CURRICULUM VITAE

Name	:	**Anil K. Tyagi**
Designation	:	Professor of the Department
Institute and Address	:	Department of Biochemistry University of Delhi South Campus Benito Juarez Road New Delhi-110021.
Phone	:	91-11-24110970 (Office) 91-11-24118434 (Dept.) 91-11-26492215 (Res.)
Fax	:	91-11-24115270
Electronic Mail Address	:	akt1003@rediffmail.com aniltyagi@south.du.ac.in
Date of Birth	:	2nd April 1951
Sex	:	Male

Education

Degree	University	Subject	Division	Year
Ph.D.	University of Delhi	Medical Biochemistry	-	1977
M.Sc.	University of Allahabad	Biochemistry	First	1972
B.Sc.	University of Meerut	Zoology, Botany, Chemistry	First	1970

Positions

Duration	Designation	Institute
August 2011 onwards	**Professor**	Department of Biochemistry University of Delhi, South Campus, New Delhi-110021
August 2008 to August 2011	**Professor & Head**	Department of Biochemistry University of Delhi, South Campus, New Delhi-110021
August 1999 onwards	**Professor**	Department of Biochemistry University of Delhi, South Campus, New Delhi-110021
August 1996 to August 1999	**Professor & Head**	Department of Biochemistry University of Delhi, South Campus, New Delhi-110021
May 1993 to August 1996	**Professor of Biochemistry**	Department of Biochemistry University of Delhi, South Campus, New Delhi-110021
August 1990 to May 1993	**Head of the Department**	Department of Biochemistry University of Delhi, South Campus, New Delhi-110021
June 1986 to August 1990	**Reader**	Department of Biochemistry University of Delhi, South Campus, New Delhi-110021
June 1983 to June 1986	**Lecturer**	Department of Biochemistry V.P. Chest Institute, Delhi-110007.
May 1980 to June 1983	**International Visiting Associate**	Laboratory of Biochemical Pharmacology, NIADDK, NIH Bethesda, MD USA.
May 1978 to April 1980	**International Visiting Fellow**	National Cancer Institute NIH, Bethesda, MD USA.
January 1973 to April 1978	**CSIR - JRF SRF, PDF**	Department of Biochemistry V.P. Chest Institute, Delhi-110007

Fellow/Member of Academies

- Fellow of the National Academy of Sciences, India
- Fellow of the Indian Academy of Sciences, India
- Fellow of the Indian National Science Academy, India
- Fellow of the Society of Immunology and Immunopathology, India
- Member of the New York Academy of Sciences, USA

Membership to professional associations

- Member of Guha Research Conference
- Life Member of the Society of Biological Chemists (India)
- Life Member of Indian Society of Cell Biology
- Life Member of Association of Microbiologist of India

Honours/ Awards

- J.C. Bose Fellowship (2010).
- Vigyan Gaurav Samman Award from CST, UP Government. (2010).
- Vice President, Society of Biological Chemists (India) from 2004-2006.
- Ranbaxy Research Award, 1999.
- Dr. Nitya Anand Endowment Lecture Award of INSA, 1999.
- Shanti Swarup Bhatnagar Prize of CSIR, 1995.
- P.S. Sarma memorial award of the Society of Biological Chemists (India), 1993.
- Dr. Kona Sampath Kumar prize of the University of Delhi, 1983.
- Fellow of the National Academy of Science, Indian Academy of Science and Indian National Science Academy.

Membership of Project Advisory Committees/Scientific Advisory Committees/ Research Councils/Academic Committees and Administrative Experience

1. Member, APEX Committee, Vaccine Grant Challenge Programme, Department of Biotechnology, Government of India, New Delhi from 2011 onwards.
2. Member, Advisory Committee of Biotechnology Teaching Programme, Jawahar Lal Nehru Univerity, New Delhi from February 2011 onwards.
3. University Representative on the Governing Body, Acharya Narendra Dev College, New Delhi, 2008 onwards.
4. Member, Governing Body, V.P. Chest Institute, University of Delhi, Delhi, 2008 onwards.
5. Member, Management Committee of Bakson Homoeopathic Medical College, Greater NOIDA, Gautam Budh Nagar, U.P., 2008 onwards.
6. Member of the International Programme Approval Committee (IPAC) for the Department of Biotechnology, Ministry of Science and Technology, New Delhi, 1998 onwards.
7. Member of the Research Area Panels and Scientific Advisory Committee, Centre for DNA Finger Printing and Diagnosis (CDFD), Hyderabad, 1999 onwards.

8. Member, Institutional Biosafety Committee, National Institute of Immunology, New Delhi, 1999 onwards.
9. Member of the Academic Committee, National Institute of Immunology, New Delhi, October 1994 onwards.
10. Member Committee of Courses for Biochemistry for designing, reviewing and modification of various curriculum of the University of Delhi pertaining to Biochemistry, 1983 onwards.
11. Member Committee of Courses for M.Phil. Biotechnology for designing, reviewing and running of various courses concerning M.Phil Biotechnology at University of Delhi, 1987 onwards.
12. Member, Board of Research Studies, Faculty of Inter Disciplinary and Applied Sciences, University of Delhi, 1986-2006 and then 2008 onwards.
13. University Representative on Governing Body, ARSD College, University of Delhi, Dhaula Kuan, New Delhi, 2008-2010.
14. Member of Scientific Advisory Committee, National Centre for Cell Sciences (NCCS), Pune, 2003 -2010.
15. Member, Expert Committee, University Grants Commission (UGC), New Delhi for evaluation of major research projects, 2003-09.
16. Member, Academic Committee, ICGEB, New Delhi, January 2008-10.
17. Member, Project Review Committee of ICMR on "Leprosy and Tuberculosis and Other Chest Diseases", 2001-07.
18. Member, Technical Advisory Committee (TAC) for advising, evaluating, reviewing and monitoring activities of National Research Development Corporation (NRDC), New Delhi for activities funded by DSIR, 2007-09.
19. Member, Task Force for Vaccines and Diagnostics in the areas of health care, Department of Biotechnology, Government of India, New Delhi, 2005-08.
20. Member, Task Force for Infectious Disease Biology, DBT, New Delhi, 2005-08.
21. Member, Task Force of DST on International Collaborations, 2001-05.
22. Member Expert, Research Council of Institute of Genomics and Integrative Biology, Delhi, 1st January 2004-2007.
23. Member, Research Area Panels and Scientific Advisory Committee, National Institute of Immunology, New Delhi, 1996-2008.
24. University Representative on the Governing Body, Dayal Singh College, New Delhi, 2005-2008.
25. Member, Advisory Committee of DRS Programme, Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, May 2007 to March 2012.
26. Member, Research Advisory Committee, Central Institute of Fisheries Technology (CIFT), Cochin, 2004-2007.
27. Member, Sectional Committee IX (General Biology), Indian National Science Academy, New Delhi, 2004-2006.
28. Member, Sectional Committee M-2 (Multidisciplinary Committee for Engineering and Applied Sciences), Indian National Science Academy, New Delhi, 2005-2007.
29. Member, Apex Committee on "New Programme Support in High Priority Area of Biology 2002-2007" at Indian Institute of Science, Bangalore.

30. Member of Special Committee, School of Life Sciences, Jawaharlal Nehru University, New Delhi, 2002-2005.
31. Member of Special Committee of the Special Centre of Molecular Medicine, Jawahar Lal Nehru University, New Delhi, 2004-2007.
32. Member of the Academic Committee, Institute of Microbial Technology, Chandigarh, 1996-2004.
33. Member of the Academic Committee, Central Drug Research Institute, Lucknow, 2002-2005.
34. University Representative on the Governing Body, Sri Venkateswara College, New Delhi, 2003-2005.
35. University Representative on the Governing Body, Maulana Azad Medical College, New Delhi, 2005-2006.
36. Member of Scientific Advisory Committee, National Institute of Nutrition, Hyderabad, 2001-2004.
37. Member of Scientific Advisory Committee, Institute of Pathology, Safdarjung Hospital, New Delhi, 1998-2003.
38. University Representative on the Governing Body, Lady Harding Medical College, New Delhi, 2000-2002.
39. University Representative on the Governing Body, Rajkumari Amrit Kaur College of Nursing, New Delhi, 2001-2003.
40. Member of the Task Force of DBT on Basic Research in Modern Biology, 2000-2004.
41. Member, Sectional Committee (General Biology), Indian Academy of Sciences, Bangalore, 2002-2004.
42. Member of Scientific Advisory Committee, Tuberculosis Research Centre, Chennai, 1998-2004.
43. Member of Academic Committee, Centre for Biotechnology, Banaras Hindu University, Varanasi, 2001-2003.
44. University Representative on the Governing Body, Acharya Narendra Dev College, New Delhi from 2000-2002.
45. Member of the Biosafety Committee for the Ranbaxy Laboratories, Gurgaon, India, 2000-2002.
46. Member of Research Council of HRDG, CSIR, New Delhi,1998-2000.
47. Member of the Academic Committee of the International Centre for Genetic Engineering and Biotechnology, New Delhi, 1997-2001.
48. Member of the Project Advisory Committee of DST on "Biochemistry, Biophysics and Molecular Biology", 1998-2001.
49. Member of the Research Committee of CSIR on "Animal Science and Biotechnology" 1998-2001.
50. Member of the Research Council of Centre for Biochemical Technology, New Delhi, 1998-2001.
51. Member of the special committee of School of Life Sciences, Jawahar Lal Nehru University, New Delhi, 1995-1998.
52. Expert Consultant to the Tuberculosis Research Programme (TBRU) of the National Institutes of Health, USA, 1993-1999.

53. External expert on the Board of Studies for Biotechnology, Banaras Hindu University, Varanasi,1995-1996.
54. Member Board of Studies, Dept. of Biochemistry, Aligarh Muslim University, Aligarh, 1993-1995.
55. External expert on the Board of Research Studies in Science, The University of Kashmir, Srinagar, 1992-1995.
56. Member of Special committee for Centre of Biotechnology, Jawaharlal Nehru University, New Delhi, 1993-1996.
57. Member of Academic Council of University of Delhi, 1990-1993; 1996-1999; 2009 onwards.
58. University representative on the governing body of the Sri Venkateswara College, University of Delhi, New Delhi, 1998-2000.
59. Member of the Biosafety Committee for the Jawahar Lal Nehru University, New Delhi, 1994-1997.
60. Member of the Biosafety Committee for the Centre for Biochemical Technology, Delhi, 1994-1997.
61. University representative on the governing body of the Maitreyi College, University of Delhi, New Delhi, 1993-1996.
62. Course Incharge for the national level teachers refresher course sponsored by the University Grants Commission, 28th June - 17th July 1993.
63. Course Incharge for the natioinal level teachers refresher course sponsored by the University Grants Commission, (28th September - 17th October 1992).
64. Member of the budget allocation committee, University of Delhi South Campus, 1993-1994.
65. University representative on the Governing Body of Moti Lal Nehru College, University of Delhi, 1995-1997.
66. Member of the Library Committee, V.P. Chest Institute, University of Delhi, 1983-1986.
67. Course Incharge for the national level teachers refresher course sponsored by the University Grants Commission, 31 March – 19 April 1991.
68. Member of academic committee for Biochemistry - Kurukshetra University, 1991-1994.
69. Member of the University - Industry interaction Cell, University of Delhi, 1991-1994.
70. Member, Employees Welfare Advisory Committee, University of Delhi South Campus, 1992-1993.

Invited lectures delivered at:

1. Indo-Swedish Conference on "Post Genomic Opportunities in Tuberculosis and Other Mycobacteria Diseases, Unchagaon Fort, Bulandshahr, 29th – 31st January 2012.
2. International Symposium on "Vaccine to Translation", Suraj Kund, Faridabad, 14th – 17th November 2011.

3. "Celebration of 100 years of Chemistry", special lecture on "Development of TB Vaccines", Hans Raj College, University of Delhi, 26th March 2011.
4. UGC-SAP workshop on "Advances in Molecular Biologyand Biotechnology", Department of Plant Molecular Biology, UDSC, New Delhi, 25th March 2011.
5. Key note Lecture delivered in the Indo-Canada symposium on "Redox Status and Control in TB: From Basic Research to Drug Development", January 30th to February 1st, 2011, Hyderabad.
6. Rama-Robbins Lecture delivered during the annual meeting of the Indo-US Vaccine Action Programme, New Delhi 17th November 2010.
7. National Symposium on "Emerging Trends in Biotechnology", Indian Institute of Advanced Research, Gandhinagar, Ahmedabad, Gurjrat, 27th-28th April 2010.
8. International symposium on "Understanding and Managing the Pathogenic Microorganisms", Institute of Microbial Technology, Chandigarh, 22-24 January 2010.
9. International symposium on Trends in Drug Discovery and Development, Department of Chemistry, University of Delhi, 5th – 8th January 2010.
10. Inaugural Lecture for the Annual Function of Biochemistry Society, Institute of Home Economics, Hauz Khas, New Delhi, 15th December 2009.
11. International symposium on Emerging Trends in Biotechnology, Banaras Hindu University, Varnasi, 4th – 6th December 2009.
12. Indo-US Tuberculosis Consultation Meeing, National Institute of Immunology, New Delhi, July 2009.
13. 77th Annual Meeting of the Society of Biological Chemists (India), IIT Madras, Chennai, 18th – 20th December 2008.
14. Ranbaxy Science Foundation's 22nd Round Table Conference on "Challenges of MDR/XDR Tuberculosis in India", New Delhi, 13th December 2008.
15. International Symposium on Emerging Trends in Tuberculosis Research: Biomarkers, Drugs and Vaccines, ICGEB, New Delhi, 1st-3rd December 2008.
16. 49th Annual Conference of Association of Microbiologists of India – International Symposium on Microbial Biotechnology: Diversity, Genomics and Metagenomics, Delhi, 18th – 20th November 2008.
17. 22nd Meeting of the Joint Working Group of INDO-US Vaccine Action Programme, New Delhi, 23rd – 24th October 2008.
18. 32nd Annual Conference of Indian Association of Medical Microbiologists (IAMM), A CME on "Vaccinology - an update", AFMC, Pune, 22nd October 2008.
19. Symposium on Industrial application of microbial proteomics, Indian Institute of Advanced Research, Gandhi Nagar, Gujarat, 2nd-4th June 2008.
20. Symposium on Recent Trends in Biotechnology, Aligarh Muslim University, Aligarh, 16th January, 2008.

21. Indo-German Workshop on infectious diseases at INSA, New Delhi, 24th November 2007.
22. B.R. Ambedkar Centre, University of Delhi, Delhi, 10th July 2007.
23. Dr. C.R. Krishnamurthy Memorial Oration, ITRC, Lucknow, 5th June 2007.
24. Foundation Day Lecture at JALMA National Institute of Leprosy and Other Mycobacterial Diseases, Agra, 17th April 2007
25. Department of Genetics, University of Delhi South Campus, New Delhi-110021, 4th April 2007
26. Department of Biochemistry, Faculty of Science, MS University, Baroda, 7th March 2007.
27. International symposium on New Frontiers in Tuberculosis Research, ICGEB, New Delhi, 4th –6th December 2006.
28. Indo-UK Meeting organized by Royal Society, London, UK, 12th –14th September 2006.
29. Indo-Europe Meeting on Infectious Diseases, Bangalore, 5th –6th June 2006
30. International Conference on Opportunistic Pathogens in AIDS, New Delhi, 27th – 29th March 2006.
31. Third Indo-Australian Conference on "Vaccines for Cancer, Infectious Diseases, Lifestyle and Degenerative Diseases" Hyderabad, 6th –8th March 2006.
32. 24th Biennial Conference of the Indian Association of Leprologists, JALMA, Agra, 12th -14th November 2005.
33. Annual Meeting of the Society of Biological Chemist(s) and Molecular Biologists, India, Lucknow, 7th –10th November 2005.
34. Brainstorming workshop on Tuberculosis, ICGEB, New Delhi, 19th – 21st May 2005.
35. Prof. S.H. Zaidi Oration at Industrial Toxicology Research Centre, Lucknow 3rd November 2005.
36. Symposium on Tuberculosis Research – An Indian Perspective (TRIP), AstraZeneca Bangalore, India, 20th October 2005.
37. INDO-Australian Symposium, "Modern Biological Approaches for the Diseases caused by Mycobacteria and Helicobacter" CDFD, Hyderabad, 5th March 2005.
38. 59th National Conference on Tuberculosis and Chest Diseases, New Delhi, 3rd-6th February 2005.
39. Asian Regional Workshop on International Training and Research in Emerging Infectious Diseases, JNU, New Delhi, 8th –11th March 2005.
40. Ranbaxy Science Foundation's 15th Round Table Conference on "HIV and Tuberculosis: Co-Infections", New Delhi, 8th January 2005.
41. International symposium on "Emerging Trends in Tuberculosis Research", 15th – 17th November 2004, New Delhi, India

42. INDO-US Workshop on "AIDS in India: A workshop-symposium on Research, Trials and Treatment", 2nd – 4th August 2004, Bangalore, India.

43. INDO-UK Tuberculosis Meeting organized by the Royal Society London and DST, India, Hyderabad, 12th –13th January 2004.

44. ICMR-INSERM Workshop on Tuberculosis, Agra, India, 12th – 14th December 2003.

45. 10th Congress of Federation of Asian and Oceanian Biochemists and Molecular Biologists, Bangalore, India, 7th –11th December 2003.

46. Global challenges in TB: An update. V.P. Chest Institute, Delhi, 6th April 2003.

47. Tuberculosis Discussion Meeting organized by Royal Society, London, UK, 9th - 10th December 2002.

48. INDO-German Workshop on Infectious Diseases, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, 11th -13th December 2002.

49. BCG Group Meeting for the development of a vaccine against AIDS, International AIDS Vaccine Initiative, New York, 19th June 2002.

50. Symposium on "The Frontiers of Molecular Medicine", Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi, 2nd February 2002.

51. Refresher Course for teachers in Biochemistry, B.R. Ambedkar Centre, University of Delhi, Delhi, 6th October 2001

52. 1st Conference of Biotechnology Society of India, "Biotecon-2001", New Delhi, 4th – 6th October 2001.

53. International symposium on "Mycobacterial Diseases: Pathogenesis, Protection and Control", Calcutta, January 2001.

54. Annual meeting of the Association of Microbiologists of India (AMI), Jaipur, November 2000.

55. ATA-Apollo Millennium Medical Conference, Hyderabad, December 2000.

56. INDO-GERMAN Workshop on Tuberculosis Braunschweig, Germany, 18th –20th September 2000

57. ILTP Workshop – INDO-RUSSIAN Collaboration in Biotechnology, Moscow, Russia, 24th – 30th June 2000.

58. The first Sir Dorabji Tata Symposium – Status of tuberculosis in India, March 11-12, 2000.

59. 5th International Conference on Emerging Infectious Diseases in the Pacific Rim, Chennai, 7th – 9th January 2000.

60. Dr. Nitya Anand Endowment Lecture 1999 (awarded by INSA), Tata Institute of Fundamental Research, Bombay, 27th December 1999.

61. International training and research in emerging infectious diseases - Asian Regional Workshop on Intracellular Pathogens, New Delhi, 6th – 10th December 1999.

62. WHO/IUIS Refresher Course on immunology, vaccinology and biotechnology applied to infectious diseases, Pune, 24th November – 10th December1999.

63. Indo-US Vaccine Action Programme, Joint workshop on Novel Vaccine Technologies, 26th – 27th October 1999.

64. Indo-French Symposium on Multiple Drug Resistance and Emerging Diseases, New Delhi, March 1999.

65. Annual Meeting of the Society of Biological Chemists, India, New Delhi, December 1998.

66. 12th International Congress of Immunology, New Delhi, November 1998.

67. HIV Vaccine Development Initiative by India - Seminar arranged by NACO and Ministry of Health, New Delhi, November 1998.

68. Department of Biological Sciences, Institute of Bacteriophages, University of Pittsburgh, Pittsburgh, USA, October 1998.

69. "Reemerging Infectious Diseases" - symposium held during the meeting of Indo-US Vaccine Action Programme, Washington, DC, USA, October 1998.

70. "Mycobacterial Genome" August - symposium arranged by : Bioinformatics Centre, JNU, August 1998.

71. Host Pathogen defences in Mycobacterium tuberculosis and HIV Infections: Emerging scenario, National Institute of Immunology, New Delhi, 1998.

72. Brain Storming Session on "Development and deployment of target molecules from New Bioactive Substances" held at CCMB, Hyderabad, 1st – 2nd August 1998.

73. Indo-European Commission Symposium on Tuberculosis Research: Into the 21st Century, Chennai, 3rd – 5th February 1998.

74. ASTRA-CCMB Symposium on Molecular Aspects of Microbial Pathogenesis, Hyderabad, 11th – 13th January 1998.

75. 38th Annual Meeting of the Indian Science Congress, Hyderabad, 3rd – 6th January 1998.

76. Centre for Genetic Engineering, MK University, Madurai, March 1997.

77. Department of Biochemistry, M.S. University, Baroda, February 1997.

78. 37th Annual Meeting of the Association of Microbiologists of India, Chennai, 4th – 6th December 1996.

79. Department of Biochemistry, North-Eastern Hill University, Shillong, September 1996.

80. International conference on Eukaryotic Expression Vector Systems : Biology and Applications, National Institute of Immunology, New Delhi, February 1996.

81. Institute of Nuclear Medicine and Allied Sciences, New Delhi, January 1996.

82. Workshop on Infectious diseases: diagnostics, prophylactics, and therapeutics, National Institute of Immunology, December 1995.

83. International Symposium on Trends in Microbiology, Bose Institute, Calcutta, December 1995.

84. Annual meeting of the Society of Biological Chemists, India, Lucknow, October 1995.

85. Symposium on Pasteur's Heritage: from Molecular asymmetry/Industrial fermentation to causality and cure of infectious diseases, Institute of Microbial Technology, Chandigarh, September 1995.

86. Albert Einstein Medical College, New York, USA, April 1995.

87. Institute of Public Health Services, New York, USA, April 1995.

88. John L. McClellan Memorial Veteran's Hospital, Little Rock, USA, April 1995.

89. XI National Symposium on Developmental Biology, Maharshi Dayanand University, Rohtak, March 1995.

90. First Congress of Federation of Indian Physiological Societies, New Delhi, March 1995

91. XVIII All India Cell Biology Conference and Symposia, National Botanical Research Institute, Lucknow, February 1995.

92. Third Asian Conference on Transcription, Bangalore, September 1994.

93. Institute of Microbial Technology, Chandigarh, August 1994.

94. Department of Biochemistry, Banaras Hindu University, Varanasi, July 1994.

95. UGC sponsored Refresher course in Biochemistry at Sri Venkateswara College, University of Delhi, April 1994.

96. Annual Meeting of the Society of Biological Chemists, India, Madurai, December 1993.

97. Department of Biochemistry, North Eastern Hill University, Shillong, December 1993.

98. UGC sponsored Refresher course in Biochemistry at Daulat Ram College, University of Delhi, July 1993.

99. Annual meeting of the Society of Biological Chemists, India, Hyderabad, December 1992.

100. National Chemical Laboratory, Pune, May 1992.

101. National Institute of Immunology, New Delhi, April 1992.

102. Department of Biochemistry, University of Allahabad - March 1992

103. Brain Storming session on Molecular Biology sponsored by TAB - CSIR Centre for Biochemicals, Delhi, March 1992.

104. Annual meeting of the Tuberculosis Association of India, New Delhi, January 1992.

105. International symposium on gene expression at Indian Institute of Science, Bangalore, December 1991.

106. Department of Plant Molecular Biology, University of Delhi, March 1991.

107. Symposium on Molecular Genetics, at the annual meeting of the Indian Science Congress, Indore - January 1991.

108. Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA, May 1990.

109. The annual meeting of the Society of Biological Chemists India, New Delhi, October 1984.

Scientific meetings/Conferences attended/work presented

- Key note Lecture delivered in the Indo-Canada symposium on "Redox Status and Control in TB: From Basic Research to Drug Development", January 30th to February 1st, 2011, Hyderabad.
- Rama-Robbins Lecture delivered during the annual meeting of the Indo-US Vaccine Action Programme, New Delhi 17th November 2010.
- National Symposium on "Emerging Trends in Biotechnology", Indian Institute of Advanced Research, Gandhinagar, Ahmedabad, Gurjrat, 27th-28th April 2010.
- International symposium on "Understanding and Managing the Pathogenic Microorganisms", Institute of Microbial Technology, Chandigarh, 22-24 January 2010.
- International symposium on Trends in Drug Discovery and Development, Department of Chemistry, University of Delhi, 5th – 8th January 2010.
- Inaugural Lecture for the Annual Function of Biochemistry Society, Institute of Home Economics, Hauz Khas, New Delhi, 15th December 2009.
- International symposium on Emerging Trends in Biotechnology, Banaras Hindu University, Varnasi, 4th – 6th December 2009.
- Indo-US Tuberculosis Consultation Meeing, National Institute of Immunology, New Delhi, July 2009.
- 77th Annual Meeting of the Society of Biological Chemists (India), IIT Madras, Chennai, 18th – 20th December 2008.

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- International Symposium on Emerging Trends in Tuberculosis Research: Biomarkers, Drugs and Vaccines, ICGEB, New Delhi, 1st-3rd December 2008.
- 49th Annual Conference of Association of Microbiologists of India – International Symposium on Microbial Biotechnology: Diversity, Genomics and Metagenomics, Delhi, 18th – 20th November 2008.
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- Symposium on Industrial application of microbial proteomics, Indian Institute of Advanced Research, Gandhi Nagar, Gujarat, 2nd- 4th June 2008.
- Symposium on Recent Trends in Biotechnology, Aligarh Muslim University, Aligarh, 16th January 2008.
- Indo-German Workshop on infectious diseases at INSA, New Delhi, 24th November 2007.
- International symposium on New Frontiers in Tuberculosis Research, ICGEB, New Delhi, 4th – 6th December 2006.
- Indo-UK Meeting organized by Royal Society, London, UK, 12th– 14th September 2006.
- International Conference on Opportunistic Pathogens in AIDS, New Delhi, 27th – 29th March 2006.
- Indo-Europe Meeting on Infectious Diseases, Bangalore, 5th – 6th June 2006.
- Third Indo-Australian Conference on "Vaccines for Cancer, Infectious Diseases, Lifestyle and Degenerative Diseases" Hyderabad, 6th – 8th March 2006.
- 24th Biennial Conference of the Indian Association of Leprologists, JALMA, Agra, 12th -14th November 2005.
- Annual Meeting of the Society of Biological Chemist(s) and Molecular Biologists, India, Lucknow, 7th – 10th November 2005.
- Symposium on Tuberculosis Research – An Indian Perspective (TRIP), AstraZeneca Bangalore, India, 20th October 2005.
- Brainstorming workshop on Tuberculosis, ICGEB, New Delhi, 19th – 21st May 2005.
- INDO-Australian Symposium, "Modern Biological Approaches for the Diseases caused by Mycobacteria and Helicobacter", CDFD, Hyderabad, 5th March 2005.
- 59th National Conference on Tuberculosis and Chest Diseases, New Delhi, 3rd - 6th February 2005.
- Asian Regional Workshop on International Training and Research in Emerging Infectious Diseases, JNU, New Delhi, 8th – 11th March 2005.

- Ranbaxy Science Foundation's 15th Round Table Conference on "HIV and Tuberculosis: Co-Infections", New Delhi, 8th January 2005.
- International symposium on "Emerging Trends in Tuberculosis Research", New Delhi, India 15th –17th November 2004
- Genetics – The Expanding Horizon, Department of Genetics, University of Delhi South Campus, New Delhi, 13th – 14th October 2004.
- INDO-US Workshop on "AIDS in India: A workshop-symposium on Research, Trials and Treatment", 2-4 August 2004, Bangalore, India.
- INDO-UK Tuberculosis Meeting organized by the Royal Society London and DST, India, Hyderabad, 12th –13th January 2004.
- ICMR-INSERM Workshop on Tuberculosis, Agra, India, 12th – 14th December 2003.
- 10th Congress of Federation of Asian and Oceanian Biochemists and Molecular Biologists, Bangalore, India, 7th – 11th December 2003.
- Global challenges in TB: An update. V.P. Chest Institute, Delhi, 6th April 2003.
- Tuberculosis Discussion Meeting organized by Royal Society, London, UK, 9th - 10th December 2002.
- INDO-German Workshop on Infectious Diseases, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, 11th - 13th December 2002.
- BCG Group Meeting for the development of a vaccine against AIDS, International AIDS Vaccine Initiative, New York, 19th June 2002.
- Symposium on "The Frontiers of Molecular Medicine", Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi, 2nd February 2002.
- Expert Advisory Group Committee Meeting under INDO-US VAP Programme, Paris, 3rd November 2001.
- 1st Conference of Biotechnology Society of India, "Biotecon-2001", New Delhi, 4th – 6th October 2001.
- International symposium on "Mycobacterial Diseases: Pathogenesis, Protection and Control", Calcutta, January 2001.
- Annual meeting of the Association of Microbiologists of India (AMI), Jaipur, (Delivered a lecture and chaired a session), November 2000.
- ATA-Apollo Millennium Medical Conference, Hyderabad, December 2000.
- INDO-GERMAN Workshop on Tuberculosis Braunschweig, Germany, 18th - 20th September 2000
- ILTP Workshop – INDO-RUSSIAN Collaboration in Biotechnology, Moscow, Russia, 24th – 30th June 2000.
- The First Sir Dorabji Tata Symposium – Status of tuberculosis in India, March 11th – 12th, 2000.

