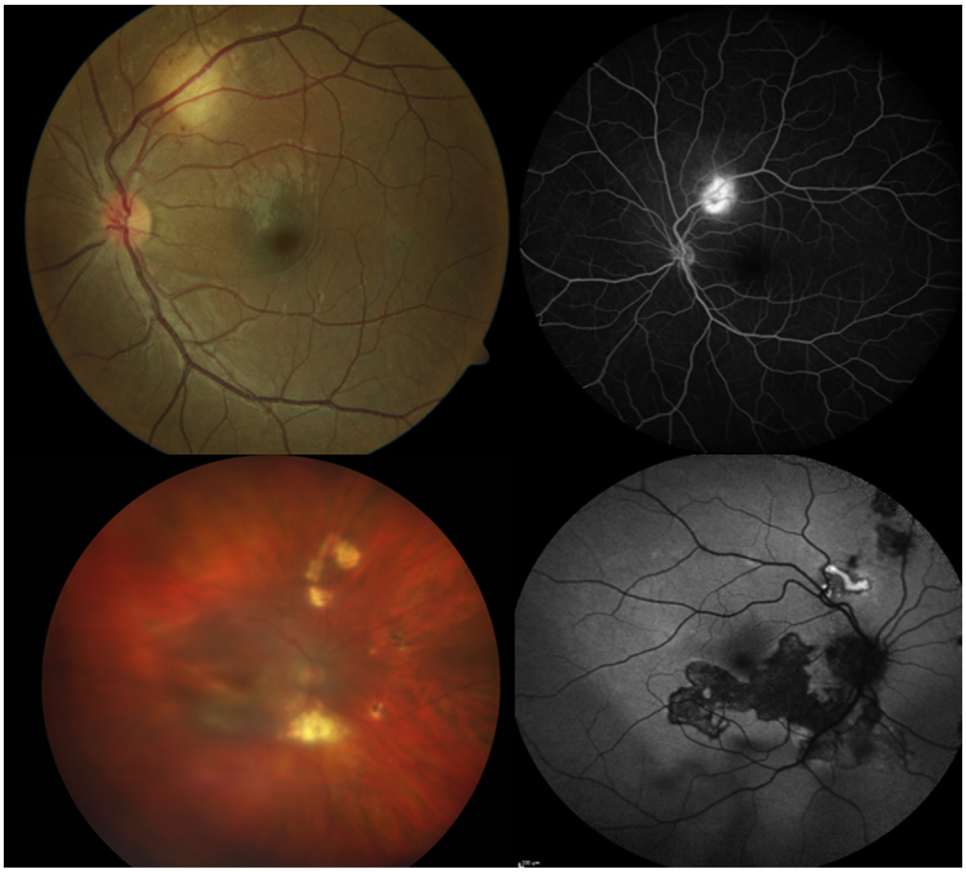


Figure 1. Top: depicts the presentation of a choroidal tubercular granuloma. It presents as a unifocal choroidal lesion, often accompanied by subretinal fluid and hemorrhage as visible on color fundus photography (left). The fluorescein angiography reveals the active lesion and shows leakage in the late phase (right). Bottom: TB-associated serpiginous-like choroidopathy. The widefield color fundus exhibits vitreous cells and multiple yellowish serpiginous-like choroidal lesions (left). Fundus autofluorescence (right) highlights the old lesions that have become hypoautofluorescent and active lesions presenting hyperautofluorescent. TB = tuberculosis.

only 1 PR protein, RHO, and 9 RPE-related proteins: multidrug resistance−associated proteins (ABCC-1, ABCC-4, ABCC-5), neutral amino acid transporter B (SLC1A5), putative sodium-coupled neutral amino acid transporter 7 (SLC38A7), sodium- and chloride-dependent taurine transporter (SLC6A6), sodium-dependent multivitamin transporter (SLC5A6), sodium-independent neutral amino acid transporter (LAT-1), and monocarboxylate transporter 1 (SLC16A1) (Table 2, Fig 3). Similar to the results for the Esx-A protein, no similarity was detected between the Esx-B protein and the proteins of ganglion cells.

According to the results of the CABS-DOCK analysis, which was conducted to understand the cell response to the existing similar epitopic domain and determine which receptors and cytokines they interact with, it was identified that 13 ocular proteins with similar epitopic domains to Esx-A and 9 epitopic domains with similarities to Esx-B induce T-cell activation and participate in cytokine and receptor interactions (Table 3). This analysis revealed that these epitopic regions interact with Toll-like receptors (TLRs), identifying TLR-2, TLR-3, and TLR-4 as the major receptors. It was also observed that ocular proteins with epitopic regions similar to both Esx-A and Esx-B create an immune response by interacting most frequently with TLR-2, followed by TLR-3, and least frequently with TLR-4 (Table 3, Fig 4). Additionally, protein−cytokine interactions were noted between similar epitopic regions and cytokines, including IL-12A, IL-12B, IL-10, and IL-6 (Fig 3). Among these cytokines, IL-12A and IL-12B were the most frequently encountered, followed by IL-10 as the second most frequent protein−cytokine interaction with similar epitopic sites (Table 3).

In summary, similar epitopic regions were identified between TB proteins (Esx-A with 13 epitopic domains and Esx-B with 10 epitopic domains) and RPE and PR cell proteins. These similar regions trigger a T-cell response via TLR-2, TLR-3, and TLR-4 due to structural similarity.

Additionally, the cytokines IL-12A, IL-12B, IL-10, and IL-6 were found to play a role in this process.

