Innate Immunity and Inflammation

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Goals and Projects:

My lab focuses on understanding the innate defense and immunoregulatory functions of corneal epithelial cells. Epithelial tissues form a protective lining of the body, including the outer surfaces that are exposed to the environment, as well as the inner body cavities, glands and ducts. Through constitutive and inducible expression of danger- and pathogen-associated molecular pattern recognition receptors, innate defense molecules, cytokines and chemokines, epithelial cells serve as an essential component of the innate immune system to stop pathogens right at the points of entry before they can cause diseases, regulate regional immune and inflammatory responses, and maintain tissue homeostasis. Recently, we discovered that the C-terminal fragments of a cytokeratin protein, K6, in corneal epithelial cells are antimicrobial [1], and that endogenous K6 regulates signaling pathways to control production of inflammatory mediators. These are unexpected functions for keratin proteins since they have long been viewed as the building blocks of cytoskeleton that maintain structural integrity and resilience of epithelial cells. Given the wide distribution of K6 among different epithelial tissues, as well as the structural and functional similarities shared among different keratins, our studies aim to advance understanding of epithelial innate immune functions of the cornea and other sites of the body, and to contribute to biocompatible anti-infective and immunoregulatory drug development in the post-antibiotic era.

Research and Innovations:

Using corneal epithelial cell culture system and conditional knockout mice in corneal infection and inflammation models, coupled with an array of modern proteomic, biochemical, molecular, cell biological, and microscopic (fluorescence and live cell imaging) tools, our ongoing basic and translational research attempts to answer important questions including:

1. What are the mechanisms for regulating keratin depolymerization, as well as production, secretion and activity of keratin-derived antimicrobial peptides (KAMPs)?
2. How does K6 regulate immune and inflammatory responses intrinsically and extrinsically?
3. Are keratin-based host defenses and immunoregulation employed by multiple sites of the body besides the cornea? If these mechanisms are dysregulated, could it lead to increased disease susceptibility and severity?
4. Can we use synthetic KAMPs and/or K6 supplementation to prevent and treat infection and inflammation of the cornea and other sites, especially those caused by multidrug resistant bacteria?

Endogenous proteolytic processes that produce antimicrobial keratin fragments. As KAMPs found in the cytosol are C-terminal fragments of K6, they should be cleaved from their full-length form by endogenous proteases. We have found that ubiquitination of K6 followed by proteasomal degradation produce KAMPs [2]. We are interested in studying the detailed molecular mechanisms underlying these processes.

Regulatory mechanisms for keratin filament depolymerization. As epithelial barriers must remain structurally resilient under various environmental challenges, the level of keratin expression and the dynamics of keratin filament turnover are both highly regulated. One of the mechanism involves phosphorylation of keratin subunits (e.g. via MAPK kinases or protein kinase C), which in turn increases keratin solubilization (filament disassembly). Triggers for phosphorylation include various stress paradigms, apoptosis and mitosis. We have found that proinflammatory bacterial components induce K6 phosphorylation, which in turn increases the solubility of its filamentous form and thus the levels of cytosolic K6 and KAMPs [2]. We are interested in studying the receptors, pathways, and kinases involved in these processes.

Microbicidal mechanisms of keratin-derived antimicrobial peptides. KAMPs have broad efficacy against both Gram-positive and Gram-negative human pathogens such as *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Escherichia coli* [1]. We have characterized the 3D structure of KAMPs by NMR, which indicated that KAMPs are the first human example of the non-$\alpha\beta$ class of antimicrobial peptides [3]. We have also demonstrated KAMPs-induced bacterial membrane disruption by high resolution transmission electron microscopy [3]. We are interested in studying the bacterial intracellular machineries that are targeted by KAMPs, and how multi-drug resistant bacteria, viruses and fungi interact with KAMPs.

Roles of K6 and keratin-derived antimicrobial peptides in protecting the cornea (and other sites) against diseases. In addition to the innate immune function, we are interested in determining if K6 and KAMPs have additional roles, e.g. their working relationships with endogenous factors in corneal epithelial cells (intrinsic) and other immune cells (extrinsic). We are also interested in determining if dysfunction of keratins and KAMPs play a role in inflammatory eye diseases.

Prophylactic and treatment use of keratin-derived antimicrobial peptides and K6-loaded nanoparticles. While infectious and inflammatory corneal diseases remain a major cause of visual impairment and blindness, we are interested in examining the activities of KAMPs against major ocular pathogens, including fungi, viruses, Acanthamoeba and antibiotic-resistant bacteria, and improving their protective/therapeutic capability against infection by optimization of dosage, administration frequency and dosage form. We are also interested in examining synergistic and additive effects of KAMPs with current antibiotics. As shortening healing time can also reduce tissue susceptibility to infection after wounding, we are interested in determining the wound healing effects of KAMPs on non-infected, injured mouse corneas. In addition to exogenous KAMPs, we will investigate the potential of intracellular delivery of K6 via nanoparticles as a new therapeutic strategy for inflammatory diseases.

Lab Staff Members:

- K. P. Connie Tam, PhD, *Principal Investigator*
- Jonathan Chan, PhD, *Research Associate*
- Yan Sun-Beck, MD, *Research Associate*

1. **Tam C**, Mun JJ, Evans DJ, Fleiszig SM. **(2012)** Cytokeratins mediate epithelial innate defense through their antimicrobial properties. *Journal of Clinical Investigation*, 122(10):3665-3677. PMC3461926. *Commentary by Michael Zasloff; recommended by Faculty of 1000.*

2. Chan JKL, Yuen D, Too PH, Sun Y, Willard B, Man D, **Tam C**. **(2018)** Keratin 6a reorganization for ubiquitin-proteasomal processing is a direct antimicrobial response. *Journal of Cell Biology*, 217:731-744. PMC5800800. *Featured as the "In Focus" article.*

3. Lee JT, Wang G, Tam YT, **Tam C**. **(2016)** Membrane-active epithelial keratin 6a fragments (KAMPs) are unique human antimicrobial peptides with a non-αβ structure. *Frontiers in Microbiology*, 7:1799. PMC5105358.