- 5th International Conference on Emerging Infectious Diseases in the Pacific Rim, Chennai, 7th – 9th January 2000.
- International training and research in emerging infectious diseases - Asian Regional Workshop on Intracellular Pathogens, New Delhi, 6th – 101th December, 1999.
- WHO/IUIS Refresher Course on immunology, vaccinology and biotechnology applied to infectious diseases, Pune, 24th November – 10th December 1999.
- Indo-US Vaccine Action Programme on Novel Vaccine Technologies, October 1999.
- Indo-French Symposium on Multiple Drug Resistance and Emerging Diseases, New Delhi, March 1999.
- Fourth International Meeting on the Pathogenesis of Mycobacterial Infections, Stockholm, Sweden, July 1999.
- Annual Meeting of the Society of Biological Chemists, India, New Delhi, December 1998.
- 12th International Congress of Immunology, New Delhi, (Delivered seminar and chaired a session), November 1998.
- HIV Vaccine Development Initiative by India - Seminar arranged by NACO and Ministry of Health, New Delhi, November 1998.
- "Reemerging Infectious Diseases" - symposium held during the meeting of Indo-US Vaccine Action Programme, Washington D.C., USA, October 1998.
- Mycobacterial Genome: Bioinformatics Centre, JNU, 25th August 1998.
- Host Pathogen defences in Mycobacterium tuberculosis and HIV Infections: Emerging scenario, National Institute of Immunology, New Delhi-110067, 10th – 11th August 1998.
- Brain Storming Session on "Development and deployment of target molecules from New Bioactive Substances" held at CCMB, Hyderabad, 1st – 2nd August 1998.
- Annual Meeting of the Tuberculosis Research Unit of NIH, Cleveland, USA, 14th – 15th June 1998.
- Indo-Eurpean Commission Symposium on Tuberculosis Research: Into the 21st Century, Chennai, 3rd – 5th February 1998. (*Delivered a seminar and chaired a session*).
- ASTRA-CCMB Symposium on Molecular Aspects of Microbial Pathogenesis, Hyderabad, 11th – 13th January 1998.
- Annual Meeting of the Indian Science Congress, Hyderabad, 3rd – 6th January 1998.

- 38th Annual Meeting of the Association of Microbiologists of India, New Delhi, 12th – 14th December 1997.
- IBY2K (Indian Biology beyond the year 2000) Symposium at CCMB, Hyderabad, 24th – 27th November 1997 (*Chaired a session*).
- Diversity in Modern Biology - An Interdisciplinary Symposium held at New Delhi, 21st – 22nd September 1997.
- WHO Meeting on the Diagnosis of Tuberculosis, Cleveland, USA 26th June 1997.
- Annual Meeting of the Tuberculosis Research Unit of NIH, Cleveland, USA, 24th – 25th June 1997.
- 32nd US-Japan Co-operative Medical Science Programme Tuberculosis-Leprosy Research Conference held at Cleveland, USA, 21st – 23rd June 1997.
- Bimal K. Bachhawat Symposium on Genomic Research Emerging Ethical, Legal, Social and Economic issues Sarovar Park Plaza Resort, Goa, 22nd – 25th May 1997.
- 37th Annual Meeting of the Association of Microbiologists of India, Chennai, 4th – 6th December 1996.
- International conference on Eukaryotic Expression Vector Systems: Biology and Applications, National Institute of Immunology, New Delhi, 4th - 8th February 1996.
- Workshop on Infectious diseases: diagnostics, prophylactics, and therapeutics, National Institute of Immunology, 21st - 22nd December 1995.
- International Symposium on Trends in Microbiology, Bose Institute, Calcutta, 4th - 8th December 1995.
- Symposium on Pasteur's Heritage: from Molecular asymmetry/Industrial fermentation to causality and cure of infectious diseases, Institute of Microbial Technology, Chandigarh, 27th - 29th September 1995.
- XI National Symposium on Developmental Biology, Maharshi Dayanand University, Rohtak, 25th - 27th March 1995.
- First Congress of Federation of Indian Physiological Societies, New Delhi, 1st - 3rd March 1995**.**
- XVIII All India Cell Biology Conference and Symposia, National Botanical Research Institute, Lucknow, 13th - 15th February 1995.
- Third Asian Conference on transcription, Indian Institute of Science, Bangalore, 25th - 27th September 1994.
- 16th International Congress of Biochemistry and Molecular Biology, New Delhi, India, 19th - 22nd September, 1994.
- 2nd International Conference on the pathogenesis of mycobacterial infections, Stockholm, Sweden, 2nd - 4th July, 1993**.**

- World Congress on tuberculosis, Bethesda, Maryland, USA**.** 16th - 19th November 1992**.**
- The annual meeting of the Tuberculosis association of India, New Delhi - 1992.
- The National Symposium on Liposome Research, University of Delhi South Campus, New Delhi, 1988, 1989, 1991, 1992**.**
- Brain Storming session on Molecular Biology, sponsored by TAB, held at the CSIR Centre for Biochemicals, New Delhi, March 1992**.**
- The annual meeting of the Clinical Biochemists of India, New Delhi, February 1992.
- The XV All India Cell Biology Conference and Symposia held at the University of Delhi South Campus, New Delhi, February 1992.
- Symposium on molecular genetics at the Annual meeting of the Indian Science Congress, Indore, India, January 1991.
- International Symposium on gene expression, Indian Institute of Science, Bangalore, December 1991
- Guha Research Conference, India, 1989, 1991, 1992, 1993, 1996, 1998, 2000, 2002, 2003. 2004, 2006, 2009, 2011.
- The International Symposium on eukaryotic cell surface macromolecules, University of Delhi South Campus, New Delhi, 1987.
- The Annual meeting of the American Society of Biochemists and Molecular Biologists, USA - 1980, 1981, 1982, 1990.
- Annual Meeting of the Society of Biological Chemists (India) - 1974, 1975, 1976, 1977, 1983, 1984, 1988, 1990, 1992, 1993, 1995, 1998, 2003, 2005, 2008.
- Gorden Research Conference on Polyamines - New Hampshire USA, 1981.
- The annual meeting of the American Association of Cancer Research, New Orleans, USA, 1979.
- International symposium on Biomembranes - Madurai Kamraj University, Madurai, December 1973.

Editorial Work

Academic Editor, PLoS ONE from 2009 onwards, published by Public Library of Science.

Member of the Editorial Board for the Journal "Indian Journal of Microbiology" from 2008 onwards published by Springer Publishing Company, New Delhi.

Member of the Editorial Board for the Journal "Current Analytical Chemistry" published by Bentham Science Publishers Ltd., USA, 2004 onwards.

Member of the Editorial Board for the Journal "Indian Journal of Medical Research" published by ICMR, New Delhi, 2003 onwards.

Member of Editorial Board for the journal "Tuberculosis" published by Elsevier Press, 2003-2007.

TEACHING EXPERIENCE

M.Sc., BIOCHEMISTRY	:	Molecular biology, Molecular genetics, Recombinant DNA technology, enzymes, carbohydrate metabolism
M.Sc., GENETICS	:	Molecular biology
M.Sc., MICROBIOLOGY	:	Molecular biology
M.Phil., BIOTECHNOLOGY	:	Molecular genetics and Molecular biology

DETAILS OF TEACHING EXPERIENCE

Total teaching experience = 33 years

M.Phil. Biotechnology	1988-2011	Molecular Biology
M.Sc. Microbiology	1994-2009	Molecular Biology
M.Sc. Genetics	1986-1989	Recombinant DNA Technology
M.Sc. Genetics	1986-2009	Molecular Biology
M.Sc. Biochemistry	1985-1989	Recombinant DNA Technology
M.Sc. Biochemistry	1985-2011	Molecular Biology
M.Sc. Biochemistry	1985-1987	Molecular genetics
M.Sc. Biochemistry	1983-1987	Enzymes, Carbohydrate metabolism
*M.D. Medical Biochemistry	1974-1978	Enzymes
*M.Sc. Medical Biochemistry	1974-1978	Enzymes, metabolism

These classes were taught while working as JRF/SRF during Ph.D. and during the post-doctoral period.

Development of curriculum for various courses

Major contribution in developing the curriculum for the following courses

- Development of new revised syllabus for B.Sc. (Hons) Biochemistry, University of Delhi, 2010.
- Development of new/revised curriculum for M.Sc. Biochemistry, University of Delhi, 2009.
- Development of revised curriculum for B.Sc. (Hons) Biochemistry for Delhi University, 1998.
- Development of revised curriculum for post-graduate diploma in Molecular and Biochemical Technology, University of Delhi, 1998.
- Development of Curriculum for M.Sc. Biochemistry, Kurukshetra University, 1991.
- Development of curriculum for postgraduate diploma course in Biochemical Technology, University of Delhi, 1990.
- Development of revised/advanced curriculum for M.Sc. Biochemistry, University of Delhi, 1989.
- Development of Curriculum for M.Phil Biotechnology, University of Delhi, 1988.
- Development of curriculum for B.Sc.(Hons) Biochemistry Course for Delhi University, 1987.
- Development of new/revised curriculum for M.Sc. Biochemistry, University of Delhi, 1985.

Meetings / Symposia / Refresher courses organized

- Co-Convenor of the symposium-cum-workshop on "Next Generation Sequencing Data Analysis" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 28th – 29th January 2011.
- Co-Convenor of the national conference on "Drug Discovery and Development" held at the University of Delhi South Campus, New Delhi, organized by Bioinformatics Centre, Sri Venkateswara College in association with Bioinformatics Centre, DISC, University of Delhi South Campus, 21st – 23rd January 2009.
- Co-Convenor of the symposium-cum-workshop on "Computational Biology – Construction of databases" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 14th - 15th March 2008.
- Co-Convenor of the symposium on "Systems Biology" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 12th - 13th March 2006.
- Chairman, Organizing Committee for Brain Storming Session on Tuberculosis held at ICGEB, New Delhi, 19th - 21st May 2005.

- Co-Convenor of the workshop entitled, "Machine Learning Techniques in Bioinformatics" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 28th - 29th March 2005.
- Co-Convenor of the Workshop entitled, "Biological databases – Mining of Information" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 28th - 29th March 2003.
- Co-Convenor of the Workshop entitled, "Applications of Genomics and Proteomics" held at the Department of Biochemistry, University of Delhi South Campus and JNU, New Delhi, 1st - 3rd February 2002.
- Convenor of the Workshop entitled, "Bioinformatics and its Application to Biology" held at the Department of Biochemistry, University of Delhi South Campus, New Delhi, 22nd - 23rd March 2000.
- Joint-convenor of the meeting - TRendys in Biochemistry, held at the University of Delhi South Campus, New Delhi, 23rd – 24th November 1999.
- Convener of the symposium on "Microbial Infections: Diagnostics, Prevention and Cure" during the 38th Annual Meeting of the Association of Microbiologists of India held at New Delhi, 12th – 14th December 1997.
- Joint-Convener of "Diversity in Modern Biology - an Interdisciplinary Symposium" held at University of Delhi South Campus, 21st – 22nd September 1997
- Course in charge for the refresher course in biochemistry sponsored by the University Grants Commission, 28th June – 17th July 1993.
- Co-convener of the Guha Research Conference held at Dalhousie, 17th – 20th May 1993.
- Course Incharge for the refresher course in Immunology sponsored by the University Grants Commission, 28th September - 17th October 1992.
- Course in charge for the refresher course in Biochemistry sponsored by the University Grants Commission, 31st March – 19th April 1991.
- Course-Incharge for the workshop on Nucleic Acid Probes held on the auspices of annual meeting of the Clinical Biochemists of India, at G.T.B. Medical College, New Delhi, February 1991.
- Convener of the Annual meeting of the Society of Biological Chemists (India), New Delhi, 1984.

DETAILS OF RESEARCH EXPERIENCE

Current Research Activities

The current research activities are focused on understanding the molecular biology of mycobacteria and developing strategies for prevention and control of tuberculosis. Techniques of molecular biology, structural biology, immunology, purification and characterization of proteins, crystallization of proteins, DNA protein interactions, gene

knock-outs and vaccine development strategies are the main tools used. Various aspects of current research activities are:

1. Development of candidate vaccines against tuberculosis by using recombinant BCG and DNA vaccine approaches.
2. New drug targets for tuberculosis: Identification, validation and applications.
3. Analysis of structure and function of mycobacterial transcriptional signals and gene expression in mycobacteria.
4. Sequencing of the complete genome of *Mycobacterium w.* (*Mycobacterium indicus pranii*) and functional genomics.

Supervision of Research Work

Ph.D. awarded	:	22
Ph.D. thesis submitted	:	1
Ph.D. students currently working	:	7
M.Phil. (Biotechnology) awarded	:	2
M.D. (Medical Biochemistry) awarded	:	1

Publications

Total	:	104
Published Research papers	:	86
Book chapters	:	15
Published Scientific Reviews	:	3

Name of the periodicals/books in which research papers/book chapters have been published

Journal of Bacteriology
Journal of Biological Chemistry
Proceeding of National Academy of Sciences (USA)
Gene
Molecular Microbiology
Methods in Enzymology
PLoS One
Nucleic Acid Research
Nature Chemical Biology
Microbiology (U.K.)
European Journal of Biochemistry
Vaccine
FEBS Letters
Cancer Research
Biochemical Biophysical Research Communications
Achieves of Biochemistry and Biophysics
Biochemical Pharmacology

Physiology and genomics
Molecular Genetics for Mycobacteria, ASM Press, Washington DC
Advances in Polyamine Research, Raven Press, New York
Advances in Pharmacology and Chemotherapy, Academic Press, New York
The Mycobacteria Cell Envelope, ASM Press, Washington DC
Trends in Pharmacological Sciences
Journal of Applied Bacteriology
BioTechniques
Biochemical Biophysical Methods
Scandinavian Journal of Immunology
Biochemical Biophysical Acta
American Journal of Biochemistry and Biotechnology
Federation Proceedings
Acta Crystallographica
Medical Microbiology and Immunology
Protein Expression and Purification
Indian Journal of Biochemistry Biophysics
Canadian Journal of Microbiology
Molecular Cellular Biochemistry

PUBLICATIONS

1. Ruchi Jain, Bappaditya Dey, Aparna Khera, Priyadarshani Srivastava, Umesh D. Gupta, V.M. Katoch, V.D. Ramanathan, **Anil K. Tyagi.** (2011). Over-expression of superoxide dismutase obliterates the protective effect of BCG against tuberculosis by modulating innate and adaptive immune responses. Vaccine. 29: 8118– 8125

2. Bappaditya Dey, Ruchi Jain, Umesh D. Gupta, V. M. Katoch, V. D. Ramanathan, **Anil K. Tyagi.** (2011). A Booster Vaccine Expressing a Latency-Associated Antigen Augments BCG Induced Immunity and Confers Enhanced Protection against Tuberculosis. PLoS ONE 6(8): e23360.

3. Garima Khare, Ritika Kar, **Anil K. Tyagi.** (2011). Identification of Inhibitors against Mycobacterium tuberculosis Thiamin Phosphate Synthase, an Important Target for the Development of Anti-TB Drugs. PLoS ONE 6(7): e22441.

4. Bappaditya Dey, Ruchi Jain, Aparna Khera, Umesh D. Gupta, V.M. Katoch, V.D. Ramanathan and **Anil K. Tyagi**. (2011). Latency antigen α–crystalin based vaccination imparts a robust protection against TB by modulating the dynamics of pulmonary cytokines. PLoSONE 6(4): e18773.

5. Garima Khare, Vibha Gupta, Prachi Nangpal, Rakesh K. Gupta, Nicholas K. Sauter and **Anil K. Tyagi**. (2011). Ferritin Structure from Mycobacterium tuberculosis: Comparative Study with Homologues identifies Extended C-terminus involved in Ferroxidase Activity. PLoSONE 6(4): e18570.

6. Purushothaman S, Annamalai K, **Tyagi AK**, Surolia A (2011) Diversity in Functional Organization of Class I and Class II Biotin Protein Ligase. PLoS ONE 6(3):e16850.

7. Nidhi Jatana, Sarvesh Jangid, Garima Khare, Anil K. Tygi and Narayanan Latha. (2011). Molecular modeling studies of fatty acyl-CoA synthetase (FadD13) from *Mycobacterium tuberculosis* – a potential target for the development of antitubercular drugs. J. Mol. Model. 17(2) : 301-313.

8. Ashish Arora, Nagasuma R. Chandra, Amit Das, Balasubramanian Gopal, Shekhar C. Mande, Balaji Prakash, Ravishankar Ramachandran, Rajan Sankaranarayanan, K. Sekar, Kaza Suguna, **Anil K. Tyagi**, Mamannamana Vijayan. (2011). Structural biology of Mycobacterium tuberculosis proteins: The Indian efforts, Tuberculosis, doi:10.1016/j.tube.2011.03.004

9. **Anil K. Tyagi**, Prachi Nangpal, Vijaya Satchidanandam. (2011). Development of vaccines against tuberculosis. Tuberculosis. Doi:10.1016/j.tube.2011.01.003.

10. Anuj Kumar Gutpa, Vineel P. Reddy, Mallika Lavania, D.S. Chauhan, K. Venkatesan, V.D. Sharma, **A.K. Tyagi** and V.M. Katoch. (2010). *jefA* (Rv2459), a drug efflux gene in *Mycobacteirum tuberculosis* confers resistance to isoniazid and ethambutol. Indian J. Med. Res. 132: 176-188.

11. Vibha Gupta, Rakesh K. Gupta, Garima Khare, Dinakar M. Salunke, Avadhesha Surolia and **Anil K. Tyagi**. (2010). Structural ordering of disordered ligand-binding loops of biotin protein ligase into active conformations as a consequence of dehydration. PloS ONE 5(2): e9222.

12. Bappaditya Dey, Ruchi Jain, Aparna Khera, Vivek Rao, Neeraj Dhar, Umesh D. Gupta, V.M. Katoch, V.D. Ramanathan and **Anil K. Tyagi**. (2010). Boosting with a DNA vaccine expressing ESAT-6 (DNAE6) obliterates the protection imparted by recombinant BCG (rBCGE6) against aerosol *Mycobacterium tuberculosis* infection in guinea pigs. Vaccine. 28: 63-70.

13. Khare, G., Gupta, V., Gupta, R.K., Gupta, R, Bhat, R. and **Anil K. Tyagi**. (2009). Dissecting the role of critical residues and substrate preferene of a fatty Acyl-CoA synthetase (FadD13) of *Mycobacterium tuberculosis*. PLoS ONE 4(12): e8387,.

14. Vibha Gupta, Rakesh K. Gupta, Garima Khare, Dinakar M. Salunke and **Anil K. Tyagi**. (2009). Crystal structure of Bfr A from *Mycobacterium tuberculosis:* Incorporation of selenomethionine results in cleavage and demetallation of Haem. PLoS One. 4(11): e8028.

15. Preeti Sachdeva, Richa Misra, **Anil K. Tyagi** and Yogendra Singh. 2009. The sigma factors of *Mycobacterium tuberculosis:* regulation of the regulators. FEBS Journal. Doi:10.1111/j.1742-4658.2009.07479.x.

16. C.M. Santosh Kumar, Garima Khare, C.V. Srikanth, **Anil K. Tyagi**, Abhijit A. Sardesai and Shekhar C. Mande. (2009). Facilitated oligomericzation of mycobacterial GroEL: Evidence for phosphorylation-mediated oligomerization. J. Bacteriol. 191: 6525-6538.

17. Vikram Saini, S. Raghuvanshi, G.P. Talwar, N. Ahmed, J.P. Khurana, S.E. Hasnain, Akhilesh K. Tyagi, and **Anil K. Tyagi.** (2009). Polyphasic Taxonomic Analysis Establishes *Mycobacterium indicus pranii* as a Distinct Species. PLoS ONE 4(7): e6263.

18. D. Basu, Garima Khare, S. Singh, **Anil K. Tyagi,** S. Khosla, S.C. Mande. (2009). A novel nucleoid-associated protein of *Mycobacterium tuberculosis* is a sequence homolog of GrotL. Nucleic Acids Res. Doi:10.1093/nar/gkp502.

19. Pooja Arora, Aneesh Goyal, Vivek T. Natarajan, Eerappa Rajakumara, Priyanka Verma, Radhika Gupta, Malikmohamed Yousuf, Omkita A. Trivedi, Debasisa Mohanty, **Anil Tyagi**, Rajan Sankaranarayanan and Rajesh S. Gokhale. (2009). Mechanistic and functional insights into fatty acid activation in *Mycobacterium tuberculosis*. Nature Chemical Biology. 5, 166-173.

20. Ruchi Jain, Bappaditya Dey, Neeraj Dhar, Vivek Rao, Ramandeep Singh, Umesh D. Gupta, V.M. Katoch, V.D. Ramanathan and **Anil K. Tyagi**. (2008). Enhanced and Enduring Protection against Tuberculosis by Recombinant BCG-Ag85C and its Association with Modulation of Cytokine Profile in Lung. PLoS ONE. 3(12): 3869.

21. Mohd Akif, Garima Khare, **Anil K. Tyagi,** Shekhar C. Mande, and Abhijit A. Sardesai (2008). Functional Studies on Multiple Thioredoxins from *Mycobacterium tuberculosis*. J. Bacteriol. 190: 7087-7095.

22. Vibha Gupta, Rakesh K. Gupta, Garima Khare, Dinakar M. Salunke and **Anil K. Tyagi**. (2008). Cloning, expression, purification, crystallization and preliminary x-ray crystallographic analysis of bacterioferritin A from *Mycobacterium tuberculosis*. Acta Cryst. F 64: 398-401.

23. Vibha Gupta, Rakesh K. Gupta, Garima Khare, Avadhesha Surolia, Dinakar M. Salunke and **Anil K. Tyagi**. (2008). Crystallization and preliminary x-ray diffraction analhysis of biotin acetyl-CoA carboxylase ligare (BirA) from *Mycobacterium tuberculosis*. Acta Cryst. F 64: 524-527.

24. A. Farhana, S. Kumar, S.S. Rathore, P.C. Ghosh, N.Z. Ehtesham, **Anil K. Tyagi,** and S.E. Hasnain. (2008). Mechanistic insights into a novel export-import system of *Mycobacterium tuberculosis* unravel its role in trafficking of iron. PLoS ONE. 3(5): e2087..

25. Shruti Jain, Garima Khare, Pushplata Tripathi and **Anil K. Tyagi.** (2008). An inducible system for the identification of target genes for a regulator in mycobacteria. American Journal of Biochemistry and Biotechnology 4(3): 226-230.

26. Ahmed, N., Saini, V., Raghuvanshi, S., Khurana, J.P., Tyagi, Akhilesh K., **Tyagi, Anil K.** and Hasnain, S.E. (2007). Molecular analysis of a leprosy immunotherapeutic bacillus provides insights into *Mycobacterium* evolution. PLoS ONE 2(10): e968.

27. Azeet Narayan, Preeti Sachdeva, Kirti Sharma, Adesh K. Saini, **Anil K. Tyagi** and Yogendra Singh. (2007). Serine threonine protein kinases of mycobacterial genus: phylogeny to function. Physiol. Genomics 29: 66-75.

28. Nisheeth Agarwal and **Anil K. Tyagi.** (2006). Mycobacterial Transcriptional Signals: Requirements for Recognition by RNA polymerase and Optimal Transcriptional Activity. Nucleic Acid Research. 34: 4245-4257.

29. Ramandeep Singh, Amit Singh and **Anil K. Tyagi.** (2005). Deciphering the genes involved in pathogenesis of *Mycobacterium tuberculosis.* Tuberculosis 85:325-335.

30. Aparna Khera, Ramandeep Singh, H. Shakila, Vivek Rao, Neeraj Dhar, P.R. Narayanan, C.N. Parmasivan, V.D.Ramanathan and **Anil K.Tyagi**. (2005). Elicitation of efficient, protective immune responses by using DNA vaccines against tuberculosis. Vaccine 23:5655-5665.

31. Parampal, Deol, Reena Vohra, Adesh K. Saini, Amit Singh, Harish Chandra, Puneet Chopra, Taposh K. Das, **Anil K. Tyagi**, and Yogendra Singh. (2005). Role of *Mycobacterium tuberculosis* Ser/Thr kinase PknF: implications in glucose transport and cell division. J. Bacteriol. 187: 3415-3420.

32. Amit Singh, Radhika Gupta, R. A.Vishwakarma, P.R. Narayanan, C.N. Paramasivan, V. D. Ramanathan and **Anil K. Tyagi**. (2005). Requirement of

mymA operon for appropriate cell wall ultrastructure and persistence of *Mycobacterium tuberculosis* in the spleens of guinea pigs. J. Bacteriol. 187: 4173-4186.

33. Vivek Rao, Neeraj Dhar, H. Shakila, Ramandeep Singh, Aparna Khera, M. Naseema, C. N. Paramasivan, P. R. Narayananan, V. D. Ramanthan and **Anil K. Tyagi.** (2005). Over-expression of the 19kDa lipoprotein of *Mycobacterium tuberculosis* obliterates the protective efficacy of BCG by polarizing the host immune responses to the Th2 phenotype. Scand. J. Immunol. 61: 410-417.

34. V.K. Chaudhary, Kulshresta Abhishek, Gupta Ghata, Verma Nitin, Sampati Kumari, S.K. Sharma, Gupta Amita, **A.K. Tyagi.** (2005). Expression and purification of 38-kDa and Mtb81 antigens of *Mycobacterium tuberculosis* for application in serodiagnosis. Protein Expr. Purif. 40: 169-176.

35. Saini, A.K., Maithal, K., Chand, P., Chowdhury, S., Vohra, R., Goyal, A., Dubey, G.P., Chopra, P., Chandra, R., **Tyagi, A.K.**, Singh, Y., Tandon, V. (2004). Nuclear localization and in situ DNA damage by *Mycobacterium tuberculosis* nucleosidediphosphate kinase. J. Biol. Chem. 279: 50142-50149.

36. Chopra, P., Koduri, H., Singh, R., Koul, A., Ghildiyal, M., Sharma, K., **Tyagi, A.K.**, Singh, Y. (2004). Nucleoside diphosphate kinase of *Mycobacterium tuberculosis* acts as GTPase-activating protein for Rho-GTPases. FEBS Lett. 571: 212-216.

37. Neeraj Dhar, Vivek Rao and **Anil K. Tyagi**. (2004). Immunogenicity of recombinant BCG vaccine strains overexpressing components of the antigen 85 Complex of *M. tuberculosis*. Med Microbiol Immunol. 193: 19-25.

38. Ramandeep Singh, Vivek Rao, H. Shakila, Radhika Gupta, Aparna Khera, Neeraj Dhar, Amit Singh, Anil Koul, Yogendra Singh, M. Naseema, P.R. Narayanan, C.N. Paramasivan, V.D. Ramanathan and **Anil K. Tyagi**. (2003). Disruption of *mptpB* impairs the ability of *Mycobacterium tuberculosis* to survive in guinea pigs. Molecular Microbiology 50(3): 751-762.

39. Chopra P, Singh B, Singh R, Vohra, R, Koul A, Meena LS, Koduri H, Ghildiyal M, Deol P, Das TK, **Tyagi AK**, Singh Y. (2003). Phosphoprotein phosphatase of Mycobacterium tuberculosis dephosphorylates serine-threonine kinases PknA and PknB. Biochem Biophys Res Commun. 311(1): 112-120.

40. Vivek Rao, Neeraj Dhar and **Anil K. Tyagi**. (2003). Modulation of host immune responses by over-expression of immunodominant antigens of *M. tuberculosis* in BCG. Scand J Immunol. 58(4): 449-461.

41. Amit Singh, Shruti Jain, Seema Gupta, Taposh Das and **Anil K. Tyagi.** (2003). *mymA* operon of *Mycobacterium tuberculosis* : its regulation and importance in the cell envelope. FEMS Microbiol. Lett. 227(1): 53-63.

42. Nisheeth Agarwal and **Anil K. Tyagi.** (2003). Role of 5`TGN3` Motif in the Interaction of Mycobacterial RNA Polymerase with a Promoter of "Extended – 10" class. FEMS Microbiol. Lett. 225: 75-83.

