Perspectives

A destiny to fulfill

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I was born on October 2, 1926. My mother died when I was 8 days old. My father remarried. I had a stepmother and a difficult childhood. I had an inclination towards Medicine but my father decided (as used to be the case in those days) that I should do a B.Sc. (Hons.) in Chemistry followed by a Master's in Chemical Engineering. He had a pharmaceutical company in Lahore, which expanded during World War-II. He wanted me to work in this factory. The partition of the country witnessed an unexpected mass migration. We came out with just our shirts and trousers. The final year of my education was in 'Camp' facilities in Delhi. I obtained first division nonetheless in the final exam in 1948. During the following two years, one had to fend for oneself. I worked in more than one company selling imported paper and other commodities at a reasonable salary but without job satisfaction. I won a scholarship for postgraduate studies in France. Arriving in Paris in September 1950, I opted for Fermentation Technology. I was sent to a Division at the Institut Pasteur which maintained the right strains of yeast for the champagnes of France. We visited the wineries every now and then and were offered to 'deguste' the brews, at that time unfamiliar to my palate. The questions that I asked during these visits and in the lab, made the Head of this Division decide to transfer my Fellowship to the Biochemistry Division of the Institut Pasteur. After Macheboeuf's death, Jaques Monod became the Head of this Division. He was not only a great scientist, original in his thinking and approach, he was a musician and played in chamber music concerts. His wife Odette had specialized in Tibetan art and worked at the Musée Guimet, the museum for oriental arts in Paris. They had a great influence on cultivating in me an appreciation for classical music and ancient cultures. I was awarded the Doctorat d'Etat, Docteur es Science (D.Sc.) in 1953, and though I was given a position in the Medical Research cadre, I moved to Germany in late 1954 as a Humboldt postdoctoral fellow. I returned to India in 1956 to join the newly established All India Institute of Medical Sciences (AIIMS) in New Delhi.

1. Introduction: Early days at AIIMS

I came to join the Institute on the afternoon of 9 August, 1956. I had problems in finding this so called "pacesetter" Institute. I was told to come beyond the Safdarjung Airport on the way to the Qutub Minar; no prominent building was visible. Passers by were few. It was largely an uninhabited area. After much searching, I came across a faded board dangling on a Neem tree which read "Office of the Member Secretary, All India Institute of Medical Sciences". About 100 yards away stood four blocks of 2 storey C-II type buildings. No formal gate or road led to these. It had rained and there were puddles of water. Bricks had been put here and there on which one could walk to reach the buildings. Inside one of them was the office of Dr K C K E Raja, the Member Secretary. It was a hot and humid day. He sat behind a huge table in his vest with a towel hanging on the back of the chair. He beamed a welcoming smile. I was the second faculty member to join; Dr Nand Keswani had joined on the forenoon of the same day as Professor of Anatomy. We shook hands and were given an office each in a C-II building. We were told that the first batch of students would join on the 25th of September and were asked to get prepared to start classes. The stores officer and his assistants had instructions to procure furniture, basic equipment and other requirements. We could recruit essential technical staff for conducting practicals. From amongst the candidates sent by the Employment Agency, I recall recruiting Lal Singh, a young, fit-looking Sardarji who was a matriculate. He was resourceful. "Not possible" or "Cannot be done" did not exist in his vocabulary. He would procure the chemicals, glassware and so on from somewhere or the other within the week for the practical class of the following week. Lal Singh stayed with me for 20 years or more, receiving promotions on the way to reach the highest level admissible in the technician category. He put his hand to any task given to him and performed it efficiently. To escape from having to carry out

the routine services of blood and urine chemistry, I had passed these to clinical pathology. Instead we offered specialized investigations on thyroid hormones, corticoids and sex steroids. We set-up also a Radio-Isotope laboratory for thyroid functions. Lal Singh was the chief technician in this lab. In his lab coat, he was addressed as Doctor Sahib by patients. With my encouragement, he studied Homeopathy as a part-time student and obtained a degree in it. He is today a reputed Homeopath with a flourishing practice and runs clinics in more than one part of Delhi.

The first 5 years of my service at AIIMS were primarily devoted to sitting with the architects for planning and building laboratories in the pre-clinical block of the main building of AIIMS, drawing out of the list of equipment within the budget, floating tenders and screening quotations. No formal research could be conducted till some wings of the pre-clinical block were ready. Of course we taught the undergraduates and had a lot of time to interact with students. This formed special bonds which have endured to this day. A large percentage of our graduates went abroad for higher degrees and specialization. Most have stayed there and almost all have done exceptionally well. They occupy important positions and are loaded with money. They count amongst the highest income professionals in the USA. They have remained attached to their Alma Mater and to the teachers who taught them initially. Nearly all are enthusiastic members of the AIIMSONIANS Association and have a reunion each year to which they flock from far and wide. Every time I have gone for a conference or to give seminars, an AIIMSONIAN in Boston, San Francisco, Utah, Houston, Washington or elsewhere (they seem to be spread everywhere) has taken the initiative to organize a dinner to which our former undergraduate and post-graduate students congregate from a distance of up to 200 km to relive old times. It is a touching experience.

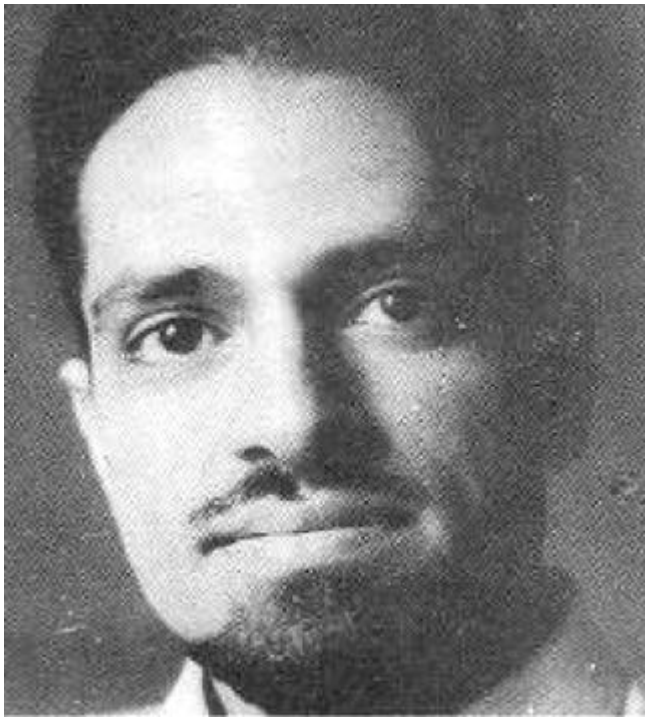

Figure 1. GPT as a research scholar at the Institut Pasteur in Paris 1953.

