

# 〔海外ラボ紹介〕 Dr. Narinder Mehra研究室

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## History

The HLA activity at the All India Institute of Medical Sciences (AIIMS) started in the early 1970's with initiation of studies on the immunogenetics of leprosy and publication of our first paper in 1975 in Tissue Antigens. By 1977, a full fledged Histocompatibility (HLA) laboratory was established in the Department of Anatomy and the first training course was conducted with Prof. M.C. Vaidya as its convenor. After spending a period of two years as Pool Officer, Prof. N.K. Mehra was appointed as Lecturer in 1979 to look after the clinical and research activities of the laboratory. The laboratory continued to thrive while being associated with the Department of Anatomy, extending hospital service to patients not only from AIIMS but from all over the country and even neighbouring countries. Being the only recognized set up in India, the laboratory served as a "referral centre" in the region and as 'Core laboratory' from India in several International Histocompatibility Workshops. In 1993, Prof. Mehra was promoted to the full Chair of Professor and the HLA laboratory was upgraded into the Department of Histocompatibility and Immunogenetics of AIIMS.

The research activities of the department reflect numerous developments in the field. Research in early years mainly involved looking for association of HLA class I and class II alleles with various diseases, identification of HLA linked control of susceptibility to leprosy and tuberculosis, studies on the role of HLA matching in renal transplantation, analysis of HLA antigen distribution at the population level and

technology development. During more recent years, when the important immunobiological function of the HLA molecules has got established, the field of research has been expanded to encompass studies of HLA restriction of immune response to mycobacterial antigens, sequence analysis of risk or protection conferring genes in various diseases, hunting for 'new alleles and novel haplotypes' in the Indian population, establishment of the unrelated donor marrow registry of voluntary donors and HLA-DNA testing for paternity determination.

## Department Structure

The Department of Histocompatibility and Immunogenetics at the AIIMS has four components:

1. **Clinical Immunogenetics:** Hospital service for patients requiring organ and bone marrow transplantation, molecular HLA matching and investigations into immunological predictors of graft rejection. HLA as a diagnostic tool in several autoimmune and rheumatological diseases, forensic service for paternity determination etc. On an average, more than 2,000 patients and their families are tested each year. In 1994, the department established the Asian Indian Donor Marrow Registry (AIDMR) which has functional links with major international voluntary donor registries and is a part of the World Marrow Donor Association.
2. **Technology Development:** Establishment of molecular methods of detecting HLA gene products, PCR-SSOP and PCR-SSP techniques, flowcytometry

cross matches for transplantation, sequencing of disease associated HLA genes, ELISA based immunological screening for prediction of graft rejection, cytokine analysis etc.

3. **Molecular Genetics** includes the study of genetic diversity of HLA using DNA based technologies, the detection and characterization of 'peptide presenting MHC molecules and susceptibility genes, and their role in controlling immune responsiveness in select disease conditions which include mycobacterial diseases like leprosy and tuberculosis, autoimmune disorders, rheumatological and cardiovascular diseases. In addition, studies have been directed to understand the immunobiology of live related donor kidney transplantation, and allogeneic bone marrow transplantation. In recent years, polymorphism at non classical HLA loci (HLA-E, F, G) as well as Hfe locus has been studied. The latter is particularly important in disorders of iron metabolism.
4. **Molecular Immunology:** Cytokine analysis and immune polarization study of TH1 and TH2 cells in healthy and disease states, immunological aspects of organ and bone marrow transplantation, molecular medicine approaches for diagnosis and treatment of infectious and autoimmune diseases.

#### Training courses conducted

- 1977 WHO sponsored 'First Training Workshop on HLA' Dec 15-17, 1977 (14 participants, 5 from South East Asian countries)
- 1979 WHO sponsored 'Second Workshop on HLA', Dec 10-19, 1979 (14 participants with 6 from South East Asian countries)
- 1989-90 Training Workshop on HLA-DNA Technology (DBT Sponsored) Dec 31, 1989-Jan 13, 1990 (15 trainee participants)
- 1996 National Workshop on 'Techniques in Donor Selection during Transplantation' (DBT sponsored), Feb 5-17, 1996 (15

participants)

#### Conferences/symposia conducted

1. First Asian Symposium on HLA in health and disease, New Delhi, December 23-24, 1977.
2. Second Asian Symposium on HLA in health and disease, New Delhi, December 20-21, 1979.
3. International Symposium on HLA : Current State of the Art, New Delhi, December 6-8, 1993.
4. 6th Asia-Oceania Histocompatibility (6AOH) Conference and Annual Scientific Meeting of the Australasian and South East Asian Tissue Typing Association (ASEATTA), New Delhi, October 28-30, 1998.
5. Indian Society for Histocompatibility and Immunogenetics (ISHI).

