BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Mackman, Nigel	John Parker Distinguished Professor of Medicine
eRA COMMONS USER NAME (credential, e.g., agency login) mackman	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Leicester, England	B.Sc	07/1981	Biological Science
University of Leicester, England	Ph.D.	07/1985	Molecular Biology

A. Personal Statement

The first 7 years of my scientific career were spent at the University of Leicester in the UK studying the role of *Escherichia coli* (*E.coli*) hemolysin toxin in bacterial pathogenesis. In 1987 I move to The Scripps Research Institute (TSRI) in La Jolla, US and began studying macrophages and blood coagulation. I spent 20 years at TSRI. In 2007, I moved to the University of North Carolina at Chapel Hill to become the John C. Parker Distinguished Professor in Medicine. In 2011 I was appointed as Director of the UNC McAllister Heart Institute. Members of the institute include basic and clinical scientists studying cardiovascular disease. I am also coDirector of the Thrombosis and Hemostasis Program. I am a member of the Immunology program within the Lineberger Comprehensive Cancer Center. For the past 28 years, I have been studying mechanisms of hemostasis and thrombosis. In particular, I study the role of tissue factor (TF), coagulation proteases, protease activated receptors (PARs) and microvesicles (MVs) in hemostasis, thrombosis, inflammation, ischemiareperfusion injury, cancer, sickle cell disease, obesity, viral infection, atherosclerosis and liver injuryMy lab has generated several unique mouse lines that have altered levels of TF, PAR1 and PAR2 for these studies. I have trained 24 postdoctoral fellows, 4 medical students and 1 graduate student. Currently, I supervise 3 postdoctoral fellows.

1/ **Mackman, N**., Tilley, R.E., and Key, N.S. Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. *Arterioscler. Thromb. Vasc. Biol*. 27:1687-1693, 2007.
2/ **Mackman, N.** Triggers, targets and treatments for thrombosis. *Nature* 451:914-918, 2008. PMCID: 2848509
3/ Owens, A.P. 3rd. and **Mackman, N**. Microparticles in hemostasis and thrombosis. *Circ. Res.* 108:1284-1297, 2011. PMCID: 3144708
4/ **Mackman, N.** New insights into the mechanisms of venous thrombosis. *J. Clin Invest.* 122:2331-2336, 2012. PMID: 22751108.

B. Positions and Honors (chronological order)

Positions

1984-1987 Postdoctoral Fellow, University of Leicester
1987-1989 Research Fellow, Dept. of Immunology, The Scripps Research Institute
1989-1995 Assistant Professor, Dept. of Immunology, The Scripps Research Institute
1995-2002 Associate Professor, Dept. of Immunology, The Scripps Research Institute
2002-2007 Associate Professor with tenure, Dept. of Immunology, The Scripps Research Institute
2007-present John Parker Distinguished Professor of Medicine, University of North Carolina at Chapel Hill
2007-present Member of the UNC Lineberger Comprehensive Cancer Center
2007-present Co-director of the Thrombosis and Hemostasis Program at UNC

2009-2011 Associate Director of the UNC McAllister Heart Institute 2011-present
Director of the UNC McAllister Heart Institute

Honors

1988-1989 Fellowship from the American Heart Association (AHA), California Affiliate.
1991 Winner, Louis N. Katz Basic Science Research Prize for Young Investigators, AHA.
1995-2000 Established Investigator, AHA.
1999 Elected Fellow of the American Heart Association (FAHA).
2004 Special Recognition Award in Thrombosis from ATVB.
2004 Treadwell Distinguished Lecture, University of California Davis.
2007 Investigator Recognition Award from the International Society for Thrombosis and Hemostasis.
2009 Sol Sherry Distinguished Lecture in Thrombosis ATVB council of the AHA.
2011 Jeffrey M. Hoeg ATVB Award for Basic Science and Clinical Research.
2014 Humphrey's Lecture at the University of Birmingham, UK.
2014 Mosesson Lecture in Thrombosis, Blood Center of Wisconsin.
2015 Distinguished Scientist Award from the International Society of Thrombosis and Hemostasis

Committees

2012-2014 Chair of the Arteriosclerosis, Thrombosis and Vascular Biology (ATVB) Council of the AHA.
2012-2014 Member of the AHA Triangle Metro Board.
2012-2014 Chair of the Missions Committee of the AHA Triangle Metro Board.