In those early years, the size of the faculty at AIIMS was small. We met frequently. At least once a month, we had a party in some home and on the roof during summer months. These parties broke barriers and we got to know each other well. We sang songs; Rama (Prof. V Ramalingaswami), Nand (Keswani) and I formed the famous trio, and as the evening progressed, there could be heard "Chal Chal re Naujavan", "Ek Bangla Bane Niara" and other film songs of our college era.

Some of us (Nand Keswani, Ranjit Roy Chaudhry and I) were bachelors when we joined AIIMS. I used to stay with my uncle and aunt who had a sprawling Bungalow at 13 Rajpur Road, Civil Lines, near the University of Delhi. I used to commute this long distance in a Fiat car, which I had bought in very good condition from a socialite friend of my aunt. It had a conspicuous emerald aqua colour and an equally notable number, 202. The result was that my sorties in the evening, normal for a bachelor with spare time, could hardly remain a secret. Knowing French and German and having lived in Europe for six years, I made friends in the Embassy circle and with each party to which I was invited, the circle widened. The invitations in those days frequently demanded black tie or National dress, and for even non-formal dinners, a suit had to be worn. As I lived at a far end, my car carried on the back seat the dress for the evening and also riding shoes, Jodhpuris and polo cap. I became a life member of the Delhi Polo Club (DPC) and enjoyed going for horse riding at least once a week if not more often until post-graduate education and research started and afternoons were no longer free. In those early post-Raj days, DPC used to organize cross-country rides on Sunday mornings starting at 5·30 or 6 AM and ending with breakfast around 8 AM. It was inpractical to go to Rajpur Road after dinner and then come back to the polo ground in the President's Estate by 5·30 AM. The outcome was that parties extending till the early hours became the preferred choice for Saturdays.

In the late nineteen sixties, Howard Goodman, Chief of Immunology at WHO (Geneva), visited India and some other south Asian countries along with Elvin Kabat, Melvin Cohn and a few other distinguished Immunologists. Their aim was to start a Centre for Research and Training (RTC) in Immunology for India and the South-

East Asian regions. Amongst others, they visited me. I was then Professor and Head of Biochemistry at AIIMS. They spoke eloquently of their mission and, surprisingly, proposed to me to Head the envisaged WHO-RTC in Immunology. How I agreed to it, and how it led to the foundation of Immunology in the country, forms a part of this story. It had also a profound effect on my research, a transition from the basic quest to learn how growth promoting and developmental hormones act on their target tissues to pursue problems of relevance to the country and devise possible solutions.

I was at that time deeply immersed in building an independent biochemistry Department at AIIMS and giving biochemistry its due place in medical teaching and research. Biochemistry in most medical institutions was then a part of physiology. Its teaching was boring and minimal compared to the strides that the subject had made in life sciences. Clinicians visualized biochemistry in limited terms of urine and blood chemistry. Auto analysers were not yet an essential part of clinical chemistry labs. Laboratory investigations were done by a half-baked, poorly motivated and reluctant "army" of technicians with the result that their reports were not reliable. Doctors made a diagnosis on basis of clinical features, whether supported or not by lab reports. My job was not only to introduce a contemporary, interesting and exciting course in biochemistry for students (who, at AIIMS, were the crème de la crème, admitted on a highly competitive basis), but also to educate my faculty colleagues on what modern biochemistry offered for their disciplines. Fortunately the AIIMS faculty had both senior professors, selected from various medical colleges in the country on grounds of eminence and experience in the practice of medicine, and bright associates recruited from abroad, who were receptive to modern biochemistry. As a result of our interaction, interdisciplinary modules for teaching were developed, where biochemistry was included alongwith pre- and para-clinical subjects for the teaching of clinical disciplines. A general course in modern biochemistry, genetics and molecular biology was instituted for all post-graduate students in various subjects of medicine and allied disciplines. This pattern of teaching not only benefited others but also the teachers in biochemistry. Most of us, including me, were non-medicals. Interdisciplinary modules made us learn relevant aspects of other subjects. The *Text Book of Biochemistry and Human Biology* (Prentice Hall of India, 1980), which I wrote and edited, has contributions from teachers in many other disciplines. Three editions of the book have appeared by now. The niche that we created for biochemistry at AIIMS, as a full fledged independent department imparting education at the undergraduate and post-graduate levels, served as a model for similar departments at the Institute of Medical Sciences, Varanasi, Postgraduate Institute of Medical Education and Research, Chandigarh and elsewhere.

2. Research during the initial years at AIIMS

2.1 *Mechanism of action of growth promoting and developmental hormones*

In the early years we had an Animal House and rudimentary lab facilities. We observed that administration of pituitary growth hormone to rats increased their body weight. It was logical to put forward the hypothesis that growth hormone enhanced the synthesis of proteins. Was it preceded by other metabolic events? The incorporation of ^{32}P into RNA, both mRNA and rRNA, was noticeably stimulated by the hormone (Talwar *et al* 1964). In a parallel project, we worked on estradiol. On the removal of ovaries, the uterus of the rat shrunk markedly. Administration of estradiol in microgram amounts caused its perceptible growth. We hypothesized that the stimulation of RNA synthesis was primary to the uterotrophic effect of estradiol. To test this hypothesis, a simple but conclusive experiment was done. Estradiol acted not only on the uterus, but also on vaginal cells. On ovariectomy, the uterus atrophied; this was quantifiable by weighing and histology, accompanied by a change of vaginal cytology observed microscopically on aspirates. Injection of the hormone restored both organs. Topical application of Actinomycin-D locally on the vagina, thereby inhibiting DNA dependent RNA synthesis, prevented the hormone-induced changes in the vagina. Uterine growth occurred normally in the same animal, implicating the critical role of RNA stimulated by the hormone for the subsequent development of the biological effects (Talwar and Segal 1963).

