

Multiple Natural and Experimental Inflammatory Rabbit Lacrimal Gland Phenotypes



AUSTIN K. MIRCHEFF, PHD,^{1,2} YANRU WANG, MD, PHD,¹ JOEL E. SCHECHTER, MD,³ MENG LI, MS,⁴ WARREN TONG, PHARM D, MS,⁵ MAYSSA ATTAR, PHD,⁵ MURTY CHENGALVALA, PHD,⁶ JOE HARMUTH, BS,⁶ AND JEFFERY J. PRUSAKIEWICZ, PHD⁷

ABSTRACT Purpose: To investigate lacrimal gland (LG) immunophysiological and immune-mediated inflammatory process (IMIP) phenotype diversity. Methods: Ex vivo matured dendritic cells (mDC) were loaded with acinar cell microparticles (M_P). Peripheral blood lymphocytes (PBL) were activated in mixed cell reactions with mDC and injected directly into autologous, unilateral LG (1°ATD-LG) of two rabbit cohorts, one naïve, one immunized with a LG lysate membrane fraction (P_i). Autoimmune IgG titers were assayed by ELISA, MCR PBL stimulation indices (SI) by [³H]-thymidine incorporation. Schirmer tests without and with topical anesthetic (STT-I, STT-I_A) and rose Bengal (RB) staining tests were performed. H&E and immunohistochemically stained sections were examined. RNA yields and selected transcript abundances were measured. Immune cell number and transcript abundance data were

submitted to Principal Component Analysis (PCA). Results: Immunizing P_i dose influenced SI but not IgG titers. STT scores were decreased, and rose Bengal scores increased, by day 118 after immunization. Previous immunization exacerbated scores in 1°ATD-eyes and exacerbated 1°ATD-LG atrophy. IMIP were evident in 2°ATD-LG as well as 1°ATD-LG. PCA described diverse immunophysiological phenotypes in control LG and diverse IMIP phenotypes in ATD-LG. IgG titers and SI pre-adoptive transfer were significantly associated with certain post-adoptive transfer IMIP phenotype features, and certain LG IMIP features were significantly associated with RB and STT I_A scores. Conclusions: The underlying variability of normal states may contribute to the diversity of experimental IMIP phenotypes. The ability to generate and characterize diverse phenotypes may lead to phenotype-specific diagnostic and therapeutic paradigms.

KEY WORDS acinar cells, autoantigens, dacryoadenitis, dendritic cells, dry eye, keratoconjunctivitis, Sjögren disease

Accepted for publication July 2016.

From the ¹Department of Physiology & Biophysics, ²Department of Ophthalmology, ³Department of Cell & Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, CA; ⁴Bioinformatics Service, Norris Medical Library, University of Southern California, Los Angeles, CA; ⁵Translational Drug Metabolism, Pharmacokinetics and Immunology, Allergan Inc., Irvine, CA; ⁶Immunology Services, Covance Research Products, Denver, PA; and ⁷Drug Metabolism, Covance Laboratories Inc., Madison, WI, USA.

Supported by an unrestricted grant from Allergan (AKM) and NIH Grant DK 048522.

The authors have no commercial or proprietary interest in any concept or product discussed in this article.

Single-copy reprint requests to Austin K. Mircheff, PhD (address below).

Corresponding author: Austin K. Mircheff, PhD, Department of Physiology & Biophysics, Keck School of Medicine, University of Southern California, 1333 San Pablo Street, BMT B11-A, Los Angeles, CA 90033. Tel: 323-442-1242. Fax: 323-442-2283. E-mail address: amirchef@usc.edu

I. INTRODUCTION

Dry eye disease, one of the most common ophthalmic morbidities, is a disorder of the physiological system that maintains the tear film as a homeostatic *milieu extérieur* for the superficial epithelial cells of the cornea and conjunctiva. A primary dysfunction in any component of the system can have consequences that ramify throughout the system. Bron et al¹ have posited four major dry eye disease phenotypes on the basis of presumed natural history: evaporative, resulting from primary Meibomian gland (MG) dysfunction; aqueous deficient, resulting from primary lacrimal gland (LG) dysfunction; primary evaporative exacerbated by secondary LG dysfunction; and primary aqueous deficient exacerbated by secondary MG dysfunction. In a retrospective study of more than 200 patients, 35% presented with MG dysfunction, 10% with LG dysfunction, 25% with both MG dysfunction and LG dysfunction, and 29% with no evidence of either MG dysfunction or LG dysfunction.²

LG dysfunction, underlying or contributing to 35% of cases, can result from a variety of etiologies, including:

© 2016 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>). *The Ocular Surface* ISSN: 1542-0124. Mircheff AK, Wang Y, Schechter JE, Li M, Tong W, Attar M, Chengalvala M, Harmuth J, Prusakiewicz JJ. Multiple natural and experimental inflammatory rabbit lacrimal gland phenotypes. 2016;14(4):460-483.

