The Ocular Surface xxx (2017) 1–12

Contents lists available at ScienceDirect

The Ocular Surface

journal homepage: www.theocularsurface.com

Pregnancy probabilistically augments potential precursors to chronic, immune-mediated or autoimmune lacrimal gland infiltrates

Austin K. Mircheff [a, b, *], Yanru Wang [a], Meng Li [c], Billy X. Pan [b], Chuanqing Ding [d]

[a] Department of Physiology & Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, CA, 90033, USA
[b] Department of Ophthalmology and Roski Eye Institute, Keck School of Medicine, University of Southern California, Los Angeles, CA, 90033, USA
[c] Bioinformatics Service, Norris Medical Library, University of Southern California, Los Angeles, CA, 90033, USA
[d] Department of Cell & Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, CA, 90033, USA

ARTICLE INFO

Article history:
Received 4 August 2017
Received in revised form
1 September 2017
Accepted 12 September 2017

Keywords:
Aging
Autoimmunity
Chronic immune-mediated inflammatory processes
Dry eye disease
Pregnancy
Prolactin
Sjögren's syndrome

ABSTRACT

Purpose: This study asked whether pregnancy, a risk factor for dry eye disease associated with both chronic, immune-mediated- and autoimmune etiologies, augments development of clusters of coordinately functioning cells (CCFC) that may be precursors to pathological lacrimal gland infiltrates.
Methods: Lacrimal glands were from six virgin- and six term-pregnant rabbits of the same age and environmental exposure history. Seventy-two immune response-related gene transcripts were assayed by real time RT-PCR. Principal component (PC) analysis identified transcript signatures of CCFC contributing negative ($^{\ominus}$) or positive ($^{\oplus}$) PC loadings and determined gland PC projections, which reflect levels of CCFC development.
Results: Three CCFC were of interest as potential precursors to pathological infiltrates. CCFC $1^{\ominus}$ was suggestive of an ectopic lymphoid structure with resting T cells and B cells. CCFC $1^{\oplus}$ was suggestive of an immune-mediated infiltrate with T_H1 cells and mature, cytotoxic B cells. CCFC $2^{\ominus}$ was suggestive of an ectopic lymphoid structure with activated T cells, mature B cells, germinal center, and plasmacytes. CCFC $4^{\ominus}$ and CCFC $5^{\ominus}$ also included plasmacytes. Pregnancy augmented CCFC $1^{\ominus}$ in some glands; augmented CCFC $1^{\oplus}$ in others; and augmented CCFC $2^{\ominus}$, CCFC $4^{\ominus}$, and CCFC $5^{\ominus}$ different combinations.
Conclusions: Potential precursors of pathological infiltrates form in the lacrimal glands by the time of sexual maturity. Pregnancy augments lacrimal gland plasmacyte populations, and it can augment development of potential precursors to either chronic, immune-mediated infiltrates or autoimmune infiltrates of various phenotypes. Systemic and strictly local, probabilistic phenomena interact with pregnancy to determine which combinatorial phenotypes are favored.

1. Introduction

Chronic, immune-mediated inflammatory diseases and autoimmune diseases can develop in most tissues, and they develop so frequently that, collectively, they are the major causes of morbidity, disability, and mortality in the developed countries. The lacrimal gland is one of the organs that can be affected as the life cycle runs its course [1–8]; the consequences, physiological dysfunction [9–13] or physical atrophy [14], contribute to severe dry eye disease, a highly prevalent inflammatory disease of the ocular system.

The chronic, immune-mediated inflammatory processes that develop in the lacrimal glands present with quite diverse histopathological phenotypes [1], each of which may map to different cellular and molecular phenotype. Diverse cellular and molecular phenotypes have been documented in labial salivary glands of patients with Sjögren's syndrome [15–19], an autoimmune disease that typically affects both the lacrimal glands and salivary glands. Other autoimmune diagnoses, i.e., sarcoidosis, granulomatosis with polyangiitis, and IgG4-related disease, also affect the lacrimal glands. However, there have been no systematic studies of the extents to which the lacrimal gland autoimmune manifestations are phenotypically diverse.

