

## **MICROBIOLOGY/ IMMUNOLOGY LABORATORY**

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- Group Members** : **Dr. Sangita Rastogi, Scientist ‘E’**
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- Collaborators** : **Dr. Sudha Salhan, Dr. Banashree Das, Dr. Jagdish Prasad, Dr. RK Jain, Prof. VK Sharma, Prof. Geetika Khanna, Dr. Sumit Batra, Safdarjung hospital, New Delhi. Dr. GP Talwar, Talwar Research Foundation, New Delhi. Dr. BC Das, ICPO, Noida. Dr. MM Misro, MOHFW, New Delhi.**
- Technical Staff** : **Madhu Badhwar, Asha Rani, Ajit Lehra, Kamal Dev**

**The Microbiology laboratory** receives samples from female patients attending Department of Gynaecology, Safdarjung hospital, New Delhi, with complaints of discharge, lower abdominal pain, cervicitis, PID and infertility for diagnosis of *Chlamydia trachomatis* by culture, PCR, Elisa and direct fluorescent assay. Further diagnosis for other sexually transmitted pathogens including *Candida* species, *Trichomonas vaginalis*, bacterial vaginosis, *Ureaplasma urealyticum*, *Mycoplasma hominis* and TORCH is performed by microscopy/ culture/ Elisa in these patients’ samples. In addition, endometrial curettage tissue samples are received from spontaneous aborters attending Department of Obstetrics & Gynaecology, Safdarjung hospital, New Delhi for the diagnosis of *C. trachomatis*. Further, the laboratory also receives synovial samples from reactive arthritis/ undifferentiated spondyloarthropathy patients attending CIO at Safdarjung hospital, New Delhi for the diagnosis of *C. trachomatis*. In addition, blood and tissue samples are received from coronary artery disease patients attending Department of Cardio-Thoracic and Vascular Surgery, Safdarjung hospital, New Delhi for detection of *C. pneumoniae* by nested PCR, real time PCR and Elisa.

**TECHNOLOGIES DEVELOPED:  
MICROBIOLOGY/ TISSUE CULTURE/ HYBRIDOMA LABORATORY:**

- ❖ Development of monoclonal antibody based diagnostic assay for detection of *C. trachomatis* using hybridoma technology.
- ❖ Development of dot blot assay using chlamydial heat shock protein 60 for detection of sequelae to *C. trachomatis*.
- ❖ Development of quantitative nested PCR for detection of *C. pneumoniae*.
- ❖ Development of an experimental model of *C. trachomatis* induced salpingitis in mice.

**For improving reproductive health of women, in-depth studies were initiated on genital *C. trachomatis* and attempts were made** towards development of an indigenous diagnostic assay and for understanding the immunobiology of *C. trachomatis*, an important sexually transmitted microorganism that was ignored till then.

**RESEARCH AREAS:**

The research is focused on female genital chlamydial infection with emphasis on the following:

- **Improving diagnosis of genital *C. trachomatis* infection.** Finding **reliable prevalence** of *C. trachomatis* in our country by **using various gold standard** diagnostic assays, chiefly, culture and later on molecular methods like PCR.
- Genotyping of *C. trachomatis* by PCR and RFLP to **know the prevalent serovar** in our region and to identify serovars of high pathogenecity.
- Development of indigenous diagnostic assays for improving diagnosis of *C. trachomatis* from Indian strains of *C. trachomatis*. The developed monoclonal antibody based diagnostic assay is going to the industry for developing a diagnostic kit. Further, development of dot Elisa for detection of antibodies **to chlamydial heat shock protein 60 has been completed** which will help in prognosis of sequelae to *C. trachomatis* in women.
- In addition to establishing culture technique for growing *C. trachomatis*, our laboratory was the **first in India to determine and publish the morphological criteria for localization of intracellular *C. trachomatis* inclusions in cervical smears stained with giemsa.** This technique for diagnosis of *C. trachomatis* has the additional advantage of being a rapid method for mass screening a large number of women in field settings in our country where sophisticated methods like culture and morphology techniques cannot be used for obvious reasons.
- **Many doctors from hospitals, scientists and researchers from R & D institutes, pharmaceuticals like Ranbaxy, etc. and technicians have also been trained in *Chlamydia* research which included culture methodology and evaluation of slides for morphological assessment of *C. trachomatis* infection.**
- Another focus has been to develop new microbicides with the ability to prevent sexual transmission of *C. trachomatis* as treatment (doxycycline, azithromycin) as failures and

recurrent chlamydial infections are common in India. In this regard, the **antimicrobial activity of novel polyherbal formulation BASANT against *C. trachomatis* was assessed and the results suggested its potential clinical utility for the prevention of *C. trachomatis* infection by the sexual route.**

