BIOGRAPHICAL SKETCH

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NAME: Duncan, Joseph A.

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POSITION TITLE: Associate Professor of Medicine and Pharmacology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
California Institute of Technology, Pasadena, CA	A.B.	06/1992	Biochemical Sciences
Univ. Texas Southwestern Medical School, Dallas, TX	M.D./Ph.D	06/2001	Medicine
University of North Carolina at Chapel Hill, Chapel Hill, NC	-	06/2003	Internal Medicine Residency
University of North Carolina at Chapel Hill, Chapel Hill, NC	-	06/2006	Infectious Diseases Fellowship

A. Personal Statement

I am an Infectious Diseases specialist and physician scientist at the University of North Carolina. I have a long-standing interest in understanding the molecular mechanisms underlying signal transduction utilizing biochemical methodologies. My Ph.D. thesis work focused on the in vitro reconstitution of a regulated lipid modification (known as palmitoylation) cycle in heterotrimeric G protein signaling. This work included the development of novel biochemical assays to assess G protein α subunit palmitoylation, the production of recombinant protein substrates, and the purification of previously unidentified enzymes involved in the process from native sources. Since training in Infectious Diseases, I have utilized my skills and experience in protein biochemistry to understand the molecular mechanisms of signaling by a family of nucleotide binding proteins involved in innate immune signaling known as NOD-like Receptors (NLRs). The NLR genes were initially identified in the human genome based on a common predicted protein domain structure and their homology to a number of immune signaling molecules and over the last 10 years have been recognized as important players in different innate immune signaling pathways recognizing a variety of pathogens. NLRP3, NLRC4, and some other NLR proteins form a Caspase-1 activating, IL-1β processing complex known as the inflammasome upon activation. Another NLR, NOD2 recognizes peptidoglycan components and initiates autophagy of cytoplasmic pathogens and subsequent presentation of antigens from those pathogens. Between my postdoctoral training and career as an independent investigator, I have led or been involved in the biochemical characterization of three NLR proteins: NLRP3, NLRP12, and NOD2. My contributions to this field include determining the critical role of ATP binding and hydrolysis to the signaling function of these proteins as well as the providing definitive evidence that these are indeed receptors for pathogen derived molecules rather than simply acting as critical intermediates in the signaling pathways activated by these pathogen derived molecules. As a physician scientist, I have had great interest in the physiologic and pathologic roles these signaling pathways can play in human diseases, particularly in infectious diseases. Over the last several years, we have determined that a number of *S. aureus* exotoxins activate the NLRP3 inflammasome and have demonstrated that this activation appears to be pathologic rather than protective in mouse models of *S. aureus* pneumonia. Additionally, we have worked with collaborators studying other pathogenic bacteria and identified virulence factors from other bacteria that also activate the NLRP3 inflammasome, suggesting that selective pressures have acted on multiple pathogenic bacteria to support the production of NLRP3-activating factors.

We discovered that *N. gonorrhoeae* is a potent activator of NLRP3, which likely contributes to the impressive inflammatory exudate observed in patients with gonococcal urethritis. Although we have yet to identify the gonococccal factor that activates NLRP3, our investigations have also shown that the gonococcus produces a lipo-oligosaccharide structure that potently activates inflammatory signaling, when compared to commensal Neisseria species. We have also shown that mutant *N. gonorrhoeae* strains lacking these structures are able to cause infection but are compromised in competitive infection with the wild type strains in both mouse model infection and experimental human infection. These studies have begun to define the selective pressures in the host that select for virulent *N. gonorrhoeae* in humans. We have also found that *N. gonorrhoeae* suppresses host antigen presenting cells' ability to stimulate T lymphocyte proliferation. We are now working to unravel the mechanisms by which *N. gonorrhoeae* evades adaptive immune responses.