43. Neeraj Dhar, Vivek Rao and **Anil K. Tyagi.** (2003). Skewing of the Th1/Th2 responses in mice due to variation in the level of expression of an antigen in a recombinant BCG system. Immunol Lett. 88(3): 175-184.

44. Puneet Chopra, Anubha Singh, Anil Koul, S. Ramachandran, Karl Drilica, **Anil K. Tyagi** and Yogendra Singh. (2003). Cytotoxic activity of nucleoside diphosphate kinase secreted from *Mycobacterium tuberculosis*. Eur. J. Biochem. 270, 625-634.

45. Koul, A., Choidas, A., **Tyagi, A.K.,** Drilca, K., Singh, Y. and Ullrich, A. 2001. Serine/threonine protein kinases PknF and PknG of *Mycobacterium tuberculosis*: Characterization and Localization. Microbiology (U.K.). 147, 2307-2314.

46. Dhar Neeraj, Rao, Vivek and **Tyagi, A.K.** 2000. Recombinant BCG approach for development of vaccines: cloning and expression of immunodominant antigens of *M.*tuberculosis. FEMS Microbiol. Lett., 190(2), 309-316.

47. Koul, Anil, Choidas, Axel, Treder, Martin, **Tyagi, A.K.**, Drlica, Karl, Singh, Y. and Ullrich, Axel. 2000. Cloning and characterization of secretory tyrosine phosphatases of *Mycobacterium tuberculosis*. J. Bacteriol. 182(19), 5425-5432.

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57. Shankar, S., and **Tyagi, A.K.** 1993. Purification and Characterization of restriction endonuclease *Mgo*I from *Mycobacterium gordonae*, Gene 131, 153-154.

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59. Shankar, S., and **Tyagi, A.K.** 1992. *Mha*AI, a novel isoschizomer of *Pst*I from *Mycobacterium habana* recognizing 5'-CTGCA/G-3'. Nucleic Acids Res. 20, 2891.

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CITATION ANALYSIS OF RESEARCH PAPERS
SEARCHED PERIOD: 1976-2008

ANIL K TYAGI

TOTAL NO. OF PAPERS ANALYSED	82
CITED PAPERS	65
TOTAL CITATIONS	1003
AVERAGE CITATIONS	12.23
PAPERS WITH >=10 CITATIONS	33
MAX. CIT. RECEIVED BY A PAPER	92

PAPERS WITH CITATIONS

1976	**TYAGI AK**	**IND J BIOCH BIOPHYS**	**13**	**43**	
	TYAGI AK	BIOCHIM BIOPHYS ACTA	485	255	1977
	RASTOGI N	INDIAN J BIOCHEM BIOPHYS	15	286	1978
1976	**TYAGI AK**	**CAN J MICROB**	**22**	**1054**	
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	RASTOGI N	INDIAN J BIOCHEM BIOPHYS	15	286	1978
	SALTVEIT ME	J AMER SOC HORT SCI	105	252	1980
1977	**TYAGI AK**	**BIOCH BIOPHYS ACTA**	**485**	**255**	
	RASTOGI N	INDIAN J BIOCHEM BIOPHYS	15	286	1978
	STORER AC	BIOCHEM J	193	235	1981
	WALI AS	CAN J BIOCHEM CELL BIOL	62	559	1984
	MASOOD R	J BIOSCIENCES	7	421	1985
	LAI YK	PHYTOCHEMISTRY	28	1579	1989
	YUEH AY	BIOCHEM J	258	221	1989
	MIKULASOVA D	FEMS MICROBIOL LETT	159	299	1998
	OIKAWA T	BIOSCI BIOTECHNOL BIOCHEM	69	2146	2005
	EPRINTSEV AT	BIOCHEMISTRY-ENGL TR	70	1027	2005
	WANG SY	J FOOD BIOCHEM	29	117	2005
	EPRINTSEV AT	BIOLOGY BULL	35	585	2008
1979	**JAYARAM HN**	**BIOCH PHARMACOL**	**28**	**3551**	
	TYAGI AK	CANCER RES	40	4390	1980
	ANANDARAJ SJ	BIOCHEM PHARMACOL	29	227	1980
	CADMAN E	BIOCHEM PHARMACOL	30	2469	1981
	POWIS G	CANCER CHEMOTHER PHARMACOL	6	1	1981
	TYAGI AK	TOXICOLOGY	21	59	1981
	TYAGI AK	BIOCHEM PHARMACOL	30	915	1981

	POWIS G	BIOCHEM PHARMACOL	30	771	1981
	BENZ C	CANCER RES	41	994	1981
	ALSTON TA	BIOCHEM BIOPHYS RES COMMUN	105	560	1982
	POWIS G	DRUG METAB REV	14	1145	1983
	ALSTON TA	ACCOUNT CHEM RES	16	418	1983
	PORTER DJT	ARCH BIOCHEM BIOPHYS	225	157	1983
	REJ R	CRC CRIT REV CLIN LAB SCI	21	99	1984
	STRAZZOLINI P	J MED CHEM	27	1295	1984
	TYAGI AK	ADVAN PHARMACOL CHEMOTHER	20	69	1984
	ALSTON TA	J BIOL CHEM	260	4069	1985
	GALLIANI G	CANCER CHEMOTHER PHARMACOL	14	74	1985
	DAMON LE	PHARMACOL THER	38	73	1988
	AHLUWALIA GS	PHARMACOL THER	46	243	1990
	JAYARAM HN	CANCER RES	53	2344	1993
1979	**TYAGI AK**	**J BIOCH BIOPHY METH**	**1**	**221**	
	KENSLER TW	CANCER TREAT REP	64	967	1980
	TYAGI AK	CANCER RES	40	4390	1980
	ARDALAN B	CANCER RES	40	1431	1980
	TYAGI AK	J BIOCHEM BIOPHYS METH	2	123	1980
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	ANANDARAJ SJ	BIOCHEM PHARMACOL	29	227	1980
	TYAGI AK	TOXICOLOGY	21	59	1981
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	KENSLER TW	CANCER RES	41	894	1981
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	TYAGI AK	ADVAN PHARMACOL CHEMOTHER	20	69	1984
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	TYAGI AK	BIOCHEM PHARMACOL	30	915	1981
	TYAGI AK	TRENDS PHARMACOL SCI	4	299	1983
	STAYTON MM	CURR TOPICS CELL REGUL	22	103	1983
	HEIMER R	BIOCHEM PHARMACOL	32	199	1983
	STRAZZOLINI P	J MED CHEM	27	1295	1984
	MISTRELLO G	J IMMUNOPHARMACOL	6	25	1984
	TYAGI AK	ADVAN PHARMACOL CHEMOTHER	20	69	1984
	WEBSTER HK	BIOCHEM PHARMACOL	33	1555	1984
	AHLUWALIA GS	BIOCHEM PHARMACOL	33	1195	1984
	WANG CC	BIOCHEM PHARMACOL	33	1323	1984
	GALLIANI G	CANCER CHEMOTHER PHARMACOL	14	74	1985
	CASEY PJ	J BIOL CHEM	261	3637	1986
	AHLUWALIA G	BIOCHEM PHARMACOL	36	3797	1987
	ARONOW B	J BIOL CHEM	262	5106	1987
	CASEY PJ	BIOCHEM PHARMACOL	36	705	1987

	JOHNSON MA	J BIOL CHEM	263	15354	1988
	DAMON LE	PHARMACOL THER	38	73	1988
	BENNETT LL	BIOCHEM PHARMACOL	37	1233	1988
	FRIDLAND A	ANN N Y ACAD SCI	616	205	1990
	ALENIN VV	VOP MED KHIM	36	59	1990
	LOWENSTEIN JM	INT J SPORT MED	11	S37	1990
	AHLUWALIA GS	PHARMACOL THER	46	243	1990
	VANDENBERGHE G	PROG NEUROBIOL	39	547	1992
	MEREDITH M	BBA-MOL CELL RES	1266	16	1995
	HOU ZL	J BIOL CHEM	274	17505	1999
	BATOVA A	CANCER RES	59	1492	1999
	HARASAWA H	LEUKEMIA	16	1799	2002
	IANCU CV	J BIOL CHEM	277	26779	2002
	EFFERTH T	BIOCHEM PHARMACOL	66	613	2003
	STRAZZOLINI P	EUR J ORG CHEM	47	10	2004
	NELSON SW	BIOCHEMISTRY-USA	44	766	2005
	GINDER ND	J BIOL CHEM	281	20680	2006
	MARCE S	CLIN CANCER RES	12	3754	2006
	BATOVA A	BLOOD	107	898	2006
	BRAND J	CHEM-EUR J	12	499	2006
1980	**KENSLER TW**	**CANCER TREATM REP**	**64**	**967**	
	TAGUCHI H	ANTICANCER RES	9	845	1989
	GREM JL	ANTI-CANCER DRUG	4	545	1993
1980	**PARK KW**	**J BIOCH BIOPHY METH**	**2**	**291**	
	TYAGI AK	TOXICOLOGY	21	59	1981
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	ALSTON TA	BIOCHEM BIOPHYS RES COMMUN	105	560	1982
	TYAGI AK	TRENDS PHARMACOL SCI	4	299	1983
	HEIMER R	BIOCHEM PHARMACOL	32	199	1983
	STRAZZOLINI P	J MED CHEM	27	1295	1984
	TYAGI AK	ADVAN PHARMACOL CHEMOTHER	20	69	1984
	ALSTON TA	J BIOL CHEM	260	4069	1985
	CASEY PJ	J BIOL CHEM	261	3637	1986
	JALAL MAF	ACTA CRYSTALLOGR C-CRYST STR	42	733	1986
	CASEY PJ	BIOCHEM PHARMACOL	36	705	1987
	HONG SS	JPN J CANCER RES	80	592	1989
	AHLUWALIA GS	PHARMACOL THER	46	243	1990

	PEETERS MA	ANN GENET PARIS	34	219	1991
	RAMACHANDRAN B	J BIOL CHEM	268	23891	1993
	PALOS TP	MOL BRAIN RES	37	297	1996
	BATOVA A	CANCER RES	59	1492	1999
	EISENBERG D	BBA-PROTEIN STRUCT MOL ENZYM	1477	122	2000
	HARASAWA H	LEUKEMIA	16	1799	2002
	STRAZZOLINI P	EUR J ORG CHEM	47	10	2004
	MARCE S	CLIN CANCER RES	12	3754	2006
	BATOVA A	BLOOD	107	898	2006
	HUANG JW	BIOCHEMISTRY-USA	45	346	2006
	BERLICKI L	MINI-REV MED CHEM	8	869	2008
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	TYAGI AK	CANCER RES	40	4390	1980
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	TYAGI AK	BIOCHEM PHARMACOL	30	915	1981
	TYAGI AK	ADVAN PHARMACOL CHEMOTHER	20	69	1984
	CASEY PJ	J BIOL CHEM	261	3637	1986
	CASEY PJ	BIOCHEM J	246	263	1987
	ALENIN VV	ZH OBSHCH KHIM	57	692	1987
	CASEY PJ	BIOCHEM PHARMACOL	36	705	1987
	SANT ME	ANAL BIOCHEM	182	121	1989
	ALENIN VV	BIOCHEMISTRY-ENGL TR	57	572	1992
	TRETYAKOV OY	BIOCHEMISTRY-ENGL TR	60	1535	1995
1981	**TYAGI AK**	**J BIOL CHEM**	**256**	**12156**	
	TABOR CW	MED BIOL	59	272	1981
	TABOR CW	ADVAN POLYAMINE RES	4	467	1982
	POSO H	ADVAN POLYAMINE RES	4	603	1982
	KUEHN GD	ADVAN POLYAMINE RES	4	615	1982
	TYAGI AK	BIOCHEM BIOPHYS RES COMMUN	109	533	1982
	MITCHELL JLA	BIOCHEM J	205	551	1982
	SEELY JE	J BIOL CHEM	257	7549	1982
	SEELY JE	BIOCHEMISTRY-USA	21	3394	1982
	PRITCHARD ML	J BIOL CHEM	257	5892	1982
	CHOI JH	J BIOL CHEM	258	2601	1983
	POSO H	BIOCHIM BIOPHYS ACTA	747	209	1983
	TYAGI AK	METH ENZYMOLOGY	94	135	1983
	ZAGON IS	METH ENZYMOLOGY	94	169	1983
	LAPOINTE DS	ARCH BIOCHEM BIOPHYS	224	515	1983
	ERWIN BG	BIOCHEMISTRY-USA	22	3027	1983
	SEELY JE	J BIOL CHEM	258	2496	1983
	FLAMIGNI F	BIOCHIM BIOPHYS ACTA	802	245	1984
	TABOR CW	ANNU REV BIOCHEM	53	749	1984
	KAYE AM	CELL BIOCHEM FUNCT	2	2	1984
	BACHRACH U	CELL BIOCHEM FUNCT	2	6	1984
	BARNETT GR	J BIOL CHEM	259	179	1984

SKLAVIADIS TK	BIOCHIM BIOPHYS ACTA	831	288	1985
DAVIS RH	PROC NAT ACAD SCI USA	82	4105	1985
TABOR CW	MICROBIOL REV	49	81	1985
FONZI WA	MOL CELL BIOL	5	161	1985
HIATT AC	J BIOL CHEM	261	1293	1986
DORAZI D	PHYSIOL PLANT	71	177	1987
BELLOFATTO V	MOL BIOCHEM PARASITOL	25	227	1987
FONZI WA	J BIOL CHEM	262	10127	1987
DIGANGI JJ	J BIOL CHEM	262	7889	1987
PANDIT M	PHYTOCHEMISTRY	27	1609	1988
EICHLER W	J PROTOZOOL	36	577	1989
FONZI WA	J BIOL CHEM	264	18110	1989
BALASUNDARAM D	EUR J BIOCHEM	183	339	1989
FONZI WA	BIOCHEM BIOPHYS RES COMMUN	162	1409	1989
SCHAEFFER JM	BIOCHEM J	270	599	1990
MATSUFUJI S	J BIOCHEM TOKYO	108	365	1990
PANDIT M	J BIOSCIENCES	15	83	1990
SMITH TA	PHYTOCHEMISTRY	29	1759	1990
COONS T	MOL BIOCHEM PARASITOL	39	77	1990
JOSEPH K	J EXP ZOOL	258	158	1991
BABY TG	BIOCHIM BIOPHYS ACTA	1092	161	1991
ROSENBERGHASSON Y	EUR J BIOCHEM	196	647	1991
SMITH TA	MYCOL RES	96	395	1992
HANSON S	J BIOL CHEM	267	2350	1992
RAJAM MV	CURR SCI	65	461	1993
YARLETT N	BIOCHEM J	293	487	1993
SCHIPPER RG	J IMMUNOL METHOD	161	205	1993
BALASUNDARAM D	J BACTERIOL	176	7126	1994
NIEMANN G	BIOCHEM J	317	135	1996
BALASUNDARAM D	J BACTERIOL	178	2721	1996
KAOUASS M	MOL CELL BIOL	17	2994	1997
HAMASAKIKATAGIRI N	GENE	187	35	1997
KAOUASS M	J BIOL CHEM	273	2109	1998
TOTH C	J BIOL CHEM	274	25921	1999
PANTAZAKI AA	MOL CELL BIOCHEM	195	55	1999
KRAUSE T	BIOCHEM J	352	287	2000
ZHU C	BIOINFORMATICS	16	478	2000
LEE YS	J BIOCHEM MOL BIOL	34	478	2001
GUPTA R	PROC NAT ACAD SCI USA	98	10620	2001
CHATTOPADHYAY MK	J BIOL CHEM	276	21235	2001
COFFINO P	BIOCHIMIE	83	319	2001
ARTEAGA-NIETO P	EXP PARASITOL	101	215	2002
POULIN R	EUR COMMISS SCI RES DEV		3	2002
MOREHEAD TA	VIROLOGY	301	165	2002
GANDRE S	BIOCHEM BIOPHYS RES COMMUN	293	139	2002
BAIS HP	PLANT CELL TISSUE ORGAN CULT	69	1	2002
BACHMANN AS	PHYSIOL MOLEC PLANT PATHOL	63	57	2003
HOYT MA	J BIOL CHEM	278	12135	2003
ZAVADA MS	NORTHEAST NAT	11	33	2004

	AOUIDA M	J BIOL CHEM	280	24267	2005
	SMIT AY	S AFR J ENOL VITIC	29	109	2008
1981	**TYAGI AK**	**TOXICOLOGY**	**21**	**59**	
	TYAGI AK	ADVAN PHARMACOL CHEMOTHER	20	69	1984
	GALLIANI G	CANCER CHEMOTHER PHARMACOL	14	74	1985
	AHLUWALIA GS	PHARMACOL THER	46	243	1990
	BRAND J	CHEM-EUR J	12	499	2006
1981	**TYAGI JS**	**TOXICON**	**19**	**445**	
	ZAIKA LL	J FOOD PROTECT	50	691	1987
	KHAN SN	J SCI IND RES INDIA	47	130	1988
1981	**TYAGI JS**	**J APPL BACTERIOL**	**50**	**481**	
	LARROYA S	IRCS MED SCI-BIOCHEM	12	1064	1984
	KAWULA TH	J INVERTEBR PATHOL	43	282	1984
	DLUGONSKI J	CAN J MICROBIOL	30	57	1984
	LYNCH PT	TRANS BRIT MYCOL SOC	85	135	1985
	BHATNAGAR RK	J APPL BACTERIOL	60	135	1986
	CLEVELAND TE	CAN J MICROBIOL	33	1108	1987
	CLEVELAND TE	APPL ENVIRON MICROBIOL	53	1711	1987
	PFEIFER TA	APPL MICROBIOL BIOTECHNOL	26	248	1987
	PEBERDY JF	MYCOL RES	93	1	1989
	DLUGONSKI J	J BASIC MICROB	31	347	1991
	AZIZ NH	MICROBIOS	89	47	1997
1981	**TYAGI AK**	**BIOCH PHARMACOL**	**30**	**915**	
	TYAGI AK	TOXICOLOGY	21	59	1981
	TYAGI AK	TRENDS PHARMACOL SCI	4	299	1983
	STRAZZOLINI P	J MED CHEM	27	1295	1984
	MISTRELLO G	J IMMUNOPHARMACOL	6	25	1984
	TYAGI AK	ADVAN PHARMACOL CHEMOTHER	20	69	1984
	AHLUWALIA GS	BIOCHEM PHARMACOL	33	1195	1984
	GALLIANI G	CANCER CHEMOTHER PHARMACOL	14	74	1985
	CASEY PJ	J BIOL CHEM	261	3637	1986
	CASEY PJ	BIOCHEM PHARMACOL	36	705	1987
	AHLUWALIA GS	PHARMACOL THER	46	243	1990
	STRAZZOLINI P	EUR J ORG CHEM		4710	2004
1982	**TABOR CW**	**FED PROC**	**41**	**3084**	
	TABOR CW	ADVAN POLYAMINE RES	4	467	1982
	LIN PPC	PLANT PHYSIOL	74	975	1984
	WEINSTEIN LH	PLANT SCI	51	311	1987

1982	**TYAGI AK**	**BIOCH BIOPH RES COMM**	**109**	**533**	
	MITCHELL JLA	BIOCHEM J	214	345	1983
	ERWIN BG	BIOCHEMISTRY-USA	22	3027	1983
	SEKAR V	METH ENZYMOLOGY	107	154	1984
	PERSSON L	BIOCHEMISTRY-USA	23	3777	1984
	TABOR CW	ANNU REV BIOCHEM	53	749	1984
	DIENEL GA	J NEUROCHEM	42	1053	1984
	LORAND L	MOL CELL BIOCHEM	58	9	1984
	GRILLO MA	INT J BIOCHEM	17	943	1985
	TABOR CW	MICROBIOL REV	49	81	1985
	FONZI WA	MOL CELL BIOL	5	161	1985
	HOLTTA E	J BIOL CHEM	261	9502	1986
	FONZI WA	J BIOL CHEM	262	10127	1987
	BALASUNDARAM D	ARCH BIOCHEM BIOPHYS	264	288	1988
	FONZI WA	J BIOL CHEM	264	18110	1989
	FONZI WA	BIOCHEM BIOPHYS RES COMMUN	162	1409	1989
	MIYAMOTO K	J BIOCHEM TOKYO	106	167	1989
	AL-SHABANAH OA	PHARMACOL RES	40	75	1999
1983	**TABOR CW**	**ADV POLYAM RES**	**4**	**467**	
	TABOR CW	ANNU REV BIOCHEM	53	749	1984
	KAYE AM	CELL BIOCHEM FUNCT	2	2	1984
	LUK GD	WEST J MED	142	88	1985
	LUK GD	GASTROENTEROLOGY	90	1261	1986
	JAIN A	MOL CELL BIOCHEM	78	3	1987
	BALASUNDARAM D	MOL CELL BIOCHEM	100	129	1991
1983	**TYAGI AK**	**METH ENZYMOL**	**94**	**135**	
	TABOR CW	ANNU REV BIOCHEM	53	749	1984
	TABOR CW	MICROBIOL REV	49	81	1985
	BIRECKA H	PLANT PHYSIOL	80	798	1986
	YAMAMOTO S	MICROBIOL IMMUNOL	32	675	1988
	BALASUNDARAM D	ARCH BIOCHEM BIOPHYS	264	288	1988
	SCHUMANN H	SYST APPL MICROBIOL	11	103	1989
	NAKAO H	J GEN MICROBIOL	135	345	1989
	KLEIN RD	EXP PARASITOL	87	171	1997
	KLEIN RD	MICROBIOL-UK	145	301	1999
	GUPTA R	PROC NAT ACAD SCI USA	98	10620	2001
	SUBHI AL	J BIOL CHEM	278	49868	2003
	CHATTOPADHYAY MK	PROC NAT ACAD SCI USA	102	16158	2005
1983	**TYAGI AK**	**TREND PHARM SCI**	**4**	**299**	
	STRAZZOLINI P	J MED CHEM	27	1295	1984
	TYAGI AK	ADVAN PHARMACOL CHEMOTHER	20	69	1984
	CASEY PJ	J BIOL CHEM	261	3637	1986

	AHLUWALIA GS	PHARMACOL THER	46	243	1990
	BRAND J	CHEM-EUR J	12	499	2006
1984	**TYAGI AK**	**ADV PHARMACOL CHEMOTH**	**20**	**69**	
	CASEY PJ	BIOCHEM PHARMACOL	36	705	1987
	HONG SS	JPN J CANCER RES	80	592	1989
	AHLUWALIA GS	PHARMACOL THER	46	243	1990
	PEETERS MA	ANN GENET PARIS	34	219	1991
	ALENIN VV	BIOCHEMISTRY-ENGL TR	57	572	1992
	RAMACHANDRAN B	J BIOL CHEM	268	23891	1993
	GUICHERIT OM	ADVAN EXPERIMENT MED BIOL	370	585	1994
	CARRERA CJ	HEMATOL ONCOL CLIN N AMER	8	357	1994
	GUICHERIT OM	J BIOL CHEM	269	4488	1994
	HORI H	CANCER RES	56	5653	1996
	BATOVA A	BLOOD	88	3083	1996
	PALOS TP	MOL BRAIN RES	37	297	1996
	BATOVA A	CANCER RES	59	1492	1999
	BATIUK TD	AMER J PHYSIOL-CELL PHYSIOL	281	C1776	2001
	HARASAWA H	LEUKEMIA	16	1799	2002
	HRABIE JA	CHEM REV	102	1135	2002
	ARULSAMY N	TETRAHEDRON LETT	44	4267	2003
	STRAZZOLINI P	EUR J ORG CHEM	47	10	2004
	LI XM	MOL CANCER THER	5	337	2006
	BATOVA A	BLOOD	107	898	2006
	BRAND J	CHEM-EUR J	12	499	2006
	BILODEAU-GOESEELS S	MOL REPROD DEV	74	1021	2007
1984	**TYAGI AK**	**P NATL ACAD SCI**	**81**	**1149**	
	SLOCUM RD	ARCH BIOCHEM BIOPHYS	235	283	1984
	TABOR CW	ANNU REV BIOCHEM	53	749	1984
	TIPPER DJ	MICROBIOL REV	48	125	1984
	ESCRIBANO MI	ENDOCYTOBIOSIS CELL RES	2	239	1985
	HANNIG EM	NUCL ACID RES	13	4379	1985
	TABOR CW	MICROBIOL REV	49	81	1985
	BENDOVA O	FOLIA MICROBIOL PRAGUE	31	422	1986
	WICKNER RB	ANNU REV BIOCHEM	55	373	1986
	LEE M	J VIROL	58	402	1986
	FUJIMURA T	MOL CELL BIOL	6	404	1986
	JAIN A	MOL CELL BIOCHEM	78	3	1987
	NESTEROVA GF	GENETIKA+	24	1141	1988
	UEMURA H	MOL CELL BIOL	8	938	1988
	ICHO T	J BIOL CHEM	263	1467	1988
	FUJIMURA T	J BIOL CHEM	263	454	1988
	BROWN GG	INT REV CYTOL	117	1	1989
	BALASUNDARAM D	MOL CELL BIOCHEM	100	129	1991
	TERCERO JC	J BIOL CHEM	267	20270	1992
	WICKNER RB	ANNU REV MICROBIOL	46	347	1992

	VANVUUREN HJJ	AMER J ENOL VITICULT	43	119	1992
1987	**JAIN A**	**MOL CELL BIOCHEM**	**78**	**3**	
	OLLER AR	BIOCHEMISTRY-USA	30	2543	1991
	BALASUNDARAM D	MOL CELL BIOCHEM	100	129	1991
	SCHWARTZ B	BIOCHEM J	312	83	1995
1987	**BHUTANI V**	**NUTR RES**	**7**	**763**	
	BHUTANI V	INT J VITAM NUTR RES	58	452	1988
	BHUTANI V	NUTR RES	9	465	1989
1988	**BALASUNDARAM D**	**ARCH BIOCH BIOPHY**	**264**	**288**	
	BALASUNDARAM D	EUR J BIOCHEM	183	339	1989
	SARKAR NK	BIOCHEM MOL BIOL INT	35	1189	1995
	TALAUE MT	J BACTERIOL	188	4830	2006
1989	**BALASUNDARAM D**	**EUR J BIOCHEM**	**183**	**339**	
	SARKAR NK	BIOCHEM MOL BIOL INT	35	1189	1995
	SANCHEZ CP	BIOCHEM BIOPHYS RES COMMUN	212	396	1995
	SETH A	J BACTERIOL	182	919	2000
	COLEMAN CS	BIOCHEM J	379	849	2004
1990	**TYAGI JS**	**TROP MED PARASITOL**	**41**	**294**	
	VERMA A	INDIAN J BIOCHEM BIOPHYS	32	429	1995
1990	**BHARGAVA S**	**J BACTERIOL**	**72**	**2930**	
	LEE MH	PROC NAT ACAD SCI USA	88	3111	1991
	KEMPSELL KE	J GEN MICROBIOL	138	1717	1992
	TYAGI JS	NUCL ACID RES	20	138	1992
	KINGER AK	GENE	131	113	1993
	GUPTA S	GENE	126	157	1993
	VERMA A	GENE	148	113	1994
	VERMA A	INDIAN J BIOCHEM BIOPHYS	31	288	1994
	VERMA A	INDIAN J BIOCHEM BIOPHYS	32	429	1995
	MISRA N	INT J LEPROSY	63	35	1995
	VASANTHAKRISHNA M	MICROBIOL-UK	143	3591	1997
	PENA CEA	J MOL BIOL	266	76	1997
	VASANTHAKRISHNA M	J BIOSCIENCES	23	101	1998
	DASTUR A	ARCH MICROBIOL	178	288	2002
	LI AH	MICROBIOL-SGM	154	2291	2008