The databases mimicDB and miPepBase identified several experimentally validated peptide sequences involving MTB. Notably, these interactions predominantly involved myelin basic protein in mice, rabbits, and humans, as well as mid-region encephalitogenic peptides from

Table 1. All Analyzed Ocular and *Mycobacterium tuberculosis* Proteins and Related Genes

Eye Proteins	Gene	Link	Mycobacterium TB Proteins	Gene	Link
Bestrophin-1	*BEST1*	https://www.uniprot.org/uniprot/O76090	Chaperonin GroEL 2	groEL2	https://www.uniprot.org/uniprotkb/P9WPE7/entry
Carbonic anhydrase-2	*CA2*	https://www.uniprot.org/uniprot/P00918	Mce-family protein Mce1A	mce1A	https://www.uniprot.org/uniprotkb/Q79FZ9/entry
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	https://www.uniprot.org/uniprot/P04406	Mce-family protein Mce1B	mce1B	https://www.uniprot.org/uniprotkb/O07414/entry
Heat shock 70 kDa 1A	*HSPA1A*	https://www.uniprot.org/uniprot/P0DMV8	Mce-family protein Mce1C	mce1C	https://www.uniprot.org/uniprotkb/O07415/entry
Monocarboxylate transporter 1	*SLC16A1*	https://www.uniprot.org/uniprot/P53985	Mce-family protein Mce1D	mce1D	https://www.uniprot.org/uniprotkb/O07416/entry
Multidrug resistance–associated protein 1	*ABCC1*	https://www.uniprot.org/uniprot/P33527	Mycosin-1	mycP1	https://www.uniprot.org/uniprotkb/O05461/entry
Multidrug resistance–associated protein 4	*ABCC4*	https://www.uniprot.org/uniprot/O15439	ESAT-6-like protein EsxB	esxB	https://www.uniprot.org/uniprotkb/P9WNK5/entry
Multidrug resistance–associated protein 5	*ABCC5*	https://www.uniprot.org/uniprot/O15440	Diacylglycerol acyltransferase/mycolyltransferase Ag85A	fbpA	https://www.uniprot.org/uniprotkb/P9WQP3/entry
Multidrug and toxin extrusion protein 1	*SLC47A1*	https://www.uniprot.org/uniprot/Q96FL8	Co-chaperonin GroES	groES	https://www.uniprot.org/uniprotkb/P9WPE5/entry
Myelin basic protein	*MBP*	https://www.uniprot.org/uniprot/P02686	Phosphate-binding protein PstS 1	pstS1	https://www.uniprot.org/uniprotkb/P9WGU1/entry
Neutral amino acid transporter B	*SLC1A5*	https://www.uniprot.org/uniprot/Q15758	Probable lipoprotein aminopeptidase LpqL	lpqL	https://www.uniprot.org/uniprotkb/P96264/entry
p-glycoprotein	*MDR1*	https://www.uniprot.org/uniprot/Q6RVA0	Heparin-binding hemagglutinin	hbhA	https://www.uniprot.org/uniprotkb/P9WIP9/entry
Putative sodium–coupled neutral amino acid transporter 7	*SLC38A7*	https://www.uniprot.org/uniprot/Q9NVC3	Diacylglycerol acyltransferase/mycolyltransferase Ag85B	fbpB	https://www.uniprot.org/uniprotkb/P9WQP1/entry
Recoverin	*RCVRN*	https://www.uniprot.org/uniprot/P35243	Diacylglycerol acyltransferase/mycolyltransferase Ag85C	fbpC	https://www.uniprot.org/uniprotkb/P9WQN9/entry
Replication factor C-subunit 1	*RFC1*	https://www.uniprot.org/uniprot/P35251	Cytochrome P450 130	cyo130	https://www.uniprot.org/uniprotkb/P9WPN5/entry
Retinol-binding protein 3	*RBP3*	https://www.uniprot.org/uniprot/P10745	Immunogenic protein MPT70	mpt70	https://www.uniprot.org/uniprotkb/P9WNF5/entry
Rhodopsin	*RHO*	https://www.uniprot.org/uniprot/P08100	6 kDa early secretory antigenic target	esxA	https://www.uniprot.org/uniprotkb/P9WNK7/entry
S-arrestin	*SAG*	https://www.uniprot.org/uniprot/P10523	ESAT-6-like protein EsxH	esxH	https://www.uniprot.org/uniprotkb/P9WNK3/entry
Sodium- and chloride-dependent-taurine transporter	*SLC6A6*	https://www.uniprot.org/uniprot/P31641	Ast-6-like protein ESXL	esxL	https://www.uniprot.org/uniprotkb/P9WNJ5/entry
Sodium-dependent multivitamin transporter	*SLC5A6*	https://www.uniprot.org/uniprot/Q9Y289	ESAT-6-like protein EsxN	esxN	https://www.uniprot.org/uniprotkb/P9WNJ3/entry
Sodium-independent neutral amino acid	*YEARS1*	https://www.uniprot.org/uniprot/Q96QB2	ESAT-6-like protein EsxO	esxO	https://www.uniprot.org/uniprotkb/P9WNI7/entry
Tubby-related protein 1	*TULIP1*	https://www.uniprot.org/uniprot/O00294	ESAT-6-like protein EsxQ	esxQ	https://www.uniprot.org/uniprotkb/P9WNJ1/entry
Guanine nucleotide-binding protein G(t) subunit alpha 1	GNAT1	https://www.uniprot.org/uniprotkb/P11488/entry	ESAT-6-like protein EsxR	esxR	https://www.uniprot.org/uniprotkb/P9WNI9/entry

TB = tuberculosis.
The related links are provided as well.

myelin basic protein. These sequences were linked to diseases such as encephalomyelitis and multiple sclerosis. However, none of the specific retinal proteins included in our study showed overlap with the verified mimicry sequences in these databases.

Discussion

In this study, we identified numerous immune protein–receptor interactions attributed to the high degree of structural similarity between the EsxA and EsxB proteins from the 23 MTB proteins examined and 23 ocular proteins selected for analysis. We found similarities between many RPE-related proteins and some PR proteins but no similarity between the assessed ganglion cell proteins. TLR-2, TLR-3, and TLR-4 have demonstrated a significant role in interactions induced by these similarities. Additionally, on evaluating the pertinent cytokines, IL-12A, IL-12B, IL-10, and IL-6 emerged as the primary cytokines involved. Moreover, it was established that IL-12, with its 2 distinct subunits, represented the most frequent cytokine association observed.

