

**IMMUNOTECHNOLOGY IN INDIA\***

**BY**

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## IMMUNOTECHNOLOGY IN INDIA

Recent advances in modern molecular biology can be attributed mainly to multidirectional advances in genetic engineering and gene cloning, protein engineering, cell fusion, cell and tissue culture, synthetic chemistry, fermentation technology etc. in the areas of Immunology, Genetics, Biochemistry, Biochemical Engineering and Molecular Biology. Biological structures and functions at tissue, cellular and molecular level have been clearly understood. Advances made in Immunology are a subject of vital importance in biology and medicine. There has been an explosion of knowledge and understanding of molecular biology and genetics of immune systems. The actions and interactions of the immune cells and molecular biology of the control of the actions of these cells have been clearly understood. Its great potential for changing human conditions has greatly increased our understanding of disease and disease processes including diagnostic, prophylactic and therapeutic procedures.

With an ever expanding world population, there are new challenges for immunology which include the immunological control of fertility, effective control of infectious diseases, including AIDS, and preventive measures and strategies for fighting epidemics. Other challenges include the immunology of aging process and of wide spread diseases such as cancer, autoimmunity and allergy. Earliest application has been in the field of pharmaceuticals. Biomedical research in development of technologies of recombinant DNA and monoclonal antibodies (MAb) have resulted in rDNA-produced insulin, interferon, and MAb based diagnostic kits. In addition production of human serum albumin,

antibiotics and vaccines for viral, bacterial and parasitic diseases are the other products being pursued. Increased knowledge about hormone growth factors, immune regulators, and neurological peptides increase their importance in the treatment of disease which eventually leads to production of such regulatory proteins and in turn has the largest application of molecular biology in immunology.

#### VACCINES:

In vaccination, two of the key elements of the immune response i.e. specificity and memory are exploited since the adaptive immune system mounts a much stronger response on second encounter with antigen. Techniques have been developed for the construction and manipulation of recombinant DNA molecules which enable viral genomes to be cloned and their genes expressed, in whole or in part, in microbial or animal cell culture to provide many viral components to be used as vaccines. Till recent times vaccines development has concentrated mainly on envelop proteins but the immunological importance of other viral components is increasingly being recognised.

There has been a great potential for use of immunotechnologies in production of more effective vaccines, against various viral, bacterial and parasitic diseases through the use of new concepts of immunisation. Emerging technologies of vaccine development such as recombinant vaccinia virus vaccines (Minor and Hruby, 1990; Tartaglia and Paoletti, 1988), recombinant DNA subunit vaccines (Murray, 1988), T-cell vaccination (Cohen and Weiner, 1988), anti-idiotypic antibodies/vaccines (Thanavala, 1989) etc. have brought in new dimensions to disease control. Anti-viral cellular immunity develops following

immunisation with monoclonal antibodies to T-cell clones from a virus infected animal. Both cytolytic T-cell and helper T-cell responses are resulted from immunization with monoclonals. Recombinant vaccinia virus vaccine with expression of foreign antigens may provide the impetus for its use in the immunoprophylaxis of infectious diseases of both human and veterinary importance. Depending on cell mediated immune response.

Vaccines are already been produced for hepatitis, Herpes and other sexually transmitted diseases, influenza, mononucleosis, malaria, cholera, filariasis, leishmaniasis, schistosomiasis, trypanosomiasis, polio, rabies, measles and other diseases. It is expected that in next 1-2 years vaccines would also be available for a number of parasitic diseases which are the cause of high mortality in the developing countries.

#### MOLECULAR PROBES:

Recent advances in hybridoma technology, genetic engineering, isotopic and non-isotopic immunoassays have allowed better understanding of the molecular genetics of many disease conditions. Studies have shown that DNA hybridization, when coupled to restriction enzyme analysis, is an essential tool in the diagnosis of a constantly growing number of genetic diseases (Kingsbury, 1987). These probes have been successfully used in the diagnosis of a number of viral, bacterial and parasitic infectious diseases and plant disease agents such as viroids, viruses and mycoplasmas.