Another feature of estradiol action was that it acted on some but not all organs of the body, though the hormone in circulation was accessible to all. We hypothesized that the selective action could be due to the presence of receptors for estradiol in some but not all tissues. This was found to be indeed the case. ^{3}H-estradiol bound to a proteinic fraction of the uterus, but not to similar extracts from lungs. The binding was highly specific. Labelled cortisone did not bind to the uterine fraction. A stereo-isomer, 17-*a*-estradiol, which has poor estrogenic property, bound poorly, whereas 17-*b*-estradiol, the active estrogen, had high binding with the uterine fraction (Talwar *et al* 1964).

A point to ponder was whether estradiol stimulated the transcription of specific genes. To answer this question, we made use of male chicks who do not normally make the egg yolk protein phosphvitin. As females made this protein, was it under the influence of the female sex hormone, estradiol? This could easily be tested by admini-

stration of estradiol to male chicks. Estradiol induced the synthesis of this egg yolk protein in birds who would have never made it otherwise (Jailkhani and Talwar 1972a,b).

Another tantalizing question was why bone and cartilage growth occurred only up to the adolescent adult stage and not thereafter, even though pituitary growth hormone continues to be made and is in circulation. We employed cartilage from young and old animals and studied the effect of growth hormone on incorporation of ^{35}S into sulphated biopolymers. The tissue from aged animals had lost responsiveness to the hormone (Talwar *et al* 1974c).

The thymus is the source of primary T lymphocytes which perform a key function in the immune response. The organ atrophies with age. We demonstrated that pituitary hormones exercise a stimulatory effects on uridine and sulphate incorporation into biopolymers at early stages of development. The receptors for growth hormone were present on thymocytes from young but not aged animals (Saxena and Talwar 1974). The hormone influences not only the metabolism of thymocytes in young animals but also the degree of immune response to T dependent antigens (Pandian and Talwar 1971).

3. The Advent of Immunology

I was immersed in and enjoying research on the mechanism of action of hormones. There was no reason for shifting to the immunology. During my Docteur es Science work at the Institut Pasteur in Paris, I had studied and passed courses in Bacteriologie and Serologie et Immunologie Generale, but during the 15 intervening years, immunology had changed vastly. Many advances had taken place. The structure of Immunoglobulins was established. The complexity and diversity of cell types constituting the immune system was apparent. A lot was happening on the differentiation and maturation of these cells, as also on the genetics of the immune response. I needed to be a student again before I could justify taking up the position of Head, Research and Training Centre (RTC), which was offered to me unexpectedly. Thus when the offer came, I was reluctant to take up the responsibility and suggested the names of others at AIIMS and other Institutions in the country. The Committee that chose me however visualized that Immunology had become a molecular and cellular science and thought that my department was appropriate for locating the RTC. They were willing to organize two teaching workshops on Advances in Immunology. A statement that floored me squarely was "India has the world's largest number of leprosy patients (3·2 million at that time). Do we expect Americans to come and work on this disease to find solutions? Scientists in India should take up the problems of India."

Figure 2. The picture recalls the discussion at AIR moderated by GPT with his 'guru' Jacques Monod (extreme right), close friend Feodor Lynen (extreme left) and R B Livingston (2nd from and left).

I agreed to Head the WHO-RTC. While seeking Government permission, Prof. P N Wahi, the then Director General of the Indian Council of Medical Research (ICMR), insisted that it should be first an ICMR and then a WHO Centre. Thus the Centre was named as the ICMR-WHO Research and Training Centre in Immunology. Our charter was to organize an average of one course each year in contemporary immunology for teachers and investigators in India and other countries in South East Asia.

4. Research on leprosy

Leprosy is a spectral disease – it has a spectrum of clinical manifestations. The vast majority of humans (~ 99%) have innate resistance and do not get the disease on exposure to *Mycobacterium leprae*. Amongst those who get it, the disease is manifest in pauci-bacillary and multibacillary forms. The spectrum varies from polar tuberculoid (TT) form of leprosy with only a single lesion containing disintegrated bacteria to polar lepromatous leprosy (LL) with disseminated lesions all over the body full of proliferating mycobacteria. The BL and LL type of multibacillary patients take 2 years or more to get cured by treatment with multi-drug therapy (MDT). They are the ones who perpetuate *M. leprae* and transmit the infection to others in the community. These patients have the maximal immune deficit. Instead of killing the bacteria, their macrophages offer a hospitable territory for them to multiply.

Our initial urge was to understand the nature of the immunological deficit in multibacillary leprosy patients and, collaterally, to learn how the tuberculoid TT patients are able to kill *M. leprae*. Though AIIMS and local hospitals in Delhi received leprosy patients, the cases were mixed up. Many came from Bihar and Nepal. I decided to go to a field area. The Danish 'Save the Children' NGO

ran leprosy homes in Aska, Orissa and Pogiri, Andhra Pradesh. I ran into Dr Blum, the Doctor incharge of these clinics, in Delhi. He was willing to receive me and my co-workers and offered us living and working space and more importantly, fresh leprosy cases. I spent two summer vacations in these clinics along with two talented Ph.D. students, A D Krishnan and Vijay Lakshmi Mehra. We took with us a plexiglass hood, spirit lamps, cell culture media and of course plenty of plasticware. The clinic had microscopes and incubators but no CO_2 incubator. We utilized dessicators for keeping the cell culture dishes and plates in which a burning candle would extinguish automatically when the atmospheric CO_2 concentration reached 5%.