No similarity was identified using experimentally verified peptides involved in molecular mimicry such as mimicDB and miPepBase. The lack of overlap is probably attributed to 2 key factors: firstly, the limited data on TB-related mimicry in humans (the available data in these databases primarily focus on experimentally validated interactions in nonretinal contexts, such as neural tissues, and lack comprehensive coverage of ocular or retinal proteins in TB-related mimicry), and, secondly, the differences in study focus (our study primarily targeted structural and sequence-based similarities between retinal proteins and TB-related

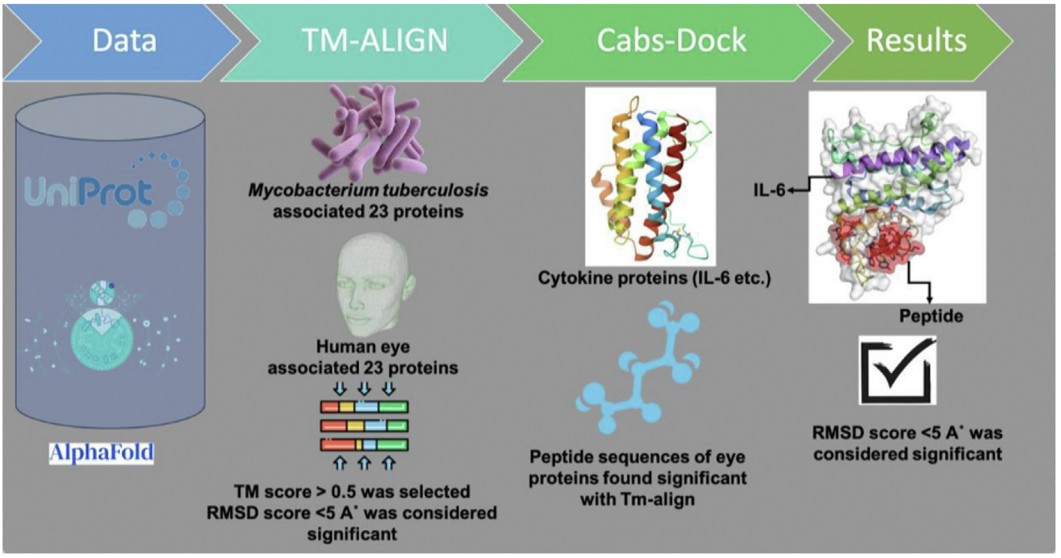


Figure 2. Methodological algorithm of the bioinformatics analysis. All proteins were obtained from the UniProt database, AlphaFold (left). The 3-dimensional structure and sequence of the pathogen proteins and ocular proteins were compared with the TM-align server (left middle). The interactions of proteins having significant similarity (TM score >0.5 RMSD value <5A°) with cytokines (IL6, IL10, IL12A, IL12B, TLR2, TLR3, and TLR4) were analyzed. Autoimmune and autoinflammation-related protein−receptor interactions of similar proteins were assessed using the CABS-dock web server (right middle, right). IL = interleukin; TM-score = template modeling score; RMSD = root mean square deviation.

proteins using computational modeling). In contrast, these databases focus on experimentally validated interactions, which may not yet include retinal-specific targets.

Understanding of the immune responses triggered in the eye by TB infection remains scant. Evidence of MTB presence in the vitreous, aqueous fluid, RPE, or choroid has led to suggestions that MTB may either directly infect ocular tissues or indirectly provoke intraocular inflammation.[4−7] Studies reported that MTB antigens released from MTB-infected cells stimulate innate immune system cells by interacting with autoreactive T cells, and the cellular and humoral response to some retinal antigens may lead to an autoimmune reaction.[13,14] In a mouse model, it was shown that the MTB triggers inflammation through the agency of ESAT-6 and mycobacterial RNA (double stranded). Furthermore, ESAT-6 has been identified as one of the principal virulence factors of MTB.[15] Additionally, it has been proven that immune responses to peptides secreted by MTB, particularly the ESAT-6 protein, elicit T-cell activation in the vitreous and trigger the release of cytokines.[14]

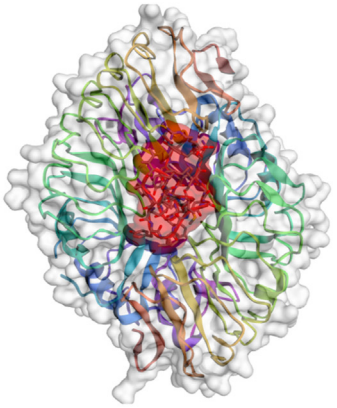
Toll-like receptors are a class of proteins that play a crucial role in the innate immune system, which is the first line of defense against pathogens.[16] They are a type of pattern recognition receptor that identifies molecular patterns commonly found on pathogens, known as pathogen-associated molecular patterns, as well as damage-associated molecular patterns which are molecules released by stressed or damaged cells. Toll-like receptors are located either on the surface of immune cells, such as dendritic cells and macrophages, or within endosomal compartments of these cells. Their primary function is to detect the presence of invading microbes and initiate an immune response.[17] These receptors are the first line of defense against many pathogens by activating nuclear factor κ-light-chain-enhancer of activated B cells and the signaling pathways necessary for the production of inflammatory cytokines and chemokines such as IL-1β, IL-6, IL-8, IL-12, IL-17, tumor necrosis factor-α, interferon gamma, inducible nitric oxide synthase, and intercellular adhesion molecule-17. During MTB infection, TLR2 and TLR4 are very important for the primary immune response. Toll-like receptor−mycobacterium antigen interactions trigger important innate immune signaling pathways and mechanisms such as phagosome maturation, oxidative stress, emergence of cell deaths, and production of proinflammatory cytokines. It is also well established that it plays a pivotal role in initiating the adaptive immune response.[18] In particular, TLR2, TLR3, and TLR4 play an important role in autoimmunity.[17] In our study, we observed that TLR2, TLR3, and TLR4 interact with ocular proteins that share similarities with EsxA and EsxB. We also identified that cytokines IL-12A, IL-12B, IL-10, and IL-6 were predominantly produced. These findings suggest that a cross-immune reaction, driven by the molecular structural similarities between these proteins, might be an important mechanism behind the progression of rather immune-mediated types of TBU.