Molecular probes in solid tumor and monoclonal antibodies have emerged as the most important tools in the new generation approaches to diagnosis, prognosis and therapy of human tumors

(Alberini, 1988). Monoclonal antibodies produced by immunization of either mice or rats with the human cancer cells or their antigenic fractions, these antibodies usually interact with Fc receptors on effector cells stimulating destruction of neoplastic cells. The monoclonal antibodies labelled radioisotope material, is principally used for diagnostic purposes (imaging) but also for possible therapeutic ends. Specific molecular probes permit study of viruses in particular human cancers.

Non-radio active probes based on ribosomal RNA provide a powerful means of microbes detection which are available in abundance as cellular molecules. DNA-anti-microbial resistance genes are important in anti-microbial therapy and control of infection.

#### IMMUNODIAGNOSTICS:

Recent advances in immunochemistry, immunocytology and immunodiagnosics have resulted in development of diagnostic procedures of considerable interest. Immunodiagnostic techniques are clinically useful for assay of immunoglobulins, components of complements, soluble and insoluble immune complexes, counting of lymphocytes subsets, detection of antibodies, proteins and human leucocytes antigens etc. Recent developments in hybridoma technology, genetic engineering, isotopic and non-isotopic immunoassays etc. have led to the development of large number of immunodiagnostic techniques for the diagnosis of physiological status or pathological and communicable disease conditions of a number of viral, bacterial and parasitic diseases. These techniques are simple, accurate, specific and cost effective and have enabled improved diagnostic accuracy and better understanding of certain diseases.

In India diagnostic kits are being developed for the early detection of pregnancy; HIV; filariasis; hepatitis-B; amoebiasis; toxoplasmosis; tuberculosis; leprosy; typhoid fever; malaria; giardiasis; leishmaniasis; rotavirus; shigellosis and reproductive hormones for humans and brucellosis; foot and mouth disease; infectious bovine rhinotracheitis, blue tongue, rinderpest, rabies for animals. In addition various tumor markers are being used for detection and monitoring of a number of human neoplastic diseases. Immunoassays have also been useful for food analysis.

#### MONOCLONAL ANTIBODIES

A significant development in immunotechnology is the production of monoclonal antibodies. Mouse myeloma cells fused to B lymphocytes from immunized mice result in continuous growing, specific monoclonal antibody secreting somatic cell hybrids or hybridoma cells. Monoclonal antibodies specific for almost all antigens can be generated today. These antibodies are being widely used as drug targetting agents, specific tools in immunodiagnosics and protein purification and immunoaffinity chromatography of therapeutic proteins. Some of the monoclonal antibodies raised to synthetic analogs of an estrolytic transition state, were also found to have catalytic properties like enzymes. Immunotechnologies are being refined to help cell fusion technology to reach its potential through advances in immunization protocol by suppression of dominant response, in vitro immunization, antigen targetting; electrofusion; laser fusion; and fusomas (Neil and Urnovitz, 1988).

#### CATALYTIC MONOCLONAL ANTIBODIES:

Catalytic monoclonal antibodies (cat-MABs), with unlimited

range of recognition site structure, provide potentially an enormous repertoire of new catalytic agent, and is becoming an important biotechnological tool (Green and Tawfik, 1989). Cat-MABs have wide applications, especially where no suitable enzymes or chemicals catalysts are currently in use. Some of its potential applications are in the synthesis of organic fine chemicals, as biosensors and in protein engineering applications such as cleavage of leader sequences and other extraneous domains in recombinant proteins or for post-translational modifications of proteins.

#### BISPECIFIC ANTIBODIES:

Bispecific antibodies (BsAbs) incorporate the binding specificities of two different antibodies into a single molecule, and are prepared by chemical crosslinking, hybridoma cell fusion or genetic engineering. The utility of bispecific mAbs in immunoassays and immuno-staining lies in increasing the sensitivity of assay. Another exciting application of fusoma technology has been the generation of bivalent mAbs with specificity for the T-cell receptor complex and tumor antigens. These reagents are able to activate and direct cytotoxic T-lymphocytes to their targets with consequent efficient cytolysis. In order to use the specificity of mAbs and yet ensure activation of cellular cytotoxicity mechanisms, redirected cellular cytotoxicity mediated by BsAbs has developed as a new form of immunotherapy for treatment of cancer and infectious disease (Fanger and Guyre, 1991).

