Akhilesh Kumar Singh

National Centre of Cell Science, Pune University Campus,
Ganeshkhind, Pune - 411 007 India
Phone: +91-20-2569-0922 Fax: 91-20- 2569-2259;
E.mail:akhil@nccs.res.in

Overview

I am doing my doctorate in the field of Biotechnology with specializations in protein chemistry, Immunology, Molecular Biology and Biophysics from National Center for Cell Science (NCCS) after obtaining Master of Science degree in Biotechnology. Owing to my versatile exposure, I am interested in the area of Immunology, signal transduction, proteomics and structural and functional aspects of proteins.

Academic

- Ph.D. " **Molecular cloning, expression, purification, characterization and structure-function analysis of *Herpesvirus saimiri* complement control protein homolog** ", National Center for Cell Science (NCCS), Pune University, Pune (2001-2006).

- M.Sc. Biotechnology, Goa University, Goa (1999-2001).

- Summer training at Agharkar Research Institute, Pune, India.
  Project undertaken:"**Characterization of metal resistant marker in *Acinetobactor* strains."(2000)**

- M.Sc. Dissertation:
  In partial fulfillment of the Master's degree. Project undertaken:
  "Search for and analysis of hemagglutinins from marine algae."
  [Lectins (Hemagglutinins) has ability to agglutinate the erythrocytes. This can be used as a standard agent for blood typing. The work mainly related to biochemical and immunological studies.]

Course Work:

M.Sc. [Biotechnology]:
Biochemistry, Molecular Biology, Biophysics, Microbiology, Bioprocess Engineering, Cell and Developmental Biology, Immunology, Genetic Engineering, Enzyme and Enzyme Engineering. Animal and Plant tissue culture.

- B.Sc. Chemistry, Botany and Zoology, Dr.R.M.L. Avadh University, Faizabad (1995-1998).

Technical Expertise

Molecular Biology and Biotechnology: DNA and RNA purification, Vector construction with desired MCS, PCR amplifications and modifications, Cloning and mutagenesis techniques etc.

Protein Chemistry and Biophysics: Standard protein purification techniques (HPLC, FPLC), protein unfolding and refolding, 1-D and 2-D Electrophoresis and Western blotting, Ligand blotting, co-immunoprecipitation as well as biophysical techniques including Fluorimetry and Circular Dichroism etc.

Cell Biology and Cell signaling: Culturing and handling of cell lines, Immunofluorescence, Si-RNA mediated studies, Transfection etc.

Research Experience

The complement system is the first line of immune defense against all foreign pathogens including viruses. In order to protect self-tissues from homologous complement, autologous tissues express complement regulatory proteins. These proteins belong to a family of structurally and functionally related proteins termed as regulators of complement activation (RCA).

Herpesvirus saimiri (HVS), a gammaherpesvirus, is a member of herpesviridae family of viruses, which causes a lethal lymphatic leukemia or reticulum cell sarcoma in owl monkey, marmosets, cinnamon and ringtail monkeys and rabbits. The deduced protein sequence of *Herpesvirus saimiri* exhibited significant homology with cellular proteins of known function. These homologous proteins include thymidylate synthase, dihydrofolate reductase, complement control protein homologs, cyclins and G protein coupled receptors.

Characterization of virulence determinants of pathogenic microorganisms provides valuable insight into the essential aspects of pathogenesis. Although a large body of literature is available on the human regulators of complement, little is known how viral homologs of complement control proteins inactivate complement. A previous report has shown that HVS-CCPH inhibits complement activation by inhibiting the C3 deposition on the cells, the exact mechanism of complement inactivation by this protein was not know. It is my conviction that characterization of HVS-CCPH would not only provide insight in understanding the HVS pathogenesis, but this information would also provide valuable information in designing SCR-complement inhibitors, as SCRs-containing complement inhibitors are being developed as clinical agents to control inadvertent complement activation that contributes to pathology in many diseases (11).