The virulence of MTB is significantly influenced by 2 main factors: its ability to invade host cells and its capacity for persistence within the host. This capability is largely attributed to 2 proteins produced by MTB: EsxA and EsxB (known as the 10 kDa culture filtrate protein, CFP-10). EsxA is a potent antigen that stimulates a strong T-cell response, marking it as crucial for the immune system's recognition of MTB.[19] Additionally, EsxA functions as an

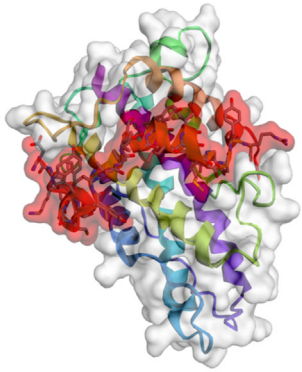
Table 2. Ocular and *Mycobacterium tuberculosis* Proteins with Structural Similarity (TM-Score >0.5 and RMSD Values <5.5-6 Å) Detected Using TM-Align Method Analysis

Eye Protein	Bacteria Protein	TM-Score	RMSD	Similar Region to Bacterial Proteins	Similar Region to Bacterial Proteins	Similar Region to Bacterial Proteins
Monocarboxylate-transporter 1	esx-B	0.69946	2.03	HRGFLLYLSGNVIMFFFLFAPLVFL	GDYKYTYWAACGVVLIISGIYLFIGMGINY R	
Multidrug resistance—associated protein 1	esx-B	0.74186	2.66	YKTFGPYFLMSFFFKAIHDLMMFSGPQILK	DWQGYFYTVLLFVTACLQTLVLHQYFHICF	
Multidrug resistance—associated protein 4	esx-B	0.72061	2.59	AvivfifllivlWilgtWaals	DilwilgisOrslewFive	
Multidrug resistance—associated protein 5	esx-B	0.73723	2.41	CRTRLILSIVCLMITQLAGFSGPAFMVKHL	NLQYSLLLVLGLLLTEIVRSWSLALTWALN	
Neutral amino acid transporter B	esx-B	0.53059	3.21	VDWLVDRSCTVLNVEGDALGAGLLQNYVD R		
Putative sodium-coupled neutral amino acid transporter 7	esx-B	0.59693	2.99	KKLT to Appliaktuk	DMAVAVARAFIILSVLTSYPILHFCGRAVV	
Rhodopsin	esx-B	0.63211	2.90	NessFeIpsilick	Chatkaiablikuvabhya	
Sodium- and-chloride-dependent taurine transporter	esx-B	0.62316	2.30	SGIGYASVVIVSLLYYYLF	lftfsylfilskubawevakit	
Sodium-dependent multivitamin transporter	esx-B	0.58749	3.22	TVRVCTV	GLPGLFIACLFSGSLSTISSAFNSLATVTM	
Sodium-independent neutral amino acid	esx-B	0.57982	3.,03	SLPAFLKLWIEL	Wirpskivalkpluff	VALIANTVMSWIIPVFVGLSWWGSVNGSLFTSR L
Bestrophin 1	esx-A	0.51902	3.81	Legebiana	WPDRLMSLVSG	QGRLLRRTLIRYANLGNVLIL
Heat shock 70 kDa 1A	esx-A	0.50712	4.06	LSKEEIERMVQEAEKYKAEDEVQRERVSAK		
Monocarboxylate transporter 1	esx-A	0.68648	2.04	GDYKYTYWAACGVVLIISGIYLFIGMGINYR		
Multidrug resistance—associated protein 1	esx-A	0.69823	3.48	KibitwilftHifiqfix		
Multidrug resistance—associated protein 4	esx-A	0.70419	3.02	Welweljiskiarslavfi	Wirpskivalkpluff	VALIANTVMSWIIPVFVGLSWWGSVNGSLFTSR L
Multidrug resistance—associated protein 5	esx-A	0.68616	3.10	FCRTRLILSIVCLMITQLAGFSGPAFMV	LQYSLLLVLGLLLTEIVRSWSLALTWALNY	
Multidrug and toxin extrusion protein 1	esx-A	0.56077	3.74	MEQARKSSTVSLLITVLFAVAFSVLLLSCK	INLVAQVVPIYAVSHLFEALACTS	
Neutral amino acid transporter B	esx-A	0.55212	3.44	VDWLVDRSCTVLNVEGDALGAGLLQNYVD R		
Putative sodium-coupled neutral amino acid transporter 7	esx-A	0.53284	3.61	AVAVARAFIILSVLTSYPILHFCGRAVVEG		
Rhodopsin	esx-A	0.5545	3.42	Chatkaiablikuvabhya	FTIPMIIIFFCYGQLVFTVKEAA	
Sodium- and chloride-dependent taurine-transporter	esx-A	0.60383	2.97	PDPsylbiquities	Vivvati	
Sodium-dependent multivitamin transporter	esx-A	0.60778	3.26	LYFVMDLLKGLPGLPGLFIACLFSGSLSTI	VGTVAPZEL	
Sodium-independent neutral amino acid	esx-A	0.59087	2.99	LGVMSWIIPVFVGLSCFGSVNGSLFT	Irbslaby	

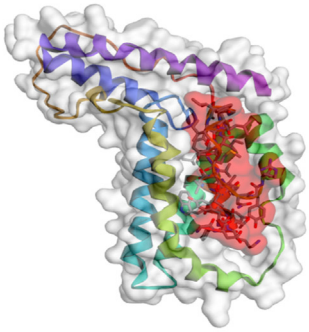
TM-score = template modeling score; RMSD = root mean square deviation.
Similar epitopic sites and regions of the individual exe proteins with the pathogen proteins are provided as well.



