Multiple Natural and Experimental Inflammatory Rabbit Lacrimal Gland Phenotypes

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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 [^{3}H]-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**

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 al[1] 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:

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OUTLINE

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 disease[10] 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