B. Positions and Honors

Positions

6/01-6/03 Internal Medicine Resident, University of North Carolina Hospitals
6/03-6/06 Infectious Diseases Subspecialty Resident, University of North Carolina School of Medicine
7/06-7/09 Clinical Assistant Professor, University of North Carolina School of Medicine
8/09-8/14 Assistant Professor of Medicine and Pharmacology, University of North Carolina
8/14-present Associate Professor of Medicine and Pharmacology, University of North Carolina
11/14-present Director Infectious Diseases Training Program, University of North Carolina

Certifications and Boards

11/04-12/2015 American Board of Internal Medicine; Certification in Internal Medicine
02/03-present North Carolina Medical License
10/07-present American Board of Internal Medicine; Certification in Infectious Diseases

Honors and Awards

1996 Haberect Wildhare-Idea Program Research Grant:
"Rapid Isolation of Small Peptide Affinity Tags"
1997 Twenty-Ninth Annual Sigma Xi Graduate Student Research Forum Poster Award:
"S-Acylation of Heterotrimeric G Protein Alpha Subunits"
1997 Alfred Gilman Memorial Award for Excellence in Research
1998 UT Southwestern Graduate School Dean's Discretionary Award
2000 Alpha Omega Alpha appointment
2002 David A. Ontjes Award (Outstanding UNC Internal Medicine Intern, 2001-2002)
2004 Pfizer Fellowship in Infectious Diseases
"Biochemical Characterization of Monarch-1, a Regulator of Signaling within the Innate Immune System"
2006 K12 Scholar - UNC Multidisciplinary Clinical Research Career Developmental Program
2007 Sexually Transmitted Infection and Topical Microbicide Cooperative Research Center Developmental Award
2008 Burroughs Wellcome Fund Career Award for Medical Scientists
2011 Fellow of the Infectious Diseases Society of America

C. Contributions to Science

1. **Molecular Mechanisms of NLR signaling.** NOD-like Receptors or NLR proteins are a family of mammalian signaling molecules that are involved in a wide variety of innate immune signaling pathways. After the identification of the gene family encoding these proteins, they were designated as "receptor" although there were no formal studies demonstrating their capacity to interact with pathogen derived molecules. Between my postdoctoral training and career as an independent investigator, I have led or been involved in the biochemical characterization of three NLR proteins: NLRP3, NLRP12, and NOD2. My contributions to this field include determining the critical role of ATP binding and hydrolysis to the signaling function of these proteins as well as the providing definitive evidence that these are indeed receptors for pathogen derived molecules rather than simply acting as critical intermediates in the signaling pathways activated by these pathogen derived molecules. We have developed unique tools to facilitate the production and biochemical characterization of the NLR protein function
    a. Mo, J. Y., **Duncan, J. A.** (2013), Assessing ATP Binding and Hydrolysis by NLR Proteins, Methods in Mol. Biol. 2013, 1040:153-68.

b. Mo, J. Y., Boyle, J. P., Howard, C. B., Monie, T. P., Davis, B. K., **Duncan, J. A.** (2012), Pathogen sensing by nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is mediated by direct binding to muramyl dipeptide and ATP. J. Biol. Chem. 2012, June 29;287(27):23057-23067

c. Ye Z., Lich J.D., Moore C.B., Duncan J.A., Williams K.L., Ting J.P., (2008) ATP binding by Monarch-1/NLRP12 is critical for its inhibitory function, Mol. Cell Biol. 2008 Mar;28(5):1841-1850.

d. **Duncan, J. A.**, Bergstralh, D. T., Wang, Y., Willingham, S. B., Ye, Z., Zimmermann, A., and Ting, J. P., (2007), Cryopyrin/NALP3 binds ATP/dATP, is an ATPase, and requires ATP binding to mediate inflammatory signaling, Proc. Natl. Acad. Sci. USA 2007 May 8;104(19):8041-8046.