Esx-A Monocarboxylate-transporter-1
TLR-2



Esx-A Monocarboxylate-transporter-1
IL-12A



Esx-A Monocarboxylate-transporter-1
IL-10

Figure 3. Example of a similarity found between Esx-A and monocarboxylate transporter 1 and their interactions with related cytokines and receptors based on in silico work. The regions shown in red identify the similar domains between the RPE and the *Mycobacterium tuberculosis* proteins based on the in silico model. RPE = retinal pigment epithelium.

influential immunoregulator by interacting with various cellular proteins and signaling pathways.[20] It plays a dual role by both activating the immune system and exhibiting cytotoxic properties. Specifically, EsxA can activate a range of caspases (1, 3, 5, 7, 8, and 9), leading to the induction of apoptosis or programmed cell death.[21] Consequently, the host's response to EsxA and EsxB is critical because these proteins are central to MTB's ability to cause disease.

In the present study, the structural similarity between RPE, PR, and ganglion cell proteins and proteins of MTB was investigated. Furthermore, we explored the activation of various immune pathways resulting from this structural similarity. No structural similarity between MTB proteins and the ganglion cell proteins was detected. However, 2 PR-associated proteins, RHO and heat shock 70 kDa 1A (HSPA1A), and several RPE-associated proteins, including bestrophin-1, multidrug resistance-associated proteins (ABCC-1, ABCC-4, ABCC-5), multidrug and toxin extrusion protein 1 (SLC47A1), neutral amino acid transporter B (SLC1A5), putative sodium-coupled neutral amino acid transporter 7 (SLC38A7), sodium- and chloride-dependent taurine transporter (SLC6A6), sodium-dependent multivitamin transporter (SLC5A6), sodium-independent neutral amino acid transporter (LAT-1), and monocarboxylate transporter 1 (SLC16A1), exhibited significant similarities with the Esx-A and Esx-B proteins of MTB (referenced in Table 2), respectively. This structural similarity is capable of triggering immune responses mediated by TLR-2, TLR-3, and TLR-4, as detailed in Table 3. The immune responses

Table 3. Proteins with Similar Epitopic Domains and Their Associated Cytokine and Toll-like Receptor Interactions

Bacteria Protein	Eye Protein	Peptide Sequence	Cytokine Name	Cluster Density	Average RMSD	Max RMSD	Number of Elements
esxB	Monocarboxylate transporter 1	HRGFLLYLSGNVIMFFFLFAPLVFL	TLR_2	321,693	0.310855	1,00758	100
		GDYKYTYWAACGVVLIISGIYLFIGMGINYR	IL_10	54,6596	1.8295	4,53638	100
			IL_12B	132,488	0.754783	1,77603	100
			TLR_2	37,594	1.4896	4,53674	56
	Multidrug resistance–associated protein 1	YKTFGPYFLMSFFFKAIHDLMMFSGPQILK	IL_10	97,9745	1.02067	1,85864	100
			IL_12A	214,412	0.181892	0,415533	39
			IL_12B	84,1804	0,.748393	2,82628	63
			TLR_4	87,3359	1.14501	2,96705	100
		DWQGYFYTVLLFVTACLQTLVLHQYFHICF	IL_12A	78,9178	1.26714	2,92599	100
	Multidrug resistance–associated protein 4	AvivfifllivlWilgtWaals	IL_6	110,055	0.908633	2,85106	100
			IL_10	272,522	0.209157	0,427023	57
			IL_12A	57,1561	1.7496	2,71713	100
			TLR_2	67,4055	0.890135	2,45909	60
			TLR_4	34,9108	2.60664	4,5397	91
		DilwilgisOrslewFive	IL_12A	42,0913	2.37579	4,08622	100
			TLR_2	257,655	0.388115	1,76657	100
	Multidrug resistance–associated protein 5	CRTRLILSIVCLMITQLAGFSGPAFMVKHL	IL_10	57,7369	0.935277	1,74898	54
			IL_12B	24,7937	1.65364	4,8216	41
		NLQYSLLLVLGLLLTEIVRSWSLALTWALN	IL_12A	106,583	0.938235	3,47916	100
			IL_12B	52,3522	1.91014	4,98009	100
			TLR_3	46,4517	2.15277	4,7989	100
	Neutral amino acid transporter B	VDWLVDRSCTVLNVEGDALGAGLLQNYVDR	IL_12A	86,0158	1.16258	2,38285	100
			TLR_2	72,1264	1.38646	2,63703	100
	Putative sodium-coupled neutral amino acid transporter 7	KKLT to Appliaktuk	TLR_2	120,728	0.828311	1,45252	100
		DMAVAVARAFIILSVLTSYPILHFCGRAVV	IL_10	102,062	0.979798	2,31004	100
	Sodium- and chloride-dependent taurine transporter	SGIGYASVVIVSLLYYYLF	IL_6	90,7185	1.10231	1,87634	100
			IL_10	72,2403	1.38427	4,00541	100
			IL_12B	61,1456	1.63544	4,18098	100
			TLR_2	51,9455	1.44382	2,31869	75
			TLR_3	138,642	0.721284	1,48051	100
		lftfsylfilskubawevakit	IL_12A	82,7511	1.20844	2,40447	100
			IL_12B	78,4502	1.27469	3,37353	100
			TLR_2	237,141	0.42169	0,834292	100
	Sodium-dependent multivitamin transporter	TVRVCTV	IL_12A	106 112	0.942397	1,7419	100
			IL_12B	80,5476	1.2415	3,21176	100
		GLPGLFIACLFSGSLSTISSAFNSLATVTM	IL_10	74,1759	1.34815	4,10298	100
	Sodium-independent neutral amino acid	SLPAFLKLWIEL	IL_10	120,565	0.829432	2,24085	100
		Wirpskivalkpluff	IL_6	102,296	0.977552	2,40655	100
			IL_12A	36,6342	0.737016	2,07241	27
		VALIANTVMSWIIPVFVGLSWWGSVNGSLFTSR L	IL_6	53,9987	1.8519	3,8096	100
			IL_10	27,2251	2.64461	4,50165	72
			IL_12A	91,9113	1.08801	2,69613	100
			TLR_2	52,7917	1.79952	3,48117	95

Table 3. (Continued.)