1991	**BALASUNDARAM D**	**MOL CELL BIOCHEM**	**100**	**129**	
	BLACHIER F	BIOCHIM BIOPHYS ACTA	1175	21	1992
	RAJAM MV	CURR SCI	65	461	1993
	WING LYC	J PHARMACOL EXP THER	266	179	1993
	MCCORMACK SA	AMER J PHYSIOL	264	G367	1993
	LINARES PN	BIOG AMINE	10	365	1994
	HUANG H	BIOG AMINE	10	259	1994
	BALASUNDARAM D	PROC NAT ACAD SCI USA	91	172	1994
	SARKAR NK	BIOCHEM MOL BIOL INT	35	1189	1995
	SHINOZAKI T	J RHEUMATOL	22	1907	1995
	HUBER M	CANCER RES	55	934	1995
	AUCHTER RM	ARCH OTOLAR-HEAD NECK SURGERY	122	977	1996
	YOUNOSZAI MK	PROC SOC EXP BIOL MED	211	339	1996
	MURLEY JS	CELL PROLIFERAT	30	283	1997
	MADESH M	BBA-LIPID LIPID METAB	1348	324	1997
	BERLAIMONT V	ANTICANCER RES	17	2057	1997
	CORRALIZA IM	BBA-GEN SUBJECTS	1334	123	1997
	SARAN S	CELL BIOL INT	22	575	1998
	MITCHELL JLA	BIOCHEM J	335	329	1998
	LEVEQUE J	ANTICANCER RES	18	2663	1998
	BOOTH VK	RADIAT RES	153	813	2000
	MCCORMACK SA	J PHYSIOL PHARMACOL	52	327	2001
	PENDEVILLE H	MOL CELL BIOL	21	6549	2001
	HAHM HA	CLIN CANCER RES	7	391	2001
	SCORCIONI F	BIOCHEM J	354	217	2001
	BAIS HP	PLANT CELL TISSUE ORGAN CULT	69	1	2002
	WORTHAM BW	ADVAN EXPERIMENT MED BIOL	603	106	2007
	BLACHIER F	AMINO ACIDS	33	547	2007
1992	**SHANKAR S**	**NUCL ACID RES**	**20**	**2891**	
	SHANKAR S	GENE	132	119	1993
	SHANKAR S	GENE	131	153	1993
	ROBERTS RJ	NUCL ACID RES	21	3125	1993
	VANSOOLINGEN D	J BACTERIOL	178	78	1996
	MANDAL P	J BIOCHEM MOL BIOL	39	140	2006
1992	**SHANKAR S**	**NUCL ACID RES**	**20**	**2890**	
	SHANKAR S	GENE	132	119	1993
	SHANKAR S	GENE	131	153	1993
	ROBERTS RJ	NUCL ACID RES	21	3125	1993
	VANSOOLINGEN D	J BACTERIOL	178	78	1996
1993	**DASGUPTA SK**	**J BACTERIOL**	**175**	**5186**	
	BASHYAM MD	BIOTECHNIQUES	17	834	1994
	TIMM JL	J BACTERIOL	176	6749	1994

VERMA A	GENE	148	113	1994
TIMM J	MOL MICROBIOL	12	491	1994
RAMESH GR	INDIAN J BIOCHEM BIOPHYS	32	361	1995
SARKAR NK	BIOCHEM MOL BIOL INT	35	1189	1995
NESBIT CE	MOL MICROBIOL	17	1045	1995
KREMER L	MOL MICROBIOL	17	913	1995
DELLAGOSTIN OA	MICROBIOL-UK	141	1785	1995
WINTER N	MOL MICROBIOL	16	865	1995
KREMER L	J BACTERIOL	177	642	1995
HATFULL GF	CURR TOPICS MICROBIOL IMMUNOL	215	29	1996
TYAGI JS	GENE	177	59	1996
PAGET E	J BACTERIOL	178	6357	1996
BEGGS ML	GENE	174	285	1996
BASHYAM MD	J BACTERIOL	178	4847	1996
MULDER MA	TUBERCLE LUNG DIS	78	211	1997
VASANTHAKRISHNA M	MICROBIOL-UK	143	3591	1997
PARISH T	MICROBIOL-UK	143	2267	1997
MOVAHEDZADEH F	J BACTERIOL	179	3509	1997
JAIN S	GENE	190	37	1997
BANNANTINE JP	MICROBIOL-UK	143	921	1997
BATONI G	FEMS MICROBIOL LETT	169	117	1998
KNIPFER N	GENE	217	69	1998
BARKER LP	MOL MICROBIOL	29	1167	1998
RAYCHAUDHURI S	MICROBIOL-UK	144	2131	1998
CHUBB AJ	MICROBIOL-UK	144	1619	1998
DASGUPTA SK	BIOCHEM BIOPHYS RES COMMUN	246	797	1998
BASHYAM MD	J BACTERIOL	180	2568	1998
PARISH A	MOL BIOTECHNOL	13	191	1999
UNNIRAMAN S	GENES CELLS	4	697	1999
HATFULL GF	METH MICROBIOL	29	251	1999
CARBONELLI DL	FEMS MICROBIOL LETT	177	75	1999
VERMA A	J BACTERIOL	181	4326	1999
BARKER LP	FEMS MICROBIOL LETT	175	79	1999
CHAWLA M	PLASMID	41	135	1999
GUPTA S	FEMS MICROBIOL LETT	172	137	1999
RUBIN EJ	PROC NAT ACAD SCI USA	96	1645	1999
TYAGI AK		109		2000
DASGUPTA N	TUBERCLE LUNG DIS	80	141	2000
DHAR N	FEMS MICROBIOL LETT	190	309	2000
UNNIRAMAN S	J BIOL CHEM	276	41850	2001
TRICCAS JA	MICROBIOL-SGM	147	1253	2001
COWLEY SC	GENE	264	225	2001
UNNIRAMAN S	NUCL ACID RES	30	5376	2002
SIRAKOVA TD	J BACTERIOL	184	6796	2002
DASTUR A	ARCH MICROBIOL	178	288	2002
UNNIRAMAN S	J BACTERIOL	184	5449	2002
MEDEIROS MA	MICROBIOL-SGM	148	1999	2002
KAMALAKANNAN V	FEMS MICROBIOL LETT	209	261	2002
BASU A	J BACTERIOL	184	2204	2002

	CHATTOPADHYAY C	J BIOCHEM MOL BIOL	36	586	2003
	RAO V	SCAND J IMMUNOL	58	449	2003
	AGARWAL N	FEMS MICROBIOL LETT	225	75	2003
	UNNIRAMAN S	BIOTECHNIQUES	35	256	2003
	SMITH I	CLIN MICROBIOL REV	16	463	2003
	SATCHIDANANDAM V	FEMS MICROBIOL LETT	218	365	2003
	SOHASKEY CD	FEMS MICROBIOL LETT	240	187	2004
	SAU S	J BIOCHEM MOL BIOL	37	254	2004
	BASU A	J BACTERIOL	186	335	2004
	BAGCHI G	MICROBIOL-SGM	151	4045	2005
	ZHU JC	THERMOCHIM ACTA	439	52	2005
	RAO V	SCAND J IMMUNOL	61	410	2005
	DEOL P	J BACTERIOL	187	3415	2005
	DATTA I	J BIOCHEM MOL BIOL	38	89	2005
	MACHOWSKI EE	INT J BIOCHEM CELL BIOL	37	54	2005
	AGARWAL N	NUCL ACID RES	34	4245	2006
	GUPTA R	BIOCHEM BIOPHYS RES COMMUN	343	1141	2006
	GALL K	FEMS MICROBIOL LETT	255	301	2006
1993	**GUPTA S**	**GENE**	**126**	**157**	
	SHANKAR S	GENE	132	119	1993
	SHANKAR S	GENE	131	153	1993
	RAMESH GR	INDIAN J BIOCHEM BIOPHYS	32	361	1995
	YOUNG DB	ANNU REV MICROBIOL	49	641	1995
	QUINN FD	CURR TOPICS MICROBIOL IMMUNOL	215	131	1996
	GORDON S	J APPL BACTERIOL	81	S10	1996
	COLLINS DM	TRENDS MICROBIOL	4	426	1996
	GALLEGOS MT	MICROBIOL MOL BIOL REV	61	393	1997
	RIVERA-MARRERO CA	MICROB PATHOG	25	307	1998
	MATSUSAKI H	J BACTERIOL	180	6459	1998
	GERRITSE G	J BIOTECHNOL	64	23	1998
	GUPTA S	FEMS MICROBIOL LETT	172	137	1999
	TYAGI AK		109		2000
	AV-GAY Y	TRENDS MICROBIOL	8	238	2000
	MONAHAN IM	MICROBIOL-UK	147	459	2001
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	RECCHI C	J BIOL CHEM	278	33763	2003
	PETTINARI MJ	PLASMID	50	36	2003
	SINGH R	TUBERCULOSIS	85	325	2005
	SINGH A	J BACTERIOL	187	4173	2005
	PAWARIA S	APPL ENVIRON MICROBIOL	74	3512	2008
1993	**SHANKAR S**	**GENE**	**131**	**153**	
	ROBERTS RJ	NUCL ACID RES	22	3628	1994
	VANSOOLINGEN D	J BACTERIOL	178	78	1996
	MANDAL P	J BIOCHEM MOL BIOL	39	140	2006
	VOSSOUGHI M	MATH COMPUT SCI ENG	7		2007

1993	**SHANKAR S**	**GENE**	**132**	**119**	
	ROBERTS RJ	NUCL ACID RES	22	3628	1994
	VANSOOLINGEN D	J BACTERIOL	178	78	1996
	MANDAL P	J BIOCHEM MOL BIOL	39	140	2006
1994	**BASHYAM MD**	**BIOTECHNIQ**	**17**	**834**	
	TYAGI JS	GENE	177	59	1996
	BANERJEE SK	BIOCHEM BIOPHYS RES COMMUN	226	362	1996
	JACKSON M	MICROBIOL-UK	142	2439	1996
	BASHYAM MD	J BACTERIOL	178	4847	1996
	FALKINHAM JO	CLIN MICROBIOL REV	9	177	1996
	CACERES NE	J BACTERIOL	179	5046	1997
	NAGY I	LETT APPL MICROBIOL	25	75	1997
	BANNANTINE JP	MICROBIOL-UK	143	921	1997
	PEIRS P	EUR J BIOCHEM	244	604	1997
	MANGAN JA	NUCL ACID RES	25	675	1997
	BERTHET FX	MICROBIOL-UK	144	3195	1998
	BANERJEE SK	FEBS LETT	425	151	1998
	HATFULL GF	METH MICROBIOL	29	251	1999
	VERMA A	J BACTERIOL	181	4326	1999
	GUPTA S	FEMS MICROBIOL LETT	172	137	1999
	ALONSO G	FEMS MICROBIOL LETT	192	257	2000
	GILOT P	J MED MICROBIOL	49	887	2000
	MANGAN JA	METH MICROBIOL	33	137	2002
	FENG ZY	J BACTERIOL	184	5001	2002
	YAO YF	J MICROBIOL METH	51	191	2002
	SUNG K	FEMS MICROBIOL LETT	229	97	2003
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	FENG ZY	ANTIMICROB AGENTS CHEMOTHER	47	283	2003
	STEPHAN J	BMC MICROBIOL	4	45	2004
	SHARBATI-TEHRANI S	INT J MED MICROBIOL	294	235	2004
	SHARBATI-TEHRANI S	MICROBIOL-SGM	151	2403	2005
	KIM BH	J PHYCOL	42	1137	2006
	JAHN CE	J MICROBIOL METH	75	318	2008
1995	**SARKAR NK**	**BIOCH MOL BIOL INT**	**35**	**1189**	
	BERGER BJ	BMC MICROBIOL	3	12	2003
	KHEDKAR SA	J MOL GRAPH MODEL	23	355	2005
1996	**BASHYAM MD**	**J BACTERIOL**	**178**	**4847**	
	MULDER MA	TUBERCLE LUNG DIS	78	211	1997
	GOMEZ JE	TUBERCLE LUNG DIS	78	175	1997
	MENENDEZ MC	J BACTERIOL	179	6880	1997
	GONZALEZYMERCHA JA	J BACTERIOL	179	6949	1997

SPOHN G	MOL MICROBIOL	26	361	1997
PLUM G	INFEC IMMUNITY	65	4548	1997
BARNES MR	J BACTERIOL	179	6145	1997
CACERES NE	J BACTERIOL	179	5046	1997
NAGY I	LETT APPL MICROBIOL	25	75	1997
MOVAHEDZADEH F	J BACTERIOL	179	3509	1997
JAIN S	GENE	190	37	1997
WU QL	J BACTERIOL	179	2922	1997
BANNANTINE JP	MICROBIOL-UK	143	921	1997
LARKIN MJ	ANTON LEEUWENHOEK INT J GEN M	74	133	1998
BERTHET FX	MICROBIOL-UK	144	3195	1998
BOSHOFF HIM	J BACTERIOL	180	5809	1998
KNIPFER N	GENE	217	69	1998
GOMEZ M	MOL MICROBIOL	29	617	1998
RAYCHAUDHURI S	MICROBIOL-UK	144	2131	1998
DHANDAYUTHAPANI S	GENE	215	213	1998
FORD ME	J MOL BIOL	279	143	1998
DASGUPTA SK	BIOCHEM BIOPHYS RES COMMUN	246	797	1998
BASHYAM MD	J BACTERIOL	180	2568	1998
PLIKAYTIS BB	J BACTERIOL	180	1037	1998
MATSUMOTO S	MICROBIOL IMMUNOL	42	15	1998
UNNIRAMAN S	GENES CELLS	4	697	1999
MULDER MA	MICROBIOL-UK	145	2507	1999
HATFULL GF	METH MICROBIOL	29	251	1999
FERNANDES ND	J BACTERIOL	181	4266	1999
VERMA A	J BACTERIOL	181	4326	1999
BARKER LP	FEMS MICROBIOL LETT	175	79	1999
DUSSURGET O	J BACTERIOL	181	3402	1999
HU YM	J BACTERIOL	181	3486	1999
HU YM	J BACTERIOL	181	1380	1999
RUBIN EJ	PROC NAT ACAD SCI USA	96	1645	1999
STOLT P	NUCL ACID RES	27	396	1999
HU JM	J BACTERIOL	181	469	1999
TYAGI AK			109	2000
DASGUPTA N	TUBERCLE LUNG DIS	80	141	2000
DHAR N	FEMS MICROBIOL LETT	190	309	2000
BIGI F	MICROBIOL-UK	146	1011	2000
PARKER AE	MICROB PATHOG	28	135	2000
ALLAND D	J BACTERIOL	182	1802	2000
RAMASWAMY SV	ANTIMICROB AGENTS CHEMOTHER	44	326	2000
TULLIUS MV	INFEC IMMUNITY	69	6348	2001
DASTUR A	TUBERCULOSIS	81	267	2001
HARRIS NB	CLIN MICROBIOL REV	14	489	2001
INGLIS NF	MICROBIOL-SGM	147	1557	2001
TORRES A	MICROB PATHOG	30	289	2001
SIRAKOVA TD	J BACTERIOL	184	6796	2002
DASTUR A	ARCH MICROBIOL	178	288	2002
KALATE RN	BIOPHYS CHEM	99	77	2002
UNNIRAMAN S	J BACTERIOL	184	5449	2002

	MAYURI	FEMS MICROBIOL LETT	211	231	2002
	TYAGI JS	TRENDS MICROBIOL	10	68	2002
	KALATE RN	COMPUT BIOL CHEM	27	555	2003
	MUSATOVOVA O	FEMS MICROBIOL LETT	229	73	2003
	CHATTOPADHYAY C	J BIOCHEM MOL BIOL	36	586	2003
	GOPAUL KK	J BACTERIOL	185	6005	2003
	BAGCHI G	MICROBIOL-SGM	149	2303	2003
	PATEK M	J BIOTECHNOL	104	325	2003
	SALA C	J BACTERIOL	185	5357	2003
	RECCHI C	J BIOL CHEM	278	33763	2003
	AGARWAL N	FEMS MICROBIOL LETT	225	75	2003
	UNNIRAMAN S	BIOTECHNIQUES	35	256	2003
	SAVIOLA B	INFEC IMMUNITY	71	1379	2003
	SATCHIDANANDAM V	FEMS MICROBIOL LETT	218	365	2003
	ROY S	RES MICROBIOL	155	817	2004
	SOHASKEY CD	FEMS MICROBIOL LETT	240	187	2004
	SHARBATI-TEHRANI S	INT J MED MICROBIOL	294	235	2004
	ROBERTS EA	J BACTERIOL	186	5410	2004
	SAFI H	MOL MICROBIOL	52	999	2004
	SAU S	J BIOCHEM MOL BIOL	37	254	2004
	LEE BR	BIOTECHNOL LETT	26	589	2004
	BAGCHI G	MICROBIOL-SGM	151	4045	2005
	JAIN V	GENE	351	149	2005
	EHRT S	FUTURE MICROBIOL	1	177	2006
	BURONI S	ANTIMICROB AGENTS CHEMOTHER	50	4044	2006
	HERNANDEZ-ABANTO SM	ARCH MICROBIOL	186	459	2006
	AGARWAL N	NUCL ACID RES	34	4245	2006
	FABOZZI G	MICROB PATHOG	40	211	2006
	GUPTA R	BIOCHEM BIOPHYS RES COMMUN	343	1141	2006
	JAIN V	J MICROBIOL	44	1	2006
	GONZALEZ-DIAZ H	BIOORG MEDICINAL CHEM LETTER	16	547	2006
	SEO JG	MICROBIOL-SGM	153	4174	2007
	CHOWDHURY RP	J BACTERIOL	189	8973	2007
	SUBBIAN S	CAN J MICROBIOL	53	599	2007
	HALBEDEL S	J MOL BIOL	371	596	2007
	BYRNE GA	J BACTERIOL	189	5082	2007
	RICHTER L	GENE	395	22	2007
	GONZALEZ-DIAZ H	CHEMOMETR INTELL LAB SYST	85	20	2007
	CHURCHILL PF	J ENVIRON SCI HEALTH B-PESTIC	43	698	2008
1997	**JAIN S**	**GENE**	**190**	**37**	
	BARKER LP	MOL MICROBIOL	29	1167	1998
	DASGUPTA SK	BIOCHEM BIOPHYS RES COMMUN	246	797	1998
	ROWLAND B	FEMS MICROBIOL LETT	179	317	1999
	HATFULL GF	METH MICROBIOL	29	251	1999
	VERMA A	J BACTERIOL	181	4326	1999
	GUPTA S	FEMS MICROBIOL LETT	172	137	1999
	TYAGI AK			109	2000

	JAIN S	MOL MICROBIOL	38	971	2000
	DASGUPTA N	TUBERCLE LUNG DIS	80	141	2000
	PINEIRO SA	CURR MICROBIOL	40	302	2000
	CHATTOPADHYAY C	J BIOCHEM MOL BIOL	36	586	2003
	KIM AI	MOL MICROBIOL	50	463	2003
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	BAGCHI G	MICROBIOL-SGM	149	2303	2003
	AGARWAL N	FEMS MICROBIOL LETT	225	75	2003
	GANGULY T	J BIOCHEM MOL BIOL	37	709	2004
	SAU S	J BIOCHEM MOL BIOL	37	254	2004
	SINGH R	TUBERCULOSIS	85	325	2005
	JAIN V	GENE	351	149	2005
	AGARWAL N	NUCL ACID RES	34	4245	2006
	RAGHUNAND TR	MICROBIOL-SGM	152	2735	2006
	AGARWAL N	MICROBIOL-SGM	152	2749	2006
	GANGULY T	PROTEIN PEPTIDE LETT	13	793	2006
	CHOWDHURY RP	J BACTERIOL	189	8973	2007
	MANDAL S	MICROBIOL-SGM	153	80	2007
	MALHOTRA M	ENVIRON MICROBIOL	10	1365	2008
1998	**BASHYAM MD**	**J BACTERIOL**	**180**	**2568**	
	PARISH A	MOL BIOTECHNOL	13	191	1999
	FERNANDES ND	J BACTERIOL	181	4266	1999
	BARKER LP	FEMS MICROBIOL LETT	175	79	1999
	BURNS HD	NUCL ACID RES	27	2051	1999
	MADSEN SM	MOL MICROBIOL	32	75	1999
	STOLT P	NUCL ACID RES	27	396	1999
	BOWN JA	J BIOL CHEM	274	2263	1999
	TYAGI AK			109	2000
	DASGUPTA N	TUBERCLE LUNG DIS	80	141	2000
	NARAYANAN S	FEMS MICROBIOL LETT	192	263	2000
	GIARD JC	J BACTERIOL	182	4512	2000
	LI MS	MICROBIOL-SGM	147	2293	2001
	HARRIS NB	CLIN MICROBIOL REV	14	489	2001
	INGLIS NF	MICROBIOL-SGM	147	1557	2001
	GAL-MOR O	J BACTERIOL	184	3823	2002
	CHATTOPADHYAY C	J BIOCHEM MOL BIOL	36	586	2003
	RECCHI C	J BIOL CHEM	278	33763	2003
	MITCHELL JE	NUCL ACID RES	31	4689	2003
	AGARWAL N	FEMS MICROBIOL LETT	225	75	2003
	UNNIRAMAN S	BIOTECHNIQUES	35	256	2003
	HAYASHI K	PLANT CELL PHYSIOL	44	334	2003
	SATCHIDANANDAM V	FEMS MICROBIOL LETT	218	365	2003
	MENENDEZ MD	J BACTERIOL	187	534	2005
	EHRT S	FUTURE MICROBIOL	1	177	2006
	AGARWAL N	NUCL ACID RES	34	4245	2006
	PASHLEY CA	MICROBIOL-SGM	152	2727	2006
	DOHERTY N	J BACTERIOL	188	2885	2006

	JEONG DW	FOOD MICROBIOL	23	82	2006
	RICHTER L	GENE	395	22	2007
	DANILCHANKA O	ANTIMICROB AGENTS CHEMOTHER	52	2503	2008
	MICK V	J ANTIMICROB CHEMOTHER	61	39	2008
1998	**DASGUPTA SK**	**BIOCH BIOPH RES COMM**	**246**	**797**	
	VERMA A	J BACTERIOL	181	4326	1999
	CHAWLA M	PLASMID	41	135	1999
	TYAGI AK			109	2000
	JAIN S	MOL MICROBIOL	38	971	2000
	DHAR N	FEMS MICROBIOL LETT	190	309	2000
	OJHA AK	INFEC IMMUNITY	68	4084	2000
	AL-ZAROUNI M	TUBERCULOSIS	82	283	2002
	UNNIRAMAN S	J BACTERIOL	184	5449	2002
	MEDEIROS MA	MICROBIOL-SGM	148	1999	2002
	BASU A	J BACTERIOL	184	2204	2002
	SINGH R	MOL MICROBIOL	50	751	2003
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	RAO V	SCAND J IMMUNOL	58	449	2003
	DHAR N	IMMUNOL LETT	88	175	2003
	VENKATESH J	J BIOL CHEM	278	24350	2003
	SATCHIDANANDAM V	FEMS MICROBIOL LETT	218	365	2003
	BASU A	J BACTERIOL	186	335	2004
	SHENOY AR	BIOCHEMISTRY-USA	44	15695	2005
	MATHEW R	J BACTERIOL	187	6565	2005
	SINGH A	J BACTERIOL	187	4173	2005
	RAO V	SCAND J IMMUNOL	61	410	2005
	DENNEHY M	VACCINE	23	1209	2005
	YU JS	CLIN VACCINE IMMUNOL	13	1204	2006
	RAO A	APPL ENVIRON MICROBIOL	73	1320	2007
	SALLAM KI	GENE	386	173	2007
1999	**GUPTA S**	**FEMS MICROB LETT**	**172**	**137**	
	TYAGI AK			109	2000
	COLLINS DM	TUBERCULOSIS	81	97	2001
	MEHROTRA J	INT J MED MICROBIOL	291	171	2001
	SINGH A	FEMS MICROBIOL LETT	227	53	2003
	RECCHI C	J BIOL CHEM	278	33763	2003
	FROTA CC	INFEC IMMUNITY	72	5483	2004
	MOSTOWY S	J BACTERIOL	186	104	2004
	MARRI PR	FEMS MICROBIOL REV	30	906	2006
	TALAAT AM	J BACTERIOL	189	4265	2007
2000	**DHAR N**	**FEMS MICROB LETT**	**190**	**309**	
	CHOUDHARY RK	INFEC IMMUNITY	71	6338	2003
	SINGH A	FEMS MICROBIOL LETT	227	53	2003

	RAO V	SCAND J IMMUNOL	58	449	2003
	DHAR N	IMMUNOL LETT	88	175	2003
	DHAR N	MED MICROBIOL IMMUNOL	193	19	2004
	KHERA A	VACCINE	23	5655	2005
	RAO V	SCAND J IMMUNOL	61	410	2005
	DENNEHY M	VACCINE	23	1209	2005
	JOSEPH J	EXPERT REV VACCINES	5	827	2006
	FAN XL	ACTA BIOCHIM BIOPHYS SINICA	38	683	2006
	SHARMA K	J BACTERIOL	188	2936	2006
	WANG LM	CHIN MED J	120	1220	2007
2000	**KOUL A**	**J BACT**	**182**	**5425**	
	KOUL A	MICROBIOL-SGM	147	2307	2001
	KENNELLY PJ	CHEM REV	101	2291	2001
	BARRY CE	TRENDS MICROBIOL	9	237	2001
	VAN HUIJSDUIJNEN RH	DRUG DISCOV TODAY	7	1013	2002
	BATONI G	SCAND J IMMUNOL	56	43	2002
	COWLEY SC	RES MICROBIOL	153	233	2002
	PRENETA R	COMP BIOCHEM PHYSIOL PT B	131	103	2002
	LI RH	J BACTERIOL	185	6780	2003
	CHOPRA P	BIOCHEM BIOPHYS RES COMMUN	311	112	2003
	SINGH R	MOL MICROBIOL	50	751	2003
	SINHA I	FEMS MICROBIOL LETT	227	141	2003
	BOITEL B	MOL MICROBIOL	49	1493	2003
	CHOPRA P	INDIAN J MED RES	117	1	2003
	ANAYA-RUIZ M	INT J PARASITOL	33	663	2003
	PRENETA R	MICROBIOL-SGM	150	2135	2004
	DEWANG PM	CURR ORG CHEM	8	947	2004
	SHARMA K	EXPERT OPIN THER TARGETS	8	79	2004
	KOUL A	NAT REV MICROBIOL	2	189	2004
	COZZONE AJ	ARCH MICROBIOL	181	171	2004
	TYAGI JS	CURR SCI	86	93	2004
	GREENSTEIN AE	J MOL MICROBIOL BIOTECHNOL	9	167	2005
	COZZONE AJ	J MOL MICROBIOL BIOTECHNOL	9	198	2005
	CASTANDET M	RES MICROBIOL	156	1005	2005
	SINGH R	TUBERCULOSIS	85	325	2005
	GRUNDNER C	STRUCTURE	13	1625	2005
	SAXENA K	J BIOMOL NMR	33	136	2005
	MANGER M	CHEMBIOCHEM	6	1749	2005
	LEI JQ	CURR MICROBIOL	51	141	2005
	BIALY L	ANGEW CHEM INT ED	44	3814	2005
	VERGNE I	PROC NAT ACAD SCI USA	102	4033	2005
	MADHURANTAKAM C	J BACTERIOL	187	2175	2005
	PRABHAKAR S	J IMMUNOL	174	1003	2005
	DEWANG PM	CURR MEDICINAL CHEM	12	1	2005
	BACH H	INFEC IMMUNITY	74	6540	2006
	LESCOP E	J BIOL CHEM	281	19570	2006
	XU HM	J BACTERIOL	188	1509	2006

	WEIDE T	BIOORG MEDICINAL CHEM LETTER	16	59	2006
	POOK SH	ONCOL REP	18	1315	2007
	SOELLNER MB	J AM CHEM SOC	129	9613	2007
	CORREA IR	CHEM-ASIAN J	2	1109	2007
	BERESFORD N	BIOCHEM J	406	13	2007
	DEGHMANE AE	J CELL SCI	120	2796	2007
	HOLTON SJ	CURR PROTEIN PEPT SCI	8	365	2007
	AGUIRRE-GARCIA MM	PARASITOL RES	101	85	2007
	JANIN YL	BIOORGAN MED CHEM	15	2479	2007
	GRANGEASSE C	TRENDS BIOCHEM SCI	32	86	2007
	O'SHEA DJ	ANAL CHIM ACTA	583	349	2007
	OKU T	J BIOL CHEM	283	28918	2008
	GRUNDNER C	FEMS MICROBIOL LETT	287	181	2008
	BACH H	CELL HOST MICROBE	3	316	2008
	MADHURANTAKAM C	PROTEINS	71	706	2008
	WEHENKEL A	BBA-PROTEINS PROTEOMICS	1784	193	2008
2000	**TYAGI AK**	**MOL GENET MYCOBACT**	**131**		
	HOTTER GS	FEMS MICROBIOL LETT	200	151	2001
	HOBSON RJ	MICROBIOL-SGM	148	1571	2002
	CLARK-CURTISS JE	ANNU REV MICROBIOL	57	517	2003
	SMITH I	CLIN MICROBIOL REV	16	463	2003
	DELOGU G	MOL MICROBIOL	52	725	2004
	MACHOWSKI EE	INT J BIOCHEM CELL BIOL	37	54	2005
2001	**KOUL A**	**MICROBIOLOGY**	**147**	**2307**	
	PALLEN M	TRENDS MICROBIOL	10	556	2002
	MADEC E	MOL MICROBIOL	46	571	2002
	CHABA R	EUR J BIOCHEM	269	1078	2002
	MOLLE V	BIOCHEMISTRY-USA	42	15300	2003
	CHOPRA P	BIOCHEM BIOPHYS RES COMMUN	311	112	2003
	SINGH R	MOL MICROBIOL	50	751	2003
	SINHA I	FEMS MICROBIOL LETT	227	141	2003
	VERMA A	INFEC IMMUNITY	71	5772	2003
	BOITEL B	MOL MICROBIOL	49	1493	2003
	MOLLE V	BIOCHEM BIOPHYS RES COMMUN	308	820	2003
	CHOPRA P	INDIAN J MED RES	117	1	2003
	ORTIZ-LOMBARDIA M	J BIOL CHEM	278	13094	2003
	YOUNG TA	NATURE STRUCT BIOLOGY	10	168	2003
	PULLEN KE	STRUCTURE	12	1947	2004
	PRENETA R	MICROBIOL-SGM	150	2135	2004
	KUMARI S	DRUGS TODAY	40	487	2004
	GOPALASWAMY R	PROTEIN EXPRESS PURIF	36	82	2004
	COWLEY S	MOL MICROBIOL	52	1691	2004
	WALBURGER A	SCIENCE	304	1800	2004
	GOOD MC	J MOL BIOL	339	459	2004
	MOLLE V	FEMS MICROBIOL LETT	234	215	2004

