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To my children Urbi, Adit, and Arit

Foreword

As a researcher in basic and translational Immunobiology and Drug Discovery and later on, hyper-specializing into lung inflammation and lung stem cell engineering, I have felt, on the one hand as an academician deficit of a text book that gives the beginner, an *in depth* grasp of the subject and its nuances and on the other hand, a comprehensive *ready reckoner* for the bench scientist, that offers insight and troubleshooting ability keeping in mind the nitty gritties of the techniques and tools necessary for investigation. Hence, the endeavour for this discourse, which shall attempt on a four pronged approach to the same basic tools and techniques to alleviate human suffering, viz. firstly introduction to the basic biological cum clinical and drug discovery aspects of pulmonary and systemic inflammation; followed by detailed discussion on preclinical models of pulmonary and systemic inflammation researcher both in academia and the drug discovery industry; suceeded by the description of studies done on the roles of some key molecules in acute allergic asthma under which focuses on two major areas- research area 1 and 2 which talk respectively about exploring the roles of enantiomers of albuterol, the OTC drug of choice for acute asthma management and studies on prophylactic and therapeutic strategies to combat some local and systemic inflammatory pathologies under which chapter separate sections or sub-chapters are dedicated to investigating the roles of integrins $\alpha 4$ and $\beta 2$, the three selectins, gp91phox and MMP-12 in acute allergic asthma. This section precedes studies on the roles of integrins $\alpha 4$ and $\beta 2$ in chronic allergic asthma and in lymphopoiesis & homing and the last chapter is dedicated to the study of their roles in aseptic peritonintis. The information, mostly trouble-shooting tips from a conceptual as well as practical standpoint, I have often found, either vague or altogether lacking in research articles or text books, but have always felt to be critical for a successful reproduction of a previous work or a continuation of an ongoing problem. I sincerely hope that this is an honest and thorough work and our effort to share the finer details shall benefit the basic in academics as well as the translational researcher in the industry in their quest as well as give students, beginner and senior, a glimpse into possibilities of the excitement that science has to offer and to choose their career carefully, something that is often beyond the scope of a standard text book and perhaps often lost in translation in the technical jargon of an erudite journal. Our research has indeed filled

lacuna in the existing literature in the relevant fields, now we hope that this discourse shall excite future students and researchers and initiate them into the art of science. Each chapter highlights recent advances in a selected domain in lung disease research. This book shall serve as a comprehensive resource for both scientists and clinicians studying various aspects of fundamental and translational health sciences and medicine and shall provide a single reference comprising both basic and specialized information.

Kolkata, India Ena Ray Banerjee

Preface

At the beginning of my research career, when I was toying with the choice of various areas of possible study, immunology had appealed to me the most, to my mind at that time, when one is usually faced with the dilemma of a choice of career that offers a quick entry into the world of professionals versus a slightly longer-drawn-out yet infinitely rewarding career of an esoteric academician. Unfortunately, I did not get many role models to follow nor very sound career advice post my completion of M.Sc. in Zoology from the University of Calcutta. The options were either applied fields strictly in Zoology, such as the much in demand fisheries or entomology, or the slightly archaic career in museology and taxonomy or at best genetics which was also rather for a basic research career, with very little possibilities of translation. In due course, I qualified the UGC NET exam and opted for research in immunobiology, steering clear of infectious diseases and choosing instead cytokine biology, and joined Indian Institute of Chemical Biology, Calcutta, and worked on immunomodulation of interleukin 8 receptor expression on human polymorphonuclear neutrophils using various immunomodulators such as PMA and LPS. My work, while exposing me to the finer techniques and tools of our trade, also gave me an insight into the possibilities of research traversing various areas of immunobiology using rather repetitive yet useful assays. In fact, the take-home message here would be that receptor biology is an all-encompassing field that can be potentially applied to one of many receptor types and should help the researcher explore receptors pertinent to various diseases with even limited exposure to tools and techniques. Once you get a feel of the subject, you are initiated, so to speak, it becomes easy to use the basics and extrapolate into realms that can foray into very diverse fields of biological and related sciences.

