

BIOGRAPHICAL SKETCHNAME: **Clifford V. Harding, MD, PhD**POSITION TITLE: **Kahn Professor and Chair of Pathology, Case Western Reserve University**

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

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Harvard College, Cambridge, MA	AB	1975-1979	Biology
Washington University, St. Louis, MO	MD/PhD	1979-1985	Cell Biology
Washington Univ Sch. of Medicine, St. Louis, MO	Resident	1985-1989	Pathology/Immunology
Washington Univ Sch. of Medicine, St. Louis, MO	Chief Res.	1989-1990	Pathology/Immunology

A. Personal Statement

Clifford V. Harding, MD, PhD, is Professor and Chair of Pathology at Case Western Reserve University. He has a long-standing productive NIH-funded research program on the cell biology of antigen presenting cells (APCs) and their regulation by Toll-like receptors (TLRs) or infection with *Mycobacterium tuberculosis* (Mtb) or HIV. He has over 190 publications on topics in immunology, cell biology and infectious diseases (>10,500 citations, h-index = 55). His early work included the discovery of exosomes and their genesis by exocytosis of multivesicular exosomes in 1983, and participation in the first study demonstrating that exosomes from APCs contain MHC molecules (1996). In recent years he has published on exosomes released from macrophages, including exosomes from Mtb-infected macrophages that bear bacterial molecules. His work from the 1980's and 1990's included fundamental discoveries concerning cell biological and biochemical mechanisms of antigen processing and antigen presentation by MHC-I and MHC-II molecules. In recent years he has studied the regulation of APCs, particularly in the context of infection and signaling by innate immune receptors. He has studied the regulation of APCs by TLR2 agonists expressed by Mtb (lipoproteins and glycolipids), and recently he has studied the signaling pathways by which Mtb regulates the balance of inflammatory mechanisms in macrophages and regulates the magnitude and differentiation of T cell responses. These mechanisms may contribute to immune evasion and the persistence of Mtb infection. Other projects have focused on regulation of immune responses by TLR9 and type I interferon, as well as regulation of signaling by these receptors. He has been a nationally-recognized leader in developing research training for PhD students, MD-PhD students and physician-scientists. He has developed both basic and translational research training programs, and he has designed and launched new training programs in Immunology and Cancer Biology (e.g. as founding Director of the Immunology Training Program at CWRU) and a new Clinical and Translational Scientist Training Program (CTSTP; CTSA TL1 supported). He has contributed to the national/international Immunology research community with service on study sections and through the American Association of Immunologists (e.g. as Chair of the AAI Committee on Public Affairs).

B. Positions

1989-1990	Instructor in Pathology, Washington University School of Medicine, St. Louis, MO
1990-1993	Assistant Professor of Pathology, Washington University School of Medicine
1989-1993	Attending Physician, Barnes Hospital, St. Louis, MO
1993-1996	Assistant Professor of Pathology, Case Western Reserve Univ. School of Medicine
1993-present	Staff Physician, University Hospitals Case Medical Center
1996-1999	Associate Professor of Pathology (primary) and Oncology (secondary), CWRU
1999-present	Professor of Pathology (primary) and Oncology (secondary), CWRU
2001-present	Director, CWRU Medical Scientist Training Program (MSTP)
2004-present	Adjunct Staff, Dept. of Immunology, Lerner Research Institute, Cleveland Clinic Foundation
2005-2013	Director, CWRU Immunology Training Program (Co-Director, 2013-present)
2006-present	Secondary appointment, CWRU Center for Clinical Investigation

2007-present Director, CWRU Clinical and Translational Scientist Training Program (CTSTP)
2008-present Chair, Department of Pathology, CWRU and UHC (Interim, 2008-2010)
2011-present Secondary appointment, Division of Infectious Diseases and HIV Medicine
2012-present Interim Chair, Department of Anatomy