	SHARMA K	FEMS MICROBIOL LETT	233	107	2004
	SHARMA K	EXPERT OPIN THER TARGETS	8	79	2004
	KOUL A	NAT REV MICROBIOL	2	189	2004
	TYAGI JS	CURR SCI	86	93	2004
	FONTAN PA	CURR SCI	86	122	2004
	GREENSTEIN AE	J MOL MICROBIOL BIOTECHNOL	9	167	2005
	PAPAVINASASUNDA KG	J BACTERIOL	187	5751	2005
	CURRY JM	INFEC IMMUNITY	73	4471	2005
	KANG CM	GENE DEVELOP	19	1692	2005
	DURAN R	BIOCHEM BIOPHYS RES COMMUN	333	858	2005
	DEOL P	J BACTERIOL	187	3415	2005
	MOLLE V	J BIOL CHEM	281	30094	2006
	MOLLE V	PROTEOMICS	6	3754	2006
	SHARMA K	FEBS J	273	2711	2006
	NIEBISCH A	J BIOL CHEM	281	12300	2006
	DASGUPTA A	MICROBIOL-SGM	152	493	2006
	SINGH A	TUBERCULOSIS	86	28	2006
	BOKAS D	APPL MICROBIOL BIOTECHNOL	76	773	2007
	COX RA	CURR MOL MED	7	231	2007
	DOVER LG	CURR MOL MED	7	247	2007
	NARAYAN A	PHYSIOL GENOMICS	29	66	2007
	ZHENG XJ	BIOCHEM BIOPHYS RES COMMUN	355	162	2007
	O'HARE HM	MOL MICROBIOL	70	1408	2008
	FIUZA M	J BIOL CHEM	283	18099	2008
	HETT EC	MICROBIOL MOL BIOL REV	72	126	2008
	THAKUR M	J BIOL CHEM	283	8023	2008
	CANOVA MJ	PROTEOMICS	8	521	2008
	WEHENKEL A	BBA-PROTEINS PROTEOMICS	1784	193	2008
2003	**SINGH R**	**MOL MICROBIOL**	**50**	**751**	
	ALZARI PM	STRUCTURE	12	1923	2004
	SAINI AK	J BIOL CHEM	279	50142	2004
	SHARMA K	EXPERT OPIN THER TARGETS	8	79	2004
	KOUL A	NAT REV MICROBIOL	2	189	2004
	GREENSTEIN AE	J MOL MICROBIOL BIOTECHNOL	9	167	2005
	COZZONE AJ	J MOL MICROBIOL BIOTECHNOL	9	198	2005
	CASTANDET M	RES MICROBIOL	156	1005	2005
	SINGH R	TUBERCULOSIS	85	325	2005
	GRUNDNER C	STRUCTURE	13	1625	2005
	MANGER M	CHEMBIOCHEM	6	1749	2005
	VILLARINO A	J MOL BIOL	350	953	2005
	SINGH A	J BACTERIOL	187	4173	2005
	RAO V	SCAND J IMMUNOL	61	410	2005
	MADHURANTAKAM C	J BACTERIOL	187	2175	2005
	MUSTELIN T	NAT REV IMMUNOL	5	43	2005
	SZOOR B	J CELL BIOL	175	293	2006
	MULLER D	J MED CHEM	49	4871	2006
	MUSTELIN T	ADVAN EXPERIMENT MED BIOL	584	53	2006

	SEIBERT SF	ORG BIOMOL CHEM	4	2233	2006
	TAUTZ L	EXPERT OPIN THER TARGETS	10	157	2006
	BRENCHLEY R	BMC GENOMICS	8	434	2007
	SOELLNER MB	J AM CHEM SOC	129	9613	2007
	CORREA IR	CHEM-ASIAN J	2	1109	2007
	BERESFORD N	BIOCHEM J	406	13	2007
	GRUNDNER C	STRUCTURE	15	499	2007
	PRUIJSSERS AJ	J VIROL	81	1209	2007
	OKU T	J BIOL CHEM	283	28918	2008
	GRUNDNER C	FEMS MICROBIOL LETT	287	181	2008
	NOREN-MULLER A	ANGEW CHEM INT ED	47	5973	2008
	WALTHER T	ORG LETT	10	3199	2008
	SHI M	VIRUS GENES	36	595	2008
	WEHENKEL A	BBA-PROTEINS PROTEOMICS	1784	193	2008
2003	**CHOPRA P**	**BIOCH BIOPHY RES COMM**	**311**	**112**	
	ALZARI PM	STRUCTURE	12	1923	2004
	PULLEN KE	STRUCTURE	12	1947	2004
	SHARMA K	EXPERT OPIN THER TARGETS	8	79	2004
	GREENSTEIN AE	J MOL MICROBIOL BIOTECHNOL	9	167	2005
	DURAN R	BIOCHEM BIOPHYS RES COMMUN	333	858	2005
	LAI SM	MICROBIOL-SGM	151	1159	2005
	THAKUR M	J BIOL CHEM	281	40107	2006
	MOLLE V	J BIOL CHEM	281	30094	2006
	MITIC N	CHEM REV	106	3338	2006
	SHARMA K	FEBS J	273	2711	2006
	SHARMA K	J BACTERIOL	188	2936	2006
	ALDERWICK LJ	PROC NAT ACAD SCI USA	103	2558	2006
	DASGUPTA A	MICROBIOL-SGM	152	493	2006
	DOVER LG	CURR MOL MED	7	247	2007
	YOOSEPH S	PLOS BIOL	5	432	2007
	SACHDEVA P	FEBS J	275	6295	2008
	HETT EC	MICROBIOL MOL BIOL REV	72	126	2008
	WEHENKEL A	BBA-PROTEINS PROTEOMICS	1784	193	2008
2003	**RAO V**	**SCAND J IMMUNOL**	**58**	**449**	
	DOHERTY TM	CLIN MICROBIOL REV	18	687	2005
	ANDERSEN P	MICROBES INFECT	7	911	2005
	RAO V	SCAND J IMMUNOL	61	410	2005
	DOHERTY TM	VACCINE	23	2109	2005
	KAUFMANN SHE	INT J TUBERC LUNG DIS	10	1068	2006
	MEHTA A	CURR SCI	93	1501	2007
	HWANG SA	VACCINE	25	6730	2007
	GUPTA UD	VACCINE	25	3742	2007
	HERNANDEZ-PANDO R	CURR MOL MED	7	365	2007
	SHI CH	ACTA BIOCHIM BIOPHYS SINICA	39	290	2007
	NAGY G	INT J MED MICROBIOL	298	379	2008

2003	**SINGH A**	**FEMS MICROBIOL LETT**	**227**	**53**	
	DANIEL J	J BACTERIOL	186	5017	2004
	SINGH A	FEMS MICROBIOL LETT	232	231	2004
	SINGH R	TUBERCULOSIS	85	325	2005
	RAHMAN MT	VET MICROBIOL	110	131	2005
	SINGH A	J BACTERIOL	187	4173	2005
	GOLBY P	MICROBIOL-SGM	153	3323	2007
	ROBACK P	NUCL ACID RES	35	5085	2007
	KING A	PLANTA	226	381	2007
	RICHTER L	GENE	395	22	2007
	NARAYAN A	PHYSIOL GENOMICS	29	66	2007
	CHERUVU M	TUBERCULOSIS	87	12	2007
	GOUDE R	FUTURE MICROBIOL	3	299	2008
	IBARRA JA	GENETICA	133	65	2008
	FONTAN P	INFEC IMMUNITY	76	717	2008
2003	**AGARWAL N**	**FEMS MICROBIOL LETT**	**225**	**75**	
	SHARMA K	FEMS MICROBIOL LETT	233	107	2004
	SHARMA K	EXPERT OPIN THER TARGETS	8	79	2004
	AGARWAL N	NUCL ACID RES	34	4245	2006
	PASHLEY CA	MICROBIOL-SGM	152	2727	2006
	GALL K	FEMS MICROBIOL LETT	255	301	2006
	SCHOEP TD	MICROBIOL-SGM	153	3071	2007
	RICHTER L	GENE	395	22	2007
2003	**DHAR N**	**IMMUNOL LETT**	**88**	**175**	
	RAO V	SCAND J IMMUNOL	61	410	2005
	KABBESH M	DIAGN MICROBIOL INFECT DIS	51	251	2005
	STORNI T	ADVAN DRUG DELIVERY REV	57	333	2005
	RAPEAH S	VACCINE	24	3646	2006
	KLEIN AB	J IMMUNOASS IMMUNOCHEM	27	61	2006
	HERNANDEZ-PANDO R	CURR MOL MED	7	365	2007
	SHI CH	ACTA BIOCHIM BIOPHYS SINICA	39	290	2007
	TANG C	J INFEC DIS	197	1263	2008
2003	**CHOPRA P**	**EUR J BIOCH**	**270**	**625**	
	DORION S	ANAL BIOCHEM	323	188	2003
	MEENA LS	BIOTECHNOL APPL BIOCHEM	38	169	2003
	SAINI AK	J BIOL CHEM	279	50142	2004
	TIWARI S	J BIOL CHEM	279	43595	2004
	KUMAR P	DNA REPAIR	3	1483	2004
	TOMIOKA H	CURR PHARM DESIGN	10	3297	2004
	CHOPRA P	FEBS LETT	571	212	2004
	SHAH YM	MOL CELL ENDOCRINOL	219	127	2004

	SHARMA K	EXPERT OPIN THER TARGETS	8	79	2004
	HAVLASOVA J	PROTEOMICS	5	2090	2005
	KUMAR P	NUCL ACID RES	33	2707	2005
	DE OLIVEIRA AHC	COMP BIOCHEM PHYSIOL PT D	1	300	2006
	MUKHOPADHYAY S	INFEC IMMUNITY	74	3853	2006
	SHARMA K	FEBS J	273	2711	2006
	COUTINHO-SILVA R	PURINERGIC SIGNAL	3	83	2007
	RUMJAHN SM	BRIT J CANCER	97	1372	2007
	ZHOU QH	BIOCHEM BIOPHYS RES COMMUN	356	348	2007
	SANSOM FM	MICROBIOL MOL BIOL REV	72	765	2008
	MATTOO AR	FEBS J	275	6237	2008
	KOLLI BK	MOL BIOCHEM PARASITOL	158	163	2008
	KREHENBRINK M	BMC GENOMICS	9	55	2008
	MATTOO AR	FEBS J	275	739	2008
2004	**SAINI AK**	**J BIOL CHEM**	**279**	**50142**	
	RICH RL	J MOL RECOGNIT	18	431	2005
	KUMAR P	NUCL ACID RES	33	2707	2005
	DE OLIVEIRA AHC	COMP BIOCHEM PHYSIOL PT D	1	300	2006
	MISRA G	ACTA CRYSTALLOGR F-STRUCT BIO	63	1084	2007
	UENO PM	MICROBIOL-SGM	154	3033	2008
2004	**CHOPRA P**	**FEBS LETT**	**571**	**212**	
	FISCHBACH MA	METH ENZYMOLOGY	407	33	2006
	SUN J	J LEUKOCYTE BIOL	82	1437	2007
	KOUMANDOU VL	BMC GENOMICS	9	298	2008
2004	**DHAR N**	**MED MICROBIOL IMMUNOL**	**193**	**19**	
	SKEIKY YAW	VACCINE	23	3937	2005
	TSENOVA L	INFEC IMMUNITY	74	2392	2006
	GUPTA UD	VACCINE	25	3742	2007
	ZHANG M	FEMS IMMUNOL MED MICROBIOL	49	68	2007
	TANG C	J INFEC DIS	197	1263	2008
2005	**SINGH R**	**TUBERCULOSIS**	**85**	**325**	
	RANJAN S	BMC BIOINFORMATICS	7	S9	2006
	SOELLNER MB	J AM CHEM SOC	129	9613	2007
	STINEAR TP	GENOME RES	18	729	2008
2005	**KHERA A**	**VACCINE**	**23**	**5655**	
	HUYGEN K	FUTURE MICROBIOL	1	63	2006
	LI H	VACCINE	24	1315	2006
	MITSUYAMA M	TUBERCULOSIS	87	S10	2007
	MEHER AK	VACCINE	25	6098	2007

	GUPTA UD	VACCINE	25	3742	2007
	WALKER KB	CURR MOL MED	7	339	2007
	LIU SG	IMMUNOL LETT	117	136	2008
2005	**DEOL P**	**J BACT**	**187**	**3415**	
	GREENSTEIN AE	J MOL MICROBIOL BIOTECHNOL	9	167	2005
	COZZONE AJ	J MOL MICROBIOL BIOTECHNOL	9	198	2005
	FERNANDEZ P	J BACTERIOL	188	7778	2006
	RAGHUNAND TR	MICROBIOL-SGM	152	2735	2006
	PEREZ J	BIOCHEM BIOPHYS RES COMMUN	348	6	2006
	CASHIN P	FEMS MICROBIOL LETT	261	155	2006
	MOLLE V	PROTEOMICS	6	3754	2006
	SHARMA K	FEBS J	273	2711	2006
	ALDERWICK LJ	PROC NAT ACAD SCI USA	103	2558	2006
	DOVER LG	CURR MOL MED	7	247	2007
	NARAYAN A	PHYSIOL GENOMICS	29	66	2007
	RAO A	APPL ENVIRON MICROBIOL	73	1320	2007
	PIMENTEL-SCHMI EF	J MOL MICROBIOL BIOTECHNOL	12	75	2007
	LEWIN A	BMC MICROBIOL	8	91	2008
	LAKSHMINARAYAN H	PROTEIN EXPRESS PURIF	58	309	2008
	HETT EC	MICROBIOL MOL BIOL REV	72	126	2008
	CANOVA MJ	PROTEOMICS	8	521	2008
	WEHENKEL A	BBA-PROTEINS PROTEOMICS	1784	193	2008
	GOPALASWAMY R	FEMS MICROBIOL LETT	278	121	2008
2005	**SINGH A**	**J BACTERIOL**	**187**	**4173**	
	RICHTER L	GENE	395	22	2007
	CHERUVU M	TUBERCULOSIS	87	12	2007
	KRUH NA	J BIOL CHEM	283	31719	2008
	RUSSELL-GOLDMAN E	INFEC IMMUNITY	76	4269	2008
	GOUDE R	FUTURE MICROBIOL	3	299	2008
	TOBIN DM	CELL MICROBIOL	10	1027	2008
	IBARRA JA	GENETICA	133	65	2008
	FONTAN P	INFEC IMMUNITY	76	717	2008
2005	**RAO V**	**SCAND J IMMUNOL**	**61**	**410**	
	HOVAV AH	MICROBES INFECT	8	1750	2006
	ARAVINDHAN V	FEMS IMMUNOL MED MICROBIOL	47	45	2006
	JUNG SB	INFEC IMMUNITY	74	2686	2006
	HUNG CY	ANN N Y ACAD SCI	1111	225	2007
	HENAO-TAMAYO M	VACCINE	25	7153	2007
	DA FONSECA DM	IMMUNOLOGY	121	508	2007
	HERNANDEZ-PANDO R	CURR MOL MED	7	365	2007
	BASTIAN M	J IMMUNOL	180	3436	2008

2005	**CHAUDHARY VK**	**PROTEIN EXPR PURIF**	**40**	**169**	
	KULSHRESTHA A	PROTEIN EXPRESS PURIF	44	75	2005
	ACHKAR JM	CLIN VACCINE IMMUNOL	13	1291	2006
	BENABDESSELEM C	J CLIN MICROBIOL	44	3086	2006
	ABEBE F	SCAND J IMMUNOL	66	176	2007
	ZHANG HM	CLIN MICROBIOL INFECT	13	139	2007
	LEE JS	RESPIROLOGY	13	432	2008
2006	**AGARWAL N**	**NUCL ACID RES**	**34**	**4245**	
	CHOWDHURY RP	J BACTERIOL	189	8973	2007
	FIELDS CJ	J BACTERIOL	189	6236	2007
	HALBEDEL S	J MOL BIOL	371	596	2007
	CHEN SC	J BACTERIOL	189	5108	2007
	PIMENTEL-SCHMI F	J MOL MICROBIOL BIOTECHNOL	12	75	2007
	GEBHARD S	MICROBIOL-SGM	154	2786	2008
	CHAUHAN S	J BACTERIOL	190	4301	2008
	PAWARIA S	APPL ENVIRON MICROBIOL	74	3512	2008
	TOUZAIN F	BMC BIOINFORMATICS	9	73	2008
	GEBHARD S	J BACTERIOL	190	1335	2008
2007	**AHMED N**	**PLOS ONE**	**2**	**968**	
	AHMED N	NAT REV MICROBIOL	6	387	2008
2007	**NARAYAN A**	**PHYSIOL GENOM**	**29**	**66**	
	BOKAS D	APPL MICROBIOL BIOTECHNOL	76	773	2007
	KULASEKARA HD	NAT CELL BIOL	9	734	2007
	O'HARE HM	MOL MICROBIOL	70	1408	2008
	MATTOO AR	FEBS J	275	6237	2008
	CANOVA MJ	PLASMID	60	149	2008
	FIUZA M	J BIOL CHEM	283	18099	2008
	WOLUCKA BA	FEBS J	275	2691	2008
	HETT EC	MICROBIOL MOL BIOL REV	72	126	2008
	MOLLE V	BIOCHEM J	410	309	2008
	CANOVA MJ	PROTEOMICS	8	521	2008
	WEHENKEL A	BBA-PROTEINS PROTEOMICS	1784	193	2008
	MATTOO AR	FEBS J	275	739	2008

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Important Research Contributions

Dr. Tyagi's research efforts since the beginning of his research career in 1973, have mainly focused on unraveling the biochemical and molecular mechanisms associated with the control and amelioration of human diseases especially tuberculosis.

Summary of the Important Research Contributions

Studies on the transcriptional signals of mycobacteria

Dr. Tyagi's group has contributed significantly to the understanding of transcriptional machinery and gene expression in mycobacteria. By isolating and characterizing, a large number of transcriptional signals from the slow growing *Mycobacterium tuberculosis* and the fast growing *Mycobacterium smegmatis*, it was demonstrated that stronger promoters occur less frequently in the case of *M.tuberculosis* compared with *M.smegmatis*. By directly comparing their relative transcriptional strength in mycobacteria and *E.coli* Dr. Tyagi and colleagues showed that most of the mycobacterial promoter elements function poorly in *E.coli.* His work has also provided evidence that RNA polymerases of *M.smegmatis, M.tuberculosis* and *M.bovis* BCG recognize mycobacterial promoter elements with comparable efficiencies. By analyzing these promoters by DNA sequencing, primer extension, and deletion experiments, Dr. Tyagi's group has shown that mycobacterial transcriptional signals differ from their counterparts in *E.coli* with respect to their -35 regions and the corresponding recognition domain of sigma factor of RNA polymerase.

Dr. Tyagi's group has also characterized "extended –10" promoters of mycobacteria. The promoter element of the *Mycobacterium tuberculosis* gene, *pknH* (Rv1266c) was used as a representative of promoters belonging to the "extended –10" class. By using in vitro transcription assays with reconstituted RNA Polymerase holoenzyme, DNase I protection assays, deletion studies and open complex formation studies, it was shown that mutation in the TGN motif of the *pknH* promoter resulted in the loss of >75% of its activity. Studies on the binding of RNA Polymerase with wild type as well as TG^- mutant derivative of the *pknH* promoter revealed that the TGN motif is required for the transition of close complex into open complex. It was concluded that the presence of TGN motif reduces the thermal energy required for the conversion of close complex into open complex, necessary for initiation of transcription.

These studies have shed significant light on divergence of mycobacterial transcriptional machinery from those of other bacteria. Also, these studies have provided a better understanding of the molecular basis of slow growth rate of *M.tuberculosis* and an explanation for the poor expression of mycobacterial genes in *E.coli*. Besides, the promoters identified in this study have been employed for the development of several vectors for expression of genes in mycobacteria.

Development of vector systems for genetic manipulations in mycobacteria

Dr. Tyagi's laboratory has developed a repertoire of vectors, which have proved to be extremely useful to several investigators in genetic manipulations of mycobacteria for the basic understanding of these organisms at a molecular level. Besides developing several vectors, for isolation of promoters, for construction of expression libraries and for trapping the promoters of structural genes under the control of a transcriptional regulator, Dr. Tyagi and colleagues have also developed an integration-proficient vector system for stable expression of genes in mycobacteria. This recombinant BCG system has been very

useful for a large number of investigators for expression of mycobacterial genes as well as antigen genes from several other pathogens for the development of BCG into a multipurpose vaccine vehicle.

Development of candidate recombinant BCG and DNA vaccines against tuberculosis

With the aim of modifying BCG into a more potent vaccine against TB, a generic approach has been developed by Dr. Tyagi's laboratory for expression of genes in mycobacteria which provides a desired level of expression of an antigen based upon the choice of mycobacterial promoter. Dr. Tyagi's group has expressed several antigens of *M. tuberculosis* by using this expression system to develop a number of candidate vaccines against TB. The evaluation of these candidate vaccines for immune responses in mice and for protective efficacy in guinea pigs has shown that two of the recombinant BCG vaccines provide more efficient protection than BCG itself against a sub-cutaneous challenge of *M. tuberculosis* in guinea pigs. In a parallel approach, Dr. Tyagi and colleagues have also developed several candidate DNA vaccines. Based on reduction in the bacillary load in lung and spleen of guinea pigs as well as associated histopathological changes, some of these candidate DNA vaccines imparted significant protection against the subcutaneous challenge of *M. tuberculosis*.

Till this point of time, no aerosol challenge facility was available in India. Hence, evaluation of the candidate vaccines was carried out by using subcutaneous infection of guinea pigs although it is only the 2nd preferred route of infection, the most popular and internationally acceptable one being the aerosol infection route. However, as the aerosol infection facility at the National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra became available, the promising candidate vaccines were evaluated against aerosol challenge of *M. tuberculosis* in guinea pigs by using heterologous prime boost approach. In this study, three regimens comprising of (i) recombinant BCG overexpressing 85C, (ii) recombinant BCG overexpressing α-crystallin as the priming agent followed by boosting with a DNA vaccine expressing the same antigen and (iii) BCG as priming agent followed by boosting with DNA vaccine expressing α–crystallin showed extremely good results and proved their superiority in comparison to the present BCG vaccine both on the basis of reduction in the bacillary load in lung and spleen as well as histopathological changes. The Tuberculosis Vaccine Clinical Trial Expert Group (TVCTEG) of the Department of Biotechnology, Government of India, has approved these vaccine regimens for human clinical trials. Currently, upstream pre-clinical work on these candidate vaccines is in progress so that the human clinical trials can be initiated.

Study of genes involved in the establishment and progression of tuberculosis – identification of new drug targets

(i) mymA operon

Dr. Tyagi's laboratory identified a new gene (*virS*) from *M .tuberculosis*. The 38 kDa VirS protein was shown to be homologous to VirF protein of *Shigella*, VirFY protein of *Yersinia* and Cfad, Rns and FapR proteins from various enterotoxigenic *E.coli* (ETEC) strains. All these homologs of VirS act as positive regulators of transcription in their respective systems and regulate the expression of structural genes required for establishment of disease.

The 7 genes (*Rv3083-Rv3089*), which are present divergently to *virS (Rv3082c)* constitute an operon designated as the *mymA* operon. Dr. Tyagi's group showed that transcription of the *mymA* operon is dependent on the presence of VirS protein. A 4-fold

induction of the *mymA* operon promoter occurs specifically in wild type *M. tuberculosis* and not in the *virS* mutant of *M. tuberculosis* (Mtb*ΔvirS*) when exposed to acidic pH. Expression of the *mymA* operon was also induced in infected macrophages by 10-fold over a six-day period. To gain an insight into the function of the proteins encoded by this operon, bioinformatic analysis was carried out, which suggested the involvement of these proteins in the modification of fatty acids required for cell envelope. This was supported by altered colony morphology and cell envelope ultra structure displayed by the *virS* mutant of *M. tuberculosis* (Mtb*ΔvirS*).

virS mutant (Mtb*ΔvirS*) and *mymA* mutant (Mtb*mym:hyg*) of *M. tuberculosis* exhibit reduced content and altered composition of mycolic acids along with the accumulation of saturated C24 and C26 fatty acids as compared to the parental strain. These mutants were markedly more susceptible to major antitubercular drugs at acidic pH and also showed increased sensitivity to detergent (SDS) and to acidic stress than the parental strain. Disruption of *virS* and *mymA* genes impairs the ability of *M. tuberculosis* to survive in activated macrophages, but not in resting macrophages, suggesting the importance of *mymA* operon in protecting the bacterium against harsher conditions. Infection of guinea pigs with Mtb*ΔvirS*, Mtb*mym:hyg* and the parental strain resulted in ~800-fold reduced bacillary load of the mutant strains as compared with the parental strain in spleens of animals at 20 weeks post infection. Phenotypic traits were fully complemented upon reintroduction of *virS* gene into Mtb*ΔvirS*. These observations show important role of *mymA* operon in the pathogenesis of *M. tuberculosis* at later stages of progression of the disease.

ii) Tyrosine phosphatases of *M. tuberculosis*

Protein tyrosine kinases and tyrosine phosphatases from several bacterial pathogens have been shown to act as virulence factors by modulating the phosphorylation and dephosphorylation of host proteins. Two tyrosine phosphatases namely MptpA and MptpB have been identified and characterized from *Mycobacterium tuberculosis*. To determine the role of MptpB in the pathogenesis of *M. tuberculosis* Dr. Tyagi and colleagues constructed a *mptpB* mutant strain by homologous recombination and compared the ability of parent and the mutant strain to survive intracellularly. It was shown that disruption of the *mptpB* gene specifically impairs the ability of the mutant strain to survive in guinea pigs but not *in vitro* or in a macrophage cell line suggesting the importance of its role in the host-pathogen interaction. Infection of guinea pigs with the mutant strain resulted in a 70-fold reduction in the bacillary load of spleens in infected animals as compared to the bacillary load in animals infected with the parental strain. Upon reintroduction of the *mptpB* gene into the mutant strain, the complemented strain was able to multiply and survive in guinea pigs at rates comparable to the parental strain. These observations demonstrate a role of MptpB in the survival of *M. tuberculosis* in the host during the progression of the disease.

To determine the role of mycobacterial tyrosine phosphatase A (MptpA, Rv 2234) in the virulence of *Mycobacterium tuberculosis*, Dr. Tyagi and colleagues constructed an *mptpA* mutant strain by homologous recombination and compared the ability of the parental and *mptpA* mutant strain of *M. tuberculosis* to survive intracellularly. It was shown that disruption of *mptpA* gene impairs the ability of *M. tuberculosis* to survive in IFN-γ activated macrophages as well as in guinea pigs. Six days after infection of activated macrophages with *M. tuberculosis*, an approximately 14-fold reduction was observed in the survival of intracellular *mptpA* mutant in comparison to the intracellular

parental strain. Infection of guinea pigs with the mutant strain resulted in 8 folds reduction in the bacillary load in the spleens and lungs of infected animals as compared to the bacillary load in animals infected with the parental strain, at 3 weeks post-infection. At 6 weeks post- infection, the bacillary load in guinea pigs infected with the *mptpA* mutant strain was reduced by 80 and 90 folds in spleens and lungs, respectively, in comparison to bacillary load in guinea pigs infected with the parental strain. Commensurate with these observations, infection of animals with the *mptpA* mutant strain showed a significantly reduced histopathological damage to lungs in comparison to infection with the parental strain. Introduction of *mptpA* operon (an operon comprised of Rv2232 – Rv2235) into the *mptpA* mutant strain restored the ability of the mutant strain to survive at levels comparable to the parental strain in IFN-γ activated mouse macrophages. These studies established the importance of *mptpA* operon in the intracellular survival of *M. tuberculosis*. These studies have provided a better understanding of the importance of tyrosine phosphatases in the survival of *M. tuberculosis* in the host tissue. Moreover, these studies have led to the identification of these two tyrosine phosphatases as attractive targets for the development of new anti-tubercular drugs.

