Immunology in India: an emerging story

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Although immunological research is of only recent origin in India, it is nevertheless rapidly becoming an area of choice for young researchers in this country.

The perennial appeal of immunology lies in the fact that it provides a window for almost every aspect of biological and biomedical research. The plasticity of lymphocytes as they switch among quiescence, active proliferation and various differentiation programs encapsulates the essence of both cell biology and the gene expression events that drive cell fate 'decisions'. The structural and functional diversity of immunity-related receptors elicits questions in evolutionary biology, genetics, biochemistry, biophysics and structural biology. Although belated, this universe of exciting opportunities offered by immunological research is also rapidly being increasingly recognized by researchers in India. And with the present availability of resources and educated manpower, the country is geared to mature into an important contributor to this field.

Susruta, the father of plastic surgery, is considered the originator of the rhinoplasty technique that has been practiced since 600 BCE. A detailed description of the procedure is provided in the Susruta Samhita. The procedure later spread from India to Persia and Egypt and, many centuries later, to Europe and is still practiced today by some families in India and Nepal. Reprinted from ref. 18 with permission from the author.

A historical perspective

The history of medicine in India goes back to remote antiquity, to a period between 4,000 and 900 BCE known as the 'Vedic period'. Although early Vedic medicine represented an amalgamation of religious, magical and empirical elements, a more rational approach known as 'Ayurveda' (or 'the art of healing') gained a foothold by around 1,000 BCE. Ayurveda continues to flourish even today as an alternative system of medicine. It was founded on the principle that the equilibrium among the bodily humors (or 'Doshas') defines the overall homeostasis of the body, which is reflected in the natural immunity of the host. Atopic and other chronic diseases, as well as susceptibility to pathogens, were all thought to derive from perturbations of this equilibrium. The Susruta Samhita (the treatise of Susruta) and the Charaka Samhita (the treatise of Charaka) represent two important pillars of Ayurveda and were compiled between 800 and 600 BCE. The Susruta Samhita provided the first systematic division of surgery into separate fields. The highlight of Susruta's achievement was the operation of rhinoplasty; Susruta is internationally regarded as the father of plastic surgery. The Charaka Samhita, in contrast, represents a treatise on medicine. It deals in elaborate detail with subjects such as fetal generation and development, the anatomy of the human body, and the function and malfunction of the human body. It also discusses the etiology, diagnosis and treatment of several human diseases, including autoimmune diseases.

The roots of modern immunology in India, however, were established only recently, occurring through the conversion of a small group of biochemists at the All India Institute of Medical Sciences (AIIMS) in Delhi. This conversion was the direct result of a series of courses in immunology sponsored by the World Health Organization and organized by G.P. Talwar in the biochemistry department, first held in 1968. The consequent exposure to the work of leading international experts in the field prompted several of the participants to enter

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this unfamiliar but exciting area. Research programs founded on immunology-oriented themes soon materialized. These themes included the development of contraceptive vaccines (G.P. Talwar), major histocompatibility complex (MHC) polymorphism (M.C. Vaidya) and an examination of immune responses in patients with leprosy (G.P. Talwar and Indira Nath). Although the early phase of the leprosy program concentrated on humoral responses[1], Nath subsequently expanded its scope to include a detailed study of cell-mediated immunity[2]. Her work showed that the generalized immune suppression in Indian patients was due to the secretion of soluble factors such as leukotrienes, prostaglandins and interleukin 10 by monocytes. In a similar vein, N.K. Mehra augmented the program on HLA polymorphisms at AIIMS, and the HLA-specific associations now being investigated range from transplantation to susceptibility to many infectious and chronic diseases. Notably, nuclei for immunological research also began to emerge in other parts of the country during this period. Thus, R.S. Kamat at the Haffkine Institute in Bombay began to look at immune responses generated against bacterial infections, whereas V.R. Muthukkaruppan and colleagues at the Madurai Kamaraj University added further strength to the leprosy program.