Honors

1979 Magna cum laude with highest honors in Biology; Phi Beta Kappa (Harvard College)
1989 American Association of Pathologists Experimental Pathologist-in-Training Award
1991 Pfizer Scholar & American Cancer Society Junior Faculty Research Award
1991-present American Association of Immunologists (member)
1996-2001 AITRC Study Section, NIH (Chair, 1999-2001)
1995-present Reviewer for NIH study sections, American Cancer Society, Wellcome Trust, Netherlands Organization for Scientific Research, Dutch Cancer Society, Israel Science Foundation
1994-2001 Editorial Boards, Traffic, Cellular Microbiology, Advances in Anatomic Pathology
2006-present Editorial Board, Biology Direct Immunology
2015-present Editorial Board, Pathogens and Immunity
2001-present Member, Faculty of 1000
2002, 04, 05 Section Editor, Current Opinion in Immunology
2008- 2010 Midwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research (MRCE) Steering Committee
2009-2012 MD-PhD Section Steering Committee for American Association of Medical Colleges (AAMC) Group on Graduate Research, Education and Training (GREAT)
2009-present American Association of Immunologists Committee on Public Affairs (Chair, 2014-2016; Chair of Peer Review and Grant Submission Subcommittee, 2012-2014).
2012-present Fellow, American Association for the Advancement of Science
2013-present Association of American Physicians

C. Contributions to Science

Dr. Harding has over 190 publications (30 in the last 5 years), >10,500 citations, average citations per paper >55, and an h-index of 55 (11/05/15). See My NCBI:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/clifford.harding.1/bibliography/41079817/public/?sort=date&direction=ascending>.

Discovery of exosomes and exploration of their roles in cell biology and immunology.

Dr. Harding published the discovery of exosomes by exocytosis of multivesicular endosomes in 1983 (1). He subsequently collaborated with Raposo and Geuze in the first report of exosomes related to immunology with the first demonstration that antigen presenting cells release exosomes containing MHC molecules (2). Recently his lab has studied the release of exosomes and microvesicles from macrophages infected with *Mycobacterium tuberculosis* (Mtb) (3). He has collaborated with Dr. Wearsch in the discovery that vesicles released from Mtb-infected macrophages comprise two distinct subsets: one derived from the host cell (exosomes) and one derived directly from Mtb (bacterial membrane vesicles that traffic out of infected cells). These distinct vesicle populations have distinct properties and functions, e.g. bacterial membrane vesicles are the primary means to export of immunomodulatory mycobacterial molecules from infected macrophages (4).

1. Harding C, Heuser J, Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *J Cell Biol.* 1983;97(2):329-39. PMID: PMC2112509.
2. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, et al. B lymphocytes secrete antigen-presenting vesicles. *J Exp Med.* 1996;183(3):1161-72.
3. Ramachandra L, Qu Y, Wang Y, Lewis CJ, Cobb B, Takatsu K, et al. *Mycobacterium tuberculosis* synergizes with ATP to induce release of microvesicles and exosomes containing major histocompatibility complex class II molecules capable of antigen presentation. *Infect Immun.* 2010;78:5116-25. PMID: PMC2981298.
4. Athman JJ, Wang Y, McDonald DJ, Boom WH, Harding CV, Wearsch PA. Bacterial Membrane Vesicles Mediate the Release of *Mycobacterium tuberculosis* Lipoglycans and Lipoproteins from Infected Macrophages. *J Immunol.* 2015; 195:1044-1053. PMID: PMC4506856.

Signaling pathways for regulation of macrophages by *Mycobacterium tuberculosis*

Dr. Harding discovered the essential role of ERK signaling in inhibition of CIITA and MHC-II expression by macrophages. He also discovered previously unknown C/EBP binding sites in the CIITA promoters I and IV and showed that TLR2-ERK signaling induces C/EBP for recruitment to these sites to mediate suppression of CIITA expression (and hence MHC-II expression) by macrophages (5-7). Recently, he has elucidated the central role of TLR2-mediated ERK signaling in induction of IL-10 and suppression of IL-12, and he discovered that these ERK-dependent mechanisms suppress Th1 responses to Mtb (7). He also demonstrated the interplay of signaling by different TLRs, e.g. the ability of TLR2 signaling to suppress TLR9 induction of type I interferon (8).