There are no compelling answers to the questions of how any of the chronic, immune-mediated and autoimmune diseases develop and why so many different chronic, immune-mediated phenotypes

* Corresponding author. Department of Physiology & Neurobiology, Keck School of Medicine, University of Southern California, 1333 San Pablo Street, BMT B11-A, Los Angeles, CA, 90033, USA.
E-mail address: amirchef@usc.edu (A.K. Mircheff).

https://doi.org/10.1016/j.jtos.2017.09.005
1542-0124/

and Sjögren's syndrome disease phenotypes develop [20]. If answers can be found, they may lead to new, effective diagnostics and therapies. Two recent studies with rabbits suggest that clusters of cells that may be precursors to many different pathological infiltrates have already formed in the lacrimal glands by the time the animals reach sexual maturity. The evidence for the cell clusters' presence derives from Pearson's analysis of variations in the abundances of up to 47 immune response-related gene transcripts in one study [21] and from principal component analysis of variations in the abundances of 31 immune response-related gene transcripts in a second study [22]. Each of the cell clusters hypothesized to be potentially pathological included several different types of immune cells, and, in some cases, also acinar- and ductal epithelial cells, all functioning in concert to express signature sets of transcripts. Both studies identified clusters of coordinately functioning cells (CCFC) that are of interest as potential precursors to autoimmune ectopic lymphoid tissues, such as those that characterize Sjögren's syndrome, because their signature transcripts included transcripts that typically are expressed in T cells, i.e., mRNAs for CD4, CD8, CTLA-4, and CD28, and transcripts that typically are expressed in B cell zones of secondary lymphoid tissues, i.e., mRNAs for CXCL13, IL-1α, IL-4, IL-6, IL-10, and BAFF. The ectopic lymphoid tissue-resembling CCFC appeared to have developed in dyadic relationships, i.e., in counterpoise, with reciprocal CCFC, and the reciprocal CCFC also were of interest, as they expressed transcripts that typically are associated with cell-mediated immune responses. The state of the counterpoise between the ectopic lymphoid tissue-suggestive CCFC and the cell-mediated immune response-resembling CCFC was the major source of variability in both studies, but both studies also identified additional CCFC that expressed transcripts that typically are associated with immune responses. In view of these findings, the extents to which the various CCFC have developed in a gland can be said to determine the gland's immunological phenotype.

Previous exposures to environmental dryness or environmental high temperatures appeared to favor development of different CCFC [21], but probabilistic phenomena must have interacted with the environmental exposures to determine levels of CCFC development in each gland, because glands from animals with the same environmental exposure histories presented with different combinatorial CCFC phenotypes. Indeed, in many cases, the glands associated with left eye and right eye of the same animal presented with different combinatorial phenotypes.

Exposure to high degrees of environmental dryness is a risk factor for both acute dry eye symptoms [23] and for chronic, immune-mediated dry eye disease [24]. Exposure to both low- and high environmental temperatures also appears to be a risk factor for exacerbation of dry eye symptoms [25]. Therefore, the findings suggest that, as certain combinations of CCFC develop over time, they begin to manifest as different pathological phenotypes. If this is the case, then one might predict that other risk factors in addition to environmental exposures also augment the development of combinations of CCFC that are of interest as potential precursors to pathological infiltrates.