Review Panels

2009-2013 NHLBI Program Project Review Committee.
2005-2007 Member, Western Review Consortium, AHA.
2004 Ad Hoc Hemostasis and Thrombosis (HT) Study Section, NIH.
2002 Member, Vascular Wall Biology II Study Group, AHA.
1997-2001 Member, Pathology A Study Section, NIH.
1995-1997 Member, Thrombosis Study Committee, AHA.
1993-1997 Member, Peer Review Committee, California Affiliate, AHA.

Senior Associate Editor Positions

2012- present Senior Associate Editor: ATVB

Associate Editor Positions

2012-present Associate Editor Journal of Clinical Investigation
2007-2012 Associate Editor ATVB
2006-2012 Associate Editor Journal of Thrombosis and Hemostasis 2002-2007
Associate Editor Thrombosis and Hemostasis

Editorial Boards

2014-present Journal of Thrombosis and Hemostasis
2007-present Thrombosis Research
2005-2007 ATVB

C. Contributions to Science

1/ *E.coli* hemolysin toxin. My PhD project was to clone and characterize the hemolysin operon from *Escherichia coli.* The size of *E.coli* hemolysin toxin was reported as 58 K daltons but this was shown to be a degradation product. In my first experiment in the lab I identified a 107 K dalton protein in the culture supernatant of hemolytic *E.coli* (1). This 107 K dalton band was subsequently shown to be *E.coli* hemolysin toxin. I next cloned the complete hemolysin operon from a plasmid present in hemolytic *E.coli* and showed that there were four genes encoding two transport proteins (HlyB anad HlyD), a protein required for activation of the toxin (HlyC) and the toxin itself (HlyA) (2). I analyzed the mechanism of toxin secretion and found that it required HlyB and HlyD that formed an ATP-regulated pore in the membrane similar to P-glycoprotein in mammals. Moreover, the toxin has a non-classical C-terminal signal for secretion (3). Importantly, I showed that this C-terminal peptide could direct the secretion of other proteins from *E.coli* (4). My discovery was patented as a novel method to generate large amounts of recombinant proteins from *E.coli.*

1/**Mackman, N.**, Holland, I.B. Secretion of a 107K dalton polypeptide into the medium from a haemolytic *E. coli* K12 strain. *Mol. Gen. Genet*. 193:312-315, 1984.
2/ **Mackman, N.**, Holland, I.B. Functional characterization of a cloned haemolysin determinant from *E. coli* of human origin, encoding information for the secretion of a 107K polypeptide. *Mol. Gen. Genet*. 196:129-134, 1984.
3/ **Mackman, N.**, Nicaud, J-M., Gray, L., Holland, I.B. Identification of polypeptides required for the export of haemolysin 2001 from *E. coli. Mol. Gen. Genet.* 201:529-536, 1985.
4/ **Mackman, N.**, Baker, K., Gray, L., Haigh, R., Nicaud, J.-M., Holland, I.B. Release of a chimeric protein into the medium for *Escherichia coli* using the C-terminal secretion signal of haemolysin. *EMBO J.* 6:2835-2841, 1987.

2/ Tissue factor. My postdoctoral project was to clone the human TF gene. The TF cDNA had been cloned by 4 labs in 1987 including the Edgington lab at TSRI. It took me more than 2 years of screening lambda libraries and manually sequencing the clones to complete the project. I published the complete sequence of the human TF gene in 1989 (1). Next, I identified the regions of the promoter required for LPS induction of TF gene expression in human monocytic cells (2). In collaboration with Dr. Carmeliet's lab we generated TF deficient murine embryos and discovered that TF was required for remodeling of the murine yolk sac vasculature (3). In parallel to this work my lab generated mice containing a minigene that expressed human TF from a short region of the human TF promoter. We then showed that the human minigene could rescue the embryonic lethality of murine TF null embryos (4). However, the level of human TF expression was only ~1% of wild-type levels and the mice were designated "low TF mice". These mice have been used extensively by us and others to study the role of TF in hemostasis, thrombosis and other processes, such as inflammation in sickle cell disease. My lab also generated TF floxed mice that allows deletion of the TF gene in different cell types by crossing these mice with mice expressing the Cre recombinase in a cell type-specific manner.