The Department took the initiative to create the Indian Society for Histocompatibility and Immunogenetics (ISHI) with the basic aim to promote encouragement and assist the study of the Histocompatibility, Immunogenetics and Transplantation Immunology in the country. The inaugural scientific meeting of ISHI is to be held at the AIIMS, New Delhi, March 1-4, 2001 with Prof. N.K. Mehra as the 'Founder President' of ISHI.

#### Important Awards and Honours

The work of the Department has been recognized nationally and internationally. Some of the important honours include the prestigious Shanti Swarup Bhatnagar Awards of the CSIR, Ranbaxy Science Foundation Award, several awards from the Indian Council of Medical Research and major scientific organizations of the country.

At the international level, the HLA activity of the Department has been recognized by the Histocompatibility organizations. Prof. N.K. Mehra has served as President of the Australasian and South East Asian Tissue Typing Association (ASEATTA), President of the 6th Asia-Oceania Histocompatibility (6AOH),

Secretary General and Vice President of the Federation of Immunological Societies of Asia-Oceania (FIMSA).

Teaching and Training of immunology and clinical immunogenetics to undergraduate and postgraduate medical and science students. Short term training for 2-3 months period is also provided.

### Graduate and postgraduate thesis

A large number of postgraduate and postdoctoral students have written their thesis in the Department.

These include:

Ph.D :18, MD/MS : 22, DM/Mch : 6, M.Sc : 3

The department has developed active links with

several clinical specialities of the AIIMS and other institutions in the country. Active International collaborators include Leiden University Medical Centre, Holland; Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, Holland; Fred Hutchinson Cancer Research Centre, Seattle USA; University of Western Australia, Perth, Australia; National Institute of Hematology and Blood Transfusion, Budapest, Hungary; Tokai University School of Medicine, Isehara, Japan and many more.

Scientific publications : 255

Books/monographs : 3



## Department of Histocompatibility and Immunogenetics All India Institute of Medical Science

The laboratories of the Department of Histocompatibility and Immunogenetics of the AIIMS, New Delhi are well equipped to undertake a range of activities including:

1. HLA Serology
2. PCR based DNA technologies
3. Flowcytometry
4. ELISA tests
5. Cell Culture Work
6. DNA Sequencing
7. Cellular Immunology parameters

The laboratories have a dedicated computer network with need based programmes that have been developed over the last ten years. All laboratory records are stored using electronic and/or paper media and are available for reference in accordance with current regulations. A specialized feature of the Department is its operational and functional links with several clinical departments of the AIIMS and development of 'clinical immunogenetics services' for the benefit of several categories of patients.

### Laboratory Tests/services provided

The current range of laboratory tests for hospital service undertaken by the department are given below:

#### 1. Serological Phenotyping Tests

- 1) HLA-ABC phenotyping  
Serological typing of HLA Class I (A, B & C) specificities using the complement dependent cytotoxicity (CDC) assay for the purpose of HLA typing in transplantation, disease association and platelet transfusion.
- 2) HLA-DR & DQ phenotyping  
Serological typing of HLA Class II (DR & DQ) specificities by methods and purposes as in (1).
- 3) HLA-ABC DR & DQ genotyping  
By methods as in (1) and (2), performed on family members for the purpose of determining

segregation and identification of HLA haplotypes, for use in HLA matching for living related transplantation of solid organs or marrow.

