
BIOGRAPHICAL SKETCH

NAME: John F. Kearney

eRA COMMONS USER NAME (credential, e.g., agency login): KEARNEY

POSITION TITLE: Professor of Microbiology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Adelaide, South Australia	B.D.S. Hons	1969	Dental Science
University of Melbourne, Victoria, Australia	Ph.D.	1973	Immunology
Univ. of Alabama at Birmingham, Birmingham, Alabama, USA	Postdoctoral studies	1973-75	Immunobiology

A. Personal Statement.

The goal of this research is to provide new insight into the interaction of adaptive immunity with the innate immune system in response to highly conserved antigens expressed by commensal and pathogenic organisms of importance in human disease. In particular, the PI has tried to understand the factors that influence emerging B cell clones which express stereotypical germ-line encoded BCRs to epitopes often shared by microbial and self antigens. The PI has amassed a tool chest of antigen-specific hybridomas, anti-clonotype markers, transgenic and gene-targeted mice with B cells specific for epitopes targeted by the antibody responses to *Streptococcus pneumoniae*, *Enterobacter cloacae*, Groups A and B streptococci bacteria. In the process, he stimulated renewed interest in the development and function of innate-like Marginal Zone, B1a and B1b B cells and their secreted antibody products. After finding that many monoclonal antibodies (mAbs) to bacterial-associated epitopes also react with the fungus *Aspergillus fumigatus* (A.f.), and other allergen-bearing organisms, the PI showed that neonatal exposure to environmental bacterial antigens in mice dampened the development of allergic-airway disease. In the long-term he has demonstrated a continuum of successful and productive research projects built on those earlier investigations, and now extended into studies of allergy and inflammatory lung diseases, which are increasing major health problems in this westernized country. Dr. Kearney's experience in these research areas and the training of 27 predoctoral students and the same number of postdoctoral fellows have prepared him to lead this proposed project.

1. John F. Kearney, Preeyam Patel, E. K. Stefanov, R. G. King. Natural Antibody Repertoires: Development and Functional Role in Inhibiting Allergic Airway Disease. *Annual Review of Immunology*. 2015, In Press. (Appendix 1, Final edited text not available on line)

B. Positions and Honors**Positions and Employment**

1973-1974	Visiting Foreign Dental Scientist, Dept. Pediatrics, University of Alabama at Birmingham, AL
1974-1976	Research Associate, Dept. of Pediatrics, University of Alabama, Birmingham, AL
1976-1980	Assistant Professor, Dept. of Microbiology, University of Alabama, Birmingham, AL
1978	Visiting Senior EMBO Fellow, Department of Genetics, University of Cologne, Germany
1980-1983	Associate Professor, Department of Microbiology, University of Alabama, Birmingham,
1983-Present	Distinguished Professor in Microbiology, University of Alabama at Birmingham
1983-Present	Senior Scientist, Comprehensive Cancer Center, University of Alabama at Birmingham
1985-1986	Member, Basel Institute for Immunology, Basel, Switzerland
1986-1990	Member, NIH Allergy and Immunology Study Section
2008-2011	Senior Fellow of the American Asthma Foundation

Other Experience and Professional Memberships

Member of the American Association of Immunologists

Member of the Australasian Society for Immunology

Honors

Dean's Award for Excellence in Mentorship, 2010

University of Alabama Health Science Center Distinguished Faculty Lecturer, 2013

UAB Distinguished Professor in Immunology, 2014

C. Contributions to Science

1. The PI's early publications directly addressed B cell isotype switching. These studies were facilitated by the development of the first model in which mouse B cells could be induced to switch immunoglobulin isotypes. This model, dubbed by others as the "low-density LPS culture system", revealed new findings on cellular aspects of the switch of IgM-bearing adult and neonatal B cells to a variety of IgG isotypes, the effects of anti-IgM antibodies on plasma cell development, and the transient production of double IgM- and IgG-bearing B cells during isotype switching. This model was then widely used in the research community to study the modulation of B cell activation by B cell growth factors, specific cytokine-directed B isotype switching, and elucidation of the genetic mechanisms involved in isotype switching.

a. **Kearney, J. F.** and Lawton, A. R.: B lymphocyte differentiation induced by lipopolysaccharide: I. Generation of cells synthesizing four major immunoglobulin classes. *J. Immunol.* 115:671, 1975.

b. **Kearney, J. F.** and Lawton, A. R.: B lymphocyte differentiation induced by lipopolysaccharide: II. Response of fetal lymphocytes. *J. Immunol.* 115:677, 1975.

c. **Kearney, J. F.**, Cooper, M. D. and Lawton, A. R.: B lymphocyte differentiation induced by lipopolysaccharide: III. Suppression of B cell maturation by anti-immunoglobulin antibodies. *J. Immunol.* 116:1664, 1976.

d. **Kearney, J. F.**, Cooper, M. D. and Lawton, A. R.: B lymphocyte differentiation induced by lipopolysaccharide: IV. Development of immunoglobulin class restriction in precursors of IgG synthesizing cells. *J. Immunol.* 117:1567, 1976.