#### HYBRID ANTIBODIES:

Antibodies and growth factors when coupled to different toxins produce cytotoxic molecules that selectively kill cells bearing appropriate antigens or receptors. Using a fragment which

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contains the heavy and light chain variable domain, immunotoxins are obtained through construction and expression of single chain antibody toxin fusion protein in *E. Coli* which are homogenous, specific and more cytotoxic in nature. Using above technique it is possible, now to create active recombinant immunotoxins with a number of other antibodies. Some of the recent applications of immunotoxins have been in the treatment of diseases involving interleukin-2 receptor positive cells, allograft rejection, AIDS and epidermal growth factor.

#### T-LYMPHOCYTE:NATURAL PROTECTORS

Nature has given sufficient protection to the human body. Among natural protectors, T-lymphocytes are the most important. T-lymphocytes are responsible for cell-mediated immunity and they are identified by special receptors for antigen found on their cell membranes. These lymphocytes on receiving activation signals from a mononuclear phagocyte that bears the exact processed antigen to which this particular T lymphocyte is specific, plus the two chains of DR, gets activated and begins clonal expansion into four distinct subsets of T lymphocytes. Helper T lymphocytes stimulate enhanced function in other lymphocytes and mononuclear phagocytes. Suppressor T lymphocytes serve as controls to dampen immunologic responses of other cells. The overall function of the cell that is being influenced will reflect the overall balance between helper and/or suppressor T-lymphocyte influences. Killer T lymphocytes are antigen-specific and kill targets bearing the antigen for which the killer cell has a receptor. Memory T lymphocytes provide memory for the cell mediated component of the immune response. Following clonal proliferation to specific antigen, T lymphocyte memory cells are established.

The assessment of peripheral blood lymphocyte numbers and ratio in different diseases helps in the understanding of the relationship between blood and tissue lymphocyte, facilitating diagnosis and is of immense therapeutic value (Westermann and Pabst, 1990). It provides a basis for developing new vaccination strategies and for predicting the spread of lymphoid tumors (Pals et al., 1989). Lymphocytes have been used as vehicles for radiating isotopes and toxic substances, to achieve tumor regression, including the intravenous use of lymphokine activated killer cells and tumor infiltrating lymphocytes (Kradin et al., 1989).

#### LYMPHOKINES:

Within a few days of nonself invasion, the T lymphocytes of an immunized individual surround the nonself invader and begin arranging for its destruction directly (via killer T lymphocytes) and indirectly (via non-specific killer cells, mononuclear phagocytes, PMN and NK lymphocytes). A variety of signal polypeptides are secreted by T lymphocytes that travel to other cells to perform specific functions. The biological response modifiers (BRM) that are known to be made by T lymphocytes are called lymphokines. These lymphokines are capable of directing the function of other cells in the area where nonself invasion is occurring. Examples of lymphokines include: (i) gamma interferon (IFN), which causes mononuclear phagocytes to have enhanced killing function; (ii) Interleukin 2, which acts as a growth factor for T lymphocytes, causing increased cellular proliferation; (iii) B-cell Growth Factor (BCGF), which causes B lymphocytes clones to proliferate and (iv) Lymphotoxin (LT), which is secreted predominantly by killer T lymphocytes and can

cause tumor target cell death. All these substances have important role to play.

The studies on the role of T-cell subsets and cytokines in regulation of infection have provides valuable information. The division of CD4 T-cells into distinct T-helper I and II cells based upon cytokine production, has helped understand the immune responses, particularly those with infectious diseases. The nature of immune response associated with stimulation of a particular T-helper subset however, depends upon the specific pathogen. It is influenced by the antigen, concentration of the antigen, route of antigen administration as well as by the antigen presenting cells (Scott and Kaufmann, 1991). CH8 cells also play a central role in protective immune response against viral, bacterial and protozoal infections. Most of the CD8 T cells express cytolytic activities and produce gamma-interferon. In addition, Human gamma-delta cells also play an important role in anti-bacterial activity. These cells produce Tumor Necrosis Factor and gamma interferon and express cytolytic activity.