5. Pennini ME, Pai RK, Schultz DC, Boom WH, Harding CV. Mycobacterium tuberculosis 19-kDa lipoprotein inhibits IFN-gamma-induced chromatin remodeling of MHC2TA by TLR2 and MAPK signaling. *J Immunol.* 2006;176(7):4323-30.

6. Pennini ME, Liu Y, Yang J, Croniger CM, Boom WH, Harding CV. CCAAT/enhancer-binding protein beta and delta binding to CIITA promoters is associated with the inhibition of CIITA expression in response to Mycobacterium tuberculosis 19-kDa lipoprotein. *J Immunol.* 2007;179(10):6910-8. PMID: PMC2631233.

7. Richardson ET, Shukla S, Sweet DR, Wearsch PA, Tschlis PN, Boom WH, et al. TLR2-dependent ERK signaling in *Mycobacterium tuberculosis*-infected macrophages drives anti-inflammatory responses and inhibits Th1 polarization of responding T cells. *Infect Immun.* 2015;83:2242-2254.

8. Simmons DP, Canaday DH, Liu Y, Li Q, Huang A, Boom WH, et al. Mycobacterium tuberculosis and TLR2 agonists inhibit induction of type I IFN and class I MHC antigen cross processing by TLR9. *J Immunol.* 2010;185(4):2405-15. PMID: PMC2990778.

Agonists and receptors involved in regulation of immune responses and host-pathogen interactions by CpG DNA via TLR9 and Mtb and its lipoproteins and lipoglycans via TLR2

Dr. Harding published the first report of CpG-DNA/TLR9 as a potent regulator of APCs and T cell responses and the discovery of the Th1 adjuvant effect of CpG DNA (9). The prior work by Krieg had only discovered a B cell stimulatory effect of CpG DNA. TLR9 was later identified as the receptor for CpG DNA. Other papers by Dr. Harding have dissected the functional differences between type A and type B CpG oligodeoxynucleotides. Dr. Harding has done much work regulation of immune responses by Mycobacterium tuberculosis lipoproteins and lipoglycans via TLR2 (over 20 publications in this area), including discovery of the lipoglycan binding function of LprG and the structural/biochemical basis of this property (10), as well as discovery of the contributions of LprG to localization of lipoarabinomannan (LAM) in the Mycobacterium tuberculosis cell envelop and its ability to inhibit phagosome maturation (11). He has characterized the role of TLR2 on T cells (12) as well as more extensive studies of its role in regulating APCs infected with Mycobacterium tuberculosis (see next section).

9. Chu RS, Targoni OS, Krieg AM, Lehmann PV, Harding CV. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (Th1) immunity. *J Exp Med.* 1997;186(10):1623-31. PMID: 2199137.

10. Drage MG, Tsai HC, Pecora ND, Cheng TY, Arida AR, Shukla S, et al. Mycobacterium tuberculosis lipoprotein LprG (Rv1411c) binds triacylated glycolipid agonists of Toll-like receptor 2. *Nature Struct Mol Biol.* 2010;17(9):1088-95. PMID: PMC2933325.

11. Shukla S, Richardson ET, Athman JJ, Shi L, Wearsch PA, McDonald D, et al. Mycobacterium tuberculosis lipoprotein LprG binds lipoarabinomannan and determines its cell envelope localization to control phagolysosomal fusion. *PLoS Pathog.* 2014;10(10):e1004471. PMID: PMC4214796.

12. Reba SM, Li Q, Onwuzulike S, Ding X, Karim AF, Hernandez Y, et al. TLR2 engagement on CD4 T cells enhances effector functions and protective responses to Mycobacterium tuberculosis. *Eur J Immunol.* 2014;44:1410-21. PMID: PMC4112943.