In my Ph.D. study, we have been focused on the mechanistic part of the inactivation of complement activation by HVS-CCPH. In brief, we have answered the following questions:
Cloning, expression and purification of HVS-CCPH using a suitable expression system. HVS-CCPH cDNA was cloned in to Pichia expression

vector, mammalian expression vector and in bacterial expression vector. Expressed protein was purified to homogeneity using various chromatographic techniques.

Characterization of the biological properties, and mechanism of complement inactivation of HVS-CCPH. Purified protein was subjected to mass spectrometry analysis and sequencing and the characterized protein was used to define the mechanism of complement inactivation using various complement assays.

Structure-function analysis of HVS-CCPH by deletion and site-directed mutagenesis. HVS-CCPH is entirely made up of four SCR domains. We have generated a series of deletion mutants to address which domain(s) are responsible for the functional activities of the protein. We have also made point mutation to fine characterize the structural determinants of the HVS-CCPH important for its activities.

As a result of the work in protein chemistry, immunology and cell biology, I am well versed with the contemporary techniques employed in Molecular Biology, Biophysics, Protein Chemistry, Immunology and Cell Biology.

Awards

- Qualified all India combined entrance examination conducted by the Jawaharlal Nehru University, New Delhi for admission to M. Sc. Biotechnology held in May 1999.

- Awardee of studentship by Department of Biotechnology, Government of India during M. Sc biotechnology, Academic year 1999-2001.

- Qualified Graduate Aptitude Test in Engineering in the year 2001 conducted by Indian Institute of Technology, on behalf of Ministry of Human Resource and Development, Government of India in Discipline: Life Sciences, percentile: 86.13

- Qualified National Eligibility Test for Junior Research Fellowship jointly organized by Council of Scientific and Industrial Research and University Grants commission held in December 2000.

- Awarded Lectureship (LS) (Dec 2000) by UGC-CSIR, Govt. of India.

Seminars/Conferences/Symposium attended:

- Oral presentation at "**8th FIMSA / IIS Advanced Immunology Course Focus on Clinical Immunology**" during the period March 1-5, 2006, All India Institute of Medical Sciences, New Delhi, India.

- Poster presented at "**8th FIMSA / IIS Advanced Immunology Course Focus on Clinical Immunology**" during the period March 1-5, 2006, All India Institute of Medical Sciences, New Delhi, India. Received Certificate for best poster.
- Poster presented in International symposium **" Emerging Viral Infections: New Frontiers & Challenges"**during the period 11th to 13th October 2004 at National Institute of Virology, Pune, India.
- Symposium attended on **"Molecule Machines and Networks"** January 5-9, 2004 at National Centre for Biological Sciences, TIFR, GKVK Campus, Bangalore-560065, India.
- Poster presented at "**First Indian Senior Fellow Meeting"** at International Centre for Genetic Engineering and Biotechnology, New Delhi, India. 2& 3rd April 2003.

Publications:

Peer Reviewed

- **Singh AK,** Mullick J, Bernet J and Sahu A 2006 "Functional characterization of the complement control protein homolog of *Herpesvirus saimiri* : R118 is critical for factor I cofactor activities." (***Communicated***)

- Mullick J,**Singh AK**,Panse Y, Yadav V, Bernet J and Sahu A 2005 "Localization of functional domains of Kaposica, the complement control protein homolog of Kaposi's Sarcoma-Associated Herpesvirus (Human Herpesvirus 8). **J. Virol.** 79 (9) 5850-5856 .

- Mullick J, Bernet J, Panse Y, Hallihosur S, **Singh A.K**, and Sahu A 2005 "Identification of complement regulatory domains in Vaccinia virus complement control protein. **J. Virol.** 79(19) 12382-12393.

