Special Article

Potentially Pathogenic Immune Cells and Networks in Apparently Healthy Lacrimal Glands

Austin K. Mircheff, PhD,[1,2] Yanru Wang, MD, PhD,[1] Chuanqing Ding, MD, PhD,[3,4] Dwight W. Warren, PhD,[4] and Joel E. Schechter, PhD[4]

ABSTRACT Lacrimal glands of people over 40 years old frequently contain lymphocytic infiltrates. Relationships between histopathological presentation and physiological dysfunction are not straightforward. Data from rabbit studies have suggested that at least two immune cell networks form in healthy lacrimal glands, one responding to environmental dryness, the other to high temperatures. New findings indicate that mRNAs for several chemokines and cytokines are expressed primarily in epithelial cells; certain others are expressed in both epithelial cells and immune cells. Transcript abundances vary substantially across glands from animals that have experienced the same conditions, allowing for correlation analyses, which detect clusters that map to various cell types and to networks of coordinately functioning cells. A core network—expressing mRNAs including IL-1α, IL-6, IL-17A, and IL-10—expands adaptively with exposure to dryness, suppressing IFN-γ, but potentially causing physiological dysfunction. High temperature elicits concurrent increases of mRNAs for prolactin (PRL), CCL21, and IL-18. PRL is associated with crosstalk to IFN-γ, BAFF, and IL-4. The core network reacts to the resulting PRL-BAFF-IL-4 network, creating a profile reminiscent of Sjögren's disease. In a warmer, moderately dry setting, PRL-associated increases of IFN-γ are associated with suppression of IL-10 and augmentations of IL-1α and IL-17, creating a profile reminiscent of severe chronic inflammation.

KEY WORDS aging, autoimmunity, chronic inflammation, dacryoadenitis, dry eye, prolactin, Sjögren's disease*

I. INTRODUCTION

Dry eye disease is one of the most common morbidities eye care specialists are called upon to treat. The etiology is widely recognized as multifactorial. Certain inflammatory processes that arise in the lacrimal glands, most notably Sjögren's disease,[1,2] graft-versus-host disease,[3] sarcoidosis,[4,5] Wegener's granulomatosis,[6] HTLV-associated dacryoadenitis,[7] and HIV-associated diffuse infiltrative lymphocytosis,[8,9] are associated with severe physiological dysfunction that leads to keratoconjunctival inflammation. Recent data suggest that many cases of dry eye disease result from age-related decreases in meibomian gland function, impairment of the tear film lipid layer, accelerated evaporation, and increased tear film osmolarity.[10-13] Accordingly, some authors have proposed that subsequently diagnosed lacrimal gland physiological dysfunction develops as an untoward consequence of the mechanisms that initially drive compensatory increases in lacrimal fluid production,[10,13] but possible cellular bases for such a phenomenon have not yet been proposed.

A glossary of terms used in this review is appended to the article.

A. Inflammation and Physiological Dysfunction: Pathophysiological Diversity

Classic histopathological findings show chronic inflammation to be a normal, almost ubiquitous, concomitant of aging in the lacrimal glands. Waterhouse found lymphocytic infiltrates in 65% of lacrimal glands from women aged 75 years and older.[14] Damato et al reported infiltrates in 70% of glands from women and men, mean age

Accepted for publication June 2014.

[1]Department of Physiology & Biophysics, [2]Department of Ophthalmology, Doheny Eye Institute, [3]Department of Pharmacology & Pharmaceutical Sciences, and [4]Department of Cell & Neurobiology, Keck School of Medicine and School of Pharmacy, University of Southern California, Los Angeles, California

Supported by unrestricted grants from Allergan and by NIH Grants EY 005801 (defunct), EY 010550 (defunct), EY 013720 (defunct), EY 017731 (defunct), and DK 048522.

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

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

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jtos.2014.06.003.

*The authors' opinion is that "Sjögren's disease" is to be preferred over the historical "Sjögren's syndrome," as the immunopathological processes underlying the classic signs and symptoms are coming to be understood.