My Ph.D. research was completed in roughly three years (1994–1997) with some fairly good publications in national and international journals, and having presented my work in some relevant conferences, I received my degree from Jadavpur University the next year as soon as my first child, a daughter, Urbi was born. Subsequently, I was told most solemnly by my mother, an East–west fellow and an accomplished anthropologist herself, that an academic career was the best career option for a woman. I therefore applied for and was duly selected for lectureship by College Service Commission and Public Service Commission as well and started teaching undergraduate Zoology which I did for 8 years in total. After my son Adit was born, we left for a postdoctoral stint in the USA where I worked on hematology,

exploring the roles of various integrins and selectins in inflammation of acute allergic asthma using various genetic knockout models of mice. From work on lung inflammation, I naturally progressed to use of cell-based therapy to ameliorate degenerative lung diseases, at the base of which lies inflammation. This took me into the realm of stem cells and regenerative medicine, and I became interested in exploring stem cell niches in the lung. We then returned home, and my entry into the world of applied biological research began with my foray into the Biotech and pharmaceutical industry where I briefly headed research with preclinical models of lung inflammation and aiming various libraries of chemical moieties toward various targets. Returning to academics after a couple of years (because the pressures and constraints of the industry are not always pleasant for us free-thinking academicians), my lab was initiated into working on the areas best identified as relevant in my experience as a university teacher and researcher as well as my exposure as an industry work force and that I felt I was best trained to do, and these are the following: (a) inflammation biology being my initiation into the world of research still remained my strong point as I understood how receptors and ligands interacted and what that meant for the overall signaling of the cell. In this I chose inflammation and drug discovery in inflammatory diseases as being the most relevant as far as translation is concerned, not, however, compromising basic research that asked fundamental questions. We work on the following primary models of inflammation where we ask questions on disease markers as well as test and screen drugs on them: allergen-induced acute and chronic allergic asthma, aseptic peritonitis, noninfectious colitis, idiopathic pulmonary fibrosis, and various transplantation models. In line with my training in Immunology, we also work on developing a platform technology for novel format camelid antibodies of diverse antigen: disease markers and cell differentiation markers. (b) Stem cell and regenerative medicine is the other area where I naturally migrated into while working with the lung as my primary target tissue for therapy. While I was trying to understand the hematological parameters and roles of various integrins in the onset of allergic inflammation as well as lymphoid homing in diseased state in the lung, regenerative medicine was the obvious transition where pharmacological intervention was not enough. So we began exploring pathways to convert embryonic stem cells with pluripotent characters into lung cells, a world of possibilities opened before us, and we began using various avenues such as endodermal differentiation, with or without embryoid body formation, varying density and giving heat shocks and co-culture with other cells, gently engineering the pluripotent cells into lineage of our choice, namely, non-ciliated alveolar epithelial tissue. In due course, validation was done with transplantation models where homing and engraftment were studied.

So here is hoping that many more curious souls like me shall enter the world of research asking questions and opening new vistas for generations to come and enjoy the thrill of science all the way.

August 15, 2012 Ena Ray Banerjee
Kolkata, India

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Kolkata, India

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Introduction

In order to begin work on a disease, with the aim to ameliorate human suffering, a researcher must first educate herself about the nitty-gritties of what is involved and what is at stake. This involves recreating the inner workings of a human disease in a nonhuman but closely related animal so as to facilitate delineation of each and every fine detail of the onset, etiology, establishment, development, progression, and exacerbations (cyclical manifestations in an exaggerated form of the disease pathology). In order to understand these inner workings, the model has to be simple enough and yet similar enough to be of any use to alleviate suffering of the human patients. The bench-to-bedside strategy therefore aims to recreate a complex disease first, part by part, outside the body (ex vivo) which enables easy dissection of nodal points of disease onset-progression, and then the entire composite disease phenotype is addressed using a whole organism by various treatments such as administration of a molecule (chemically induced), be it a drug, a polymer, an antibody or a peptide, or even an allergen or toxin, by various routes of administration, which, over various time intervals, usually manifest in a complex human disease in the subhuman primate or non-primate.