2. During an early-career sabbatical with Dr. Klaus Rajewsky, the PI isolated the first non-secreting plasmacytoma P3x63.Ag.653. This invaluable cell line was immediately released to investigators globally for hybridoma production. This line, without functional H+L antibody genes, facilitated the isolation of pre-B cell hybridomas providing the best evidence that mu chain synthesis preceded light chain synthesis at this stage of B cell development. These pre B cell hybridomas were then shared with multiple laboratories and helped establish the genetic mechanisms associated with the asynchronous expression of immunoglobulin μ heavy chains and of light chains. The isolation of μ -only hybridomas and production of a monoclonal antibody also lead directly to the isolation and functional definition of the first known ER-associated mammalian molecular chaperone, binding immunoglobulin protein (BiP) also known as 78-kDa glucose-regulated protein (GRP-78). These findings were greeted skeptically by reviewers (e.g. "If this is so important why hasn't a lab more important than yours discovered it"). This publication went on to be chosen among the 50 most important papers in the first 50 years of *J. Cell. Biol.* This discovery and our early sharing of the monoclonal anti-BiP antibody helped pave the way for early investigations in the elucidation of molecular interactions in the unfolded protein (UPR) stress response.

a. **Kearney, J. F.**, Radbruch, A., Liesegang, B., and Rajewsky, K.: A new mouse myeloma cell line which has lost immunoglobulin expression but permits the construction of antibody secreting hybrid cell lines. *J. Immunol.* 123:1548-1550, 1979.

b. Burrows, P. D., LeJeune, M. and **Kearney, J. F.**: Evidence that murine pre-B cells synthesize μ heavy chains but no light chains. *Nature* 280:838-841, 1979.

c. Perry, R. P., Kelley, D. E., Coleclough, c. and **Kearney, J. F.**: Organization and expression of immunoglobulin genes in fetal liver hybridomas. *Proc. Natl. Acad. Sci. USA* 78:247-251, 1981.

d. Bole, D., Hendershot, L. and **Kearney, J. F.**: Post-translational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. *J. Cell. Biol.* 102:1558-1566, 1986

3. After these studies on development of pre B cells lacking a conventional two-component BCR, the PI's interest shifted to studies on the molding and establishment of the neonatal and adult antibody repertoires after conventional BCRs were expressed. P3X63Ag8.653 facilitated the construction of monoclonal anti-B cell idiotype (Id) antibodies panels to trace the development and functional activation of B cells responding to selected bacterial and self-associated antigens. These anti-Id reagents were shared with multiple investigators at the time and his lab still provides selected hybridomas to outside investigators. The use of anti-Id antibodies showed that there was an early flux of clonal development so that antigen-specific clones present early in life were replaced by alternate clones in the adult. Introduction of appropriate bacterial antigen early in life, but not later, could permanently alter these clonal profiles. Although our interpretation of these findings has altered, these results have been replicated 30 years later and are key to our hypothesis that neonatal exposure to bacteria in our environment can also affect allergic responses in the adult. These reagents are key to tracing and manipulating B clones in our experimental models and extending our studies into molecular analysis of B cell Ig repertoires at the single-cell level.

- a. Stohrer, R., Lee, M. C. and **Kearney, J. F.**: Analysis of the anti- α 163 dextran response with monoclonal anti-idiotype antibodies. *J. Immunol.* 131:1375-1379, 1983.
- b. Stohrer, R. and **Kearney, J. F.**: Fine idiotype analysis of B cell precursors in the T-dependent and T-independent responses to α -3 Dextran in BALB/c mice. *J. Exp. Med.* 158:2081-2094, 1983
- c. Stohrer, R. and **Kearney, J. F.**: Ontogeny of B cell precursors responding to α 163 dextran in BALB/c mice. *J. Immunol.* 133:2323-2326, 1984.
- d. Benedict, C. L. and **Kearney, J. F.**: Increased junctional diversity in the fetal B cells results in a loss of protective anti-phosphorylcholine antibodies in adult mice. *Immunity* 10:607-617, 1999

4. Studies from the PI and others described in reference 2.a (above) showed that VH81x, the most DH-proximal gene of the mouse immunoglobulin heavy-chain locus, was expressed at a high frequency in perinatal life, but later most VH81x rearrangements in the adult bone marrow were non-functional. Because IgM hybridomas from neonatal mice expressing rearranged VH81x genes were highly self-reactive to intracellular components, a VH81x Heavy-chain transgenic (tg) mouse was constructed. In this mouse, there was a remarkable enrichment of B cells expressing the transgene with an identical Vk light chain in the spleen marginal zone (MZ). Hybridomas expressing this light chain from the VH81x transgenic mice were also highly reactive to self intracellular antigens. Based on this unexpected finding, transgenic mice expressing rearranged VH genes from anti-phosphorylcholine plasmacytomas and other VH transgenic mice were made in his laboratory from anti- α 1-3 dextran, anti-GlcNAc, and anti-sialyl-lacto-N-tetraose hybridomas showed similar MZ enrichment. These unique findings were greeted by reviewers skeptically at first, but they eventually lead to a resurgence of interest in MZ B cells and their importance in handling blood-borne infections. In turn, MZ-like human B cell subsets were identified by other investigators and led to the defining of human IgM memory B cells which could have germline or somatically mutated immunoglobulin V genes.