Studies on the biosynthesis, regulation and function(s) of polyamines

In order to delineate the key target points for inhibition of mycobacterial growth, Dr. Tyagi initiated work to understand the biosynthesis of polyamines. His work led to demonstration that for polyamine biosynthesis in mycobacteria, putrescine is generated by both ornithine decarboxylase and arginine decarboxylase pathways contributing in the ratio of 1:6. He discovered a unique mode of regulation of ornithine decarboxylase in mycobacteria wherein its activity is modulated by a specific RNA inhibitor. This novel mode of control of ornithine decarboxylase opened an exciting new vista in the regulation of polyamine biosynthesis. Arginine decarboxylase from mycobacteria was also purified and characterized to show a pH dependent modulation of the enzyme. He demonstrated that pH dependent changes in the polarity of the active site environment, cause pyridoxal 5'-phosphate to form different Schiff-base tautomers at pH 8.4 and pH 6.2 with absorption maxima at 415 nm and 333 nm, respectively. These separate forms of Schiff-base were shown to confer different catalytic efficiencies to the enzyme.

Dr. Tyagi's work has also shown that polyamines influence transcription by facilitating binding of RNA polymerase to template as well as movement of enzyme along the template. In the milieu of the cell wherein the concentration of polyamines changes with the growth status, these molecules can impose a remarkable regulatory effect on the transcriptional activity of cell.

Earlier studies of Dr. Tyagi on the regulation and functions of polyamines in *S.cerevisiae* showed that polyamine induced inactivation of ornithine decarboxylase in yeast results from post-translational modification of existing enzyme protein. He also showed that in yeast, double stranded RNA plasmids (of the yeast killer system) specifically require polyamines for their replication and maintenance.

Elucidation of mechanism of action and of resistance to L-alanosine - a novel antibiotic anticancer agent

During his post-doctoral tenure at the National Institutes of Health, Dr. Tyagi studied the metabolism, mechanism of action and of resistance to L-alanosine an antibiotic anticancer agent. He showed that L-alanosine acts by inhibiting

adenylosuccinate synthetase (ASS) and demonstrated that L-alanosine does not inhibit this enzyme directly but forms an adduct with 5-amino-4-imidazole carboxylic acid ribonucleotide (AICOR). Alanosyl-AICOR inhibits ASS with a K_i of 0.228 μM thereby restricting the concentration of adenine nucleotides required for the synthesis of DNA in the actively dividing cells of leukemia.

His work also demonstrated that two mechanisms played a dominant role in the state of resistance to L-alanosine : (i) a significantly diminished ability to accumulate L-alanosyl-AICOR (due to depression in the activity of the enzyme SAICAR synthetase) and (ii) a significantly enhanced ability to reutilize preformed purines (due to elevation in the levels of enzymes of purine salvage pathway).

Besides, at each stage of his career, Dr. Tyagi has significantly contributed `state-of-the-art' methodology/technology by developing methods for assay of purine biosynthesis enzymes - adenylosuccinate lyase and succino amino imidazole carboxylic acid ribonucleotide synthetase, for automated separation of nucleic acid bases, nucleosides, nucleotides and their precursors, for assay of ornithine decarboxylase, for RNA isolation from mycobacteria and developing mycobacterial plasmid vectors for a range of purposes.

Details of Research Contributions

Some important research contributions of Dr. Tyagi are described below in a chronological order.

1973-1977

During these formative years, Dr. Tyagi worked on mycobacteria. This group of microorganisms comprises various pathogenic and non pathogenic organisms including the pathogens that cause tuberculosis and leprosy. The special emphasis was on understanding the mechanism of oxidative phosphorylation in mycobacteria in order to understand the slow growing nature of mycobacteria.

Role of various dehydrogenases in energy production in *M. tuberculosis* BCG

The levels of all the dehydrogenases associated with electron transport chain in *M.tuberculosis* BCG were investigated. NAD-dependent malate dehydrogenase was found to be the most active, and was exclusively present in the soluble fraction. Isocitrate dehydrogenase was fairly active; however, other enzymes like malate vitamin K reductase, succinic dehydrogenase, alpha-ketoglutarate dehydrogenase and pyruvate dehydrogenase were present in low levels. Malic enzyme and beta-hydroxy-butyrate dehydrogenase could not be detected in BCG. Localization and specific activity of some enzyme complexes of the electron transport chain like NADH oxidase, NADH-cytochrome *c* oxidoreductase, succinate cytochrome *c* oxidoreductase, NADH-DCIP oxidoreductase and cytochrome oxidase in BCG was also studied.

Spectal studies using the ETP from *M.tuberculosis* BCG revealed the occurrence of cytochromes *a, b* and *c*. The carbon monoxide difference spectrum, however, demonstrated the presence of only cytochrome a but not cytochrome O.

Studies on the specificity of phosphate acceptor system in BCG revealed that only ATP but not AMP, could replace ADP. Judged by the rates of oxidation and phosphorylation it was concluded that the organism could utilize only malate, succinate and isocitrate for its energy requirements. Malate was shown to be oxidized only by NAD dependent (MAL_{NAD} pathway) malate dehydrogenase. All three sites of phosphorylation were found functional in *M.tuberculosis* BCG.

Conclusion

Of the dehydrogenases associated with electron transport chain of *M.tuberculosis* BCG, malate dehydrogenase is the most active one. The organism could generate energy only by oxidizing malate, succinate and isocitrate. Only MAL_{NAD} pathway was operable for malate oxidation. All three sites of ATP production were functional in slow growing *M.tuberculosis* BCG.

Determination of site of action of non heme iron protein in electron transport chain

Studies were also carried out on the site of action of nonheme iron protein in the malate vitamin K reductase pathway of *Mycobacterium phlei*. Irradiation with ultraviolet light destroyed malate oxidase activity of both cell free extracts as well as reconstituted system and the loss of activity could not be significantly restored by vitamin K1 alone, which suggested the participation of another light sensitive component. Using the techniques of irradiation with ultraviolet light (360 nm), o-phenanthroline and electron acceptors like MTT, it was shown that nonheme iron protein combination with flavin

(metalloflavoprotein) acts at a site prior to vitamin K in the MAL_{FAD} pathway of *M.phlei*.

To support the above view, electron paramaganetic resonance studies were carried out. Electron transport particles from *M.phlei* upon reduction with malate exhibited electron paramagnetic resonance signals at g=2.002 and 1.94, characterisitic of napthosemiquinone and nonheme iron protein respectively. Upon irradiating the particles with ultraviolet light (360 nm) these signals were not observed suggesting that ultraviolet irradiation destroyed the environment around the metal in such a way that malate failed to reduce the metal.

Conclusion

Site of action of non heme iron protein in the electron transport chain of *M.phlei* (in the MAL_{NAD} pathway) was established. It was found that non heme iron protein participates before or in combination with flavin in electron transport chain of *M.phlei*.

Purification and characterisation of malate dehydrogenase

Malate dehydrogenase (EC 1.1.1.37) was purified from *M.phlei* to homogeneity. The enzyme was found to be composed of four subunits of equal molecular weight (21,554). Tyrosine and isoleucine were identified as the N- and C-terminals of the malate dehydrogenase of *M.phlei*. Amino acid composition of the malate dehydrogenase was determined to understand the chemical structure of the protein molecule. Studies on the effect of acid and urea on the structure of malate dehydrogenase demonstrated that treatment of the enzyme with acid and urea results in the dissociation of the enzyme followed by loss of catalytic activity. This dissociated enzyme could however be reconstituted by bringing the pH back to neutrality or by removing the urea from the enzyme solution. Slow removal of urea by dialyzing in cold proved a better extent for reconstitution.

Conclusion

The native enzyme probably has only one active site and the catalytic monomer is the tetrameric form of the protein. Inactivation followed by dissociation of protein by acid and urea treatment therefore reveals that for making up a single active site cooperative interaction and folding of the four polypeptide chains is essential.

1978-1980

Studies on a novel, natural and unique anticancer agent

The scientific literature during these years marked the blossoming of knowledge concerning the treatment of cancer specially the designing, biological effects, mechanism of action and application of cancer drugs. Dr. Tyagi's efforts during this period focussed on studying the pharmacology, metabolism and mechanism of action of an antiviral,antimicrobial and antitumor antibiotic L-alanosine 2-amino-3- [(N-hydroxy-N-nitroso) amino] propionic acid. A natural product L-alanosine is structurally distinctive as this compound was unique among natural compounds to have both N-nitroso functionality and a hydroxy group on a single nitrogen atom and it had already shown very promising anticancer activity.

Interaction of L-alanosine with enzymes metabolizing L-aspartic acid, L-glutamic acid and their amides.

First a comprehensive analysis was made of the manner in which L-alanosine interacts with the enzymes responsible for the metabolism of the dicarboxylic amino acids and their amides. It was found that the drug impedes the transport of L-aspartic acid and, to a lesser degree, than of L-glutamic acid, L-asparagine and L-glutamine by lymphoblasts, *in vitro*. In each of these instances, inhibition was apparently competitive in type. Of the enzymes involved in the metabolism of L-aspartic acid, adenylosuccinate synthetase, SAICAR synthetase (5-amino-4-imidazole-N-succino-carboxamide ribonucleotide synthetase) L-aspartyl tRNA synthetase L-aspartate transcarbamylase and L-aspartate aminotransferase were inhibited by L-alanosine; moreover, each of these enzymes except L-aspartyl tRNA synthetase accepted the antibiotic as substrate, although at substantially diminished rates. Of the enzymes involved in the metabolism of L-glutamic acid, L-alanosine inhibited only L-glutamine synthetase and L-glutamate decarboxylase to a prominent degree. Although L-alanosine provoked a rise in the concentration of inosinic and (IMP) *in vitro*, pointing to the conclusion that the drug was capable of inhibiting adenylosuccinate synthetase under these circumstances, no such rise was seen *in vivo* either in tumor or in liver. However, 1 and 5 hr after administration L-alanosine depressed hepatic ATP and NAD pools, an effect which indicated that the drug is, in fact, restricting the intracellular concentration of adenine nucleotides. Of the metabolites of L-alanosine *in vitro*, α-decarboxy alanosine, α-keto alanosine, α-hydroxy alanosine, alanosyl IMP and N-carbamyl L-alanosine did not inhibit adenylosuccinate synthetase to any prominent degree, whereas the metabolite generated by SAICAR synthetase powerfully inhibited this enzyme, with a K_i of 0.3 μM. Parenteral therapeutic doses of L-alanosine produced striking increases in the concentrations of L-aspartic acid in tumor and liver as well as of L-aspartic and L-glutamic acid in urine.

Conclusion

In quantitative terms, transamination of L-alanosine and reduction of the resultant α-ketocarboxylic acid appeared to be the principal metabolic fate of the antibiotic. In qualitative terms, with therapeutic, toxicologic and enzymologic actions as end points, the most important metabolic fate of L-alanosine was its condensation with 5-amino-4-imidazole carboxylic acid ribonucleotide to yield a fradulent anabolite capable of powerfully inhibiting adenylosuccinate synthetase (K_i 0.3 μM).

Studies on the mechanism of action of L-alanosine

L-alanosine, like azaserine, is a derivative of L-alanine and contains a negatively charged nitrogenous α-substituent. However, azaserine arrests the synthesis of all purines, but L-alanosine interrupts the synthesis of adenine alone. Now, the pathway of the synthesis of adenine is common upto the step at which 5-formamidoimidazole-4-carboxamide ribonucleotide undergoes ring closure to form IMP. Thus, L-alanosine's site of action seemed likely to be subsequent to this step, most probably at the level of the reactions involved in the conversion of IMP to AMP. Consequently, the two steps of primary concern became (1) the addition of L-aspartate to IMP and (2) the removal of fumarate from the adenylosuccinate thus formed to yield AMP.

The finding that L-aspartic acid, even at high concentrations, wholly failed to alleviate the effects of L-alanosine on cell replication or AMP formation suggested that the antibiotic was not behaving as a competitive inhibitor of that amino acid in the

adenylosuccinate synthetase reaction, or that the drug might require conversion to the formally competitive species.

Further, support for the latter alternative was provided by an examination of the chronology of inhibition of adenylosuccinate synthetase. When a therapeutic dose of L-alanosine (500 mg/kg) was given to mice bearing nodules of leukemia L5178Y and the inhibition of adenylosuccinate synthetase was followed over time, it was observed that there was a 30-minute lag before inhibition became prominent, but, thereafter, the drug inhibited tumoral adenylosuccinate synthetase for an 8-hour period. Subsequently, a gradual restitution of activity was observed. Virtually all the inhibition seen was reversible by dialysis.

When the inhibition of adenylosuccinate synthetase produced by L-alanosine *in vitro* was compared to that exerted by the drug *in vivo* marked disparities emerged: *in vitro*, the inhibition by L-alanosine of adenylosuccinate synthetase, partially purified from leukemia L5178Y cells, using L-aspartic acid as a variable substrate, was non-competitive and weak, with a K_i of 57 mM; with GTP, and IMP as variable substates, inhibition was also non-competitive and feeble, with K_i of 30 mM and 37 mM, respectively.

Since L-alanosine itself can inhibit adenylosuccinate synthetase, it became important to determine whether the nodules of L5178Y cells used in these studies contained the antitumor agent *in vivo* at a concentration commensurate with the kinetics of inhibition measured *in vitro*. It was found that the concentration of L-alanosine in these tumors fell to 110 μM within 2 hours after the administration of the drug, and to 170 μM within 8 hours, despite the fact that inhibition of adenylosuccinate synthetase had been found to persist at approximately 70% over this time span. Obviously, these concentrations of L-alanosine were incapable of exerting the magnitude of enzyme inhibition observed, a finding that ruled out the possibility that the antibiotic itself was functioning *in vivo* as the proximate inhibitor of adenylosuccinate synthetase.

In spite of the postulation that the active metabolite of L-alanosine was the adduct of the antitumor agent with AICOR. Our early attempts to demonstrate this molecule in the tumors of mice treated with the drug met with failure. However, because its identification was central to any explanation of the mechanism of action of L-alanosine, more comprehensive studies on the *in vivo* formation of L-alanosyl-AICOR were undertaken using L5178Y cells growing as subcutaneous nodules in mice. L-alanosyl-AICOR was prepared from L-alanosine and AICOR by the catalytic action of a preparation of SAICAR synthetase partially purified from avian liver. This compound was a strong inhibitor of adenylosuccinate synthetase, and Bratton-Marshall reaction positive.

Studies on the inhibition by L-alanosyl-AICOR of partially purified adenylosuccinate synthetase from leukemia L5178Y showed that the anabolite was a formally competitive inhibitor versus IMP, with an apparent K_i of 0.228 μM.

In as much as it was possible to condense L-alanosine with AICOR *in vitro*, and in view of the fact that the resulting antimetabolite, L-alanosyl-AICOR, was a very potent inhibitor of adenylosuccinate synthetase, a search was mounted to demonstrate the occurrence of this anabolite in living tumors. To this end, mice bearing L5178Y nodules were given a very large dose (50 μCi) of radioactive L-alanosine along with nonradioactive L-alanosine (500 mg/kg); tumors were excised, flash frozen, and

extracted, and the extracts subjected to high resolution chromatography. A prominent radioactive peak, co-eluting with L-alanosyl-AICOR and unique to the tumors of treated recipients, was detected at concentrations of 70, 53 and 20 μM at 2, 4, and 8 hours respectively.

To confirm that this material was indeed L-alanosyl-AICOR, all chromatographic fractions from these studies were tested for inhibition of partially purified adenylosuccinate synthetase and for Bratton-Marshall positively. Fractions corresponding to the peak coeluting with L-alanosine had no effect on the enzyme; however, those corresponding to the peak coeluting with L-alanosyl-AICOR strongly inhibited the partially purified preparation of adenylosuccinate synthetase. Only the fractions corresponding to this peak were observed to be Bratton-Marshall reaction positive.

Conclusion

These studies led to the understanding of mechanism of L-alanosine. L-alanosine acts as anticancer agent not directly but after being converted to L-alanosyl-AICOR. The later inhibits adenylosuccinate synthetase very potently resulting in depletion of purine nucleotides.

Mechanism of Resistance against L-alanosine

Sublines of P388 and L12010 leukemia were rendered resistant to L-alanosine and designated P388/LAL and L1210.LAL. Assessments were made to certain biochemical and pharmacological determinants of the sensitivity or resistance to L-alanosine of these sensitive and resistant lines. It was observed that the antibiotic strongly inhibited adenylosuccinate synthetase and DNA synthesis only in the parent or sensitive lines; moreover, after a therapeutic dose of the drug, the concentration of L-alanosyl-AICOR, the putative active anabolite of L-alanosine, was dramatically higher in these parent ines as compared with the resistant variants. Enzymologic studies established that, in P388/LAL, the specificity activity of the enzyme SAICAR synthetase was depressed significantly. In both resistant lines, however, the enzymes of purine salvage were present at levels about 200 per cent higher than those measured in the native strains.

Conclusions

The two mechanisms were found to be dominant in the state of resistance to L-alanosine - a significantly diminished ability to accumulate L-alanosyl-AICOR and significantly enhanced ability to re-utilize preformed purines which are responsible for the development of resistance against L-alanosine.

1981-1983

This period saw the extension of Dr. Tyagi's research activities into the area of polyamines. Polyamines play a crucial role in various cellular processes. Cell growth and differentiation does not occur in the absence of polyamines. For this reason polyamine biosynthesis has gained widespread importance as a target for metabolic and pharmacological intervention. His investigations during this period focussed on regulation and role of polyamines in *Saccharomyces cerevisiae*.

Regulation of ornithine decarboxylase in *S.cerevisiae*

Ornithine decarboxylase (ODC) was purified to homogeneity (1500 folds) from yeast and characterized. It was discovered that the enzyme is synthesized as a precursor

of 86 kDa and then is converted to 68 kDa form during purification. This conversion was inhibited by proleolytic inhibitors. We were also able to isolate this 86 kDa form of the enzyme using an antibody - sepharose column with antibodies against 68 kDa form.

In view of these new findings, it was decided to study the effect of addition of spermine and spermidine to the growth medium on the amount of ornithine decarboxylase protein found in the yeast cells. It was shown that addition of amines to the medium resulted in the complete loss of ornithine decarboxylase activity within 6 hours; this inactivation required protein synthesis. In contrast to the loss of enzymatic activity, there was no significant loss of immunoreactive 68 kDa protein. When this experiment was repeated with our improved immunoprecipitation procedure, complete retention of the 86kDa protein, despite complete loss of enzyme activity was observed. Thus, we found evidence that a post-translational modification of the 86 kDa form occurs following growth in amine-supplemented medium. This modification is unrelated to the proteolytic cleavage of the native enzyme.

Immunoprecipitates from one of the *spe*10 mutants which lack ornithine decarboxylase activity were prepared, to determine if these strains contain residual inactive protein. It was found that these inactive extracts contained an amount of 86 kDa protein equal to that found in the very active extracts obtained from the derepressed *spe*2 strain. This was an evidence for regulation of the enzyme activity by a modification which is not related to the proteolytic changes.

Conclusion

The addition of polyamine causes loss of ODC activity by negative control and this loss which is dependent on protein synthesis results from post translational modification of the enzyme.

Requirement of polyamines for the replication and maintenance of dsRNA plasmids (killer plasmids) of yeast

Double-stranded RNA (ds RNA) genomes are found in all major groups of organisms such as viruses of mammals, insects, plants, fungi and bacteria etc. Of the stably maintained ds RNA systems, the best studied one is the killer system of *S.cerevisiae*. Certain strains of yeast secrete protein toxins, also called killer toxins to which they are resistant but that kill other members of the same species. Atleast two distinct killer specificities have been recognized which are known as K1 and K2 killers. These are encoded by two double-stranded RNAs namely M1 and M2. *S.cerevisiae* is of increasing interest as model eukaryote and the killer systems permit detailed study of genetics of model eukaryote. Thus we had undertaken to study whether polyamines are required for the replication and maintenance of these killer plasmids.

The killer systems involve a group of cytoplasmic or non mendelian genetic elements. Most of them are located on ds RNA molecules, which are encapsulated in virus like particles called VLPs but they are not 'autonomously replicating' elements, as both virus and plasmids are often described. Studies have defined 39 chromosomal genes and six plasmids involved in various ways in the maintenance, replication and expression of various components of killer system.

Various strains of yeast were taken which are mutants and thus are defective in one of the steps of polyamines biosynthesis and either by mating these strain with the strains that carry a specific killer component and selecting the sergeants or by the process of cytoduction generated strains which are mutants for a specific step of polyamine

biosynthesis and at the same time carry a killer component of interest such as M2 dsRNA, EXL, HOK, NEX, L-A HN or combination of any of these.

After testing for both these characteristics these mutants were depleted of polyamines by growing them on a polyamine free medium. On this medium, *spe*2 mutants, which contain putrescine but lack spermidine and spermine grow indefinitely but with a 3-4 times longer doubling time. *spe*10 mutants which lack all - putrescine, spermidine and spermine stop growing after several colony isolations on this medium. At this stage these mutants were again replica plated onto a polyamine containing medium, they were grown and again tested whether they still contain the killer specificity in question or have lost it during polyamine depletion.

The strains containing the KIL-K1 or KIL-K2 plasmid and *spe*2 and *spe*10 mutation are killers in nature when they are grown on a rich YPAD medium which contains polyamines but when they were grown in the absence of polyamines and had exhausted their polyamine contents they became non-killers and sensitive to killer toxin thus showing that polyamines are required for the maintenance and replication of these plasmids. Also, it showed that Putrescine is not enough to maintain these plasmids and spermidine or spermine are specifically required, because *spe*2 mutant continue to make large amounts of putrescine and lack only spermidine and spermine yet they lose both M1 and M2 dsRNAs. When 100 μM spermidine was included in the polyamine free medium during the growth of these strains then neither *spe*2 nor *spe*10 strains showed any loss of killer plasmids but ones the killer plasmid is lost from either *spe*2 or *spe*10 strains it could not be restored back by growing these strains in the presence of polyamines. After polyamine deprivation both M1 and M2 dsRNAs were lost from these strains.

Both these *spe*2 and *spe*10 strains carry EXL plasmid which prevents replication of KIL K2. When either of these was mated with strain 1387 which carries KIL-K2, the diploids generated did not show any killing because KIL-K2 is excluded in the presence of EXL. When the *spe*10 strain was depleted of polyamine contents by extended growth on polyamine free medium and was again mated with strain 1387 the diploids now clearly show killing. This indicates that *spe*10 strain has lost EXL and that polyamines are required for the replication of EXL. Once lost, EXL could not be restored by addition of polyamines.

When *spe*2 strain was depleted of spermidine and spermine by extended growth on polyamine free medium, the EXL is not lost, as the diploids generated did not show any killing.

The *spe*2 strains in contrast to spe10 strains continue to make putrescine in greater than wild type amount when grown on a polyamine free medium. Thus, these results showed that putrescine alone in the absence of spermidine and spermine was sufficient to maintain the EXL plasmid. It was also observed that addition of 100 μM putrescine to polyamine free medium during growth prevented the loss of EXL. The polyamine requirement for another variety of dsRNA that is designated L-A-HN were then studied. It carries two cytoplasmic genes HOK i.e. helper of killer and NEX i.e. neutralizer of EXL. A detailed study of polyamine requirement of this plasmid showed that this plasmid does not require polyamines for its maintenance and replication.

Conclusion

M1, M2 and L-A-E dsRNAs all require polyamines for their replication and maintenance. These requirements are not identical for all these dsRNAs. M1 and M2

require spermidine or spermine but putrescine alone is of no help. However, for L-AE any of the polyamine, putrescine, spermidine or spermine is good enough. While L-A-E requires polyamines another variety of L-dsRNA i.e. L-A-HN does not require any of the polyamines. This is rather striking because these two RNA molecules have 99% sequence homology. This data showed that polyamines are important in the replication of KIL-K1, KIL-K2 and EXL for specific steps and that these steps were not involved in the replication or maintenance of HOK and NEX.

1984-1989

During these years Dr. Tyagi's efforts were refocussed on mycobacteria - to develop strategies which could lead to prevention and control of tuberculosis. It was thought that polyamine biosynthesis would be a very useful target for this purpose and thus he initiated work to understand the biosynthesis of polyamines and its regulation in mycobacteria in order to delineate the key target points for inhibition of polyamine biosynthesis. In addition, the work was also started on the role of polyamines in transcription in mycobacteria to understand whether polyamines might have a special role in gene expression in mycobacteria as the latter has highly GC rich genome and polyamines have been shown to exert their effect by transition of B-DNA to Z-DNA apart from the condensation of DNA. Work was initiated on the promoter regions of slow and fast growing mycobacteria in order to understand their involvement, if any, in slow growth of some mycobacterial species and also to study their structure and function and use strong mycobacterial promoters for generation of more soluble expression vectors to study molecular genetics of mycobacteria and for expression of specific protective antigens for tuberculosis and leprosy.

Regulation of putrescine biosynthesis in mycobacteria

It was found that activities of both arginine decarboxylase and ornithine decarboxylase are closely associated with mycobacterial growth polyamines were required during the period of high metabolic activity. Conversely, polyamines were not required by resting or non-proliferating cells. This work represented the first report on the activities of arginine decarboxylase and ornithine decarboxylase during the growth of *M.smegmatis* and their relationship to polyamine biosynthesis. Both ornithine decarboxylase and arginine decarboxylase exhibit highest activities during the log phase of growth curve, however, the maximal activity of arginine decarboxylase is four time higher than the maximal activity exhibited by ornithine decarboxylase, leading to a situation hitherto unknown in bacteria.

Assay of arginine decarboxylase using both, 1-^{14}C arginine or U-^{14}C arginine exhibited that while decarboxylastion of the 1-carboxy group of arginine would result in the formation of agmatine, (a decarboxylated guanidino compound) the guanidino group of arginine was further metabolized to labelled CO_2. Hence, for every arginine molecule, two molecules of CO_2 will be formed.

Labelling of ornithine decarboxylase and arginine decarboxylase products showed that the putrescine formed as a result of the above two activities in dialysed crude extracts of *N.smegmatis* corresponded to the activities of the two enzymes measured *in vitro*.

Conclusion

The results indicate that for polyamine biosynthesis the contribution of putrescine from ornithine decarboxylase : arginine decarboxylase is in the ratio, 1:6.

Studies on arginine decarboxylase from *M.smegmatis* TMC 1546

In an attempt to study the enzyme arginine decarboxylase in order to evaluate its role in putrescine biosynthesis in *M.smegmatis*, its purification and study of its properties were undertaken. Arginine decarboxylase was purified by a new, hitherto unpublished procedure resulting in 311 fold purified preparation with a specific activity of 2577 nmoles CO_2/mg protein/hour and a yield of 10.0 per cent. The purified enzyme had a molecular weight of 232,000 and a subunit Mw. of between 58,000 to 59,000. The results indicated the native tetrameric enzyme to be made up of four equivalent subunits. Purified arginine decarboxylase exhibited a pH optimum at pH 8.4, an optimum temperature for decarboxylation at 37^{o} to 40^{o}C and was moderately labile to heat denaturation.

The holo-arginine decarboxylase was completely resolved into its apoenzyme form by dialysis of the former against hydroxylamine. The apoenzyme form showed negligible activity at pH 8.4 in the absence of added pyridoxal-5' -phosphate and regained almost 100 per cent of its activity, in the presence of 0.5 mM pyridoxal-5'-phosphate. However, the activity of the reconstituted preparation at pH 6.2 was observed to be only 30 per cent of that shown at pH 8.4. These results demonstrated a strong correlation with results obtained when the holoenzyme activity was determined as a function of pH and that arginine decarboxylase from *M.smegmatis* was strongly dependent on pyridoxal-5'-phosphate for its activity. Unlike the enzyme from *E.coli* arginine decarboxylase from *M.smegmatis* did not require Mg^{++} for activity at pH 8.4. However, at pH 6.2, Mg^{++} enhance enzyme activity by 23.0 per cent.

The holo-arginine decarboxylase at pH 8.4 showed, a characteristic absorption maximum at 415 nm, whereas the apo-arginine decarboxylase showed a characteristic absorption of protein at 280 nm, along with a minor peak at 333 nm, absorption of holo-arginine decarboxylase from *M.smegmatis* with a peak at 415 nm was consistent with the formation of a Schiff-base through an azomethine linkage. Addition of 0.5 mM pyridoxal-5'-phosphate to the apoarginine decarboxylase at pH 8.4 resulted in the appearance of a peak indicating the formation of an azomethine bond vis-a-vis Schiff base. The formation of such an absorption species is concomitant with a 99.0 percent regain of enzyme activity. Further, the reconstitution studies with apo-arginine decarboxylase indicated that at pH 6.2 pyridoxal-5'-phosphate is involved in a different type of Schiff base formation with an absorption at 333 nm. The addition of Mg^{++} apparently creates a more favourable conformation. We then carried out differential spectrometry at pH 8.4 on apo-arginine decarboxylase at pH 8.4. These studies indicated that pyridoxal-5'-phosphate induced positive co-operativity at optimal pH leading to a conformational change resulting in an increased catalytic activity.