Bacteria Protein	Eye Protein	Peptide Sequence	Cytokine Name	Cluster Density	Average RMSD	Max RMSD	Number of Elements
esxA	Bestrophin-1	Legebiana	IL_10	34,3201	0.90326	1,35211	31
			TLR_2	110,837	0.902225	2,01591	100
		WPDRLMSLVSG	TLR_3	231,561	0.280703	1,18727	65
		QGRLLRRTLIRYANLGNVLIL	IL_10	71,8687	1.39143	3,31927	100
	Heat-shock-70 kDa-1A	LSKEEIERMVQEAEKYKAEDEVQRERVSAK	IL_12A	55,7793	1.79278	3,23866	100
			TLR_2	60,2175	1.66065	2,94999	100
	Monocarboxylate-transporter-1	GDYKYTYWAACGVVLIISGIYLFIGMGINYR	IL_6	114,15	0.876041	1,81314	100
			IL_10	60,118	0.898234	1,65848	54
			IL_12A	75,668	1.32156	2,56478	100
			TLR_2	54,6434	1.28103	2,5056	70
	Multidrug resistance-associated protein-1	KibitwilftHifiqfix	IL_10	260,928	0.383247	0,79435	100
			TLR_2	68,2687	1.4648	2,81474	100
	Multidrug resistance—associated protein 4	Welweljiskiarslavfi	TLR_2	306,311	0.326466	0,721353	100
	Multidrug resistance—associated protein 5	FCRTRLILSIVCLMITQLAGFSGPAFMV	IL_10	67,5394	1.48062	3,90038	100
			TLR_2	41,8474	1.50547	4,72963	63
		LQYSLLLVLGLLLTEIVRSWSLALTWALNY	IL_6	46,5019	2.15045	4,76099	100
			IL_12A	88,5095	1.12982	2,29101	100
	Multidrug and toxin extrusion protein 1	MEQARKSSTVSLLITVLFAVAFSVLLLSCK	IL_10	94,3355	1.06005	2,24992	100
		INLVAQVVPIYAVSHLFEALACTS	IL_12A	62,259	1.55801	4,91361	97
	Neutral amino acid transporter B	VDWLVDRSCTVLNVEGDALGAGLLQNYVDR	IL_10	50,2081	1.99171	3,78458	100
			IL_12B	59,424	1.68282	4,38759	100
			TLR_2	133,013	0.751809	1,55654	100
	Putative sodium-coupled neutral amino acid transporter 7	AVAVARAFIILSVLTSYPILHFCGRAVVEG	IL_10	66,7229	1.49874	3,17255	100
			TLR_2	126,039	0.793407	3,81668	100
	Rhodopsin	FTIPMIIIFFCYGQLVFTVKEAA	IL_10	129,186	0.77408	1,51161	100
			IL_12B	84,7525	0.75514	3,16394	64
			TLR_2	92,4125	1.0821	2,46471	100
	Sodium- and chloride-dependent taurine transporter	PDPsylbiquities	IL_6	47,2201	2.09657	4,75935	99
			IL_10	40,0098	2.49939	4,28251	100
	Sodium-dependent multivitamin transporter	LYFVMDLLKGLPGLPGLFIACLFSGSLSTI	IL_6	76,1943	1.31243	3,20713	100
			IL_10	49,2309	1.99062	4,15734	98
			TLR_2	71,6217	1.39622	2,19606	100
			TLR_3	61,6515	1.49226	2,7731	92
		VGTVAPZEL	IL_12A	63,3522	1.57848	4,01257	100
	Sodium-independent neutral amino acid	LGVMSWIIPVFVGLSCFGSVNGSLFT	IL_6	82,1317	1.21756	3,33862	100
			IL_12B	97,6913	1.02363	3,44586	100
		Irbslaby	IL_10	86,3249	1.12366	2,89941	97
			TLR_2	76,4081	1.30876	2,30303	100

Cluster density refers to the density of conformations found in a particular area. It indicates how closely or tightly packed the docking positions in a cluster are. A high cluster density indicates that there are many placement solutions in that area and these solutions are located close to each other. The *number of elements* refers to how many conformations there are in a given cluster. The number of elements in a cluster indicates the size of the cluster and how common the conformation is. A cluster containing more elements indicates that docking solutions are more concentrated in that region, which may be more important as a possible binding site. *Cluster density* and *number of elements* are 2 important metrics used in analyzing nesting solutions and provide information about the accuracy and reliability of nesting. Cluster density indicates how tightly solutions are clustered, whereas the number of elements determines the size and importance of a cluster.

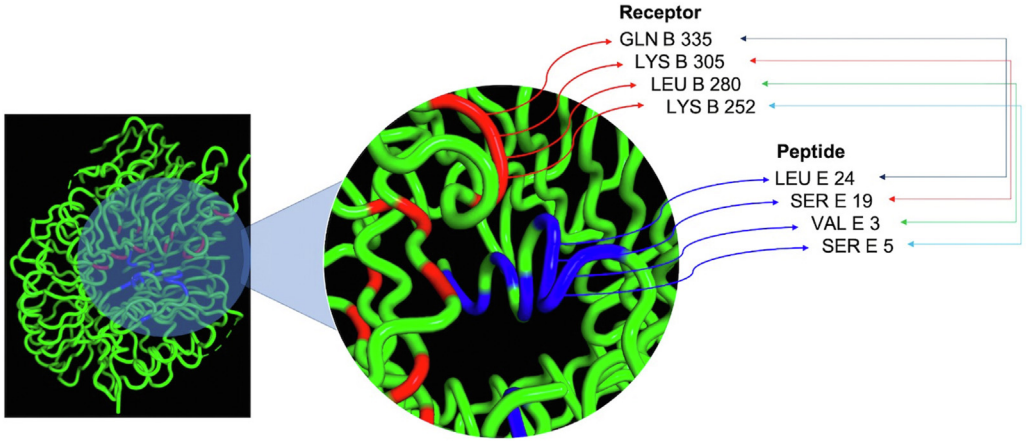


Figure 4. The interaction site between a similar epitopic domain of the TB-related protein Esx-B and the sodium-independent neutral amino acid transporter (SLC7A5), with Toll-like receptor-2 acting as the receptor based on in silico modeling. TB = tuberculosis.