- Mullick J, Bernet J, **Singh AK**, Lambris JD and Sahu A 2003 "Kaposi's Sarcoma-Associated Herpesvirus (Human Herpesvirus 8) open reading frame 4 protein(Kaposica) is a functional homolog of complement control proteins"; **J. Virol.** 77(6) 3878-3881.

- Bernet J, Mullick J, **Singh A K** and Sahu A 2003 "Viral mimicry of the complement system"; **J. Biosci.** 28 249-264.

Abstract:

- **Singh, A.K**., Mullick, J. and Sahu, A. Molecular cloning and expression of Herpesvirus saimiri complement control protein homolog. First Indian Senior Fellow Meeting (2003), ICGEB, New Delhi.

- **Singh, A.K.**, Jayati Mullick, Arvind Sahu, "Mechanism of complement inactivation by Herpesvirus saimiri complement control protein homolog" presented at Emerging Viral Infections: New Frontiers and Challenges, Pune, October 11-13, 2004.

- **Singh, A.K.**, Jayati Mullick, Arvind Sahu, "Functional charaterization of complement control protein homolog of *Herpesvirus saimiri*" 8th FIMSA / IIS Advanced Immunology Course Focus on Clinical Immunology" during the period March 1-5, 2006, All India Institute of Medical Sciences, New Delhi, India.

- **Singh, A.K**., Panse, Y., Yadav, V., Bernet, J. and Sahu, A. Characterization of Kaposica, an immune evasion protein of Kaposi's sarcoma-associated herpesvirus. First International Conference on Natural Products & Molecular Therapy (2005), Cape Town, South Africa.

- Mullick, J., **Singh, A.K.,** Panse, Y., Yadav, V, Bernet, J. and Sahu, A. Identification of domains important for complement regulatory activity in viral complement regulators VCP and Kaposica. 10th European Meeting On Complement in Human Diseases, (2005), Heidelberg, Germany.

- Mullick, J., Bernet, J., **Singh, A**., Lambris, J. and Sahu, A. Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) open reading frame 4 protein (kaposica) is a functional homolog of complement control proteins. XIX International Complement Workshop (2002), Paleremo, Italy.

- Mullick, J., Bernet, J., **Singh, A**., Lambris, J. and Sahu, A. Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) open reading frame 4 protein is a functional homolog of complement control proteins. First Indian Senior Fellow Meeting (2003), ICGEB, New Delhi.

- Mullick, J., Bernet, J., **Singh, A.K**., Lambris, J.D. and Sahu, A. Kaposi's sarcoma-associated herpesvirus open reading frame 4 protein (kaposica) targets C3 and C4 to inactivate complement. *Mol. Immunol*. (2003), 40(2-4):pp 175. 9th European Meeting On Complement in Human Diseases, (2003), Trieste, Italy.

- Mullick, J., Bernet, J., **Singh, A.K**. and Sahu, A. Kinetic analysis of the interactions of Kaposica, the Kaposi's sarcoma-associated herpesvirus open reading frame 4 protein, with human complement components C3b

and C4b. *Mol. Immunol.* (2004), 41 (2-3) pp. 283. XX International Complement Workshop (2004), Honolulu, Hawaii.

Reference:

1. **Dr. Arvind K. Sahu,** Scientist 'E', (Senior Research Fellow, Wellcome Trust, U.K.)

National Center for Cell Science, Pune University Campus,

Ganeshkhind, Pune – 411007.

Phone: 020-25690922 Fax: 020-25692259

E-mail: arvindsahu@nccs.res.in

2. **Dr. Debashis Mitra**, Scientist 'E',

National Centre for Cell Science, Pune University Campus,

Ganeshkhind, Pune – 411007

Phone: 020-25690922 Fax: 020-25692259

Email: dmitra@nccs.res.in

3. **Dr. Jomon Joseph,** Scientist 'D',

National Centre for Cell Science, Pune University Campus,

Ganeshkhind, Pune – 411007

Phone: 020-25690922 Fax: 020-25692259

Email: josephj@nccs.res.in