2. **Role of the NLRP3 inflammasome in *S. aureus* infection pathogenesis.** *S. aureus* is the primary cause of severe skin and soft tissue infection in the United States requiring emergency medical attention and can cause life-threatening necrotizing infections with very high mortality rates even among healthy young patients. *S. aureus* produce a number of exotoxin virulence factors with cytolytic properties. These include several secreted pore forming toxins: α toxin (also called α hemolysin), γ toxin, and bicomponent leukocidins (lukAB/GH, lukED, and Panton Valentine Leukocidin or PVL). We demonstrated that highly purified pore forming toxins are potent activators of the NLRP3-inflammasome. We also recently discovered that LukAB activates the NLRP3-inflammasome, demonstrating that LukAB is the primary toxin responsible for *S. aureus*-induced NLRP3-inflammasome activation in a wide array of *S. aureus* strains. Overall, these findings implicate activation of the NLRP3-inflammasome as a point of convergence for multiple *S. aureus* toxins implicated in the bacteria's virulence. In murine models of infections, *S. aureus* lacking α toxin either fail to cause disease or lead to attenuated infections. We have studied how activation of the NLRP3-inflammasome by α toxin influences *S. aureus* disease pathogenesis. *Nlrp3$^{-/-}$ mice* are protected from death and hypothermia associated with intratracheal delivery of heat killed *S. aureus* and α toxin administration. Compared to wild type C57BL/6 mice, *Nlrp3$^{-/-}$* mice have reduced airway neutrophilia and pulmonary inflammation in response to treatment with α toxin. Overall, our lab has demonstrated that these *S. aureus* virulence factors act through host NLRP3 inflammasome activation and that activation of NLRP3 can be pathologic rather than protective during *S. aureus* infections.

a. Melehani, J.H., James, D. B. A., DuMont, A. L., Torres, V. J., and **Duncan, J. A.**, *Staphylococcus aureus* leukocidin A/B (LukAB) kills human monocytes via host NLRP3 and ASC when extracellular, but not intracellular PLoS Pathog. 2015 Jun 12;11(6):e1004970.

b. Holzinger, D., Gieldon, L., Mysore, V., Nippe, N., Taxman, D. J., **Duncan, J. A.**, Broglie, P. M., Marketon, K., Austermann, J., Vogl, T., Foell, D., Niemann, S., Peters, G., Roth, J., and Löffler, B. (2012), Staphylococcus aureus Panton-Valentine Leukocidin Induces an Inflammatory Response in Human Phagocytes via the NLRP3-Inflammasome. J. Leukoc. Biol. 2012, Nov;92(5):1069-81. Epub 2012 Aug 14.

c. Kebaier, C., Chamberland, R. R., Allen, I. C., Gao, X., Hall, J. D., Jania, C., Doerschuck, C. M., Tilley, S. L., and **Duncan, J. A.** (2012), Staphylococcus aureus α-Hemolysin Mediates Virulence in a Murine Model of Severe Pneumonia Through Activation of the NLRP3 Inflammasome. J. Infect Dis. 2012 Mar;205(5): 807-817.

d. Craven, R. R., Gao, X., Allen, I. C., Gris, D., Wardenburg, J. B., McElvania-TeKippe, E., Ting, J. P., and **Duncan, J. A.** (2009) Staphylococcus aureus α-Hemolysin Activates the NLRP3-Inflammasome in Human and Mouse Monocytic Cells. PLoS ONE. 2009 Oct 14;4(10): e7446.

3. **Mechanisms of innate and adaptive immune evasion by *N. gonorrhoeae*.** *Neisseria gonorrhoeae* (*Ng),* the most common sexually transmitted bacterial pathogen worldwide*,* is an exclusive human pathogen well adapted to life in the human genital tract. At the mucosal surface, *Ng* can cause localized inflammation. In men, this leads to copious urethral discharge. However, in ~50% of infected women, *Ng* is clinically silent and seemingly undistinguished from other vaginal commensal bacteria by its host. In both cases, *Ng* evades the development of protective immune responses, retaining the capacity to re-infect the same host. We used a combination of bacterial genetics, mouse infection models, and a unique experimental human infection to show that specific modifications of *Ng* lipooligosaccharide can control the fitness and symptomatic presentation of *Ng* infection. We have also found that *Ng* has immune-suppressive effects on dendritic cells (DCs), which are the cells that facilitate $CD4^+$T-cell proliferation and polarization in response to pathogen-derived antigens. Our data indicates *Ng* sheds factors that suppress DC-induced T-cell proliferation and shift T-cell polarization towards non-protective phenotypes. Combined our studies have

begun to understand the complex relationship between innate immune responses to *Ng* and protective immunity to the pathogen.