OUTLINE

- I. Introduction
- II. Methods
 - A. Rabbits
 - B. Acinar Cells
 - C. Autoantigen Preparations
 - 1. P_i and S_j Fractions
 - 2. M_p Fraction
 - D. Protein Determination
 - E. Systemic Immunization
 - 1. Immunization
 - 2. ELISA
 - 3. Western Blotting
 - F. Ex Vivo Maturation, Loading, and Activation of Dendritic Cells (mDC)
 - G. Ex Vivo Mixed Cell Reactions
 - H. Adoptive Transfer by Injection of Activated PBL
 - I. Ocular Surface Assessments
 - J. Tissue Collection
 - K. Real-Time RT-PCR
 - L. Immunohistochemistry and Image Analysis
 - M. Data Analysis
- III. Results
 - A. Autoantigen Preparations
 - B. Ex Vivo Mixed Cell Reactions
 - C. Systemic Immunization
 - 1. Humoral Autoimmune Responses
 - 2. Cellular Autoimmune Responses
 - D. Ocular Surface Manifestations after Adoptive Transfer and after Systemic Immunization
 - 1. Protocol 1
 - 2. Protocol 2
 - 3. Protocol 3
 - E. Histopathology and Immune Cell Infiltration after Adoptive Transfer and after Systemic Immunization and Adoptive Transfer
 - 1. Atrophy: Protocol 1 and Protocol 3
 - 2. Histopathology
 - a. Protocol 1
 - b. Protocol 3
 - 3. Infiltration by Bone Marrow-Derived Cells and T Cells: Protocol 1 and Protocol 3
 - F. Immune Response-Related Gene Transcript Expression, Protocol 1 and Protocol 3
 - G. Principal Component Analysis
 - 1. Principal Component Projections, Control LG, 1°ATD_N LG, 2°ATD_N LG, 1°ATD_{Imm} LG, and 2°ATD_{Imm} LG
 - 2. Functional Clusters, Control LG, 1°ATD_{Imm} LG, and 2°ATD_{Imm} LG
 - a. Control LG
 - b. 1°ATD_{Imm} LG
 - c. 2°ATD_{Imm} LG

- H. Functional Clusters and Mediators Associated with LG Atrophy, Ocular Surface Inflammation, and Altered Tear Fluid Production
 - 1. LG Atrophy
 - 2. Ocular Surface Inflammation
 - 3. Altered Tear Fluid Production
- I. Promoting Expression of Selected IMIP Phenotypes
- IV. Discussion
- V. Conclusions

impairment of corneal sensory innervation; side effects of systemic medications; infection-associated inflammatory processes; atrophic changes; graft-versus-host processes; autoimmune processes; or noninfectious immune-mediated inflammatory processes (IMIP). The atrophic and fibrotic changes frequently found in LG from elderly individuals may be sequelae of IMIP that resolved earlier in life.³ Graft-versus-host disease is associated with severe LG histopathology and dysfunction.⁴ The recognized autoimmune processes include those of Mikulicz's- or IgG4-related disease and Sjögren disease. Sjögren disease is associated with severe LG dysfunction, often in a context of focal infiltrates but otherwise mild parenchymal and stromal histopathology. Two categories of Sjögren disease, primary and secondary, are recognized, and the primary disease manifests in at least 4 non-interconvertible phenotypes.^{5,6} Classical IMIP diagnoses include sarcoidosis and granulomatosis with polyangiitis (Wegener's granulomatosis).⁷ Notably, however, evidence of IMIP not attributable to the recognized diagnoses is seen in a large majority of *post mortem* LG.^{8,9} Moreover, the histopathological presentations that have been described in *post mortem* LG are remarkably diverse,⁹ implying that there may be considerably more IMIP phenotypes than current classification and diagnostic paradigms envision.

The diversity of LG autoimmune and IMIP phenotypes suggests that ocular surface disease phenotypes related to LG dysfunction may be similarly diverse. One implication is that some LG and IMIP phenotypes may be responsive to current therapeutic modalities, while others are not. Thus, historical failure to recognize the diversity of LG autoimmune and IMIP phenotypes may have contributed to the present dearth of pharmacotherapies for dry eye disease¹⁰ by handicapping interpretation of data from clinical trials of prospective modalities. Another implication is that some LG IMIP phenotypes may not be associated with LG dysfunction.

A recent study of LG from young adult female rabbits indicates that healthy LG are immunophysiologically diverse by the time animals reach sexual maturity. Characteristics of the natural diversity suggest new insights into the diversity of IMIP phenotypes that develop later in life. They also have implications for the design of animal models that might be used to study IMIP phenotype-specific mechanisms and to develop IMIP phenotype-specific therapeutic modalities. In addition to the parenchymal cells, i.e., the

Download English Version:

<https://daneshyari.com/en/article/8591347>

Download Persian Version:

<https://daneshyari.com/article/8591347>

[Daneshyari.com](https://daneshyari.com)