BIOGRAPHICAL SKETCH

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NAME: Rafeul Alam, M.D., Ph.D.

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

POSITION TITLE: Professor

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
Dacca College, Dacca, Bangladesh	H.S.C.	1972	Science
Lodz Medical College, Lodz, Poland	M.D.	1980	Medicine
Lodz Medical College, Lodz, Poland	Ph.D.	1984	Allergy/Immunology

A. Personal Statement

My lab studies the mechanism of persistence of inflammation in asthma. We have been exploring signaling mechanisms of various inflammatory cells that allow persistence of inflammation. We studied blood-derived inflammatory cells (T cells, eosinophils and basophils) and airway tissue and sputum-derived cells from asthmatic patients. Lately we have been characterizing T cells from bronchoalveolar lavage (BAL) obtained from refractory asthmatic patients. Based upon these studies we have identified a new endotype of asthma that is characterized by the dominant presence of dual positive Th2/Th17 cells. In this application we propose to further characterize BAL Th2/Th17 CD4 T cells, examine their differentiation pathway and delineate the mechanism of their steroid resistance. I have a broad-based background in signaling studies in T cells and in asthma. I have been studying mechanism of allergic inflammation for more than 20 years. My specific expertise is in activation of tyrosine kinases and MAP kinases. I have been funded by NIH and other national organizations throughout my research career. I believe that I have the necessary expertise and resources to conduct the proposed experiments in the application. This is a multiple PI project with Dr. Richard Martin as anther PI. I have a track record of collaborating and publishing with Dr. Martin under a previous NIH-funded PPG.

B. Positions and Honors

Professional Experience

1985-1985 Research Fellow, University of London Charing Cross & Westminister Medical School, London, UK
1985-1986 Research Fellow, National Institute for Occupational Safety & Health, Morgantown, WV
1986-1988 Research Fellow, University of Texas Medical Branch at Galveston, TX
1988-1992 Assistant Professor, University of Texas Medical Branch, TX
1992-1997 Associate Professor, University of Texas Medical Branch, TX
1995-2002 Director, Allergy & Immunology Division, Univ. of Texas Medical Branch, TX
1997-2002 Professor of Medicine, Microbiology & Immunology, Univ. of Texas Medical Branch, TX
2002- Professor and Chief, Division of Allergy & Immunology, National Jewish Health, and Professor, University of Colorado Denver School of Medicine, Denver, CO

Honors

1970 Scholar Gold Award, Government of Pakistan
1979 Copernicus Gold Medal, Government of Poland
1985 Annual Award of the Polish Society of Allergists for the best Ph.D. dissertation
1987 Clemens Von Pirquet Award of the American College of Allergists
1988 James McLaughlin Award of the University of Texas Medical Branch
1988 Presidential Award from the American Academy of Allergy & Immunology
1993 Pharmacia Allergy Research Foundation Award
1997 Election to American Society for Clinical Investigation (ASCI)
2000 Sealy and Smith Distinguished Chair in Medicine at the University of Texas
2002 Veda H. & Chauncey H. Ritter Chair in Immunology at National Jewish Health
2012 Election to Association of American Physicians (AAP)

Study Section Activities

2014 ZRG1 IMM-N (02) M, Special Study Section, April 2014
2013 ZRG1 IMM-N(03), Special study section, July 2013
2012 HAI Study Section, Ad Hoc, October 2012
NIAID ZAI1-LGR-I-S2, Special Study Section, May 2012
IAID ZAI1-JTS-I-M2, Special Study Section (Chair), February 2012
2011 NIAID ZAI1 QV-I (J1), Special Study Section (Chair), September 2011
NIAID ZAI1-QV-I-S2, Special Study Section, June 2011
2009 NIAID ZRG1 IMM-H 03M, Special Study Section
2007 NIAID ZAI1 QV-I J2 Special Study Section (Chair)
2006 NIAID ZAI1 QV-I (M3) Special Study Section
2005 NIAID ZRG1 HAI KO8 Special Study Section
2000-2004 NIAID Allergy, Immunology & Transplantation Research Committee, AITRC Study Section, standing member
2001 NHLBI Special Study Section for SCOR Grants
2000 NIEHS Program Project Study Section
1999 NIH Allergy & Immunology (ALY) Study Section, ad hoc member
1998 NIEHS Special Study Section for RFA-97-004
1996 NIH Study Section, Member of Immunology, Virology & Pathology (IVP) Study Section-ZRG-2
Ad hoc member of the Veterans Administration Immunology Study Section