#### DRUG DELIVERY:

Majority of new generation of therapeutic drugs, are peptides or proteins which are easily broken down by the body unless they are protected in some way. New technologies for delivering drugs, however, are addressed to the need of maximising the time the drugs's active ingrediants are present (Brunt, 1989). With the exception of monoclonal antibodies, most of the new delivery vehicles are synthetic. Some new drug delivery systems include: Bioerodable non-toxic synthetic polymers: Transdermal medicaments; Microsponges; and Liposomes

## IMMUNOLOGICAL APPROACHES TO CONTRACEPTION

Efforts continue to develop safe, efficacious cost-effective, long lasting and reversible contraceptive vaccines using immunological approaches (Talwar and Raghupathy, 1989). Vaccines are under development for the control of fertility in males and females against Follicle Stimulating Hormone (FSH), Leutinising Hormone (LH), egg antigens, human chorionic gonadotropin (hCG), GnRH and sperm antigens. Candidate vaccines based on hormone human chorionic gonadotropin, follicle stimulating hormone antibody and vitamin carrier proteins are under trial in India. Vaccines inducing antibodies against hCG are undergoing phase II clinical trials after undergoing phase I clinical trials in females with satisfactory results at NII. The oFSH vaccine developed at IISc, Bangalore has entered clinical at four different centres in the country after successful experiments in animals. The vaccine with vitamin carrier proteins being developed again at IISc is still under experimental stage. Research to identify sperm antigens for incorporation into second generation vaccines is in progress at NII.

### TRANSPLANT TOLERANCE:

Recent application of recombinant DNA technology to studies of the major histocompatibility complex (MHC) has not only yielded important information about the structure of these genes, but has also raised the possibility of manipulating the immune response to transplanted tissue via genetic engineering (Sundt and Sachs, 1988). Studies have been carried out in which MHC genes have been introduced into bone marrow stem cells in an attempt to induce specific transplant tolerance across MHC barriers. Grafts survival has been prolonged by pretreatment with

whole blood, lymph node cells, splenocytes, bone marrow, purified erythrocytes, platelets and subcellular membrane preparations bearing specific antigens.

#### IMMUNOLOGICAL TOLERANCE:

. There has been a rapid advance in the understanding of the phenomenon of immunological tolerance and the mechanism by which it is achieved. In this context, the study of the products of the minor lymphocyte stimulating genes, nick named "superantigens" has been more pronounced since they stimulate an extremely large fractions of T cells (Robey and Urbain, 1991). T cells recognise these genes in combination with major histocompatibility complex (MHC) class II molecules to undergo deletion or anergy or even clonal excretion. Small resting B cells, as also the tolerant B cells that lack the costimulatory signal, can anergize T cells. Suppressor cells could therefore be anergic cells themselves and the persistence of anergic lymphocytes could serve to maintain the state of tolerance.

The extraction of naturally processed peptides from MHC Class-I glycoproteins has paved the way for a major advance in the understanding of the antigen processing pathway that ultimately induces cytotoxic T-cell responses (Rotzschke and Falk, 1991). Naturally occurring peptide antigens derived from the MHC Class-I restricted processing pathway.

#### CYTOKINES AND TUMOR THERAPY:

Developments in the field of cellular immunology and recombinant DNA technology have suggested new possibilities for effective immunotherapy of cancer in man. Isolation, genetic modification, expression and purification of lymphokines and growth factors have enabled more selective approaches to

stimulate anti-tumor immune reactions in the tumor-bearing host (Rosenberg, 1988; Packard, 1989).

#### T-Cell TARGETED IMMUNOTHERAPY:

There has been major advances in immuno intervention strategies in allergic, inflammatory and autoimmune diseases through specific targeting of T-Cells. Different approaches to using peptides for immune regulation have been described which include approach to the specific modulation of T-Cell activity through T-Cell receptors, which recognize peptide epitopes bound to MHC class-II molecules (Feldman et al., 1992). Anti-cytokine therapy, particularly directed against interleukin-I (IL-I) and tumor necrosis factor alfa (TNF-alfa) provide yet another approach to immunotherapy. Anti-T-Cell antibodies like the use of anti-CD4 and/or anti-CD8 is already being applied to the treatment of rheumatoid arthritis.