Our studies in the field clinics during summer and in our lab at AIIMS during the rest of the year indicated that the multibacillary patients did not have a generalized immune defect. They reacted normally to most antigens and responded normally to cholera and tetanus toxoid vaccines (Jha *et al* 1971). Their lymphocytes did not react to *M. leprae* and did not undergo antigen driven blast transformation (Talwar *et al* 1972). Depending on the stage of the disease and the category to which the patient was classifiable, the defect was either intrinsic and or acquired as a consequence of *M. leprae* infection. Acquired immunosuppression was relieved by treatment when the patients became bacillary negative (Nath *et al* 1977).

A key question concerned the determinants which made macrophages competent to kill *M. leprae*, which was the case in polar tuberculoid TT patients, whereas the same cell in polar lepromatous LL cases offered a hospitable territory for the proliferation of *M. leprae*. To answer this, we needed an *in vitro* system. *M. leprae* does not grow in any culture medium and requires obligatorily a host cell for multiplication. Monocytes from human peripheral blood cells can be isolated and made to differentiate in culture to macrophages. We required a quantifiable method to determine the growth of *M. leprae*. Bacterial counts could not be employed, as the bacillus is a very slow grower and takes 13 days to divide. The bacilli form globi and the initial inoculum coming from the patients has an unknown number of living and dead bacilli. We hit on the idea of employing ^{3}H-thymidine for incorporation into DNA. In 'permissive' conditions, bacilli would make DNA preparatory to division. On the other hand, the host cell, the macrophages, lack thymidine kinase and hence would not incorporate radiolabelled thymidine (Talwar *et al* 1974a). Employing monocyte derived macrophages and lymphocytes from TT and LL patients in various combinations, we could determine that the defect in LL patients was in both macrophages and lymphocytes and in their interaction via cytokines generated to respond to *M. leprae* (Talwar *et al* 1974b). This results in a failure to recognize some key component(s) of *M. leprae*.

5. Can there be a vaccine for leprosy?

Traditionally, vaccines have been made with killed or attenuated micro-organisms. This approach was not tenable for leprosy as, inspite of dead and live *M. leprae* in the body, no immunity develops. We looked for cross-reactive mycobacteria; sixteen were investigated and their ability to cause blast transformation of lymphocytes from tuberculoid (TT) and LL patients determined. The rationale was that TT patients have an autoregressive disease; thus there should be a positive reaction with lymphocytes of these patients. However, in addition, the lymphocytes of LL patients who do not respond to *M. leprae* should respond to the cross-reactive mycobacteria. Also their ability to generate cytokines such as macrophage migration inhibitory factor (MIF) was measured. On basis of these studies, five mycobacteria got shortlisted. In the next stage, their ability to cause the Mitsuda/Dharmendra reaction, which is a delayed hypersensitivity skin reaction *in vivo* was investigated in five centres located in different parts of the country (to minimize the influence of environmental mycobacteria to which patients may be sensitized). From the ensemble of these studies an atypical, fast growing, non-pathogenic mycobacterium, bearing the code "w" was identified. Fourteen papers in the Golden Jubilee issue of *Leprosy in India* 1978 describe this work.

After pre-clinical toxicology, permissions were obtained from the Drugs Controller of India and Institutional Ethics Committee to carry out phase-I safety trials in LL patients cured by drugs. As their specific immune deficit persists, the study was to see whether by immunization with autoclaved Mycobacterium w (Mw), they became positive to *M. leprae* lepromin. Sixteen of the 19 patients converted to lepromin positivity, and the conversions were stable on retest after 9–11 months (Chodhuri *et al* 1983).

The next task was to investigate the efficacy of the vaccine. By the very nature of the disease (99% being protected naturally and 2–10 years required for the disease to manifest after infection), prophylactic trials had to be of a long duration and involve a large number of subjects. We therefore undertook immuno-therapeutic trials in multibacillary patients. When detected, such patients had to be put on a multi-drug regime (MDT) on ethical and public health grounds. Thus all patients received supervised MDT and every alternate patient was given the vaccine or a placebo intradermally once every 3 months. Trials were conducted at the Ram Manohar Lohia and Safdarjung Hospitals by Drs S A Zaheer and A K Walia under Profs H K Kar and R S Misra. These were highly systematic and thorough trials. Bacterial index (BI) determination and histopathology on each biopsy was done by two independent laboratories and a clinical

assessment was made at each stage by more than one experienced leprologists. These phase-II/III trials conducted in 388 patients in Delhi and 1100 patients in Kanpur Dehat demonstrated that the BI declined significantly faster in patients receiving the vaccine. Their clinical improvement was better. Surprisingly, no significant untoward reactions were observed. In fact the vaccine cleared the granulomas expeditiously. Figure 3 is a representative illustration of two patients receiving MDT + Mw vaccine. At the end of 24–29 months of treatment 57·3% LL patients could be released from treatment in the MDT + vaccine group, whereas their number was 11·9% on MDT alone.

What was interesting in other independent trials was the finding that with Mw vaccine, no viable *M. leprae* remained in patients skin after 6 months, whereas it took around 18 months for all *M. leprae* to be dead in patients receiving MDT alone (Katoch *et al* 1995).