Professional Societies

American Society for Clinical Investigation (ASCI)
Association of American Physicians (AAP)
Southern Society for Clinical Investigation
American Academy of Allergy and Immunology
American Association of Immunologists
American Thoracic Society
American College of Allergists
American College of Physician
Colorado Allergy Society

C. Major Contribution to Science

1. **Identification of Basophil Histamine-Releasing Factors (HRFs)**: The activation of basophils and mast cells is fundamental to allergic inflammation and host defense. I have initially identified and characterized a non-IgE mediated mechanism of histamine release that we and others have initially termed histamine releasing factor(s) (1, 2). These studies were conducted before the era of cloning of cytokine/chemokine genes. Once the chemokine genes were cloned, I demonstrated that CC chemokines, especially to CCL2 (MCP1/MCAF),CCL3 (MIP1a), CCL5 (RANTES) and CCL7 (MCP3) represented most of the histamine releasing activity of lymphocyte/monocyte culture supernatants (3, 4). These chemokines represents a non-IgE mechanism of mediator release from basophils and mast cells and are frequently elevated in inflammatory diseases including asthma.
    1) Alam R, Roznieckі J, & Selmaj K. A mononuclear cell derived histamine releasing factor (HRF) in asthmatic patients. Histamine release from basophils in vitro. Ann Allergy. 1984; 53:66-69. PMID: 6204562.

2) Alam R, Kuna P, Rozniecki J, & Kuzminska B. The magnitude of the spontaneous production of histamine releasing factor (HRF) by lymphocytes in vitro correlates with the state of bronchial hyperreactivity in patients with asthma. J Allergy Clin Immunol. 1987; 79:103-108. PMID: 3805541.
3) Alam R, Lett-Brown MA, Forsythe PA, Anderson-Walters DJ, Kenamore C, Kormos C, and Grant JA. Monocyte chemotactic and activating factor is a potent histamine-releasing factor for basophils. J. Clin. Invest. 1992, 89:723-728. PMCID: PMC442914.
4) Alam R, Forsythe PA, Lett-Brown MA, and Grant JA. Macrophage inflammatory protein-1 alpha activates basophils and mast cells. J. Exp. Med. 1992; 176:781-786. PMCID: PMC2119365.

2. **Delineation of the Signaling Mechanism of Interleukin-5 and Eotaxin in Eosinophils**: IL5 and eotaxin represent two most important cytokines that regulate the development and function of eosinophils. The latter cell type plays a crucial role in allergic inflammation and host defense. I delineated the major signaling pathways in human eosinophils that were activated by IL5 and eotaxin. Our studies have shown the activation of the tyrosine kinase—Lyn, Hck, Jak1 and Jak2 by IL5 (1-3). This led to the activation of Raf-MEK-ERK1/2 and STAT5 signaling. These pathways controlled eosinophil differentiation, survival, chemotaxis and degranulation (3, 4).
   1) Pazdrak K, Schreiber DS, Forsythe PA, Justement L, and Alam R. The intracellular signal transduction mechanism of interleukin 5 in eosinophils: the involvement of lyn tyrosine kinase and the Ras-Raf-1-MEK-microtubule-associated protein kinase pathway. J. Exp. Med. 1995; 181:1827-1834. PMCID: PMC2192005.
   2) Pazdrak K, Adachi T, Alam R. Src homology 2 protein tyrosine phosphatase (SHPTP2)/Src homology 2 phosphatase 2 (SHP2) tyrosine phosphatase is a positive regulator of the interleukin 5 receptor signal transduction pathways leading to the prolongation of eosinophil survival. J. Exp. Med. 1997; 186:561-568. PMCID: PMC2199030.
   3) Pazdrak P, Olszewska-Pazdrak B, Stafford S, Alam R. Lyn, Jak2, and Raf-1 kinases are critical for the antiapoptotic effect of interleukin 5, whereas only Raf-1 kinase is essential for eosinophil activation and degranulation. J. Exp. Med. 1998; 188:421-429. PMCID: PMC2212466.
   4) Kampen G, Stafford S, Adachi T, Jinquan T, Quan S, Grant JA, Skov P, Poulsen L, Alam R. Eotaxin induces degranulation and chemotaxis of eosinophils through the activation of ERK2 and p38 mitogen-activated protein kinases. Blood. 2000; 95:1911-1917. PMID: 10706854.