The growing interest in immunology soon led to the establishment of the National Institute of Immunology (NII) in Delhi in 1986, with G.P. Talwar as its founding director. At that time, Talwar was at the forefront of international research aimed at developing antifertility vaccines, and his demonstration that β-human chorionic gonadotropin, a self antigen, could be used as a contraceptive vaccine in women remains a landmark contribution to the field[3]. Talwar is also credited with the first isolation of a new strain of mycobacteria now called '*Mycobacteria indicus pranii*'. A heat-killed form of this mycobacteria has since proven effective as an adjunct to standard multidrug therapy for patients with leprosy and is commercially available as an immunotherapeutic vaccine formulation.

Research into the more basic aspects of immunology began to gain ground by the late 1980s. This was initiated by a small group of young researchers who had recently returned from postdoctoral stints abroad. G.C. Mishra at the Institute of Microbial Technology in Chandigarh began his career by exploring the still relatively new phenomenon of T cell costimulation. The novel approach adopted by his group led to the identification of an entirely new class of costimulatory molecules that selectively induced T helper type 1 or type 2 differentiation. Notably, these molecules were not 'new' proteins but instead represented post-translationally modified isoforms of well-known 'housekeeping' gene products[4].

Studies of the thresholds that regulate activation versus anergy and proliferation versus differentiation of T cells were jointly initiated by Satyajit Rath and Vineeta Bal at the NII. So far, several previously unknown pathways have been delineated; examples of these include the function of protein kinase A in mediating the commitment of activated T cells to secondary responsiveness, as well as the function of inducible nitric oxide synthase in regulating T cell death and immune memory[5,6]. My group at the International Centre for Genetic Engineering and Biotechnology (ICGEB) in Delhi has pursued studies of antigen recognition by primary B lymphocytes and their subsequent activation and differentiation in T cell–dependent responses. A highlight of this research has been the identification of a previously unknown subset of receptors for immunoglobulin D on primary cells and the elucidation of their function in defining antigen-affinity thresholds for memory B cell differentiation[7].

The Indian Immunology Society was established in 1971 and began to hold annual meetings in 1973. It now boasts about 1,000 registered members and has recently launched new initiatives to increase active participation from its student community. To promote the exchange of ideas, an informal platform known as the 'Molecular Immunology Forum' was also conceived in 1993. Its membership is restricted to active researchers who meet once a year. The objective of this forum is to serve as a peer-review group for ongoing work so investigators may benefit from this collective input. The Molecular Immunology Forum has contributed considerably toward enhancing the quality of Indian research efforts in immunology.

Research funding and institutions

The independence of India from colonial rule in late 1947 saw the emergence of a national mindset that was determined to be self-reliant. A natural outcome of this was an emphasis on strengthening indigenous abilities in science and technology. India's first prime minister, Jawaharlal Nehru, also visualized science as a vehicle for qualitatively transforming a society still rooted mostly in tradition. The Council of Scientific and Industrial Research was one of the first institutional structures to be set up with the mission of promoting scientific and industrial research and development. This council functions under the auspices of the Ministry of Science and Technology and has grown over the years to its present tally of over 38 research institutions, 11 of which focus on research in various aspects of biology, including immunology.

Education has always occupied a preeminent place in Indian history, and the earliest known universities of Nalanda and Taxila flourished here as far back as in the fifth century BCE. To reexamine the educational requirements of an independent India, however, the Indian government set up a University Education Commission in 1948. The report of this committee led to the establishment of the University Grants Commission in 1953. This commission was charged with the mandate of promoting and coordinating university education and research in India; so far, there are over 230 universities spread across the country. In addition, the Indian government has recently announced the creation of thirty new central universities, five new Indian institutes of science education and research, eight new Indian institutes of technology and twenty new institutes of information technology.