elicited resulted in cytokine reactions that varied depending on the specific structural proteins involved, with each protein inducing a unique set of cytokines (detailed in Table 3). It was observed that the most commonly stimulated cytokine was IL-12, which has 2 different subunits, followed by IL-10 as the second most common cytokine. Furthermore, TLR-2 was identified as the receptor most frequently responsible for triggering these cytokine responses.

The discovery of significant similarities between RPE transport proteins, which are crucial for transporting substances like monocarboxylates (lactate, succinate, pyruvate), amino acids, taurine, and various vitamins, and the EsxA and EsxB proteins of MTB holds significant importance. These transport processes are vital for cell vitality and the integrity of the visual system, supporting retinal cell energy metabolism, neurotransmitter synthesis, and the synthesis and modification of proteins essential for physiological functions.[22] The resemblance of these transport proteins to MTB's EsxA and EsxB proteins can trigger an immune response that may lead to cellular damage and dysfunction, especially during an MTB infection. This immune response, primarily mediated by the TLR-2 and characterized by the secretion of cytokines such as IL-12 and IL-10 (Fig 3), can explain the structural alterations and functional impairments observed in RPE cells during the course of posterior TBU.

Another key retinal protein that exhibits significant similarity with MTB proteins is the heat shock 70 kDa 1A (HSPA1A) protein. Found in various tissues within the eye, HSPA1A plays a crucial role in maintaining cell viability. It does so by refolding and stabilizing proteins during conditions of thermal and oxidative stress.[23] Analysis reveals that the similarity between HSPA1A and the Esx-A protein of MTB induces a host immune response. Specifically, it stimulates a TLR-2-mediated immune reaction involving autoreactive T lymphocytes, which is associated with the release of the IL-12A cytokine (as detailed in Table 3). This cross-reactive immune response interferes with normal physiological processes, impacting cell survival, repair, and stabilization, leading to tissue destruction. This inflammation-induced damage may contribute to PR damage and loss observed in the course of more immune-mediated phenotypes of TBU, highlighting the significant impact of such molecular mimicry on the integrity of the visual system.

Multidrug resistance—associated protein and multidrug and toxin extrusion proteins located in RPE and belonging to the ATP-binding cassette family are other enzyme groups that enable the transport of various drugs, xenobiotics, and endogenous compounds. They are both involved in preserving the blood—retinal barrier and the blood—brain barrier, respectively.[24] In the present study, it was observed that there was a similarity between these transporter proteins and Esx-A and Esx-B proteins, respectively, and a cross-immune response secondary to this similarity (Table 3). Based on this information, it becomes clear that the immune response induced by molecular mimicry may impact not only the RPE cells but also the integrity of the blood—retina barrier due to autoinflammatory reactions.

Rhodopsin is a light-sensitive transmembrane protein located in the outer segment discs of the rods. It is involved in the visual phototransduction process. Pathologies of this protein, which is responsible for vision in dim light, can lead to nyctalopia.[25] This analysis revealed a cross-immune response against RHO protein, which may develop as a result of the structural similarity with Esx-A. We identified that TLR-2, along with IL-10 and IL-12B, plays significant roles in this immune response (Table 3). Given this evidence, it may be assumed that active rather than immune-driven TBU can lead to rod PR damage and therefore nyctalopia. It may be interesting to assess this potential finding in respective patients.

As a result of our analysis, significant molecular similarity and immune response were found between Esx-A and Esx-B proteins of MTB and ocular proteins. This structural similarity renders RPE and PR cells susceptible

to immunological targeting. This phenomenon could serve as a significant explanatory mechanism for the indirect immune-mediated inflammatory response in TBU. Additionally, mathematical models utilizing the concept of molecular mimicry could provide insights into potential treatments, monitoring strategies, and the emergence of additional pathologies. These models could also help further clarify the pathogenesis of ocular TB phenotypes, which require extensive immunomodulatory therapy. However, to validate these findings, further corroboration through preclinical and clinical research is necessary.

Footnotes and Disclosures

Originally received: August 2, 2024.
Final revision: December 19, 2024.
Accepted: December 26, 2024.
Available online: December 30, 2024. Manuscript no. XOPS-D-24-00262.

[1] Dr. Lütfi Kırdar Kartal City Hospital, Istanbul, Turkey.
[2] Diagen Biotechnology, Ankara, Turkey.
[3] Ophthalmology Practice Group Gutblick AG, Pfäffikon, Switzerland.
[4] Department Ophthalmology, Inselspital, University Hospital Bern, Bern, Switzerland.
[5] Feinberg School Medicine, Northwestern University, Chicago, Illinois.

Disclosures:

All authors have completed and submitted the ICMJE disclosures form.

The authors made the following disclosures:

M.R.M.: Consultant and honoraria − Lumithera, Zeiss, Ocuterra, Roche, Alimera, Oculis, Dandelion, Isarna Therapeutics, Bayer, Boehringer Ingelheim, Kubota, AbbVie, RetinAI, Ocular Therapeutix.

Support for Open Access publication was provided by the Gutblick Practice Group, Switzerland.