These impressive results called for phase-III/IV field trials, which were conducted in over 4 lakh subjects in a

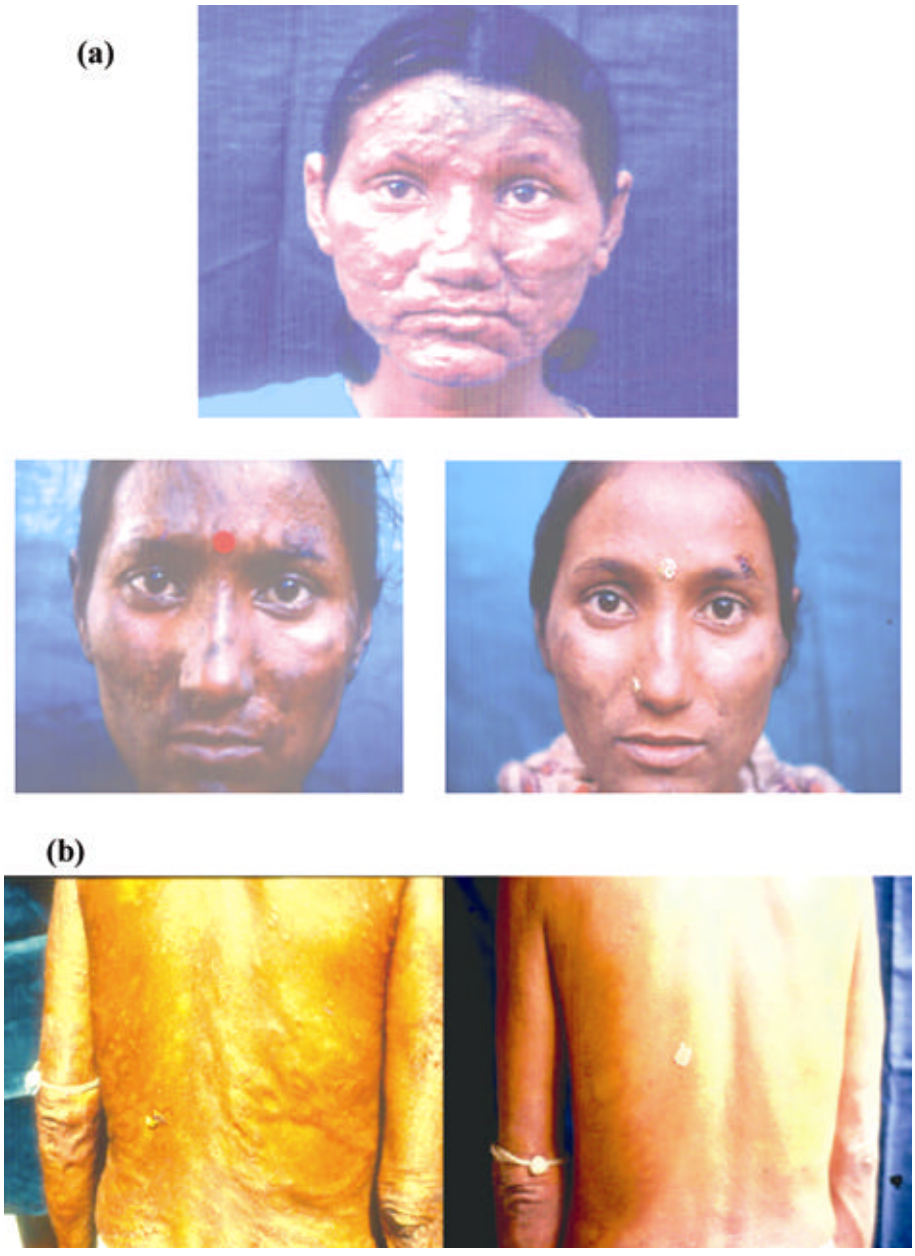

Figure 3. Effect of combining immunotherapy with chemotherapy on clinical improvement of multibacillary patients. (**a**) MD, a lepromatous leprosy patient after 4 doses of vaccine and after 8 doses of the vaccine. (**b**) RPS, a BL patient at the time of enrollment and after 2 doses of the vaccine.

community block of Kanpur Dehat as part of the National Leprosy Control Programme. These were double blind trials and the code was made and kept with ICMR. There were four groups, two of patients receiving MDT ± vaccine/placebo. Another two groups comprised the healthy household contacts of leprosy patients, who received either the Mw vaccine or placebo (1/8 dose of tetanus toxoid vaccine). Three surveys were carried out by Dr Kiran Katoch after 3, 6 and 9 years of vaccination. The vaccine conferred protection against leprosy in 68%, 60% and 28% of household contacts as per these surveys indicating a fairly good protection up to 6 years after which a booster of this killed vaccine would be desirable (Sharma *et al* 2005).

Mw vaccine is the first leprosy vaccine to reach the public. It has received the approval of the Drugs Controller General of India for marketing. It is made by M/s Cadila Pharma and is marketed as "Immuvac". Immuvac has also the approval of the US Federal Drugs Administration.

6. Ancilliary benefit of the vaccine

While transferring the technology to Cadila, the company asked for a biological assay to confirm that it was Mw and not a mutant that had been grown. A Ph.D. student of ours, Indira Guleria Singh, had done studies during my time at NII (see below) on 4 genetic strains of mice, 2 of them Balb/c and C57BL/6 were responders and 2 others C_3H and CBA non-responders to BCG. Mw vaccine however protected all 4 strains of mice against tuberculosis (Singh *et al* 1991). We also noted that guinea pigs, highly sensitive to tuberculosis, can resist challenge with *M. tuberculosis* $H_{37}Rv$ on prior immunization with Mw. This is a test routinely performed by Cadila on the batches of Mw grown by them. Immuvac accelerates significantly the clearance of acid-fast (presumably tubercular) bacilli in the sputum of the patients. More interestingly, the benefit is more seen in chronic and relapse cases. Based on these preliminary results, the Department of Biotechnology has funded a multi-centre trial for the utility of employing Immuvac for tuberculosis.

7. Necessity to persevere and withstand onslaughts

My work in leprosy and also on beta hCG vaccine (see below) was faced with innumerable hurdles. The "Crab Syndrome" is often cited, where you are pulled back at various stages of your work by your own peers. Phase-I trials of the Mw vaccine were delayed. Newspapers published, with prominent headlines, that Mw was in fact a different bacillus and I was guilty of unethical practices. None of these charges have stood the test of time, but they were distasteful. Gene sequencing was to reveal that Mw is distinct from 33 other mycobacteria listed in the Data Bank (Reddi *et al* 1994).