Research in India is also actively pursued in national research and development institutions. These institutes, such as the Council of Scientific and Industrial Research, are supported by various governmental branches. Another organizational structure for health-related research is the Indian Council of Medical Research (ICMR), formed in 1949 and funded by the Ministry of Health. The ICMR directly supports over 25 research institutes in the country covering all aspects of biomedical sciences, including infectious disease, autoimmune disease, oncology, nutrition, epidemiology and medical statistics. The now-famous Chingleput trials for bacille Calmette-Guerin vaccination, which first established the principles of double-blind randomized trials in humans, was conducted by ICMR's Tuberculosis Research Centre (Chennai). Notably, this study also highlighted the inadequacy of this vaccine in countries in which tuberculosis is endemic[8]. The ICMR also actively supports projects of scientists working in other institutions and universities through its extramural program. Research in clinical, basic and 'translational' aspects of immunology in various laboratories has received strong support from the ICMR. To further accelerate the growth of science, the Ministry of Science and Technology established the Department of Science and Technology in 1971 to support basic and applied research in the various institutions and universities in the country.

But perhaps the strongest stimulus for rejuvenating research in the life sciences came with the setting up of a separate Department of Biotechnology (DBT) in the Ministry of Science and Technology in 1986 to actively promote the field of modern biology in India. Since its

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Learning under the Bodhi tree. Students debate the merits of the hygiene hypothesis at a recent annual meeting of the IIS. Such open-air sessions, which provide an opportunity for students to engage in self-driven debate on important ideas in immunology, is now an integral component of these annual meetings. Another such initiative of the IIS is the annual publication of a student newsletter. Both the contributing authors and the editorial board of this newsletter are exclusively PhD students and postdoctoral fellows. Photograph courtesy of Madhuri Thakar, National AIDS Research Institute, Pune.

creation, the DBT has contributed considerably to the growth of biology research; it has achieved this, at one level, through the generous funding of projects in individual laboratories. In addition, the DBT has also been actively involved in establishing new institutes and upgrading the infrastructure of existing ones. The NII represents the first new institute to be established by the DBT, and at least seven more institutes focusing on broad areas such as cell biology, infectious disease biology, brain research, plant biotechnology, genomics and biodiversity have been added to the DBT's portfolio.

Extramural funding of research by the DBT is mediated by several expert committees of peers, called 'task forces', that review and fund project proposals in specific areas of expertise. Although there is not yet a specific task force for immunology, immunologists can seek funding from those dealing with basic science, medical biotechnology, infectious disease biology, human genetics or animal biotechnology or the task force on interdisciplinary research. In addition, there are other committees specifically set up to promote infrastructure improvements and to review and fund 'big-budget' research programs that involve many collaborating partners. In the current 5-year plan period, special emphasis is being given to multi-institutional and multidisciplinary programs, including the active promotion of collaborative partnerships among academic laboratories and biotechnology and pharmaceutical industries.

Although a strong framework for governmental funding of science has emerged in a short time span, the government's capacity to galvanize scientific research was initially tempered by economic constraints. The available resources were limited and therefore too thinly spread. The economic turnaround in the mid-1990s that led to a healthy national growth rate of between 8% and 9% in the past decade, however, has reenergized the scientific ambitions of the country. A substantial increase in science funding by the Indian government (for example, the budget allocation for DBT has increased by about fourfold over the past 5 years) has led to aggressive investment in new institutions and universities, upgrading of infrastructure, new research programs of an interdisciplinary nature and much greater funding for individual projects. In addition, several attractive programs have also been initiated to induce scientists of Indian origin who are working abroad to return to India. A good example is the joint initiative between the DBT and the Wellcome Trust to offer 70 fellowships annually for biomedical research, at various levels, for the next 5 years. The cumulative effect of all these initiatives on scientific output is sure to be felt in the coming years.

Present research

Although unintended, the introduction of immunology to India through exercises in vaccine development led to a general perception that immunology was mainly a 'translation-oriented' science. New vaccines were the desired end goal, and diagnostic kits were considered the 'low-hanging fruit' to be picked along the way. The enormous public health burden, coupled with the limited resources available during the formative years of Indian immunology, also contributed to this layperson's view. Asking questions about more basic issues related to the functioning of the immune system was considered less important and, perhaps, even socially irresponsible.