Corresponding author: Austin K. Mircheff, PhD, Department of Physiology & Biophysics, Keck School of Medicine, University of Southern California, 1333 San Pablo Street, BMT B-11A, Los Angeles, CA 90033. Tel: 323 442-1242. E-mail address: amirchef@usc.edu

OUTLINE

I. Introduction
  A. Inflammation and Physiological Dysfunction: Pathophysiological Diversity
  B. Epithelial Factors and Development of Immunopathological Infiltrates
  C. Recent Insights from Rabbit Studies
II. New Findings
  A. Materials and Methods
    1. Animals
    2. Maximal Dryness and High Temperature
    3. Designation of Groups and Individual Glands
    4. Tissue Collection and Processing
    5. Immunohistochemical Staining and Image Analysis
    6. Laser Capture Microdissection
    7. RNA Extraction, Reverse Transcription, and Real-Time RT-PCR
    8. Data Analysis and Presentation
      a. Glands that Appeared as Outliers
      b. Correlation Analyses
      c. Mapping Correlation Clusters to Cells and Networks
      d. Judging Whether Networks Might be Intact in Glands that Appear as Outliers
      e. Relationships between Transcript Abundances and Meteorological Variables
  B. Histoarchitectural Organization of Transcript Expression
  C. Intergroup Variations, Intragroup Variations, Correlation Clusters, Cells, and Networks
    1. A Core, Adaptive-Reactive Network
    2. Core, Adaptive-Reactive Network Transcripts in $V^{58\%,17^\circ}$
    3. Additional Transcripts Associated with the Core, Adaptive-Reactive Network in $V^{82\%,29^\circ}$
    4. A T_H3-Reminiscent Cluster and Network Associations in $V^{61\%,27^\circ}$
    5. A T_H2-Reminiscent Profile in Gland $V^{61\%,27^\circ}$02.OS
    6. The Core, Adaptive-Reactive Network and the Inflammatory Process in Gland $V^{61\%,27^\circ}$02.OS.
    7. Crosstalk between Cells Expressing mRNAs for PRL, IL-4, IFN-γ, and IL-10
    8. Positive- and Negative Crosstalk to a T_H1/NKT-Reminiscent-TNF-α Network in $V^{72\%,32^\circ}$
    9. Emergence of Novel Profiles in Glands $V^{68\%,37^\circ}$03.OS and $V^{72\%,32^\circ}$01.OD
    10. BAFF mRNA-Expressing Cells, the PRL-Mediated Epithelial-T_H2 Network, and the PRL-Mediated Epithelial-T_H1 Network
    11. Crosstalk between the Core, Adaptive-Reactive Network and Other Cells and Networks in Glands $V^{68\%,37^\circ}$02.OS and $V^{68\%,37^\circ}$03.OS
    12. Correlation Clusters Reminiscent of Immune Inductive Tissues
  D. Associations between Environmental Variables and Network Functions
    1. Empirical Descriptions
    2. Exponential Growth Heuristics and Crosstalk
III. How Determined, Adaptive Network Responses May Lead to Diverse Immunopathological States
  A. Possible Adaptive Value of the Core Network and of T_H2-Reminiscent Cells
    1. Counter-regulatory, Anticipatory, or Corollary?
    2. Physiological Signals Related to Dryness
    3. Physiological Signals Related to High Temperature
  B. T_H3-Reminscent Cells and Constitutive, Adaptive Immunoregulation
  C. Crosstalk and Inflammatory Pathogenesis
  D. Crosstalk Promoting Elements of Ectopic Immune Inductive Tissue
  E. Crosstalk Promoting IL-17-Mediated Inflammation
  F. Physiological Dysfunction without Frank Inflammation: Stochastic Processes and Determined Responses
IV. Conclusions
V. Acknowledgments
VI. Glossary

62 ± 17 years.[15] They also suggested that cases in which atrophic and fibrotic changes are not associated with notable infiltrates may be the sequelae of previous inflammatory processes. Obata et al reported infiltrates in 69% of palpebral glands and 83% of orbital glands from women and men between 40 and 87 years of age.[16] Waterhouse found infiltrates in fewer of glands from elderly men (20%), but, strikingly, he also found that infiltrates begin to appear early in adult life and that they occur at similar rates (22% and 18%, respectively) in glands from women and men younger than 40 years of age. These numbers point to the conclusion that the prevalence of lymphocytic infiltrates is substantially greater than the prevalence of clinical dry eye disease, implying that the infiltrates that commonly develop are immunopathologically diverse, with diverse impacts on lacrimal physiological function. Moreover, as dry eye disease is several-fold more prevalent in women than in men, the disparity between the processes that do and do not cause clinically significant physiological dysfunction must be greater in men.

The contrast between Sjögren's disease and Mikulicz's disease[17,18] illustrates the principle that some immune cell infiltrates are associated with physiological dysfunction, while others are relatively benign, at least with respect to lacrimal gland physiological function. Moreover, findings from laboratory studies suggest a general principle to account for the difference: Some mediators known to be produced by inflammatory infiltrates cause physiological dysfunction in ex vivo models, some do not, and some may augment fluid production. Nitric oxide (**NO**) impairs stimulus-secretion coupling in human labial salivary gland acinar cells,[19] interleukin (**IL**)-1 suppresses protein secretion