Conclusion

It was evident from our studies that at pH 8.4 tautomeric form I is the preferred Schiff base resulting in maximum catalytic activity. At pH 6.2 the preferred tautomer is form III with absorption maxima at 333 nm which does not promote positive cooperativity induced by pyridoxal 5'-phosphate. The addition of Mg^{2+} at pH 6.2 apparently creates a more favourable conformation. This pH-induced change in the preferred tautomeric form is most likely mediated through a pyridoxal-5'-phosphate-dependent conformational change in the enzyme. Spectrophotometric analyses indicate

that the pH-labile active-site polarity may have a role to play in the regulation of enzyme activity.

Role of polyamines in transcription and its implication in gene regulation

RNA polymerase was purified from *M.phlei* to a 467 fold purified preparation. All three polymines i.e. putrescine, spermidine and spermine stimulated the RNA synthesis in a dose-dependent manner. Spermidine and spermine showed a biphasic effect on RNA synthesis. Both inhibition as well as stimulation of transcription could be observed depending upon the concentration of polyamines employed. Thus, in a growing cell where the concentration of polyamines is changing with the growth status, these molecules can have a regulatory effect on transcription of various genes.

To study whether these effects were the result of polyamine interactions with DNA template or with the enzyme, experiments were performed in which the enzyme concentration was kept constant with varying concentration of template at two different (i.e. a suboptimal and an optimal) concentrations of polyamines. The result of this study suggested that this modulation results from a change in the conformation of the DNA as a result of interaction with polyamines.

Stimulation of RNA synthesis by dilution of reaction mixture after attainment of plateau suggested that the product of the reaction might inhibit the RNA synthesis. More pronounced stimulation was obtained by addition of polyamines to the reaction mixture at plateau point. These results were further confirmed by the fact that addition of RNA isolated from *M.phlei* or yeast inhibited RNA synthesis and this inhibitory effect was significantly reversed by polyamines. This suggests that the hybrid formed between nascent RNA and DNA may act as a barrier for movement of the enzyme along the template. Polyamines can destabilize the RNA-DNA hybrid, thus, effecting the smooth movement of the enzyme along the template.

RNA synthesis by polyamines could be influenced either by affecting initiation or elongation of RNA chains. The initiation was studied by following incorporation of [gamma-^{32}P] labelled ATP and elongation was studied in the presence of rifampicin/sarkosyl to block further initiation of RNA chains. This study showed that influence on RNA synthesis by polyamines resulted from their effect on both initiation as well as elongation of RNA chains.

Conclusion

Polyamines influence transcription by facilitating binding of enzyme to template as well as movement of enzyme along the template. These effects result from conformational changes in the template. More significantly, however, it appears that different concentrations of polyamines can have a variable effect on the transcriptional activity, as also a given concentration of polyamines can exert diverse effect on the transcription of various genes. Thus in the milieu of the cell wherein the concentration of polyamines is changing with the growth status, these molecules can impose a remarkable regulatory effect on the transcriptional activity of the cell.

Discovery of an RNA inhibitor to ornithine decarboxylase

A study was undertaken to demonstrate the presence of an inhibitor to ornithine decarboxylase in *M.smegmatis* as our preliminary studies indicated the presence of such a non-dialysable inhibitor in the crude extracts. The results of these studies carried out to isolate and characterize the inhibitor of ornithine decarboxylase demonstrated that (i) It

was a ribonucleic acid, 0.194 kb in size (ii) It was specific for ornithine decarboxylase from *M.smegmatis* and did not inhibit ornithine decarboxylase from *E.coli* and *S.cerevisiae* (iii) The concentration of this inhibitor increases four fold when cells of *M.smegmatis* were grown in medium supplemented with 0.5 mM putrescine and 1.0 mM spermidine (iv) Studies carried out on the mode of interaction of the inhibitor with ornithine decarboxylase showed that inhibition was linear upto 40 per cent, however, a maximum of 70 per cent may be achieved. The inhibition was independent of temperature and time.

Conclusion

Based on these results a unique mode of regulation of ornithine decarboxylase in mycobacteria was apparent wherein its activity is modulated by a specific RNA inhibitor. It seems that transcription of a particular gene in mycobacteria is controlled by the level of polyamines in the cell, the RNA product of which in turn regulates the activity of ornithine decarboxylase. This novel mode of control of ornithine decarboxylase wherein an RNA specifically inhibits mycobacterial ornithine decarboxylase opened an exciting new vista in the regulation of polyamine biosynthesis.

1990-1998

Studies on the pathogenesis of *M.tuberculosis* - identification and characterization of virulence associated genes.

The establishment of infection by a pathogen depends upon its ability to enter, survive and multiply within the host cell. Pathogens usually employ several mechanisms which may act individually or in concert to produce infection and disease. We still seem to be far from knowing anything definite about the nature of genes that are responsible for the pathogenesis of *M .tuberculosis*. Several attractive approaches are being pursued to identify such genes in *M .tuberculosis*.

virS* and *mymA* genes of *M. tuberculosis

Dr. Tyagi's laboratory identified a new gene (*virS*) from *M. tuberculosis* H_{37}Rv, the 38 kDa protein product of which shows homology with virF protein of *Shigella*, virFY protein of *Yersinia* and Cfad, Rns and FapR proteins from various enterotoxigenic *E.coli* (ETEC) strains. All of these proteins act as positive modulator of transcription. VirF and VirFy proteins of Shigella and Yersinia, respectively, regulate the transcription of structural genes required for host invasion and intracellular survival. VirF in addition, also controls the infection of adjacent cells. Likewise Cfad, Rns and FapR, which constitute a family of analogous regulatory proteins from different enterotoxigenic strains of *E.coli*, regulate transcription of structural genes required for adhesion and colonization of epithelial cells. The protein product of the gene from mycobacteria, like in the cases of its homologs, contains a helix-turn-helix motif in the C-terminal region. This gene was found to be present only in the species belonging to the *Mycobacterium tuberculosis* complex. The sequence and structural homology of VirS with virulence regulating proteins along with its presence exclusively in the organisms of MTB complex strongly suggest its involvement in the establishment of disease.

Another gene designated as *mymA* (for mycobacterial monooxygenase) was divergently arranged to *virS* and codes for a 55 kDa protein that exhibits homology with cyclohexanone monooxygenase from *Acinetobacter* sp. and N,N-dimethylaniline monooxygenase from mammals. PCR and Southern blot analysis of genomic DNAs from

several mycobacterial species show that this gene is present exclusively in the members of the *M .tuberculosis* complex. Expression of *mymA* in *M .tuberculosis* was detected by immunoblotting with antibodies against the *mymA* protein. Deletion analysis of the upstream region of *mymA* showed that its expression is subjected to regulation through the possible involvement of trans-acting factor(s) specific to *M .tuberculosis* that are absent in *M. smegmatis. mymA* and the *virS* gene are located divergent to each other.

mymA could be detected both in the avirulent and virulent strains of *M .tuberculosis* by using specific polyclonal antiserum, its expression being dependent on the growth status of cells, and showed a maximum at an A_{600nm} of 3.0 representing the log phase in the growth curve. However, the overall expression was very weak suggesting that *mymA* is not expressed well under the *in vitro* culture conditions. The observed expression could possibly represent the basal level of *mymA* expression which could be induced to optimal level under specific environmental and physiological conditions.

The analysis of the upstream region of *mymA* revealed that *mymA* is under the transcriptional control of both down- and up- regulating elements in *M .tuberculosis* possibly with the involvement of trans- acting factors. *M.smegmatis* which lacks *mymA* coding sequence appears to lack one or more of these trans-acting regulators. None of the constructs with the upstream DNA sequences of *mymA* showed any transcriptional activity in *M.smegmatis*.

Studies on the transcriptional signals of Mycobacteria

E.coli and *Streptomyces lividans* have been used to study expression of mycobacterial genes. The efficiency of these heterologous systems is, however, variable and does not permit the expression of majority of mycobacterial genes. In addition, to understand the genetic responses elicited by mycobacteria during host pathogen interactions it is important to study the regulation of mycobacterial gene expression in homologous systems that would respond faithfully to various physiological constraints imposed by the host environment. Although various excellent vectors have been developed for this purpose, the repertoire of such systems is limited. A major obstacle in the development of such vectors has been the lack of information on mycobacterial transcriptional signals. Moreover, the rate of transcription in mycobacteria has been found to be relatively very low and the initiation of transcription has been found to be specially poor although studies have shown that these differences can not be attributed to inherent low activity of RNA polymerase. The answer presumably lies in the promoter regions of mycobacteria. Hence, it was proposed that a detailed study of mycobacterial promoters may not only shed light on the divergence of mycobacterial transcriptional machinery from those of other bacteria, it may also provide a basis for the observed differences in the growth rate of various mycobacteria. More significantly, it promised the availability of tools to generate versatile expression systems for mycobacteria.

A promoter selection vector was constructed for mycobacteria to analyze the sequences involved in mycobacterial transcriptional regulation. The vector pSD7 contains extrachromosomal origins of replication from *Escherichia coli* as well as from *Mycobacterium fortuitum* and a kanamycin resistance gene for positive selection in mycobacteria. The promoterless chloramphenicol acetyltransferase (CAT) reporter gene has been used to detect mycobacterial promoter elements in a homologous environment and to quantify their relative strengths. Using pSD7, Dr. Tyagi and colleagues isolated 125 promoter clones from the slow growing pathogen *Mycobacterium tuberculosis* H37Rv and 350 clones from the fast-growing saprophyte *Mycobacterium smegmatis*. The

promoters exhibited a wide range of strengths, as indicated by their corresponding CAT reporter activities (5 to 2,500 nmol/min/mg of protein). However, while most of the *M.smegmatis* promoters supported relatively higher CAT activities ranging from 100 to 2,500 nmol/min/mg of protein, a majority of those from *M. tuberculosis* supported CAT activities ranging from 5 to only about 100 nmol/min/mg of protein. These results indicate that stronger promoters occur less frequently in the case of *M.tuberculosis* compared with *M. smegmatis*.

The extent of divergence of mycobacterial promoters has been studied *vis a vis* those of *E.coli*. Of the 100 promoter clones tested from *M.smegmatis* only 12 transformed *E.coli* for chloramphenicol resistance and out of 100 promoter clones tested from *M.tuberculosis* none of the clones transformed *E.coli* for chloramphenicol resistance. The CAT activities of mycobacterial promoters was found to be very low in *E.coli* exhibiting differences of several hundred fold in their activities in mycobacteria and *E.coli*. In order to dissect the specific sequence requirements for transcription initiation in mycobacteria, we have carried out the DNA sequencing and promoter-mapping and *in vitro* studies. Dr. Tyagi's group has shown that the recognition of mycobacterial promoters is similar in the fast growing saprophyte *M.smegmatis* and the slow growing *M.tuberculosis* and *M.bovis* BCG. Analysis of sequences of these promoters shows that promoters of *M.tuberculosis* are more GC rich (56%) than the promoters of *M.smegmatis* (41%). Higher GC content of *M.tuberculosis* promoters may contribute to a relatively lower transcription observed in this species. Alignment of promoter sequences based on the transcriptional start points shows that the -10 regions of mycobacteiral and *E.coli* promoters are highly similar. However, the absence of TTGACA like sequences in the -35 region of most of the mycobacterial promoters seems to be their distinct feature. The degeneracy of sequences in the -35 region of mycobacterial promoters places them close to *Streptomyces* promoters. Comparison of sequences in the -10 and -35 binding regions of MysA, HrdB and RpoD (the principal sigma factors of *M.smegmatis*, *Streptomyces* and *E.coli*, respectively) shows that (i) all three sigma factors have identical -10 binding domain, (ii) the -35 binding domain of MysA is identical to HrdB but is very different compared to the corresponding region of RpoD. Thus mycobacterial transcriptional machinery may be highly similar to Streptomyces but different from that of *E.coli* and the major cause for this difference lies in the -35 region of the promoters and the corresponding binding domain of sigma factor.

Further, a detailed analysis was carried out to identify what other sequences/ features apart from –10 region contribute to the activity of mycobacterial promoters. Since majority of the known housekeeping promoters of mycobacteria are weak and are unlikely to carry consensus / nearly consensus recognition sequences, it required to generate strong promoters, which bind efficiently with the RNAP of mycobacteria, which was obtained by following a strategy, similar to the saturation mutagenesis. However, due to lack of sufficient knowledge about the mycobacterial promoter elements (except the Pribnow Box), Dr. Tyagi and colleagues started with background information about the promoters from other prokaryotic systems. A DNA sequence library harboring ~100 bp long DNA fragments containing random sequences in a stretch of 29 bases was generated, which represented the number of bases acquired between –35 and –10 positions in a typical prokaryotic promoter (number of bases in 2 hexamers separated by a distance of 17 bp = [2x6]+17. Despite using the incomplete library of DNA sequences, it was possible to select a few strong promoter sequences. A_{37} from this library based on its extremely high activity and near-perfect score was chosen for further characterization.

Thorough analysis of A_{37} revealed that its extremely high activity could be subscribed to cumulative effect of several features such as a purine at +1, a conserved –10 sequence along with an extended –10 motif. It was observed that replacing the base at +1 by any of the purine residues resulted in ~2-fold increase in the promoter's activity in mycobacteria. In the DNase I footprinting experiments, hyperactivities of DNase I at –24/–25 positions of A_{37} indicated overexposure of the bases to DNAse I due to the presence of RNAP. This suggested that interaction of RNAP with A_{37} may result in the generation of a favorable conformation of the promoter possibly due to bending at –24/–25 positions for a better binding of holoenzyme to both the –35 and the –10 sequences.

It was further shown that for the optimal activity and recognition of RNAP, a sequence at –35 region, 5'-TTGCGA-3' was preferred by mycobacterial transcriptional machinery. Significant changes in the activities of the promoters, A_{37TG-}, *sigA*, *mmsA* and *gcvH* on the substitution of their respective –35 regions substantiated the importance of –35 region in the activity of a mycobacterial promoter. Further evidence for the role of –35 sequence in promoter function was provided by enhanced binding of the mycobacterial RNAP with A_{37TG-}*con* and *sigAprocon* promoter derivatives containing 5'-TTGCGA-3' sequence at –35 region. However, substitutions of various individual bases at –35 site still resulted in substantial promoter activities, indicating that mycobacterial transcriptional machinery can tolerate variety of sequences at –35 position, as was reported by Dr. Tyagi and colleagues in their previous studies.

Alterations in the distance between –35 and –10 sequences revealed that unlike *E. coli* RNAP (where the optimum distance between –35 and –10 sequences is 17 bp), mycobacterial enzyme requires an 18 bp long spacer sequence for optimal promoter activity. Around 40% of the putative promoter sequences in 5'UTRs, obtained by pattern search analysis, exhibited a distance of 18 bp between putative –35 and –10 sequences, suggesting that a distance of 18 bp between –35 and –10 sequences represents an optimal spacer length for mycobacterial promoters. Further support for this comes from analysis of several known mycobacterial promoters, which revealed the presence of a spacer of 18 bp in most of the strong promoters.

Despite the similarities with *E. coli* promoters, the mycobacterial promoters do not function efficiently in *E. coli*. Recently, in a study, it was shown that the presence of GC rich sequences in the spacer region drastically influences the strength of promoters in *E. coli*. This observation was further substantiated by the fact that majority of strong *E. coli* promoters have an AT content of >75-80%. Analysis of A_{37TG-}*con E. coli* promoter derivative indicated the presence of high GC content (~60%) in the spacer region. Hence, the GC-rich spacer sequence of this promoter (from position –13 to –20) was replaced by a sequence resulting in 75% AT richness in the spacer region. This enhanced AT richness resulted in 15-fold higher activity of this promoter in *E. coli*. It has been observed that the inter-domain distance between regions 2.4 and 4.2 of *E. coli* σ^{70} is much shorter than the distance between –10 and –35 promoter elements. Hence, the AT rich spacer sequence may be better suitable for appropriate binding of this region required by RNAP to establish optimal contacts with –10 and –35 hexameric sequences. Although, σ^{A} from mycobacteria has not been crystallized as yet, possibly, it may have a more appropriate distance between 2.4 and 4.2 regions, thus, making it less dependent on the maneuvering of promoter region affected by bending of the spacer sequence. This may provide an explanation as to why mycobacterial promoters may function with highly GC rich spacer regions but exhibit significantly reduced activity in *E. coli*.

Dr. Tyagi has also analyzed the role of the TGN motif present immediately upstream of the -10 region of mycobacterial promoters. Sequence analysis and site-specific mutagenesis of a *Mycobacterium tuberculosis* promoter and a *Mycobacterium smegmatis* promoter revealed that the TGN motif is an important determinant of transcriptional strength in mycobacteria. It was shown that mutation in the TGN motif can drastically reduce the transcriptional strength of a mycobacterial promoter. The influence of the TGN motif on transcriptional strength is also modulated by the sequences in the -35 region. Comparative assessment of these extended -10 promoters in mycobacteria and *E.coli* suggested that functioning of the TGN motif in promoter of these two species is similar.

Designing and construction of vectors for study of mycobacterial molecular genetics and for expression of genes in mycobacteria

The nodal expression vector

During the past decade considerable progress has been made to develop systems for studying molecular genetics of mycobacteria, yet many limitations in the study of mycobacterial genetics still remain to be overcome. The existing vectors mostly depend on mycobacterial hsp60 and hsp70 gene promoters for expression and this has obstructed the development of versatile expression systems that would permit modulation of gene expression in mycobacteria. Using the mycobacterial promoters of different strength isolated in Dr. Tyagi's laboratory a system has been developed that will permit the expression of genes in mycobacteria at a desired level. A shuttle vector pSD5 has been constructed which can propagate in both mycobacteria and *E.coli*. It carries a modular expression cassette which provides site for cloning of promoters, ribosome binding site with an appropriately placed initiation codon and multiple cloning site for cloning of genes. The expression level of any gene can be altered as desired by the use of mycobacterial promoters of different strength.

Blue-white selection based promoter trap vector

Another derivative of pSD5 contains promoterless ß-galactosidase gene for isolation of transcriptional signal from mycobacteria. The vector provides a rapid selection for mycobacterial promoters in a homologous environment by simple blue white selection. Secondly, the chronological order of appearance and colour intensity of the blue colonies provides an index of the strength of cloned promoter. Furthermore, this selection strategy permits cloning of a wide range of promoters without incorporating any bias towards the promoters of a certain range as can occur in the vectors using drug resistance genes as basis for promoter selection.

Vector for construction of expression libraries in mycobacteria

Another derivative of pSD5 namely pSD5C has been designed to construct mycobacterial genomic libraries and express the cloned inserts as fusion proteins with maltose binding protein in mycobacteria. The expression of fusion proteins is controlled by the *Ptac* promoter thereby allowing regulation of expression with the inducer IPTG in *E.coli* XL1-Blue strain, whereas in mycobacteria the gene is expressed in a constitutive manner. This vector works as an excellent vector system for generating expression libraries of mycobacteria, which can be screened in *E.coli* by a nucleic acid or antibody probe using induction of *Ptac* promoter by IPTG. The clone so selected can be directly subjected to expression studies in mycobacteria wherein its expression can be achieved without any further subcloning step. Such libraries in addition can be useful for genetic

complementation of nonpathogenic mycobacterial species with genomic libraries of pathogenic species such as *M. tuberculosis* $H_{37}Rv$ for identifying the genetic determinants responsible for the disease causing ability of the latter. The vector can also be used for expression of heterologous DNA fragments from other pathogenic organisms in mycobacteria.

Integration proficient vector

In one of the pSD5 derivatives the origin of replication of mycobacteria and the gene for kanamycin resistance have been excised out and the integration specific sequences of L5 bacteiophage have been cloned. This vector can stably express a gene under a mycobacterial promoter by integrating site specifically into mycobacterial genome. Such a vector should serve as an excellent tool for stable expression of a mycobacterial or foreign gene in *Mycobacterium bovis* BCG for the purpose of producing recombinant DNA based improved BCG vaccines.

1999 onwards

Use of Recombinant BCG based approach for the development of vaccine against infectious diseases

BCG represents the most extensively used vaccine with a record 3 billion doses administered during the last several decades. While the efficacy of BCG as a vaccine against TB can be a matter of debate, what has been proven beyond doubt is that BCG is an extremely immunogenic, safe and stable vaccine, which is given at the time of birth to elicit long term immunity with a single administration. These factors have made large number of investigators focus their efforts on approaches based on recombinant DNA technology to modify BCG not only into a recombinant BCG vaccine against tuberculosis but also to employ it as a multipurpose vaccine vehicle against several other microbial infections.

Dr. Tyagi has carried out important ground work and has taken lead by developing an expression system, which with its capacity to modulate gene expression, holds very good promise as a tool for development of BCG into a multipurpose vaccine delivery vehicle.

Six different promising immunodominant antigens of *M. tuberculosis* namely 85A, 85B, 85C, 19 kDa antigen, 38 kDa antigen and ESAT-6 were cloned under different mycobacterial promoters and over expressed in BCG. The evaluation of immune responses elicited by different recombinant BCG strains separately expressing the antigens 85A, 85B, 85C, 19 kDa antigen, 38 kDa antigen and ESAT-6 was carried out. Humoral immune responses and cell-mediated immune responses were measured by ELISA and splenocyte proliferation assays, respectively. The Th1/Th2 bias of the immune responses was measured by isotyping the antibody responses as well as by analyzing the cytokine profiles. The protective efficacy of the recombinant BCG strains expressing the above antigens was evaluated in the guinea pig model of tuberculosis. Immunizations were carried out by intradermal injections with $1x10^6$ cfu of BCG or rBCG. The protective efficacy of the rBCG strains was evaluated at various doses of subcutaneous challenge with *M. tuberculosis* viz. $3.5x10^2$ cfu, $5x10^4$ cfu and $7.5x10^5$ cfu. The animals were euthanized 3 and 8 weeks post-challenge and post-mortem virulence scores were assigned. Bacterial load in spleen was determined and histopathological analysis of liver and lung tissue was performed to determine the percentage of granuloma in the organs and cellular composition of the granuloma.

Immunization with wild type BCG (WtBCG) elicited a Th1-Th2 or Th2 type of T cell response against purified mycobacterial antigens (antigens of the 85 complex, 19 kDa antigen and 38 kDa antigen) as well as against BCG sonicate. In general, the recombinant BCG constructs elicited immune responses of higher magnitude as compared to the wild type BCG and the response was markedly shifted towards either Th1 or Th2 phenotype. Overexpression of the antigens 85A, 85B and 85C and the 38 kDa antigen resulted in a predominantly Th1 response characterized by increased titres of antibodies of IgG2a isotype and preferentially increased secretion of IFN-γ against individual purified proteins as well as BCG sonicate. Overexpression of ESAT-6 in BCG resulted in a mixed Th1-Th2 or Th2 type of T cell response against the purified antigen as well as BCG sonicate as observed in the case of immunization with WtBCG although the magnitude of these responses was significantly higher. In contrast, overexpression of the 19 kDa antigen in BCG induced a very predominant, Th2 type immune responses against BCG sonicate although the response against the purified 19 kDa antigen was predominantly Th1 type. It was observed that modulation of the immune responses was dependent on the level of expression of the antigen with highest level of expression usually inducing maximal immuno-modulation.

In case of each antigen, the recombinant BCG strain expressing the antigen at the highest level was evaluated for its protective efficacy in guinea pigs. The BCG vaccination was quite effective in reducing the bacillary load in the spleen of the animals. Some recombinant BCG strains reduced the bacillary load more efficiently than BCG, others did not show any significant improvement over BCG. The immunization with rBCG-19 overexpressing the 19kDa antigen did not provide any protection. In fact, it abrogated even the protective efficacy of BCG completely. In spite of statistical variations within a particular group, it was observed that recombinant BCG strains overexpressing either ESAT-6 or antigen 85C conferred better protection to animals as compared to the protection imparted by BCG. The immunization with rBCG strains overexpressing either antigen 85A or 85B did not show very clear results although overexpression of 85B seemed to provide slightly better protection than BCG.

Development of candidate DNA vaccines against tuberculosis and their evaluation in mice and guinea pigs

DNA inoculation represents a novel approach to vaccine and immune therapeutic development. The direct introduction of gene expression cassettes into a living host transforms a number of cells into factories for production of the introduced gene products. Expression of these delivered genes has important immunological consequences and results in a specific immune activation of the host against the novel expressed antigens. The recent demonstration by several laboratories that these immune responses are protective in infectious disease experimental models as well as cancers is viewed with optimism. Further, the relatively short development times, ease of large-scale production, low development, manufacturing and distribution costs all combine with immunological effectiveness to suggest that this technology will dramatically influence the production of a new generation of experimental vaccines and immune therapies.

Development and evaluation of candidate DNA vaccines for protection against tuberculosis

Expression of the antigens:

The genes encoding the three selected mycobacterial antigens namely ESAT-6, α-crystallin and Superoxide dismutase were cloned in the eukaryotic expression vectors indigenously developed in Dr. Tyagi's laboratory and expression was analysed in the COS-1 cell line. All three antigens were expressed in the mammalian cells.

Evaluation of protective efficacy of candidate DNA vaccines in guinea pigs:

Immune responses elicited by these candidate DNA vaccines were evaluated by immunization of mice with plasmid DNA and measuring humoral immune responses as well as cellular immune responses.

For the evaluation of the protective efficacy of the candidate DNA vaccines, guinea pigs were immunized with the vaccine constructs and later challenged with *M. tuberculosis*. The protective efficacy was evaluated by measuring the bacillary load in lung and spleen homogenates and histopathological analysis of liver and lung tissues.

The DNA vaccine expressing the gene for ESAT-6 was effective in decreasing the bacterial CFU in spleen and lung by about 1.0 log and 0.5 log, respectively as compared to sham immunized animals. The results of histopathology also revealed a reduction in the percentage of granuloma in liver and lung.

Immunization of mice with alpha-crystallin DNA vaccines resulted in a reduction in the spleen CFU by about 1.0 log, However, this plasmid DNA immunization was not effective at reducing the lung CFU. The histopathological analysis suggested a decrease in granuloma in liver as well as lung.

The plasmid DNA encoding the Superoxide dismutase was found to be the most effective one in decreasing the CFU in lung as well as in spleen. The mice immunized with this plasmid DNA exhibited a 1.6 log reduction in the spleen CFU and a 1.0 log reduction in the lung CFU. The histopathological analysis also revealed that immunization with this vaccine resulted in maximum reduction in the lung granuloma when compared to the other two vaccine constructs.

Heterologous prime boost approach with aerosol challenge model

It may be stated here that the aerosol route of infection, which is usually employed for infection of guinea pigs, leads to extensive colonization of the bacilli in the lung and further spread of this infection in lung as well as to the other organs such as spleen. The subcutaneous route of infection employed in these studies (it is supposedly the second best route for infection after the aerosol route) on the other hand, leads to a different pattern of initial bacillary distribution among different organs (~90% to liver, ~10% to spleen and only 1-2% to lungs) within 24 hours. Secondly, BCG is known to protect animals more efficiently against hematogenous spread of the tubercle bacilli from the lungs of an infected animal (Dissemination TB). The infection by subcutaneous route does not draw much analogy with dissemination TB for which BCG supposedly acts as a relatively more potent vaccine. Thus, subcutaneous route of challenge does not result in a significant load of bacilli in lungs unless very high dose of *M. tuberculosis* is used for infection as seen in these studies. This is in sharp contrast to the proceedings in the case of aerosol challenge, wherein just a few bacilli can result in extensive colonization of bacilli in lungs. Thus, in spite of use of guinea pigs as a challenge model, the

subcutaneous route of infection does not mimic the infection and its progression in a manner similar to humans.

Based on these observations, it was proposed that the protective efficacy of a candidate vaccine in guinea pigs should be evaluated i) by using aerosol route of challenge and ii) by employing an appropriate dose of *M. tuberculosis* for infection in order to determine the exact merit of the candidate vaccine in question.

Standardization of parameters for aerosol challenge

With the aerosol challenge facility at the National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Agra, being a new facility, it was incumbent on us to standardize various parameters such as the procedure of infection itself, the dose of *M. tuberculosis* challenge and the time period for evaluation of protective efficacy.

BCG is known to protect guinea pigs against low dose of aerosol challenge of *M. tuberculosis*. Thus, to determine the true potential of candidate vaccines, it was important to determine an appropriate dose of M. tuberculosis for infection at which BCG would act sub-optimally, that would provide suitable setting for evaluation of protective efficacy of candidate vaccines.