3. **Cloning and Characterization of Unc119 as an Activator of Src Family Kinases and Establishment of its Association with Immunodeficiency**: Because of the importance of IL5 for eosinophilic inflammation we sought to identify molecules that were associated with the IL5 receptor alpha subunit. Using a yeast two-hybrid approach we cloned a novel gene called Unc119 (1). We characterized the function of this protein and demonstrated that Unc119 activated Src family kinases including Lyn and Hck in eosinophils (1), and Lck and Fyn in T cells (2). Through the activation of these receptor proximal tyrosine kinases Unc119 played a crucial role in activation of eosinophils and T cells. Through knockdown experiments we demonstrated that Unc119 played an important role in allergic asthma (3). Clinical relevance of Unc119 was established by the finding that an Unc119 mutation was associated with idiopathic CD4 lymphopenia, a human immunodeficiency disease (4). Lck activation was impaired in T cells from the immunodeficient patient.

   1) Cen O, Gorska, MM, Stafford SJ, Sur S, Alam R. Identification of UNC119 as a novel activator of SRC-type tyrosine kinases. J. Biol. Chem. 2003; 278(10):8837-8845. PMID: 12496276.
   2) Gorska MM, Stafford S, Cen O, Sur S, Alam R. Unc119, a novel activator of Lck/Fyn, is essential for T cell activation. J. Exp. Med. 2004; 199:369-379. PMCID: PMC2211793.
   3) Gorska MM, Goplen N, Liang Q and Alam R. Uncoordinated 119 preferentially induces Th2 differentiation and promotes the development of asthma. J. Immunol. 2010; 184:4488-96. PMID: 20220094.
   4) Gorska M, and Alam R. A mutation in the human Uncoordinated 119 gene impairs TCR signaling and is associated with CD4 lymphopenia. Blood. 2012; 119:1399-406. PMCID: PMC3286207.

4. **Development of a Mouse Model of Chronic Asthma and the Delineation of the Role of ILC2 and ERK1/2 Bistability in Persistence of Asthma in this Model**: Human allergic asthma is a chronic

disease and persists without regard to allergen exposure. Existing mouse models of asthma largely represent an acute form of asthma. Some investigators chronically expose mice to an allergen before experimentation and label this a chronic model. However, the pathology and lung function are typically studied 1-3 days after the last allergen exposure. Hence this also represents acute asthma occurring after chronic allergen exposure. To address this unmet need we developed a mouse model where asthma persists longer than 6 months after the last allergen exposure (1, 2). Thus, this model more closely mimics human asthma. Using this model we demonstrated that persistence of asthma required sustained production of IL33, which was driven by ILC2 but not Th2 cells. ILC2 mediated this effect through IL13. IL13 induced epithelial memory by generating a process called signaling bistability. A signaling pathway is called bistable when it continues to generate output in the absence of the inciting input signal. We demonstrated that repetitive IL13 stimulation induced epithelial memory through ERK1/2 bistability (3, 4). ERK1/2 continued to generate intracellular signals despite the cessation of IL13 stimulation. ERK1/2 bistability represented a unique mechanism of epithelial memory formation. These studies established a crucial role for ILC2 and IL13-driven ERK1/2 bistability in persistence of asthma.

1) Goplen N, Karim Z, Liang Q, Gorska MM, Morimoto Y, Rozario S, Guo L, and Alam R. Combined sensitization of mice to extracts of dust mite, ragweed, and Aspergillus species breaks through tolerance and establishes chronic features of asthma. J Allergy Clin Immunol. 2009; 123:925-932. PMCID: PMC2683988.
2) Christianson CA, Goplen NP, Zafar I, Irvin C, Good JT Jr, Rollins DR, Gorentla B, Liu W, Gorska MM, Chu H, Martin RJ, Alam R. Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33. J Allergy Clin Immunol. 2015 Jan 21. pii: S0091-6749(14)01740-0. PMID: 25617223.
3) Liu W, Tundwal K, Liang Q, Goplen Q, Rozario S, Quayum N, Gorska M, Wenzel S, Balzar S and Alam R. Establishment of extracellular signal-regulated kinase 1/2 bistability and sustained activation through Sprouty 2 and its relevance for epithelial function. Mol Cell Biol. 2010; 30:1783-99. PMCID: PMC2838067.
4) Liu W, Liang Q, Balzar S, Wenzel S, Gorska M, Alam R. Cell-specific activation profile of extracellular signal-regulated kinase 1/2, Jun N-terminal kinase, and p38 mitogen-activated protein kinases in asthmatic airways. J Allergy Clin Immunol. 2008; 121:893-902. PMID: 18395552.

5. **Identification of a Th2/Th17 Endotype Representing Severe Refractory Asthma**: Many experimental drugs have failed in asthma primarily because of the heterogeneous nature of this disease. There is an unmet need to mechanistically define asthma subphenotypes. A mechanistically defined phenotype is called endotype. We described a new endotype of asthma that is characterized by the predominant presence of dual positive Th2/Th17 cells in the airways (1). Asthmatic patients with this endotype have more severe disease (lower FEV1, PC20 for methacholine, and asthma control test score) as compared to Th2 high and Th2/Th17 low endotypes. Th2/Th17 cells are steroid resistant due to high level expression of MEK and the transcription factors c-Fos and JunB (1-3). Inhibition of MEK reverses steroid resistance. These findings have implications for identification and clinical management of severe asthmatic patients.