Regulation of MHC-II antigen presentation by Mycobacterium tuberculosis (Mtb). Dr. Harding demonstrated processing of Mtb to produce peptide:MHC-II complexes in phagosomes and the ability of viable Mtb to resist processing (13). He discovered the inhibition of MHC-II expression and antigen presentation by Mtb signaling through TLR2, (14-16).

13. Ramachandra L, Noss E, Boom WH, Harding CV. Processing of Mycobacterium tuberculosis antigen 85B involves intraphagosomal formation of peptide-major histocompatibility complex II complexes and is inhibited by live bacilli that decrease phagosome maturation. *J Exp Med.* 2001;194(10):1421-32.

14. Noss EH, Pai RK, Sellati TJ, Radolf JD, Belisle J, Golenbock DT, et al. Toll-like receptor 2-dependent inhibition of macrophage class II MHC expression and antigen processing by 19 kD lipoprotein of *Mycobacterium tuberculosis*. *J Immunol*. 2001;167:910-8.
15. Pai RK, Convery M, Hamilton TA, Boom WH, Harding CV. Inhibition of IFN-gamma-induced class II transactivator expression by a 19-kDa lipoprotein from *Mycobacterium tuberculosis*: a potential mechanism for immune evasion. *J Immunol*. 2003;171(1):175-84.
16. Harding CV, Boom WH. Regulation of antigen presentation by *Mycobacterium tuberculosis*: a role for Toll-like receptors. *Nature Rev Microbiol*. 2010;8(4):296-307. PMID: PMC3037727.

Earlier studies of antigen processing mechanisms. Dr. Harding has published numerous papers on the mechanisms of antigen processing and presentation by both MHC-I and MHC-II molecules. He demonstrated that late endocytic compartments mediate antigen processing and formation of peptide:MHC-II complexes, contrary to the earlier model that this was done in early endosomal compartments (17, 18). He published the first demonstration that antigens expressed by bacteria that do not penetrate into the cytosol can be processed for MHC-I presentation, demonstrating vacuolar contributions to cross processing/cross priming (19). This provided new cell biological insight into the antigen processing mechanisms implicated by the discovery of cross priming by Bevan. He published the first quantitation of the number of peptide:MHC-II complexes needed to be expressed on an antigen presenting cell to stimulate a T cell response (20).

17. Harding CV, Collins DS, Slot JW, Geuze HJ, Unanue ER. Liposome-encapsulated antigens are processed in lysosomes, recycled, and presented to T cells. *Cell*. 1991;64(2):393-401.
18. Harding CV, Geuze HJ. Class II MHC molecules are present in macrophage lysosomes and phagolysosomes that function in the phagocytic processing of *Listeria monocytogenes* for presentation to T cells. *J Cell Biol*. 1992;119(3):531-42. PMID: 2289672.
19. Pfeifer JD, Wick MJ, Roberts RL, Findlay K, Normark SJ, Harding CV. Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. *Nature*. 1993;361(6410):359-62.
20. Harding CV, Unanue ER. Quantitation of antigen presenting cell MHC class II/peptide complexes necessary for T-cell stimulation. *Nature*. 1990;346:574-6.

D. Research Support

Current research grants:

NIH R01 AI034343 (Harding, PI) 09/01/1994 – 06/30/2018
 “Regulation of macrophages by *Mycobacterium tuberculosis*” (previously “Bacterial and Liposomal Antigen Processing”)

Goals: To investigate receptors and signaling pathways that direct macrophage inflammatory states and antigen presentation. Aim 1: Investigate roles of receptors (e.g. TLR2) in regulating macrophage inflammatory balance and antigen presentation. Aim 2: To investigate roles of the ERK pathway and KLF4 in regulation of macrophages *in vitro*. Aim 3: To investigate the roles of ERK and KLF4 *in vivo* during infection with *M. tuberculosis*.