8. The National Institute of Immunology

As head of the Jawaharlal Nehru Fund, Prime Minister Indira Gandhi used to meet the Jawaharlal Nehru Fellows for about half a day every year. She used to listen to what we were doing and ask for suggestions on what could be done to promote science and other scholastic endeavours in the country. I made a case for immunology, specially the beneficial aspects of the subject. Just the introduction of a handful of vaccines for immunization of children has brought down drastically the infant mortality. More vaccines have to be developed for infections prevalent in the country, and also diagnostic assays, for which hybridoma technology has ushered in a new era. She asked for a proposal. I remember to have written the proposal for a "Centre for Immunology" during a transatlantic flight, helped by lavish servings of champagne and caviar. Mrs Gandhi sent the proposal to her advisor Prof. M G K Menon, who consulted many experts. He also referred the proposal to the then Director-General Indian Council of Medical Research, Prof. V Ramalingaswami, as well as the Director-General of the Indian Council of Agricultural Research. All assessed the proposal positively and added to its scope. This is how the National Institute of Immunology proposal blossomed. I was asked to join as the first Director. I did so in an honorary capacity, retaining my professorship at AIIMS till I could, because I needed a working laboratory base. I was insistent on locating NII in a University. Jawaharlal Nehru University in Delhi allotted us a piece of land sandwiched between the Vice-Chancellor's residence and the Institute of Mass Communication. It had rocks and ravines with three rain water drains. Many considered me crazy for accepting this site, instead of getting several acres across the Jamuna river. The Institute was inaugurated by Prime Minister Rajiv Gandhi on October 6, 1986. In less than 3 years, the main central building, auditorium, experimental animal house, primate facility, hostel for post-graduates, and 20 residences for faculty were built and made functional. This may well be the fastest pace at which a composite research institute has been built, nationally or internationally, without compromising on quality of construction. To achieve this a number of strategies were adopted. To save costs, no leveling was done. Innovatively designed buildings would hug the landscape. The environment was vastly improved by planting trees. Water harvesting and reuse of water for vegetation was done. Hume pipes were laid in drains to create space for resi-

dences and other amenities. Electricity was drawn from more than one grids to minimize central breakdowns in supply. Generators and stabilizers were installed to ensure uninterrupted energy supply of constant voltage and frequency. A non-Central Public Works Department route was taken to reduce the cost and also assure a good quality of construction.

We created about 30 positions of staff scientists. Each had the inbuilt provision of rising to the highest level without the necessity of obtaining fresh sanctions. I had to argue and insist on this structure. I thought it would discourage politics and internal rivalries and encourage merit and performance as the yardstick to move upwards. Thanks to an enlightened expenditure secretary, Mr Eswaran, and the visionary support given by Prof. M G K Menon, these regulations were accepted, as was the recommendation that the salary and status of the Director, NII be equivalent to those of a Secretary to the Government of India. Each scientist was initially appointed on contract for 5 years. His or her performance was to be reviewed in the 4th year and for those on a fast track, the review could be requested after 3 years. Five floating positions were created for scholars, who were selected on a highly competitive basis, so as to accommodate them as staff scientists after they had completed their Ph.D. Invariably the scholars would go abroad as Post-doctoral fellows. The floating positions were meant to give them a place to return and remain active before they found better positions in other institutions, universities or industry.

NII was planned in its staffing pattern and facilities as a multidisciplinary Institute, offering inhouse, the possibility of inter-laboratory collaboration for taking a research lead to a potential product. Laboratories with competent staff were established for recombinant DNA technology, microbiology, virology, reproductive immunology, embryo biotechnology, structural biology, organic biochemistry, molecular genetics and product development. Scientists with productive backgrounds and high caliber contributions in each field were the initial appointees. Rather than deprive Institutions within India of their talented staff, the opportunity of joining was given to Indian scientists abroad to the extent possible.

9. Immunological approaches to control of fertility

Few will disagree that population growth is a major national problem. With an annual increment of one Australia equivalent on an already large base exceeding a billion, our resources are under high stress. India has 2% of the world's land area and 16% of the population. The message of family planning has got across, but there is a need for additional, more suitable methods for persons to use. Tubal or vasal ligation are, for all practical purposes, terminal methods and are accepted only after the birth of several children (including at least one son). Intra-Uterine Devices entail extra bleeding, which already anaemic women can not support for long. Steroid pills demand daily motivation and are essentially an urban proposition. They do have side effects though acceptable for the benefit of protection they offer. Implants cause irregular bleeding and are not acceptable to all. With this background, we thought of developing a reversible method, which may require periodic intake, may not demand daily motivation, and ideally, should not disturb the normal physiology of the woman (as is the case with contraceptive steroids which block ovulation). We thought of a vaccine against human chorionic gonadotropin (hCG), a dimer made of *a* and *b* subunits. HCG is made by the woman at a very early stage of conception. Eggs fertilized *in vitro* make it. It is critical to the implantation of the embryo; marmoset embryos exposed to anti-*b*-hCG antibodies fail to implant.

b-hCG being a 'self' protein, the woman would not normally make antibodies reacting with it. To make it immunogenic, we linked it with tetanus toxoid (TT). The choice of TT was deliberate. In seventies, tetanus accounted for the largest number of deaths of women at parturition. *b*-hCG-TT was thus a double function vaccine to induce immunity against tetanus, besides mobilizing T cells to help make antibodies against hCG. The proof of concept was obtained by the demonstration that it indeed generated anti-hCG and anti-tetanus antibodies in women (Talwar *et al* 1976). The antibody response was reversible. It did not impair ovulation, nor cause any noteworthy change in reproductive and other hormones (*Contraception* 1976).