The first criterion therefore, for a researcher of biomedicine, is the choice of the animal once the disease phenotype she is particularly interested in is chosen in its finer nuances. The choice of the animal and the strain is important because there are some that are refractory to the treatment and do not satisfactorily express the disease phenotype and some that moderately express the disease. In both cases, it is a waste of time since the data from the control animal will not appreciably differ from that of the treated. Review on such strains or original research articles on this topic comparing and contrasting how disease manifestation differs depending on strain and treatment routes are very valuable. We shall discuss the same in detail for the disease models we are particularly interested in.

The next yardstick in choosing the model will be the readouts based on subtle variations in treatment. For example, brief initial adjuvant-aided sensitization and follow-up that prolonged local challenge in an allergic model is likely to invoke a cytokine-mediated pathway more powerfully than if slightly prolonged sensitization initiates the model and local challenges are somewhat curtailed (to half or two third of the earlier regimen) when a more pronounced B-cell-IgE-directed allergic response is seen. This also will be addressed in the following chapter.

The overall assessment of the "satisfactoriness" of the disease shall be based on the following features:

(a) That the disease expression is significantly distinguishable from the negative control or placebo-treated subject
(b) That the set criteria for simulation with the human counterpart are appreciably quantifiable

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(c) That the reversal of the disease (posttreatment intervention of a known pathway) is easily detectable (either by objective quantitative evaluation or by blinded qualitative estimation)

To fulfill these checkpoints, a disease model has to be extremely well characterized so that it is easy to interpret should there be even subtle shifts posttreatment. Well-characterized genetic knockout models of mice are therefore preferred for models of diseases as information on key regulatory molecules are already unambiguously available in the public domain.

Our work mainly involves pulmonary and systemic models of inflammatory diseases. We also work on disease models of degenerative diseases (will be discussed in detail in Chap. 2 of this book). Suffice it to say that most diseases have both inflammatory and degenerative components and while one scientist may be interested to explore the inflammatory component of it, another may be interested to study regeneration. Keeping this in mind, we shall endeavor to share in detail the models of interest to us and those that are used regularly in our laboratory and those, which may we modestly say, "have a handle on!"

Pulmonary Models of Inflammation

What Is Inflammation?

Inflammation may be defined as the sequential chain of events that herald the clearance of pathogens, rogue cells, and insult to the homeostasis of the system and is characterized by complex biological and biochemical response mainly via blood-borne inflammatory cells and the soluble mediators. Receptors and their interaction with these ligands orchestrate the complicated tango of cells that stage the drama of inflammation. In the absence of inflammation, an infection would never heal. This however necessitates the correct and timely control of the phenomenon; otherwise, loss of this master control by Inflammation Regulatory Elements (IRE), the drama becomes a saga of chronic and cyclical exacerbation leading ultimately to tissue degeneration and consequent structural changes. While pharmaceutical intervention may interfere with inflammation locally, systemic inflammation has to be managed more carefully as this is also a vital requirement by the body and any consequent break in signaling may harm the entire homeostasis. The challenge therefore for the researcher is to seek ways to limit the field of operation by anti-inflammatory agents.

It is a complex biological response of vascular tissues to appropriate stimuli in which there is constant and ever-changing interplay among cells of the circulation, local resident cells, soluble mediators, and genetic factors that form a myriad of signaling networks. The role players in specific diseases change as do their particular contribution to the response to the specific pathogen, namely, a pathogen, a damaged cell, or an irritant such as an allergen. It is a protective attempt by the organism to remove the insult and initiate the healing process. So this is an essential phenomenon. However, if it were to run unchecked, it would jeopardize the survival of the organism itself and therefore need careful monitoring and therein lies the importance of the study of the pathways regulating the initiation, establishment, and progression of pathophysiology, and the resulting information generated may be used as weapons to counter and control such unchecked inflammation during the development of a disease.