1/ **Mackman, N.**, Morrissey, J. H., Fowler, B., Edgington, T.S. Complete sequence of the human tissue factor gene, a highly regulated cellular receptor that initiates the coagulation protease cascade. *Biochemistry* 28:1755-1762, 1989.
2/ **Mackman, N.**, Brand, K., Edgington, T.S. Lipopolysaccharide-mediated transcriptional activation of the human tissue factor gene in THP-1 monocytic cells requires both AP-1 and NF-κB binding sites. *J. Exp. Med.* 174:1517-1526, 1991.
3/ Carmeliet, P., **Mackman, N**., Moons, L., Luther, T., Gressens, P., Vlaenderen, I., Demunck, H., Kasper, M., Breier, G., Evrard, P., Müller, M., Risau, W., Edgington, T., Collen, D. Role of tissue factor in embryonic blood vessel development. *Nature* 383:73-75, 1996.
4/ Parry, G.C.N., Erlich, J.H., Luther, T., **Mackman, N**. Low levels of tissue factor are compatible with development and hemostasis in mice. *J. Clin. Invest.* 101:560-569, 1998.

3/ TF, coagulation proteases and PARs in cardiac injury. TF has both coagulant activity and signaling activity. The latter is mediated via its ability to bind FVIIa and to generate downstream coagulation proteases, such as thrombin. These proteases activate PARs on different cells, including platelets. We hypothesized that TF, coagulation proteases and PARs contribute to cardiac ischemia-reperfusion (I/R) injury. We found that inhibition

of TF or thrombin reduce the infarct size in a rabbit model of cardiac I/R injury (1). We also found that PAR2 deficient mice had reduced infarct size compared with controls (2). In contrast, PAR1 deficiency was not associate with a change in infarct size but these mice exhibited reduced remodeling after I/R injury (3). These studies suggest that inhibition of coagulation protease and/or PARs would reduce cardiac injury and heart failure after myocardial infarction (4). We are currently determining if administration of rivaroxaban reduces cardiac remodeling in mice after I/R injury. An ongoing clinical trial called COMMANDER HF is analyzing the effect of rivaroxaban on heart failure patients.

1/ Erlich, J.H., Boyle, E.M., Labriola, J., Kovacich, C., Santucci, R.A., Fearns, C., Morgan, E.N., Yun, W., Luther, T., Kojikawa, O., Martin, T.R., Pohlman, T.H., Verrier, E.D., **Mackman, N.** Inhibition of the tissue factorthrombin pathway limits infarct size after myocardial ischemia-reperfusion injury by reducing inflammation. *Am. J. Pathol*. 157(2):1849-1862, 2000. **(Cover)**
2/ Pawlinski, R., Tencati, M., Hampton, C.R., Shishido, T., Bullard, T.A., Casey, L.M., Andrade-Gordon, P., Spring, D., Kotzsch, M., Luther, T., Abe, J., Pohlman, T., Verrier, E.D., Blaxall, B., **Mackman, N.** Proteaseactivated receptor-1 contributes to cardiac remodeling and hypertrophy. *Circulation* 116:2298-2306, 2007. PMCID: 2848478
3/ Antoniak, S., Rojas, M., Spring, D., Bullard, T.A., Verrier, E.D., Blaxall, B.C. **Mackman, N.,** Pawlinski, R. Protease-activated receptor 2 deficiency reduces cardiac ischemia/reperfusion injury. *Arterioscler. Thromb. Vasc. Biol.* 11:2136-2142, 2010. PMCID: 2959126
4/ Antoniak, S., Pawlinski, R., **Mackman, N**. Protease-activated receptors and myocardial infarction. *I.U.B.M.B. Life* 63:383-389, 2011. PMCID: 3121912