a. Hobbs, M. M., Anderson, J. A., Balthazar, J. T., Kandler, J. L., Carlson, R. W., Ganguly, J., Begum, A. A., **Duncan, J. A.**, Lin, J. T., Sparling, P. F., Jerse, A. E., and Shafer, W. M., Lipid A structure mediates Neisseria gonorrhoeae fitness during experimental infection of mice and men, mBio 2013 Nov 19;4(6):e00892-13

b. Zhou, X., Gao, X., Broglie, P. M., Kebaier, C., Anderson, J. E., Thom, N., Apicella, M. A., Gregory D. Sempowski, G. D., and **Duncan, J. A.** Hexa-acylated lipid A is required for host inflammatory response to N. gonorrhoeae in experimental gonorrhea, Infection and Immunity 2014 Jan;82(1):184-92. Epub 2013 Oct 14.

c. Zhu, W., Ventevogel, M. S., Knilans, K. J., Anderson, J. A., Oldach, L. M., McKinnon, K. P., Hobbs, M. M., Sempowski, G. D., and **Duncan, J. A.** (2012), Neisseria gonorrhoeae Suppresses Dendritic Cell-induced, Antigen-Dependent CD4 T Cell Proliferation. PLoS ONE 2012, July 23;7(7): e41260

d. **Duncan, J. A.**, Gao, X., Huang, M., O'connor, B.P., Thomas, C. E., Willingham, S. B., Bergstralh, D. T., Jarvis, G. A., Sparling, P. F., and Ting, J. P., (2009), Neisseria gonorrhoeae activates the proteinase Cathepsin B to mediate the signaling activities of the NLRP3 and ASC - containing inflammasome, J. Immunology 2009 May 15;182(10):6460-6469..

4. **Role of NLR proteins in autoinflammatory disorders and innate immune responses to viral infections.** Although my lab's work has largely focused on molecular mechanisms of NLR protein signaling and innate immune responses to bacterial infections, we have also contributed to important studies of innate immune responses to viral infection by this family of signaling molecules. We have also contributed to studies of autoinflammatory disorders that involve mutations in NLR genes. Our lab added studies requiring protein isolation and biochemical characterization of signaling complexes that included NLRC4, NLRX1, and NLRC3 in these settings.

a. Canna, S. W., Almeida de Jesus, A., Gouni, S., Brooks, S. R., Marrero, B., Liu, Y., Dimattia, M., Zaal, K. J. M., Montealegre-Sanchez, G., Kim, H., Chapelle, D., Plass, N., Huang, Y., Biancotto, A., Fleisher, T. A., **Duncan, J. A.**, O'Shea, J. J., Benseler, S., Grom, A., Deng, Z., Laxer, R. M., Goldbach-Mansky, R., An activating *NLRC4* inflammasome mutation causes a novel autoinflammatory syndrome presenting with recurrent Macrophage Activation Syndrome, Nat. Genet. 2014 Oct;46(10):1140-6.

b. Zhang, L., Mo, J., Swanson, K. V., Wen, H., Petrucelli, A., Gregory, S. M., Zhang, Z., Schneider, M., Jiang, Y., Fitzgerald, K. A., Ouyang, S., Liu, Z. J., Damania, B., Shu, H.B., **Duncan, J. A.**, Ting, J. P., NLRC3, a member of the NLR family of proteins, is a negative regulator of innate immune signaling induced by the DNA sensor STING. Immunity 2014 Mar 20;40(3):329-41. Epub 2014 Feb 20.

c. Ting, J. P., **Duncan, J. A.**, and Lei, Y., (2010), How the noninflammasome NLRs function in the innate immune system. Science 2010 Jan 15;327(5963);286-290.

d. Moore, C. B., Bergstralh, D. T., **Duncan, J. A.**, Lei, Y., Morrison, T.E., Accavitti-Loper M. A., Madden, V. J., Sun, L., Lich, J. D., Heise, M. T., Chen, Z., Ting, J. P., (2008), NLRX1 is a regulator of mitochondrial antiviral immunity, Nature 2008 Jan 31;451(7178):573-577.

Complete List of Published Work in MyBibliography
http://www.ncbi.nlm.nih.gov/sites/myncbi/joseph.duncan.1/bibliography/41147364/public/?sort=date&direction=descending