The main drawback of *b*-hCG-TT vaccine given with the mild human-use-permissible adjuvant aluminum hy-

Figure 4. The picture shows a pioneer in the vaccine field, Jonas Salk, along with Françoise Gilot, associate of Picasso and herself a known artist, and Prof. and Mrs G P Talwar at a symposium reception at Delhi.

drochloride was that it raised antibodies of respectable titre in only 25–30% of women. How could we augment its immunogenecity? As bio-effective antibodies are conformation reading antibodies, the antigen to be presented should retain the native conformation of hCG, so that the immuno-reactivity of the antibodies would be high. *b*-hCG has 12 half cysteines linked to form 6 s-s bonds; these have to be intact. For binding to the receptor on the target tissue, *b*-hCG combines with *a*-hCG and the dimer not only fits in the receptor but also transduces the signal for biological action. So ideally we should use the dimer as the antigen. However as the *a* subunit of hCG is common to the human follicle stimulating, thyroid stimulating and lutenizing hormones, it may evoke cross-reactions. We thought of employing a heterospecific (ovine) *a* subunit. The ability of *a* subunits to combine with the *b* subunit has been retained through evolution, and the ovine *a* forms a heterospecies dimer (HSD) with *b*-hCG. HSD had higher immunogenecity and superior bioneutralization capacity (Talwar *et al* 1988).

After toxicology, Drugs Regulatory and Ethical approvals, we carried out phase-I comparative safety and immunogenecity studies with the initial vaccine (*b*-hCG-TT), and the new HSD-TT (Kharat *et al* 1990; Talwar *et al* 1990).

We had thus an improved immunogen. The next and the key question was would this vaccine prevent pregnancy in women? Nobody had provided any direct evidence for anti-hCG antibodies counteracting pregnancy *in vivo*, even though the sera from women immunized in phase-I clinical studies had bio-efficacy *in vitro* systems. How much of the antibody would be minimally necessary to prevent pregnancy was unknown. By various calculations, we fixed a threshold of 50 ng/ml bio-neutralization capacity titre for determining whether at these antibody titres or higher, women are protected.

The phase-II efficacy trials on the HSD-TT/DT vaccine were undertaken in three centres. Fertile, sexually active women with at least two live children were enrolled for the trial. Many of them had had MTP (medical termination of pregnancy) done more than once as the available methods for birth control were not suitable for them and they used the methods irregularly. They readily gave their informed consent to enroll in the trial. In all, 148 women were immunized with the vaccine. None of them experienced any inconvenience. After the initial three doses, they had the option to take boosters as and when the titres declined below 50 ng/ml. Many remained in the trial for over 2½ years taking boosters now and then. All women continued to have regular cycles. These were ovulatory as shown by the luteal phase progesterone levels. Their libido remained intact. The method was highly effective and only one pregnancy occurred in 1224 cycles at or above 50 ng/ml. It was perfectly reversible, women conceived in the first cycle below 35 ng/ml (Talwar *et al* 1994). The trials were described as a landmark (Aldhous 1994).

The immunogenecity of this improved version of the vaccine was much better than the initial prototype *b*-hCG-TT. 110/148 women (~ 74%) raised antibody titres above 50 ng/ml and in 60% of them persisted for 3 months or more above the protective threshold. The adjuvants used were the permissible alhydrogel and SPLPS in the first injection only.

The feasibility of preventing pregnancy in a reversible manner was clearly demonstrated by this and follow up work (Talwar *et al* 1997; see also the Editorial on the article in the same issue). The protection was achieved without blocking ovulation or impairing bleeding profiles. The antibodies acted by interception of embryo implantation. The luteal phase was not lengthened of those who ovulated and should normally have conceived with high frequency of intercourse. 60–80% of recipients with protective titres for an infectious disease vaccine would be highly acceptable. However, for a fertility control vaccine more than 90–95% of protection is required. Thus the vaccine has to undergo further product development, although the proof of concept was largely provided.

10. Cordon ablated

The day in October 1994, when I was told politely but bluntly the decision of the governing body that my term at NII would finish on the 30th of October and that I would have to leave by the end of the month, left me feeling injured. The Institute that I had built from scratch to a high standing nationally and internationally was unable to provide me working space. The Institute authorities could not extend my emeritus position, even though I was actively involved in the vaccine project and the Institute wanted to continue further work on the vaccine. An International Development Research Centre (IDRC) Canada grant for developing the vaccine was to be left behind with the Institute; the patents for the vaccine granted in many countries of the world were of course assigned to the Institute. These thoughts troubled my mind. I was in a further quandary. With great effort, the 10th International Congress of Immunology, due in 1998, was allotted to New Delhi. This was the first time ever that this Congress was to be held in a developing country. I was the President of the Congress. I was even denied space for the congress secretariat. One day when I was going for lunch, I ran into Dr Krishan K Tiwari, at that time the Director of the International Centre for Genetic Engineering and Biotechnology (ICGEB), Delhi. He asked me why I looked grave and depressed unlike the Talwar he had known since many years. On hearing of

my predicament he offered, subject to the approval of Dr Flaschi, the Director General of ICGEB, the position of a senior consultant at ICGEB. He would provide space for the Secretariat of the Congress – in his eyes, an honour to India. I would get laboratory space but would have to fend for research money. I could join on the day after I left NII. The Rockfeller Foundation transferred the grant on Female Reproductive Health to ICGEB on the principle that their grants were given to investigators, not to institutions. Thus started another phase of my professional life.

11. Praneem polyherbal and Basant for reproductive tract infections (RTIs) and sexually transmitted diseases (STDs)

The prevalence of RTIs causing abnormal vaginal discharge (AVD) is about 30% in women attending hospitals in Delhi. A report by Bang *et al* (1989) puts the figure at 50% in rural Maharashtra. The syndrome is caused by a variety of organisms ranging from *Trichomonas*, *Gardenerella vaginalis*, *Candida*, aerobic and anaerobic microbes, viruses, *Chlamydia*, etc. Depending on the diagnosis of the causative micro-organisms, local medication and/or antibiotics are given. Facilities for diagnosis exist in only few Institutions and the treatment is mostly empirical. Could we make a formulation with a wide-spectrum action? We looked for ingredients with action on various genital pathogens. Neem (*Azadirachta indica*) leaf has been used traditionally as a general antiseptic for wounds and its twig for oral hygiene. A number of tri, tetra terpenoids have been characterized from neem with actions on viruses, fungi, microbes and nematodes. Thus neem leaf extracts were prepared and purified to a point that they do not become unstable and their spermicidal action on human sperm assessed. To expand the range and potency of action, purified saponins from *Sappindus mukerosi*, quinine hydrochloride and *Mentha citrata* oil were added to the formulation. Praneem polyherbal (PPH) inhibited the growth of *N. gonorrhea*, multi-drug resistant (MDR) urinary tract *Escherichia coli* and MDR Staph. aureus. It acted against *Candida albicans*, *C. krusei* and *C. tropicalis*. At Johns Hopkins, it was observed to prevent the transmission of Herpes simplex-2 and *Chlamydia trachomatis* by the vaginal route in progestin sensitized mice. At the Institut Pasteur in Paris, Francoise Barré Sinoussi and Annie David demonstrated its potent virucidal action against HIV-I (Talwar *et al* 2000).