Acute and Chronic Inflammation

Inflammation can be classified as either *acute* or *chronic*. *Acute inflammation* is the initial response of the body to harmful stimuli and is achieved by the increased movement of *plasma* and *leukocytes* from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local *vascular system*, the *immune system*, and various cells within the injured tissue. Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous

destruction and healing of the tissue from the inflammatory process.

Acute inflammation is a short-term process which is characterized by the classic signs of inflammation – swelling, redness, pain, heat, and loss of function – due to the infiltration of the tissues by plasma and *leukocytes*. It occurs as long as the injurious stimulus is present and ceases once the stimulus has been removed, broken down, or walled off by scarring (*fibrosis*). The process of acute inflammation is initiated by the blood vessels local to the injured tissue, which alter to allow the exudation of *plasma* proteins and *leukocytes* into the surrounding tissue. The increased flow of fluid into the tissue causes the characteristic swelling associated with inflammation, and the increased blood flow to the area causes the reddened color and increased heat. The blood vessels also alter to permit the extravasation of leukocytes through the *endothelium* and *basement membrane* constituting the blood vessel. Once in the tissue, the cells migrate along a *chemotactic* gradient to reach the site of injury, where they can attempt to remove the stimulus and repair the tissue.

Chronic inflammation is a pathological condition characterized by concurrent active inflammation, tissue destruction, and attempts at repair. Chronic inflammation is not characterized by the classic signs of acute inflammation listed above. Instead, chronically inflamed tissue is characterized by the infiltration of mononuclear immune cells (*monocytes*, *macrophages*, *lymphocytes*, and *plasma cells*), tissue destruction, and attempts at healing, which include *angiogenesis* and *fibrosis*.

Asthma and COPD

Asthma and COPD are chronic conditions that take an enormous toll on patients, healthcare providers, and society. In the context of disease management, acute exacerbations are important clinical events in both illnesses that largely contribute to an increase in mortality and morbidity. Although these diseases are treated with the same drugs, they differ significantly in their underlying etiology. The underlying characteristics of both conditions however involve inflammatory changes in the respiratory tract, while the specific nature and the reversibility of these processes largely differ in each entity and disease stage. Both are characterized by lung inflammation; however, patients with asthma suffer largely from reversible airflow obstruction, whereas patients with COPD experience a continuous decline in lung function as disease progresses.

Asthma and chronic obstructive pulmonary disease (COPD) together form the third leading cause of death in both developed and developing countries, and annual direct and indirect cost of healthcare is more than $50 billion in the USA alone. It is estimated that there were about 45 million patients with asthma in the seven major markets in 2006, with a stabilizing prevalence. These inflammatory disorders are increasing in prevalence, and while most asthmatic patients respond well to current therapies, a small percent of nonresponders (10 %) account for greater than 50 % of healthcare costs. By 2020, India alone will account for 18 % of the 8.4 million tobacco-related deaths globally [1]. In China, COPD is one of the high frequency causes of death followed closely by ischemic heart disease and cardiovascular disease [2].

Key Role of Inflammation in Respiratory and Systemic Immune Disorders

Inflammation is key to etiology of most respiratory disorders, and while it is critical for the body's defense against infections and tissue damage, it has increasingly become clear that there is a fine balance between the beneficial effects of inflammation cascades and potential for tissue destruction in the long term. If they are not controlled or resolved, inflammation cascades lead to development of diseases such as chronic asthma, rheumatoid arthritis, psoriasis, multiple sclerosis, and inflammatory bowel disease. The specific characteristics of inflammatory response in each disease and site of inflammation may differ, but recruitment and activation of inflammatory cells and changes in structural