4/ TF+ microvesicles and thrombosis. Shortly after moving to UNC-CH I became interested in MVs, which are small membrane vesicles released from activated or apoptotic cells. The currently available assays to measure MV TF activity did not give reproducible results so my lab developed a new assay in which MVs were isolated from plasma and TF activity measured in a 2 stage FXa generation assay (1). We have measured levels of plasma MV TF activity in many different human diseases. We found that MV TF activity was increased in patients with elevated cholesterol levels and experiments with animal models indicated that these MVs were generated by activated monocytes (2). Interestingly, we found that simvastatin reduced monocyte TF expression and MV TF activity in hypercholesterolemic mice and monkeys. Cancer patients have a high rate of venous thrombosis and we speculated that these patients may have elevated levels of MV TF activity. In collaboration with Dr. Khorana we found in a small study that levels of MV TF activity increased in pancreatic patents prior to venous thrombosis (3). Similar results have been observed in several larger studies (4). We have analyzed the role of TF+ MV in cancer-associated thrombosis in mice. Injection of pancreatic cell-derived TF+ MVs into mice increased thrombosis in an inferior vena cava stenosis model. These studies suggest that plasma MV TF activity may be a useful biomarker to identify patients at risk for thrombosis.

1/ Lee, R.D., Barcel, D.A., Williams, J.C., Wang, J.G., Boles, J.C., Manly, D.A., Key, N.S., **Mackman, N.** Preanalytical and analytical variables affecting the measurement of plasma-derived microparticle tissue factor activity. *Thromb. Res.* 129:80-85, 2012. PMCID: 3272762
2/ Owens, A.P. 3rd, Passam, F.H., Antoniak, S., Marshall, S.M., McDaniel, A.L., Rudel, L., Williams, J.C., Hubbard, B.K., Dutton, J.A., Wang, J., Tobias, P.S., Curtiss, L.K., Daugherty, A., Kirchhofer, D., Luyendyk, J.P., Moriarty, P.M., Nagarajan, S., Furie, B.C., Furie, B., Johns, D.G., Temel, R.E., **Mackman, N.** Monocyte tissue factor-dependent activation of coagulation in hypercholesterolemic mice and monkeys is inhibited by simvastatin. *J. Clin. Invest.* 122:558-568, 2012. PMCID: 3266787. (A commentary was written on this paper by Roy Silverstein entitled Teaching an old dog new tricks: potential antithrombotic use for statins *J. Clin Invest.* 122:558-568, 2012. PMCID: 326687.
3/ Khorana, A.A., Francis, C.W., Menzies, K.E., Wang, J-G., Hyrien, O., Hathcock, J., **Mackman, N.**, Taubman, M.B. Plasma tissue factor may be predictive of venous thromboembolism in pancreatic cancer. *J. Thromb. Haemost.* 6:1983-1985, 2008. PMCID: 2848502
4/ Geddings JE, **Mackman N**. Tumor-derived tissue factor-positive microparticles and venous thrombosis in cancer patients. *Blood*. 122(11):1873-1880, 2013. PMID: 23798713.

5/ Role of the clotting cascade and PAR1 in the innate immune response to viral infection. The clotting contributes to host defense by immobilizing pathogens and facilitating their destruction. We hypothesized that PAR1 would enhance inflammation after viral infection. We tested the hypothesis by determining the effect of PAR1 deficiency on coxsachievirus B3-induced myocarditis. Contrary to our hypothesis, PAR1 deficient mice exhibited more cardiac injury than wild-type controls (1). We found that there was increased virus in the PAR1 deficient mice and hypothesized that PAR1 may play a role in the innate immune response to viral infection. Inhibition of either TF or thrombin also increased viral levels and cardiac injury. Studies with murine cardiac fibrobasts isolated from PAR1 deficient and wild-type mice revealed that PAR1 activation enhanced TLR3dependent IFNβ expression and downstream CXCL10 expression. These proteins are key components of the innate immune response to viral infection. Similarly, we observed more viral genomes and inflammation in the lungs of PAR1 deficient mice infected with influenza A virus compared with wild-type controls. Our results suggest that during viral infection TF-dependent activation of the coagulation cascade not only increases clot formation but also produces thrombin, which activates PAR1 and enhances the innate immune response (2).

1/ Antoniak S, Owens AP 3rd, Baunacke M, Williams JC, Lee RD, Weithäuser A, Sheridan PA, Malz R, Luyendyk JP, Esserman DA, Trejo J, Kirchhofer D, Blaxall BC, Pawlinski R, Beck MA, Rauch U, **Mackman N**. PAR-1 contributes to the innate immune response during viral infection. *J Clin Invest. 123*(3):1310-22, 2013. PMID: 23391721.
2/ Antoniak S, **Mackman N.** Multiple roles of the coagulation protease cascade during virus infection. *Blood*, 123(17):260502613, 2014. PMID: 24632711.

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