#### 2. Serological Crossmatching and Antibody Screening Tests

- 1) Lymphocyte crossmatch  
Complement dependent cytotoxicity crossmatch (CDCXM) to detect donor-specific cytotoxic antibodies in recipient sera using donor lymphocytes.
  - 2) T-Lymphocyte crossmatch  
As in (iv) but using purified donor T-lymphocytes.
  - 3) B-Lymphocyte crossmatch  
As in (iv) but using purified donor B-lymphocytes.
  - 4) Autologous lymphocyte or T or B lymphocyte crossmatch  
CDCXM using recipients own lymphocytes, or separated T or B lymphocytes and sera, to detect autoreactive lymphocyte-specific antibodies.
  - 5) DTT crossmatch  
CDCXM using DTT (dithiothreitol) treated sera to determine the IgG or IgM class of the antibody.
  - 6) Flow cytometry crossmatch (FCXM)  
Sensitive crossmatch using donor lymphocytes and recipient sera using a fluochrome labelled second antibody in order to detect IgG donor-specific antibodies against T and/or B cells.
  - 7) Panel Reactive Antibody (PRA) screening for HLA Class I and/or Class II antibodies: using random cell panel  
CDC assay to determine the presence of HLA-A, -B and -Cw and/or HLA-DR, -DQ antibodies using a randomly selected panel(s) of cells. Useful for organ and marrow transplant patients.
- #### 3. DNA-based phenotyping tests
- 1) HLA Class I DNA typing: low resolution by PCR using sequence specific primers (PCR-SSP)  
Determination of HLA-A, -B, -Cw alleles or groups of alleles by DNA analysis with a range of

PCR primers giving definition comparable to serological typing, for the purpose of HLA typing in transplantation, disease association and platelet transfusion.

- 2) HLA Class I DNA typing: high resolution by PCR using Reverse Line Strip Hybridization (RLS) technique  
Determination of HLA-A, -B, -Cw alleles by DNA analysis with a range of PCR primers or sequence based methods giving high resolution definition for the purpose as in (xi).
- 3) HLA Class II DNA typing: low resolution  
Determination of HLA-DR, -DQ and DP alleles as in (xi).
- 4) HLA Class II DNA typing: high resolution  
Determination of HLA-DR, -DQ and DP alleles as in (xii)

#### 4. Additional Tests Conducted

- 1) HLA testing for paternity determination  
Depending upon the case history, tests in Sr.Nos. i) to iii) and xi) to xiv) are conducted. Only these cases that are specifically referred by honourable courts are accepted.
- 2) HLA-B27 typing  
Determination of HLA-B27 (positive or negative)
- 3) HLA-B27 molecular subtypes  
Differentiation of the common HLA-B27 subtypes B2701, B2702, B2704, B2705, B2706, B2707 etc by PCR-SSP technique.

#### 5. Cellular Immunology

- 1) Immunophenotyping of lymphocytes  
Quantitation of T cell subsets (CD4, CD8) and B cells by Flowcytometry.
- 2) Determination of lymphokines  
Like IL-2, IL-4, IFN- $\gamma$  (and other lymphokines using intracellular cytoplasmic staining and flowcytometry - determination of TH1 and TH2 cells.
- 3) Cell culture assays

#### 6. Additional Specialized Tests

- 1) Testing for HFE gene mutations associated with iron disorders  
Identification by PCR using sequence specific primers (PCR-SSP), the Hfe (HLA-H) alleles (encoding for Cys282Tyr and His63Asp mutations) that show a strong association with haemochromatosis and other iron disorders.
- 2) HLA allele typing by DNA sequencing
- 3) Post BMT chimerism studies to evaluate donor marrow engraftment

#### 7. Asian Indian Donor Marrow Registry (AIDMR) Service

A comprehensive service for the provision of potential unrelated bone marrow donor transplants including searches for prospective donors plus appropriate advice on donor selection from the AIDMR and all International panels that are part of Bone Marrow Donors Worldwide. We can liaise and co-ordinate requests from the appropriate panels and undertake confirmatory typing plus any additional typing and other tests deemed necessary. The AIDMR currently has data on nearly 2000 voluntary donors of Asian Indian ethnic origin.

#### 8. Organ transplantation using cadaveric donors

Provision of a 24 hour, 365 days / year service for the typing, patient selection, HLA matching and cross-matching of cadaveric donors, includes:

- \* HLA-A, -B, -C, -DR, -DQ typing on peripheral blood or donor spleen.
- \* Organ matching and patient selection information including: mismatching grade, clinical urgency, transfusion status, previous transplant history, current antibody status, matching status, preliminary cross-matching results on sensitized patients.
- \* Preliminary donor/recipient cross-matching of sensitized (>49% PRA) patients (most recent serum).

- \* Telephone and fax report of phenotype, cross-matching and patient selection information to local transplant coordinators/surgeons.
- \* Post-transplant monitoring of patient's antibody status as required.