Praneem polyherbal tablets have successfully undergone phase-I safety and phase-II efficacy trials. Taken intravaginally for seven nights with soap washed fingers, 95% of women with AVD get fully or partially relieved of their symptoms. Speculum examination indicates complete cure in 69% of women. The PPH tablet is now poised for phase-III large-scale trials in 10 Centres of the Indian Council of Medical Research. Meanwhile PPH has been licensed to M/s Panacea Biotech who will supply the large number of PPH tablets for the phase-III trials. They will also make available the requisite tablets manufactured under Good Manufacturing Practices (GMP) conditions for the ongoing phase-II trials at National AIDS Research Institute (NARI) Pune, where its ability to prevent the transmission of HIV-AIDS by the hetrosexual route is being investigated.

Basant is another polyherbal formulation developed by us, which is dispensed as a cream. It contains purified curcumin, amla extracts, Aloe vera, quinine hydrochloride and *Mentha citrata* oil. It has also a wide spectrum action against genital pathogens including HIV-III and human papilloma viruses. It will be undergoing trials for its ability to eleminate $HPV_{16,18}$ and other types of papilloma viruses in women with cervical dysplasia who are positive for HPVs. If found effective, this will be a valuable therapeutic for elimination of a virus which is associated with carcinoma of the cervix.

12. Registration of a non-profit Trust

I was a Jawaharlal Nehru Fellow (JNF) from December 1979 to November 1981. This unique Fellowship relieved me from the routine duties of teaching and administration and gave freedom to write, study or do research of one's own liking. I wrote a monograph, "*Immunology of Contraception*", which was published by Edward Arnold London, The Year Book in the USA and, a translation in Russia.

The pioneering work of Köhler and Milstein in 1975 on fusion of an antibody making cell with a cell capable of multiplying perpetually, fascinated me greatly and we generated a hybridoma secreting a monoclonal antibody (MoAb) against hCG. This MoAb was rated amongst the best on grounds of its high affinity and high specificity. The fellowship rules gave full rights to the Fellows of the royalties on account of scholastic work and of research done during the Fellowship years. I was thus given rights on this MoAb, which I licensed to M/s Carter Wallace/ Wampole Laboratories as the key reagent for their pregnancy diagnosis kits. To my knowledge, this was the first Biotech product from India licensed to a US company. From this, over 3 years I received the unexpectedly high royalty of US$ 780,000 after cutting US taxes. I used the amount to create a non-profit charitable Trust named the Talwar Research Foundation (TRF). The returns from the TRF corpus were initially spent on monetary support to help bright women scientists whose career is disrupted by

marriage or children, to return to research and complete a Ph.D. Over 35 women scientists have availed of TRF grants. Money has also been given for education and boarding of orphans and stipends for bright children coming from economically deprived backgrounds. The TRF has also given generously for the rehabilitation of leprosy patients.

In the late 1990's, the trustees of the Foundation (Prof. V Ramalingaswami, M S Swaminathan, R A Mashelkar and N K Ganguly besides a distinguished lawyer, R K P Shankardass, UNESCO goodwill Ambassador Madanjeet Singh, who is my old classmate and friend since the 1940s, my aunt and daughter) asked me to seek recognition for TRF as a Scientific and Industrial Research Organization which, if obtained, would enable the Foundation to claim exemption from customs duty in addition to income tax. I was told that such recognition was given only to organizations conducting research themselves. Giving fellowships to women scientists or charities to orphans and leprosy patients did not entitle the Foundation to be considered. It so happened that at that time it was decided that the laboratory space given to me in ICGEB had to be vacated to house a National Brain Research Centre. Dr Flaschi gave me several months to make alternate arrangements. Faced with imminent ouster, I wondered whether to call it a day, as far as research goes. I was 73 on October 2, 1999. My projects were midway, however, and I was reluctant to be compelled to close. I was able to convert an adversity to a challenge. I decided to build a laboratory for TRF. Using my own money I bought 500 square yards of land near my residence in Neb Valley. We started building a laboratory there at the fastest possible pace. My son Pratap Raj is an architect based in Boston. He felt that I needed more land to make a Polyhouse (polymer covered Green House) etc. and he sent me the money to buy another 700 square yards. We were functional within a year. The Department of Scientific and Industrial Research gave TRF recognition as SIRO, to be renewed every 3 years and currently valid till 2007.

13. Epilogue

It is my intention to continue teaching and research till my last days. There will be no 'retirement' from TRF. Praneem and Basant are on clinical trials and may find use in the management of RTIs and prevention of STDs including HIV, and elimination of HPV infections leading to carcinoma of the cervix. We have humanized 4 monoclonal antibodies. These recombinant chimeric antibodies are expected to be employed for therapy of advanced stage tumours, often refactory to available drugs. There is a possibility of revival of the hCG vaccine on a platform evoking immune response in 99% of women. An Indo-US grant application pertaining to it is awaiting approval. Finally we have perfected a multimer recombinant vaccine against luteinizing hormone-releasing hormone (LHRH) (Gupta *et al* 2004; Raina *et al* 2004;

Figure 5. GPT in 2004 receiving DSc (hc) from Dr A P J Abdul Kalam, President of India.

Talwar *et al* 2004). LHRH is a unique decapeptide common to both males and females which controls the generation of sex steroids (besides gametes). The vaccine may find application in hormone-dependent cancers (prostate and breast).

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