- a. Chen, X., Martin, F., Forbush, K. A., Perlmutter, R. M., and **Kearney, J. F.**: Evidence for selection of a population of multi-reactive B cells into the splenic marginal zone. *Int. Immunol.* 9:27-41, 1997.
- a. Martin, F. and **Kearney, J. F.**: Positive selection from newly-formed to marginal zone B cells depends on the rate of clonal production, CD19 and *btk*. *Immunity* 12:39-49, 2000.
- b. Martin, F., Oliver, A. M., and **Kearney, J. F.**: Marginal zone B cells rapidly generate plasmablasts in T-independent responses to blood-borne particulate antigens. *Immunity* 14:617-629, 2001.
- c. Balazs, M., Martin, F., Zhou, T., and **Kearney, J. F.**: Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. *Immunity* 17:341-352, 2002.
- d. Martin, F. and **Kearney, J. F.**: Marginal-zone B cells. *Nat. Rev.* 2:323-335, 2002.

5. Many T-cell independent antibody responses to conserved antigens are considered Natural Antibodies. There is good evidence in mice that B1 and MZ B cell subsets with BCR VH rearrangements with little or no N additions and lacking somatic mutations contribute to the homeostatic levels of these antibodies. However, analysis of BCR-encoding genes in some responses revealed low TdT activity, whereas the dominant anti-PC antibody response was destroyed by fetal forced TdT expression. Results from the PI's lab have added to the growing evidence that B cell memory is generated by previous exposure to bacterial-associated polysaccharide and phospholipid antigens. This exposure is manifested by permanent increases in the frequency of such B cells, both in adult and neonate. More importantly, molecular analysis of antigen-specific B

cells after perinatal, but not adult, exposure to bacterial antigens results in permanent alterations in clonotype expression. These and earlier studies from the PI provided reagents and background knowledge for the repeated demonstrations that antibodies to oxidized lipids can protect against atherosclerosis. In the PI's new studies, a role for B cells and antibodies has been shown in dampening of allergic diseases and provides additional support for the Hygiene Hypothesis.

a. Foote, J. B., and **Kearney, J. F.** Generation of B Cell memory to the bacterial polysaccharide alpha 1-3 dextran. *J. Immunol.* 183:6359-6368, 2009

b. Benedict, C. L. and **Kearney, J. F.** Increased junctional diversity in the fetal B cells results in a loss of protective anti-phosphorylcholine antibodies in adult mice. *Immunity* 10:607-617, 1999

c. Mahmoud, T. I., and **Kearney, J. F.** Terminal deoxynucleotidyl transferase is required for an optimal response to the polysaccharide alpha 1-3 dextran. *J. Immunol.* 184: 851-8. 2010

d. Kin N. W., Stefanov E. K., Dizon B. L., **Kearney J. F.** 2012. Antibodies generated against conserved antigens expressed by bacteria and allergen-bearing fungi suppress airway disease. *J Immunol.* 189:2246-56

Selected from 217 peer-reviewed publications, full Pubmed list:

<http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41158784/?sort=date&direction=ascending>

D. Research Support

Ongoing

Kearney (PI) 10/01/14 – 9/30/16

Juvenile Diabetes Research Foundation

Analysis of Human and Mouse antibodies to beta cell antigens bearing N-acetyl glucosamine post-translational modifications and their potential to prevent Human Type 1 Diabetes.

The goal of this study is to determine correlates of anti-GlcNAc antibodies detected in serum of children and the development of Type 1 diabetes.

1R01AI100005-03 Kearney (PI) 3/1/12 – 2/28/17

NIH/NIAID

Response *RFA-AI-11-010 The Infant Immune System: Implications for Vaccines and Response to Infections.* Effects of neonatal microbial exposure on anti-polysaccharide B cell development and response to infections.

The goal of this study is to determine molecular and cellular aspects of B cell clonal repertoire changes induced by early exposure to bacterial antigens.

R01 AI14782-36 Kearney (PI) 3/1/01-2/28/16

NIAID

Regulation of B Cell Clonal Diversity and Its Role in Disease

The major goal of these studies is to study the environmental and genetic factors affecting the development of allergic airway disease in mice.

King (PI), Kearney (Co-PI) 6/1/14-5/30/15

UAB Immunology, Autoimmunity, and Transplantation Strategic Planning

To develop expression systems and amplification strategies for ligation-independent cloning and expression of IgV genes from single sorted antigen-specific cells.

Completed

Kearney (PI) 6/1/12 – 5/31/14

Juvenile Diabetes Research Foundation

Antibodies to beta cell GlcNAc-modified autoantigens blocks T1D in NOD mice

R01AI083449-01 Kearney (PI) 7/1/09-6/30/13

NIAID

Immunobiology of *Bacillus anthracis* Spore-host Interactions.