Secondly, in the guinea pig model, the kinetics of *M. tuberculosis* infection and disease progression follows a specific pattern, wherein, the innate immune response is followed by the emergence of adaptive immunity ~ two weeks post infection, which guides the concomitant disease progression with development of distinct pulmonary and extra-pulmonary pathology at different time points post infection. Immunization with a vaccine in a prophylactic mode results in the generation of a specific adaptive immune response, which in tern modulates the kinetics of bacterial multiplication and disease progression. Thus, for the evaluation of protective efficacy of a vaccine, it is necessary to choose an appropriate time point at which (a) various stages of disease progression are apparent and (b) the effect of vaccination on bacillary burden correlates well with the pathological situation.

Observations from various standardization experiments carried out during the initial phase of this study by employing different doses of infection, with various time points of euthanasia resulted in the following conclusions:.

- At two different doses of 50-100 CFU and 500-1000 CFU of *M. tuberculosis* - on day 1 post-infection, there was an apparent concordance between the dose of *M. tuberculosis* and the pathological damage caused to various organs such as lung, liver and spleen and total score. Moreover, at these two doses BCG provided sub-optimal protection leaving behind sufficient scope for improvement by an effective vaccine candidate.
- The magnitude of infection at 10-week time point correlated well with the resultant pathological development encompassing all the stages of disease progression

Hence, the above experimental settings, which provided appropriate conditions, were used for the evaluation of protective efficacy of candidate vaccines in this phase of study by employing heterologous prime boost approach.

In these studies, rBCG and DNA vaccines expressing various antigens were used individually and in various combinations. BCG, saline and vector immunized animals were used as control groups. Six weeks after the last immunization, guinea pigs were

infected with two different doses of *M. tuberculosis* H37Rv (50-100 and 500-1000 CFU by aerosol route) and euthanized 10 weeks post infection. After dissecting the animals, lung, liver and spleen were examined for gross pathological damage and post mortem scores were allotted to these organs, depending upon the number and size of tubercles. Lower lobe of right lung and spleen were taken for homogenate preparation to determine the bacillary load and right upper lobe was stored in 10% buffered formalin for histo-pathological analysis. In another sets of experiments, the time period between immunization and *M. tuberculosis* infection was extended to 12 weeks and the time period between infection and euthanasia was extended to 16 weeks.

In this final phase of study with the aerosol route of *M. tuberculosis* infection, three regimens showed excellent results involving of (i) recombinant BCG overexpressing antigen 85C, (ii) BCG as priming agent followed by boosting with DNA vaccine expressing α–crystallin, (iii). recombinant BCG overexpressing α-crystallin as the priming agent followed by boosting with a DNA vaccine expressing the same antigen

Recombinant BCG overexpressing antigen 85C

This regimen demonstrated a significant enhancement in the protective efficacy of BCG by over expression of Ag85C- an immuno-dominant antigen of *M. tuberculosis.* The parameters used for the evaluation of protective efficacy following an aerosol challenge with *M. tuberculosis* were, (i) bacillary load in lung and spleen and (ii) pathological changes in lung, liver and spleen. At 10 weeks post-infection, vaccination with rBCG85C resulted in a significantly reduced bacillary load in the lungs (~87 folds) along with a marked reduction in hematogenous spread to the spleen (~360 folds) in comparison to vaccination with the parental BCG strain. This reduced bacillary load was also accompanied by a marked reduction in the pulmonary, splenic and hepatic pathology. On extending the interval between vaccination and challenge (to 12 weeks) and between challenge and euthanasia (to 16 weeks), rBCG85C continued to impart a relatively superior protection with a remarkably greater control on bacillary multiplication in the lungs (~9 folds) and a successful restriction of the hematogenous spread of tubercle bacilli to spleen (~100 folds) in comparison to immunization with the parent BCG strain.

In the absence of vaccination, the clinical manifestation of progressive end-stage TB in guinea pigs is known to be associated with a strong inflammatory response to the persistent antigens or bacilli leading to extensive necrosis and progressive fibrosis. However, an efficient vaccine is expected to prime the immune system to generate an efficiently regulated and targeted response for an effective microbial and antigenic clearance, minimizing the collateral damage to the host. Immuno-localization of Ag85 complex proteins – some of the most abundant proteins of *M. tuberculosis*, as a marker of the mycobacterial antigen load, showed elevated levels of these antigens in the granulomas as observed in case of saline treated animals. This increased antigen load was found to be associated with the production of superfluous amount of TNF-α, unwarranted inflammation, tissue destruction and excessive collagen deposition. However, in addition to the bacillary clearance, rBCG85C mediated immune responses resulted in reduced antigen load indicating an effective removal of mycobacterial antigens and/or the bacillary remnants. A corresponding reduction in the extent of granulomatous inflammation and fibrosis in this group further substantiated the fact that an effective removal of the residual antigenic depots from the sites of infection is essential for the resolution of granulomatous lesions. More over, reduction in the levels of IFN-γ and TNF-α, towards the later stage of disease in case of the rBCG85C-immunized animals

further signifies the fact that, although, induction of these cytokines following *M. tuberculosis* infection is known to be essential for the initial containment of the bacilli, a subsequent reduction in the levels of these cytokines is crucial for the resolution of granulomatous lesions, as observed in this study.

BCG as priming agent followed by boosting with a DNA vaccine expressing α–crystallin

In view of the enormous number of individuals vaccinated with BCG, it becomes imperative to develop efficient booster vaccines in order to enhance the BCG induced immunity and sustain protection even in the old age. Besides, due to lack of adequate immune response to latency-associated antigens, BCG is often unable to provide sterilizing immunity against primary *M. tb* infection leading to occurrence of latent TB. Thus, in this study an attempt was made to enhance the protective immunity of BCG by heterologous boosting with a DNA vaccine-expressing α-crystallin – one of the most prominent antigens recognized during latency. The demonstration of a significantly reduced bacillary load in lung (~ 37 fold) and spleen (~ 96 fold) at 10 weeks post-infection by the 'BCG prime DNAacr boost' regimen, provides substantial evidence for its superiority over BCG. More over, a rigid control on bacillary multiplication (~100 fold and ~47 fold reduced bacillary load in lung and spleen, respectively) along with a significant reduction in pathological damage up to an extended period of 16 weeks post-infection suggests a robust and sustained enhancement in the protective efficacy of B/D regimen in comparison to classical BCG vaccination.

On histological analysis, unvaccinated animals showed extensive multi-focal coalescing granulomas with prominent central coagulative necrosis occupying more than 60% of the lung sections at 10 weeks post-infection. BCG immunization significantly reduced granulomatous infiltration in the lungs characterized by the presence of well-organized granulomas covering ~35% of the lung sections. However, animals vaccinated with B/D regimen showed well-preserved alveolar spaces with only a few scattered areas of diffused infiltration in peribronchial and perivascular areas (~5%). Corresponding to the aggravated pulmonary pathology, unvaccinated animals showed widespread infiltration with scattered areas of necrosis occupying more than 40% of the liver sections. However, all the BCG based regimens irrespective of the boosting agent, remarkably reduced the hepatic inflammation with a very few or no influx of inflammatory cells. At 16 weeks post-infection, both BCG vaccinated as well as unvaccinated animals showed a considerable increase in the pulmonary pathology. However, a booster dose of DNA vaccine significantly reduced the granulomatous inflammation in lung, when compared to a solitary immunization with BCG as well as B/V regimen. Moreover, B/D regimen conferred complete protection in liver with no evident sign of infiltration in comparison to the animals belonging to both BCG and B/V regimens, which showed scattered areas of granulomatous inflammation in liver.

Commensurate with the negligible granulomatous inflammation, B/D group showed no evident signs of collagen staining in the lungs other than the usual occurrence of collagen in the peri-bronchial and peri-vascular areas at both the time points. In contrast, widespread fibrosis was observed in and around the pulmonary granulomas in the unvaccinated animals causing loss of alveolar and micro-vasculature structure. BCG immunized animals, in comparison to significantly reduced collagen deposition at 10 weeks showed a relatively increased collagen staining at 16 weeks. Examination of

relationship between the extent of collagen deposition, bacillary load and granulomatous inflammation revealed a strong positive correlation among these parameters.

Although, the importance of heterologous prime boost immunization in the context of TB has been reported by several investigators, in this study, for the first time a latency-associated antigen (α-crystallin) was successfully employed as a booster DNA vaccine subsequent to BCG. The superior protection imparted by 'BCG prime and DNAacr boost' heterologous prime boost regimen provides several advantages, when viewed in clinical context. BCG, according to WHO guide lines, is given only once after the birth. However, the immunomodulatory effect of boosting the BCG induced immunity by employing an effective booster vaccine remains unaltered irrespective of the time span between the primary BCG vaccination and boosting. Thus, a booster dose of DNAacr to BCG immunized and unexposed individuals at any time can be expected to enhance immunity against perceived *M. tuberculosis* infection. Moreover, since, BCG protects against childhood TB, replacing it with a vaccine regimen that does not include BCG would be neither ethical nor practical, thus, employing DNAacr as a booster vaccine would simplify the matters related to the clinical testing of this regimen without hampering the child hood immunization program. In addition, the α-crystallin based memory immunity elicited by this regimen would help circumvent the occurrence of latent and reactivation TB due to enhanced recognition and clearance of the latent bacilli. However, a separate study to evaluate the effect 'DNAacr boost' on the reactivation of latent TB in a suitable animal model would be necessary to further strengthen this particular hypothesis.

Recombinant BCG overexpressing α-crystallin as the priming agent followed by boosting with a DNA vaccine expressing the same antigen

Over expression of α-crystallin in BCG imparted a significantly improved protection against *M. tuberculosis* infection, when compared to the parental BCG vaccination. However, a booster dose of this latency antigen in the form of a DNA vaccine subsequent to rBCG priming (R/D), resulted in a far superior protection. Even up to an extended period of 16 weeks post-infection, the R/D regimen was able to exhibit a rigid control on bacillary multiplication as was evident from 750 fold and 65 fold fewer bacilli in the lungs and spleen of animals immunized with R/D regimen, when compared to BCG vaccinated animals. Histopathological analysis of animals vaccinated with R/D regimen also exhibited a commensurate lesser granulomatous inflammation and associated pathological damage.

Vaccination induced alterations in the cytokine milieu dictate the variations in the disease trajectories. Measurement of immune responses at the later stages of disease in this study and their correlation with disease progression, provided an understanding about how the dynamic changes in the cytokine milieu of the lungs influence the fate of an infection. The increased levels of inflammatory cytokines such as IFN-γ and TNF-α along with reduced levels of immuno-suppressive cytokines like TGF-β and IL-10 corresponded well with the increased disease severity as observed in the case of unvaccinated animals. Both the heterologous prime boost regimens (R/D and D/R) elicited apparently similar immune responses marked by enhanced but comparable levels of inflammatory as well as immunosuppressive cytokines, however, the protection imparted by these regimens varied – while the R/D regimen provided sustained protection till 16 weeks post-infection, protection afforded by D/R regimen declined considerably after 10 weeks. This suggested that merely the measurement of levels of cytokines may

not provide appropriate correlations with disease severity and/ or level of protection, which led us to analyze the cytokine milieu based on the relative proportions of various cytokines in addition to their individual levels. As can be seen from Fig. 4B, the analysis based on the relative proportions of cytokines, guided us to draw better correlations between the distribution of cytokines and their consequential influence on protection. While, the R/D regimen with a superior protection showed a considerably increased relative proportion of IL-12 along with proportionate decline in IL-10 with time, the D/R regimen, in contrast, showed an exactly opposite trend resulting in a decline in protection after 10 weeks post-infection. Moreover, rBCGacr-immunized animals, which showed enhanced protection in the lungs at 16 weeks, also exhibited increased proportion of IL-12 along with a concomitantly reduced proportion of IL-10 as observed in case of R/D regimen. These observations from various vaccinated groups suggest that the increase in the proportion of IL-12 and decrease in the proportion of IL-10 at 16 weeks in comparison to their relative proportions observed at 10 weeks time point may be critical for the observed protection against the disease and a concomitantly reduced pathology.

Also, this study further demonstrated a close association of *M. tuberculosis* antigen load and extent of collagen deposition with the bacillary load and granulomatous inflammation observed in lung, suggesting that an efficient vaccine regimen in addition to providing protection against the initial infection should also prevent development of pathological lesions allowing the restoration of normal lung architecture.

An important corollary of these results pertains to their clinical relevance. The superior protection imparted by α-crystallin based 'BCG prime - DNA boost' and 'rBCG prime - DNA boost' regimens provides multiple advantages and possibilities in terms of their clinical relevance as stated below:

BCG, according to WHO guide lines, is given only once after the birth. However, it has been recently reported that the immunomodulatory effect of an efficient booster vaccine remains unaltered irrespective of the time span between the primary BCG vaccination and boosting. It has been observed that there was no significant difference in the magnitude of immune responses generated, when the booster is administered shortly after, or many years after BCG vaccination. In light of this, a booster dose of DNAacr to the BCG immunized individuals as described in this dissertation under "Boosting BCG" strategy, at any time, can be expected to enhance protective immunity against a perceived M. tb infection. Hence, this regimen could provide an effective strategy to boost the immunity of BCG immunized individuals.

The 'rBCG prime - DNA boost' regimen, on the other hand, can be effectively useful for the child hood immunization program. Firstly, in this regimen, the use of rBCG in place of BCG in the newborn children will not only preserve the valuable attributes of BCG, but will also result in an efficient immune response and superior protection against pulmonary TB. Secondly, a booster dose of DNA vaccine would further enhance and sustain the rBCG-induced immunity.

Since, production of α-crystallin is up regulated by M. tb during its transition from actively dividing to latent phase, prevalence of α-crystallin specific memory immunity in case of both BCG/DNAacr and rBCGacr/DNAacr regimens will aid in the enhanced recognition and clearance of latent bacilli. Hence, vaccination with these regimens is likely to reduce the incidence of latent and reactivation TB.

Conclusions

In all TB vaccine related studies, BCG has been used as the gold standard to pronounce the worthiness of a new vaccine candidate, because it is the failure of BCG in the adult human population that has necessitated the development of a new TB vaccine in the first place. However, this convention suffers from a caveat – a new vaccine is required for protection in humans, wherein, BCG does not work well; on the other hand, a new vaccine cannot progress to human trials without proving its superiority to BCG in animal models in which BCG works rather efficiently. Hence, it has been difficult to develop vaccines, which would ensure a superior protection over BCG in animal models. It is thus not surprising that in spite of a large number of vaccine related studies, merely 9 vaccine regimens have progressed to various stages of human clinical trials. These vaccines have shown a better or equal performance in comparison to BCG in their ability (i) to reduce the bacillary load in lung and spleen and/or (ii) to reduce pathological damage and/or (iii) to perform better in time to death assay. The 16 weeks assay carried out in this study to evaluate protective efficacy in a highly relevant guinea pig model of TB showed that on the basis of their comparison with all the vaccines that have already progressed to clinical trials, these three regimens imparted a remarkable protection. These vaccine regimens have been approved for human clinical trials by the Tuberculosis Vaccine Clinical Trial Expert Group (TVCTEG) of the Department of Biotechnology, Government of India. Currently, some upstream pre-clinical work on these candidate vaccines is in progress so that the human clinical trials can be initiated.

Study of *M. tuberculosis* genes involved in the establishment and progression of tuberculosis - identification of new targets for the development of anti-tubercular drugs

Dr. Tyagi's group has been working on genes involved in the establishment and progression of tuberculosis to understand the mechanism of pathogenesis and identification of new targets for the development of novel anti-tubercular drugs.

MymA operon

Dr. Tyagi and colleagues have identified and characterized the *mymA* operon (*Rv3083-Rv3089*) of *M. tuberculosis*, which is arranged in a divergent manner to *virS* (*Rv3082c*) which was identified by Dr. Tyagi's labroatory earlier. The investigations by his group showed that the transcription of the *mymA* operon is dependent on the presence of VirS protein. To identify the environmental cues that might trigger an up-regulation of the *mymA* operon, its expression under various *in vitro* conditions that simulate those faced by *M. tuberculosis* in the host environment was studied. It was observed that VirS is essential for transcription from the *mymA* operon promoter. However, a 4-5 fold induction of the promoter of the *mymA* operon by VirS occurs specifically at acidic pH. This may be due to increased synthesis of VirS at acidic pH. Alternatively, the acidic pH might change the phosphorylation state of VirS, which could improve its affinity for the promoter region of the *mymA* operon. The primary sequence analysis of VirS shows the presence of 9 putative protein kinase C phosphorylation motifs, [ST]-x-[RK]. However, induction of *mymA* operon at acidic pH and on infection of macrophages with *M. tuberculosis* underscores the importance of the encoded gene products, in processes that are important during the mycobacterial residence in the host environment.

An extensive analysis of the conserved domains and the core motifs present in the gene products encoded by *mymA* operon suggested that mycobacteria might use it for

modification, activation and transfer of fatty acids to the appropriate acceptor(s) in their cell wall. *mymA*, a monooxygenase encoded by *Rv3083* could potentially oxygenate mycobacterial fatty acids. The oxygenated fatty acids could be further modified by the acetyl hydrolase/esterase (*Rv3084*), short chain alcohol dehydrogenase (*Rv3085*) and zinc containing alcohol dehydrogenase (*Rv3086*). Finally, the acyl CoA synthase homologue (*Rv3089*) could then activate the fatty acids (modified by the products of genes *Rv3083-Rv3086*), which could subsequently be transferred to an acceptor in the cell wall of mycobacteria by acyl transferases (*Rv3087* and *Rv3088*).

It is known that under acidic conditions there is a two-fold reduction in the expression of genes present in the FAS II operon. FAS II operon that are responsible for the biosynthesis of meromycolic acids in *M. tuberculosis* by elongating long chain fatty acid precursors like C24 and C26 generated by the FAS I system. Down-regulation of the FAS II system at low pH would be expected to decrease fatty acid elongation, leading to an accumulation of C24 and C26 fatty acids. However, since the *mymA* operon is up-regulated at acidic pH, it can utilize the C24 or C26 fatty acids and as suggested above, modify and transfer them to appropriate biological acceptor(s) on the mycobacterial cell wall. Thus, induction of the *mymA* operon can play an important role in remodeling the envelope of intracellular *M. tuberculosis* under acidic conditions in the macrophages.

Dr. Tyagi and colleagues showed that MtbΔ*virS* and Mtb*mym:hyg* have an altered cell wall structure. Both strains exhibited a much denser and darker staining of cell surface, indicating an alteration in the electron transparent zone (ETZ), which is thought to be composed primarily of mycolic acids arranged perpendicular to the plane of cell surface. Such dense staining of the cell wall has also been observed after treatment of *M. avium* with isoniazid resulting from the inhibition of mycolic acids synthesis by the drug. The alterations in the cell surface of MtbΔ*virS* and Mtb*mym:hyg* strains were further substantiated by the HPLC profiles of mycolic acids from the mutants and the parental strains. Furthermore, both mutants produced less mycolic acids in comparison to the parental strain as analyzed by TLC. These findings suggest that the observed alterations in the cell wall ultrastructure result from the altered mycolic acid composition although the effect of latter on the arrangement of other cell surface lipids and proteins and their consequent contribution on the observed phenotype cannot be completely ruled out. On exposure to acidic pH, the reduction in mycolic acids synthesis was markedly more prominent in the MtbΔ*virS* and Mtb*mym:hyg* strains in comparison to the parental strain. The accumulation of fatty acids (C24:0/C26:0) at acidic pH was also observed to be higher in the mutants as compared to the parental strain. Although, a general reduction in the synthesis of mycolic acids at acidic pH can be expected to stem from the repression of Fas II operon, a much sharper decline in mycolic acid synthesis in case of both the mutant strains implicates *mymA* operon in the synthesis of mycolic acids on exposure of the pathogen to acidic pH. The emergence of new mass peaks corresponding to C88-C92 chain length of mycolic acids (1328, 1356 and 1384) in the parental strain, but not in the mutants clearly suggested the role of *mymA* operon in the synthesis of these mycolic acids at acidic pH. Further, the enhanced accumulation of C24:0/C26:0 fatty acids in the mutant strains substantiates their role in the synthesis of mycolic acids by *mymA* operon. Conventionally mycolic acids are believed to be synthesized by elongating long chain fatty acids (C16-C26) to meromycolic acids by Fas II operon of *M. tuberculosis* and the final Claisen type condensation of C24:0/C26:0 fatty acid with meromycolates results in the production of full length mycolic acids. However, an alternate approach of mycolic acid synthesis by "head–to- tail" condensation of long chain fatty acids has also been

suggested. The synthesis of mycolic acids by this approach involves the condensation of three common fatty acids. First, two of these are subjected to the omega-oxidation followed by condensation to produce meromycolic acids which in turn condenses with C24:0/C26:0 fatty acids to produce mycolic acids. This approach of mycolic acid synthesis requires enzymes that can carry out omega oxidation of fatty acids and their subsequent condensation. Interestingly, analysis of gene products of *mymA* operon revealed that Rv3083 (*mymA*) is a homologue of flavin containing monooxygenases, which can carry out omega-hydroxylation of fatty acids - the first step in omega oxidation of fatty acids, while Rv3085 and Rv3086 show homologies with dehydrogenases and could possibly carry out subsequent steps to convert terminal methyl groups of fatty acids to carboxylic groups for condensation as described. Release of acly carrier protein (ACP) esterified to the fatty acids by thioesetrase, LipR (Rv3084) leads to generation of diaacids for the condensation. Rv3087 and Rv3088 contain HHxxxDG motif required for the thioesterification or Claisen type condensation of fatty acids, the last gene Rv3089 is an acyl- CoA synthase and can activate the fatty acids. Thus, Rv3087 and Rv3088 can carry out "head to tail" condensation of fatty acids which were previously omega oxidized by Rv3083-Rv3086 gene products and further activation of the condensed fatty acids by Rv3089 can yield long chain fatty acids (keto acids). These keto acids can then be subjected to functional group modification like methylation, decarboxylation, cyclopropanation to generate meromycolic acids. The condensation process described above can produce long chain fatty acids that are indistinguishable from mycolic acids. Thus, the genes present in *mymA* operon can assemble meromycolic acids beginning from the omega oxidation of fatty acids followed by their condensation with fatty acids (C24:0/C26:0) to produce mycolic acids.

Both the mutants showed increased sensitivity to major antitubercular drugs along with enhanced susceptibility to SDS and acidic pH. Enhanced susceptibility of *M. tuberculosis* to antibiotics, detergents and environmental stresses has been shown to be associated with the alterations in the mycolic acid contents and composition.

The induction of *mymA* operon at acidic pH and a significantly reduced ability of MtbΔ*virS* and Mtb*mym:hyg* to survive in the activated macrophages as compared to the parental strain supports the hypothesis that *mymA* operon may play an important role in the survival of *M. tuberculosis* upon exposure to severely acidic conditions in activated macrophages or caseating granuloma in the later stages of infection. This was substantiated by a drastic reduction (~2.8log) observed in the ability of the mutant strains to specifically survive in spleen as compared to the parental strain at 20 weeks post infection. The genes present in the *mymA* operon apparently are involved in remodeling the cell wall integrity required for the persistence of *M. tuberculosis* in the host.

Conclusion

The involvement of *mymA* operon in the persistence of *M. tuberculosis* together with its role in maintaining appropriate mycolic acid composition to resist antitubercular drugs at acidic pH indicate that precise targeting of *mymA* operon gene products may increase effectiveness of combination chemotherapy and impede the mechanisms involved in the persistence of *M. tuberculosis*.

Tyrosine phosphatases of *M. tuberculosis* and their role in the survival of *M. tuberculosis* in the host tissue

Protein phosphorylation and dephosphorylation play a significant role in transducing signals involved in cellular processes such as adhesion, internalization and killing of pathogens. The analysis of the genome of *M. tuberculosis* revealed the presence of two genes for tyrosine phosphatases designated as MptpA and MptpB.

To investigate the role of MptpB in the pathogenesis of *M. tuberculosis,* Dr. Tyagi's group constructed a mutant strain of *M. tuberculosis* lacking the activity of MptpB. The gene encoding MptpB was inactivated in *M. tuberculosis* genome by homologous recombination using a non-replicative suicidal vector, pBKΔB. Southern blot and immunoblot analysis confirmed the verity of the mutant strain. Disruption of *mptpB* had no significant effect on the morphology and growth of *M. tuberculosis* in defined liquid culture medium suggesting that MptpB is not required for the growth of *M. tuberculosis* under *in vitro* conditions. Similar results were also observed when macrophage cell line was infected with the mutant and wild type strains. Both the strains were comparable in their ability to infect and survive in the mouse macrophage cell line J774A.1. To evaluate the role of MptpB in pathogenesis of *M. tuberculosis,* the survival of mutant strain in the guinea pig model of tuberculosis was studied. In this model of infection, a significant reduction was observed in the ability of the mutant strain to survive in the host organs. An approximately 70-fold (1.7 log) reduction in bacillary load was observed in the spleen of the animals infected with mutant strain as compared to the bacillary load from the animals infected with wild type strain at 6 weeks post-infection. This difference in the splenic bacillary load in both the groups of animals was not observed at the earlier time point of sacrifice (3 week post-infection). These observations suggest that initially both the strains (mutant and wild type) of *M. tuberculosis* are capable of establishing the infection to a similar extent. However, the ability of the strains to withstand the assault by the host was significantly different. The host was able to clear the mutant strain more efficiently than the parental strain. The influence of disruption of *mptpB* gene on survival of *M. tuberculosis* specifically in guinea pigs but not in macrophages suggests that although experiments involving infection of a macrophage cell line by *M. tuberculosis* have yielded useful information about several aspects related to the survival of pathogen in the host, a macrophage cell line may not represent the exact context encountered by mycobacteria in the host.

In order to demonstrate that the loss of virulence of *M. tuberculosis* was a direct consequence of disruption of *mptpB,* the gene was reintroduced in the mutant strain and the complemented strain was evaluated for its ability to survive in the guinea pigs. The complemented strain could establish an infection and survive in the host tissues even at the 6-week time point at levels comparable to those observed in the case of wild type *M. tuberculosis*. These observations clearly suggest that MptpB plays an essential role in the survival of *M. tuberculosis* in host.

Dr. Tyagi's group has also investigated the role of *mptpA* operon in the virulence of *M. tuberculosis* by constructing a mutant strain of *M. tuberculosis* inactivated in *mptpA* locus. Disruption of *mptpA* in the *M. tuberculosis* genome was confirmed by Southern blot and immunoblot analysis. Similar growth characteristics in MB 7H9 media and colony morphology on MB 7H10 plates suggested that MptpA is not required for *in vitro* growth of *M. tuberculosis*. Next, the ability of *mptpA* mutant and parental strain to survive in IFN-γ activated macrophages was compared. At 2 days post-infection, an

approximately 2-fold reduction in the survival of intracellular *mptpA* mutant (30% survival) was observed in comparison to the intracellular parental strain (55% survival). However, this difference in survival increased to approximately 10-folds and 14-folds at 4 and 6 days post-infection, respectively. At six days post-infection, the intracellular *mptpA* mutant showed 2% survival in comparison to the internalized parental strain that showed 28.4% survival suggesting that the *mptpA* mutant strain was impaired in its ability to survive in the activated macrophages.

Disruption of *mptpA* also impaired the ability of *M. tuberculosis* to survive in lungs and spleens of infected guinea pigs. An approximately 8-fold difference was observed in the bacillary load in spleens and lungs of guinea pigs infected with the *mptpA* mutant strain in comparison to the bacillary load in the spleens and lungs of guinea pigs infected with the parental strain of *M. tuberculosis* at 3 weeks post-infection. At 6 weeks post-infection, this difference in the bacillary load increased from 8-fold to 80 folds in case of spleen and 90 folds in case of lungs in comparison to bacillary load in spleens and lungs of animals infected with the parental strain.

Upon histopathological analysis of lung at 3 weeks post-infection, it was observed that tissue damage was comparable among the animals infected with the parental or *mptpA* mutant or *mptpA* complemented strain of *M. tuberculosis*, with similar extent of granulomatous tissue present in all cases. However, at six weeks post-infection, a significantly reduced pathological damage was observed in the lungs of animals infected with the *mptpA* mutant strain in comparison to the parental strain. This reduction in the extent of tissue damage in animals infected with the *mptpA* mutant strain suggested a healing response of the host, which was commensurate with impaired survival and reduced number of *mptpA* mutant strain in the lungs.

An 80 and 90 folds reduced bacillary load in spleens and lungs, respectively, along with markedly reduced pathological damage in lungs of animals infected with the *mptpA* mutant strain as compared to infection with the parental strain clearly implies an essential role of *mptpA* operon in the virulence of *M. tuberculosis.*

Conclusion

Thus, both MptpA and MptpB are important genes that are required for the survival of pathogen in the host tissue. Hence, both these phosphatases represent attractive targets for the development of new anti-tubercular drugs.