Recent years, however, have witnessed a 'sea change' in this perspective, and several robust programs on basic immunology have since evolved. Lymphocyte activation and differentiation and the associated processes of antigen presentation and costimulation represent key areas of interest to immunologists in India. Collaborative efforts among the groups of Anna George, V. Bal and S. Rath (NII) have identified many important mechanisms involved in regulating the activation-apoptosis balance in T cells, as well as in the differentiation of T cells into effector versus memory populations. Their studies of antigen presentation pathways have led to the identification of a previously unknown pathway in which cytoplasmic proteins processed by the proteasome are then transported to the endo-lysosomal compartment for presentation by MHC class II molecules[9]. Samit Chattopadhyay at the National Centre for Cell Science (NCCS) in Pune has shown that transcriptional activation of the transcription factor T-bet during T helper type 1 differentiation is regulated by the matrix attachment region–binding protein SMAR1, whereas Sanjeev Galande from the same institute is exploring the function of another MAR-binding protein, SATB1, in regulating gene expression from the MHC class I locus. Others groups studying various facets of T cell activation, death and differentiation include those of G.C. Mishra (NCCS), Apurva Sarin (National Centre for Biological Sciences, Bangalore), Dipankar Nandi (Indian Institute of Science, Bangalore), Satish Devdas (Institute of Life Sciences, Bhubhaneswar) and Gobardhan Das (ICGEB).

In addition to studies of T cells, the activation and differentiation of B lymphocytes is another area from which substantial contributions have emerged. A. George and her collaborators originally identified CD27 as a critical participant in driving the differentiation of memory B cells[10]. The present focus of this group is to delineate the 'downstream' signaling and gene expression events that are involved. My group has identified a previously unknown feedback loop that regulates the amplification of signaling from the B cell antigen receptor[11], and we are now using systems biology to 'decode' the mechanisms of signal processing to understand how context-specific cellular responses are achieved. In collaboration with the structural biologist Dinakar Salunke (NII), we have also addressed the question of how the germline-encoded repertoire of the B cell antigen receptor has a vast potential for recognizing diverse antigens, which is achieved through the structural plasticity of the antigen-combining site of the receptor[12]. In addition to this, Salunke is also attempting a structural resolution to determine why some antigens are allergenic in nature. Devender Sehgal's group at the NII is focusing on examining somatic mutation and the affinity maturation of antibody responses to model antigens.

Although there has been considerable 'broadening of the base' in recent years, infectious diseases continue to dominate immunology research in India. G.C. Mishra and his group are examining the involvement of dendritic cells in regulating the host response to dengue infection, and S. Bandopadhyay (Indian Institute of Chemical Biology, Kolkata) is testing the possibility of using antigen-primed dendritic cells as a vaccination strategy for leishmanaisis. At ICGEB, K. Natarajan and Pawan Sharma are investigating the regulation of host innate immune responses by the secretory antigens of *Mycobacterium tuberculosis*. Studies from Natrajan's group suggest that these antigens facilitate the creation of a 'safety niche' that allows the pathogen to evade host immune responses[13]. In contrast, H. Krishna Prasad and his team at AIIMS are concentrating on identifying mycobacterial antigens that are immunogenic in humans. The regulation of host immunity by *M. tuberculosis* also constitutes the research theme of Joyoti Basu (Bose Institute, Kolkata) and Sangita Mukhopadhyay (Centre for DNA Fingerprinting & Diagnostics, Hyderabad). Basu's work has provided important information on the pathogen-mediated suppression of innate immune responses in the host[14]. Ayub Qadri (NII), studying the regulation of inflammatory and innate immune responses during infection with pathogenic salmonella, has shown that the synthesis and secretion by salmonella of monomeric flagellin, a ligand for Toll-like receptor 5, is induced through the direct sensing by the bacteria of host-produced lysophospholipids[15]. Such host-specified induction of a Toll-like receptor ligand by the pathogen represents a previously unknown regulatory mechanism for the activation of inflammatory and innate immune responses in the host. Arvind Sahu (NCCS), in contrast, is investigating the function of viral complement control proteins in mediating immune evasion.