- **To understand mucosal host immune responses locally in persistent and recurrent *C. trachomatis* infection in order to have a mechanistic understanding of immunopathogenesis of *C. trachomatis* infection which will help in vaccine development against *Chlamydia*.**
- Studies are being undertaken on the association of *C. trachomatis* infection with **adverse obstetric outcome**, viz.: stillbirths, preterm delivery, LBW, etc. Research work was done with particular emphasis on immunological mechanisms responsible for early stage pregnancy loss leading to spontaneous abortion in women infected with *C. trachomatis* as this has clinical relevance for better prognosis and management of miscarriage, particularly in recurrent spontaneous aborters. The other focus is on cytokine production, chsp60 expression, oxidative stress, cyclooxygenase production and prostaglandin synthesis in response to *C. trachomatis* infection in women with failed pregnancy as these factors interfere with embryo implantation/ regulatory mechanisms that protect the foetus from attack by the maternal immune system.
- **Animal experimentation:** *C. trachomatis*-induced salpingitis is one of the most significant complications in women because of its impact on infertility. However, human studies on *C. trachomatis*-induced salpingitis/ infertility have limitations in terms of repetitive sampling of tubal tissues due to which, studies on the early stages of the inflammatory response are difficult. Hence, ***in vivo* experimental model of salpingitis was developed in C3H/ Jax mice by unilateral genital inoculation with human serovar of *C. trachomatis* isolated from our patients.**
- ***C. trachomatis*-associated reactive arthritis:** *C. trachomatis* has emerged as a major STD worldwide including India and its association with Reactive Arthritis (ReA) has further implications on human health. Although ReA induced due to enteric pathogens has been reported from India, yet, to the best of our knowledge, studies on ReA patients with symptoms suggestive of genitourinary infection (gReA) due to *C. trachomatis* are scanty. The research study proposes to understand the magnitude of the problem so that effective therapeutic strategies can be planned. Intra-articular diagnosis of *C. trachomatis* MOMP/ DNA in ReA patients is currently underway. This will pave the way for a better understanding of the mechanism of joint pathogenesis elicited by persistent *Chlamydia* at that site.
- ***C. pneumoniae* in coronary artery disease:** Coronary Artery Disease (CAD) is a major cause of morbidity and mortality in humans and is predicted to be the leading cause of death in the world particularly in developing countries such as India. *C. pneumoniae* has a large amount of factual data that suggests that the organism plays a contributory role in atherosclerosis. Therefore, we initiated our study in this direction and detected high prevalence of *C. pneumoniae* (29.6%) in CAD patients in India. We also found that the seropositivity for *C. pneumoniae*-specific IgA and *H. pylori* IgA was higher than the *C. pneumoniae* IgG and *H. pylori* IgG in CAD patients and that heavy smokers and non-alcoholics were more prone to these bacterial infections, which may exacerbate the process of atherogenesis. Our study suggested that specific detection of *C. pneumoniae*-IgA was

significantly higher in CAD patients compared to CAD first-degree relatives. Overall, this study suggests the major role of *C. pneumoniae* in CAD progression.

#### **PATENTS:**

1. Patent for development of serovar specific monoclonal antibody to *Chlamydia trachomatis* (File no. 792/Del/2003 and is in the process of going to the industry).
2. Patent filed for development of primary cervical epithelial cell line from cervical lavage (File no. 44/Del/2010).
3. Patent filed for development of dot-blot assay for prognosis of sequelae to *Chlamydia trachomatis* infection in women using chlamydial heat shock protein 60 (File no. 186/Del/2008).

#### **TECHNOLOGIES FOR COMMERCIALIZATION:**

1. Development of serovar specific monoclonal antibody to *Chlamydia trachomatis* to BCIL (In process).

#### **INTERNATIONAL COLLABORATIONS:**

1. Diagnostic r-RNA probes for detection of *C. trachomatis*: **Indo-German. German collaborator:** Dr. G. Krupp, Director, Arthus Biotech (2001-02).
2. Effect of sex hormones on induction of immunity by dendritic cells in female genital tract during *Chlamydia trachomatis* infection: **Indo-US Contraceptive Research and Reproductive Health, Funded by DBT (2005-09). US collaborators:** Dr. Paul Wallace, Director, Flow cytometry Laboratory, Roswell Park Cancer Institute, Buffalo, USA & Dr. Charles Wira, Dartmouth Medical School, Lebanon, Hanover, USA.
3. Australia-Canada-India *Chlamydia* Research Alliance for improved detection, treatment and control of chlamydial infection: Funded by Queensland Government, Australia (2010-13). **Australian collaborators:** Prof. Peter Timms, Prof. Ken Beagley **Canadian collaborators:** Prof. Robert Brunham, Prof. Jim Mahony, Prof. Volker Gerds.

#### **EXTRAMURAL PROJECTS :**

1. Collection of well characterized isolates and development of for diagnostic assay for *Chlamydia trachomatis*. **Funded by DBT (May 2000-Oct. 2003).**
2. Cytokine regulation in the immunopathogenesis of salpingitis/infertility due to *Chlamydia trachomatis* infection in women. **Funded by DST (July 2002-Dec. 2005).**
3. Role of chlamydial heat shock protein in the pathogenesis of genital tract infection in women. **Funded by DRDO (Nov. 2003-07).**
4. Study of virulence genes in Indian strains of *Chlamydia trachomatis*- **Funded by DBT (March 2005-09).**

5. Role of *Chlamydia pneumoniae* in coronary artery disease patients. **Funded by DST (Jan. 2007-May 2009).**
6. Immune response to *Chlamydia trachomatis* in spontaneous aborters. **Funded by DRDO (July 2004-07).**

#### **MEMBERSHIP OF NATIONAL/ INTERNATIONAL BODIES:**

- ❖ **Member-** International Union Against Sexually Transmitted Infections (Asia-Pacific)
- ❖ **Fellow-** Indian College of Allergy & Applied Immunology
- ❖ **Life member-** Indian Immunology Society
- ❖ **Member-** Indian Association of Pathologists and Microbiologists

#### **PANEL OF ANTIBODIES:**

**Monoclonal antibody to *Chlamydia trachomatis* species specific & serovar specific**