NIH R01 AI27243 (Boom) 04/15/13-03/31/17
 “Heterogeneity of T-Cells in *Mycobacterium tuberculosis* Infection”

This project focuses on the human T cell response to *M. tuberculosis* with the following aims: Aim 1. To determine the molecular mechanism of inhibition of TCR signaling by ManLAM in terms of how ManLAM is delivered to T cells, how and where it inserts in the T cell membrane and how it interferes with TCR function and T cell-APC conjugate formation, and ManLAM’s structural requirements for inhibition; Aim 2. To determine the functional consequences of ManLAM inhibition of CD4+ T cell activation in terms of recovery of inhibition, induction of apoptosis or anergy, differential effects on CD4+ T cell subsets, synergy with inhibition of MHC-II antigen processing and effects on the activated T cell proteome; Aim 3. To determine the ability of ManLAM to inhibit T cell responses to MTB *in vivo* using the murine MTB infection model and determining if there is variable sensitivity to ManLAM among humans.

Role: Co-Investigator

NIH R21 AI104480 (Sieg, Hardy, multi-PI) 05/10/13-04/30/16 (no cost extension)
 “Type I interferon as a mediator of immune failure in HIV disease”

Goals: To investigate the role of type I interferon in the regulation of interleukin-7 mediated T cell homeostasis in HIV infection. Aim 1. To investigate mechanisms of IFN-I-mediated suppression of IL-7 and S1P responsiveness in primary human T cells. Aim 2. To investigate IFN-I, IL-7 and S1P responsiveness in immune failure.

Role: Co-Investigator

NIH R21 AI103443 (Wearsch) 04/15/13-03/31/16 (no cost extension)

"Roles for exosomes in adaptive immunity to *M. tuberculosis*"

Goals: To investigate the nature of exosomes released from macrophages infected with *M. tuberculosis* and their roles in immune regulation.

Role: Co-Investigator

Current training Grants:

NIH T32 GM007250 (Harding, PI). Period: 07/01/1975-06/30/2019

"Medical Scientist Training Program"

Goal: Training of MD/PhD students for successful careers in biomedical research.

UL1 TR000439 and TL1 TR000441 (Konstan/Harding TL1 Director). Period: 06/01/2007-05/31/2017

"Case Western Reserve University/Cleveland Clinic CTSA" (Harding, Director for TL1 component)

Goal: Training in clinical and translational research.

Completed:

NIH R01 AI069085 (Harding, PI). Period: 12/01/2006-11/30/2011 (no cost extension to 11/30/12)

M. tuberculosis lipoprotein-TLR2 interactions

Aims: Determine receptor dependence (e.g. TLR1/2/6, CD14, CD36) of recombinant MTB lipoproteins.

Construct recombinant TLR2, TLR1 and TLR6 for direct binding assays with recombinant lipoproteins to define structural requirements for ligand binding to TLR2.

NIH RO1 AI035726 (Harding, PI) 05/01/1994-12/31/2010 (no cost extension to 12/31/2011)

"Class II MHC transport and function"

Aims: 1. Characterize immune regulation by PAMPs, e.g. MTB lipoproteins, including in vivo adjuvant function, induction of dendritic cell maturation, cytokine secretion by macrophages and dendritic cells. 2. Dissection of intracellular signaling and transcriptional control mechanisms whereby prolonged PAMP exposure inhibits macrophage APC function, including mechanisms of inhibition of CIITA. 3. Studies of PAMP-knockout bacteria and APC modulation in vivo. Goals include ex vivo analysis of APCs from mice infected with BCG or MTB and testing lipoprotein PAMP-deficient bacteria for ability to inhibit macrophage APC function.

P01 AI076174 (Lederman, PI; Harding, Co-Investigator) Period: 08/01/08-07/31/13

Defining the Pathogenesis of Immune Deficiency in Chronic HIV Infection

The Cleveland Immunopathogenesis Consortium (CLIC), including investigators at 10 academic and research institutions in the United States and Canada, will study mechanisms whereby HIV infection results in progressive immune deficiency. Project 1: Bystander activation drives T cell losses in chronic HIV infection (Lederman). Project 2: Loss of intestinal barrier function in HIV infection (Levine).

Role, Harding: Co-Investigator in Projects 1 and 2.