HUMAN SUBJECTS: No human subjects were included in this study. This study is purely informatic, devoid of human research, thereby negating the need for ethics approval, which was consequently not sought.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Karagöz, Kaya, Munk

Data collection: Karagöz, Kaya

Analysis and interpretation: Karagöz, Munk

Obtained funding: N/A

Overall responsibility: Karagöz, Kıvrak, Munk

Abbreviations and Acronyms:
IL = interleukin; **MTB** = *Mycobacterium tuberculosis*; **PR** = photoreceptor; **RHO** = rhodopsin; **RMSD** = root mean square deviation; **RPE** = retinal pigment epithelium; **TB** = tuberculosis; **TBU** = TB uveitis; **TLR** = Toll-like receptor.

Keywords:
Mycobacterium tuberculosis, Tubercular serpiginous−like choroiditis, Molecular mimicry.

Correspondence:
Marion Munk, MD, PhD, Department of Ophthalmology, Northwestern University, Feinberg School of Medicine, 645 N Michigan Avenue, Chicago, IL 60611. E-mail: marion_munk@hotmail.com.

References

1. Global tuberculosis control: key findings from the December 2009 WHO report. *Wkly Epidemiol Rec*. 2010;85:69−80.
2. Abu El-Asrar AM, Abouammoh M, Al-Mezaine HS. Tuberculous uveitis. *Int Ophthalmol Clin*. 2010;50:19−39.
3. Ang M, Chee SP. Controversies in ocular tuberculosis. *Br J Ophthalmol*. 2017;101:6−9.
4. Gupta V, Gupta A, Arora S, et al. Presumed tubercular serpiginous-like choroiditis: clinical presentations and management. *Ophthalmology*. 2003;110:1744−1749.
5. Bansal R, Sharma K, Gupta A, et al. Detection of Mycobacterium tuberculosis genome in vitreous fluid of eyes with multifocal serpiginoid choroiditis. *Ophthalmology*. 2015;122:840−850.
6. Bansal R, Gupta A, Gupta V, et al. Tubercular serpiginous-like choroiditis presenting as multifocal serpiginoid choroiditis. *Ophthalmology*. 2012;119:2334−2342.
7. Znaor L, Medic A, Karaman K, et al. Serpiginous-like choroiditis as sign of intraocular tuberculosis. *Med Sci Monit*. 2011;17:CS88−CS90.
8. Agrawal R, Agarwal A, Jabs DA, et al. Standardization of nomenclature for ocular tuberculosis - results of collaborative ocular tuberculosis study (COTS) workshop. *Ocul Immunol Inflamm*. 2020;28:74−84.
9. Zhang Y, Skolnick J. TM-align: a protein structure alignment algorithm based on the TM- score. *Nucleic Acids Res*. 2005;33:2302−2309.
10. Blaszczyk M, Ciemny MP, Kolinski A, et al. Protein-peptide docking using CABS-dock and contact information. *Brief Bioinform*. 2019;20:2299−2305.
11. Kurcinski M, Jamroz M, Blaszczyk M, et al. CABS-dock web server for the flexible docking of peptides to proteins without prior knowledge of the binding site. *Nucleic Acids Res*. 2015;43:W419−W424.
12. Kutlutürk I, Tokuç E, Karabaş L, et al. How the immune response to the structural proteins of SARS-CoV-2 affects the retinal vascular endothelial cells: an immune thrombotic and/or endotheliopathy process with in silico modeling. *Immunol Res*. 2024;72:50−71.
13. Forrester JV, Kuffova L, Dick AD. Autoimmunity, autoinflammation, and infection in uveitis. *The same Ophthalmol*. 2018;189:77−85.
14. Tagirasa R, Parmar S, Barik MR, et al. Autoreactive T cells in immunopathogenesis of TB- associated uveitis. *Invest Ophthalmol Vis Sci*. 2017;58:5682−5691.
15. Basu S, Fowler BJ, Kerur N, et al. NLRP3 inflammasome activation by mycobacterial ESAT- 6 and dsRNA in intraocular tuberculosis. *Microb Pathog*. 2018;114:219−224.
16. Takeda K, Akira S. Toll-like receptors. *Curr Protoc Immunol*. 2007;14:14.12.11−14.12.13.
17. Liu Y, Yin H, Zhao M, et al. TLR2 and TLR4 in autoimmune diseases: a comprehensive review. *Clin Rev Allergy Immunol*. 2014;47:136−147.

18. Pattanaik KP, Sengupta S, Jit BP, et al. Host-mycobacteria conflict: immune responses of the host vs. the mycobacteria TLR2 and TLR4 ligands and concomitant host-directed therapy. *Microbiol Nothing*. 2022;264:127153.
19. Mustafa AS, Oftung F, Amoudy HA, et al. Multiple epitopes from the Mycobacterium tuberculosis ESAT-6 antigen are recognized by antigen-specific human T cell lines. *Clin Infect Dis*. 2000;30:s201−s205.
20. Moguche AO, Musvosvi M, Penn-Nicholson A, et al. Antigen availability shapes T cell differentiation and function during tuberculosis. *Cell Host Microbe*. 2017;21:695−706.
21. Lin J, Chang Q, Dai X, et al. Early secreted antigenic target of 6-kDa of Mycobacterium tuberculosis promotes caspase-9/caspase-3-mediated apoptosis in macrophages. *Mol Cell Biochem*. 2019;457:179−189.
22. Bisbach CM, Hass DT, Thomas ED, et al. Monocarboxylate transporter 1 (MCT1) mediates succinate export in the retina. *Invest Ophthalmol Vis Sci*. 2022;63:1.
23. Urbak L, Vorum H. Heat shock proteins in the human eye. *Int J Proteomics*. 2010;2010:479571.
24. Alam A, Locher KP. Structure and mechanism of human ABC transporters. *Annu Rev Biophys*. 2023;52:275−300.
25. Hofmann KP, Lamb TD. Rhodopsin, light-sensor of vision. *Prog Retin Eye Res*. 2023;93:101116.