Research in clinical immunology has also gained ground in recent years, with a steady increase in the number of groups working in this area. At the NII, Rahul Pal is exploring the link between antilymphocyte antibodies and autoimmunity, whereas Rajni Rani is probing the association of diabetes with polymorphisms in gene loci encoding cytokines and MHC molecules. A systematic delineation of the genetic basis of susceptibility to asthma is underway in the laboratory of Balram Ghosh at the Institute for Genomic and Integrative Biology in Delhi. A strong association with many previously unknown polymorphic loci has already been identified, the most notable being the gene encoding transforming growth factor-β1 (ref. 16). At the Sanjay Gandhi Postgraduate Institute of Medical Sciences in Lucknow, Sonya Nityanand has developed a strong program for identifying the mediators of inflammatory responses in Takayasu's arteritis. A group headed by Bhaskar Saha (NCCS) is exploring the possibility of manipulating CD40 signaling to optimize T cell–dependent antitumor responses. This strategy is based on his own earlier finding of a functional dichotomy of CD40 whereby it naturally limits antitumor responses by inducing interleukin 10 (ref. 17). Immune dysfunction in cancer and exploiting natural killer and γδ T cells for cancer therapy is the theme of Shubha Chiplunkar's research (Advanced Centre for Treatment, Research and Education in Cancer, Mumbai), whereas researchers at the National Aids Research Institute in Pune are characterizing innate and adaptive immune responses in both early infection with human immunodeficiency virus and coinfection with human immunodeficiency virus and tuberculosis.

True to its roots, vaccine development continues to represent a strong component of the immunology research scenario in India. A rotavirus vaccine developed at AIIMS by M.K. Bhan is now undergoing clinical trials, whereas the technology for large-scale production of a vaccine for Japanese encephalitis virus developed at the NII has been transferred to an Indian biotechnology company. The groups of V.S. Chauhan and Chetan Chitnis at ICGEB have separately developed candidate vaccines for *Plasmodium falciparum* and *Plasmodium vivax*, respectively. Preparations are underway for their phase I clinical trials. Finally, in terms of new and more potent vaccines for tuberculosis, promising leads are emerging from the laboratories of Anil Tyagi (Delhi University), S. Vijaya (Indian Institute of Science, Bangalore) and Javed Agrewala (Institute of Microbial Technology).

Future prospects

It is evident from sampling of ongoing activities presented here that there has been a gratifying intensification of immunological research in India. Nonetheless, the 'critical mass' of immunologists continues to be very restrictive, limiting the extent of Indian contributions to the field. Although important discoveries have emanated from Indian immunologists, these have been sporadic in nature. It will be important to transform these occasional bursts into a more steady and sustained steam. The present governmental efforts to strengthen the scientific infrastructure give reason for optimism that this will also affect immunology research both by expanding the pool of Indian immunologists and by providing them with the state-of-the-art tools for their research.

The paradox of India is that although its cultural identity dates back to the origins of recorded history, it is still in its infancy in terms of its identity as an independent and sovereign nation. India is only now beginning to emerge as a state with the wherewithal and confidence to chart its own future. The present availability of economic resources, coupled with a firm political commitment to invest for the future in knowledge-based arenas also bodes well for the future of immunology in India. The timing of this is particularly fortuitous, as it coincides with the emergence of India, with close to half a billion of its people below 30 years of age, as one of the world's 'youngest' nations. Thus, the burden of responsibility for fruitfully exploiting ongoing initiatives will be shouldered by a new generation that is unencumbered by the trappings of either history or the mitigating circumstances experienced by its predecessors. For immunology in India, then, this could well mean a considerably enlarged work force asking bolder and more risky, but original, research questions. To what extent this favorable alignment will eventually 'translate' into scientific leadership 'on the ground' is something that only time will tell.

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