Several additional risk factors for dry eye disease have been identified. Sjögren's syndrome may be as much as 16-fold more prevalent in women than in men [26]. Dry eye disease associated with chronic, immune-mediated inflammation also is more prevalent in women [27–29]. The steroid reproductive hormones influence expression of numerous genes in murine lacrimal glands and other ocular surface tissues, and such influences are thought to mediate the sex-associated risks [30,31]. Pregnancy is characterized by increased levels of chorionic gonadotropin, estradiol, progesterone, prolactin, and bioavailable testosterone [32], as well as glucocorticoids, mineralocorticoids, and angiotensin, and studies indicate that pregnancy increases risks for dry eye signs or symptoms [33–35], for chronic dry eye disease associated with immune-mediated inflammation [33,36], and for Sjögren's syndrome [37,38]. Pregnancy also has been shown to be associated with corneal inflammatory changes [39] and with lacrimal gland physiological [40,41] and immunological [42] changes in rabbits. Therefore, the goal of this study was to learn whether and how pregnancy influences the development of CCFC of interest in lacrimal glands of healthy, young adult female rabbits.

Abundances of 72 transcripts were determined by real time RT-PCR, and the abundance data were submitted to principal component analysis and to tests for significant differences. Principal component analysis once more identified a CCFC (CCFC $1^{\ominus}$) that expressed a set of transcripts suggestive of an ectopic lymphoid tissue which contained a B cell zone and a T cell zone and which developed in a reciprocal relationship with a CCFC (CCFC $1^{\oplus}$) that expressed a set of transcripts suggestive of T_H1 cells and cytotoxic B cells. The analysis identified a third CCFC (CCFC $2^{\ominus}$) that expressed a set of transcripts suggestive of an ectopic lymphoid tissue, which contained a germinal center, an active T cell zone, mature B cells, and plasmacytes, and it identified two additional CCFC that expressed sets of transcripts suggestive of plasmacytes together with other cell types. Pregnancy augmented CCFC $1^{\ominus}$ in some glands and CCFC $1^{\oplus}$ in others. It also augmented the plasmacyte-containing CCFC, but in different combinations in different glands. In all, pregnancy augmented eight different combinatorial CCFC phenotypes of interest.

2. Methods

Housing of rabbits and euthanasia with Euthasol after ketamine/xylazine anesthesia conformed to the ARVO Resolution on the Use of Animals in Ophthalmic and Vision Research and were approved by the University of Southern California Animal Care and Use Committee.

Study animals consisted of two cohorts of 20 week-old (i.e., young adult) female New Zealand white rabbits that had been exposed to the same warm, dry environment in a barrier-free facility at Irish Farms, in Norco, CA. Six rabbits were virgin, and six were pregnant. The pregnant animals were euthanized on the 29th day of gestation, i.e., 1-day short of term. Partial data from the virgin animal have been reported previously [21,22].

Rabbits were necropsied immediately after euthanasia, and lacrimal glands were collected and divided into two equal portions. One portion was placed in RNALater® for mRNA extraction, and one portion was embedded in OCT for sectioning and immunohistochemical staining (IHC). Most of the OCT-embedded material was consumed for other studies. The subsets of samples available for this study were stained for the rabbit T lymphocyte antigen (RTLA) and CD18, typically expressed by bone marrow-derived cells, as described in previous reports [21,22]. The mRNA extracts from each gland were individually subjected to reverse transcription and real time RT-PCR with rabbit-specific primer-probe sets [43]. A total of 72 transcripts were assayed.

Transcript abundances were submitted to principal component analysis, performed with the Partek Genomics Suite (Partek Inc., St. Louis, MO). Principal component analysis isolates the independent sources of variability (the “principal components”) in complex data sets, and it identifies the contribution (the “loading”) that each variable makes to each principal component. The sets of transcripts that contributed strong loadings to principal components were attributed to clusters of cells that functioned coordinately (CCFC). According to this paradigm, each gland's set of principal component projections is a measure of the extents to which the respective CCFC had developed in the gland.

Please cite this article in press as: Mircheff AK, et al., Pregnancy probabilistically augments potential precursors to chronic, immune-mediated or autoimmune lacrimal gland infiltrates, The Ocular Surface (2017), https://doi.org/10.1016/j.jtos.2017.09.005