#### APPLICATION IN NEW TECHNOLOGIES:

**Polymerase Chain Reaction:** Using the technology of Polymerase Chain Reaction (PCR), DNA target fractions can be amplified which are detectable by a probe (Hayden et al., 1991). The polymerase chain reaction is an in vitro method for producing large amounts of specific DNA fragments of defined length and sequence from small amounts of a complex template. Specific target fragment can be increased upto several millionfold in a few hours. This rapid, sensitive and automated amplifications reaction can be combined with simple non-radioactive methods for detecting the amplified target sequences and, therefore promises to play a crucial role in all DNA based diagnostics procedure, including diagnosis of cancer and infectious disease. In addition Polymerase Chain Reaction finds application in (a) altering of

sequences (b) selection of DNA sequence (c) amplifying external sequences and (d) complementary DNA amplification. Thus, the emergence of a new technique has transformed the approach towards fundamental and applied biological problems.

**DNA finger printing:** There is one region of the genome of the human and some other vertebrate species that is genetically unique in every individual (except identical siblings, clones, etc.). This segment of the genome is called satellite DNA which can be isolated by a cDNA probe and then examined in further details by RFLP analysis, known as DNA finger printing. The technique has a great potential in fields of agriculture, animal husbandry and social problems like medico legal cases especially disputed parentage linkage, blood relatedness, and analysis in disease condition etc. DNA finger prints are stable and transmitted from generation to generation.

#### INDUSTRIAL TECHNOLOGIES:

**Immunoaffinity chromatography:** Since now pure monoclonal antibodies to almost all proteins are available, their use in immunoaffinity chromatography for purification of therapeutic proteins has revolutionised the healthcare on the whole. Proteins of potential therapeutic value which have been purified by immunoaffinity chromatography on monoclonal columns include Human interferons, Interleukins, Human factor X, Tissue plasminogen activators, recombinant hepatitis B antigen etc. (Jack and Wade, 1987).

#### BIOSENSORS

In the area of bioelectronics, technologies have been used to develop improved biocensors or new conducting devices called biochips. A number of biosensors which use enzymes for

detecting specific substances are already in use. More versatile sensors which use enzymes or monoclonal antibodies are under development. These sensors would be specially useful in control of industrial bioprocesses (Turner, 1990).

#### ANIMAL HUSBANDRY:

Biotechnology offer new opportunities for genetic improvement of traditionally important livestock. Fish production and prawn aquaculture through biotechnological innovations help in production of fish with better growth rate. Current rDNA projects include transgenic chickens, pigs, cattle and fish with growth hormone genes that promote faster growth and larger animals, cows that excrete pharmaceutically valuable products in their milk, and silkworm caterpillars that produce human insulin.

In animal and veterinary products, biotechnology is being used to develop products similar to those being developed in the pharmaceutical industry. Since agricultural products are not of high value, products are limited to animals such as pets and breeding stock only. However, most important products are likely to be vaccines and growth promotants. The regulatory requirements for animal health products, especially for vaccines and diagnostics, are significantly less stringent than for human health products. Distribution and delivery systems are different. Because of these features veterinary products have become an attractive field for application of biotechnology.

#### CONCLUSION:

Advances in modern biology, specially in relation to development of new technologies in the areas of immunology have far reaching effect in improving the general condition of health

in developing countries through better vaccines, diagnostic techniques and improved drug delivery systems and the understanding of disease and disease processes. Development and application of immunotechnologies have provided us an insight to the knowledge of basic immunology, processes unique to the immune systems, the discrimination between the self and nonself and response to foreign antigens, cellular interactions that regulate immune system and studies on receptor properties of major histocompatibility complexes etc. New challenges facing immunology with an expanding global population include new tools for immunological control of fertility, effective control of infectious diseases including AIDS, control of epidemics, new approaches to the control of cancers, autoimmune diseases, immunodeficiencies and transplantation etc. In order to achieve meaningful results and applying them to large scale benefit to the community, it is necessary to create facilities and capabilities for basic and applied research in immunology and allied sciences on multi-disciplinary integrated basis.

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