1) Irvin C, Zafar I, Good J, Rollins D, Christianson C, Gorska MM, Martin RJ, Alam R. Increased frequency of dual-positive TH2/TH17 cells in bronchoalveolar lavage fluid characterizes a population of patients with severe asthma. J Allergy Clin Immunol. 2014 Nov; 134(5):1175-1186.e7. PMCID: PMC4254017.
2) Guo L, Chen C, Liang Q, Karim MZ, Gorska MM and Alam R. Nuclear translocation of MEK1 triggers a complex T cell response through the corepressor silencing mediator of retinoid and thyroid hormone receptor. J Immunol. 2013; 190:159-167. PMCID: PMC3530839.
3) Liang Q, Guo L, Gogate S, Karim Z, Hanifi A, Leung D, Gorska M and Alam R. IL-2 and IL-4 stimulate MEK1 expression and contribute to T cell resistance against suppression by TGF-beta and IL-10 in asthma. J Immunol. 2010; 185:5704-13. PMCID: PMC3367768.
4) Goplen N, Karim MZ, Guo L, Zhuang Y, Huang H, Gorska MM, Gelfand G, Pagés G, Pouysségur J**,** and Alam R. ERK1 is important for Th2 differentiation and development of experimental asthma. FASEB J. 2012; 26:1934-1945. PMCID: PMC3336776.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/1DC0y34YPMPAP/bibliography/47537738/public/?sort=date&direction=ascending

D. Research Support

List both selected ongoing and completed research projects for the past three years (Federal or non-Federally-supported). *Begin with the projects that are most relevant to the research proposed in the application.* Briefly indicate the overall goals of the projects and responsibilities of the key person identified on the Biographical Sketch. Do not include number of person months or direct costs.

Ongoing Research Support

RO1 AI102943-01A1 (Alam-PI) 07/15/2014 - 06/30/2018
NIH
Sprouty-2 Regulation of Signaling in Asthma
The Major goals of this project are to: 1). To examine the role of spry 2 in generating sustained signaling in T cells; 2). To study the importance of spry 2 for T helper cell differentiation; 3). To delineate the role of spry 2 in the development of asthma; 4). To establish the relevance of spry 2 for human asthma.

RO1 AI091614-01 (Alam-PI)
NIH 07/01/2011 - 6/30/2016
Role of MEK1 in T cell function in asthma
The major goals of this project are to: 1). Study the mechanism of increased MEK1 expression in T cells from asthma; 2). Investigate the effect of nuclear MEK1 on the gene repressor SMRT in T cells from asthma; and 3). Examine the contribution of MEK1, SMRT and IL-2 to persistence of inflammation in asthma.

N01 HHSN272200700048C (Alam PI of the subcontract) 11/01/2007 - 10/30/2016
NIAID, NIH
The Identification of potentially pathogenic and therapeutic epitopes from common human allergens
PI: Alessandro Sette (La Jolla Institute for Allergy & Immunology, San Diego, CA)
Task 1. Develop human subjects protocols and conduct clinical research. Task 2. Identify and validate allergen-specific T cell epitopes. Task 3. Develop and operate a database management system, and submit data to the Immune Epitope Database and Analysis Resource.
Role: PI of the subcontract

Completed Research Support

PPG HL 36577 (Alam Project leader, Project 3) 8/01/2007 - 7/31/2012
NIH
Signaling Memory in Chronic Asthma
The major goals of this project are to: 1). Delineate the mechanism of self-perpetuated activation of ERK1/2 in epithelial cells; 2). Investigate the mechanism of preservation of the memory-related ERK1/2 pool in epithelial cells in asthma; 3). Study the biological relevance of sustained ERK1/2 activation in the lung; and 4). To determine whether CD8+BLT1+ lymphocytes have a higher propensity to sustain ERK1/2 activation and whether airway cells with increased ERK1/2 activity manifest steroid-resistance in the chronic asthma model.
Role: PI

R56 AI077535-01A1 (Alam PI) 05/22/2009 - 12/31/2012
NIH
Mechanism of T Cell Resistance against Treg-mediated Suppression in Asthma
The major goals are to: 1). Define and characterize T cell resistance to regulatory T cell-mediated inhibition in asthma and 2). Investigate the role of the ERK1/2 signaling pathway in T cell resistance.
Role: PI