CONTINUING MEDICAL EDUCATION

RELEVANCE OF ADVANCE IN THE GENETIC ENGINEERING IN LEPROSY

V M Katoch

Introduction

Advances in molecular biology have influenced every discipline of life sciences including modern medicine. Among these advances the application of recombinant DNA technology or genetic engineering is the most important. Broadly it encompasses the ability to recombine the genes with appropriate vectors (plasmids, phages and others), make them enter suitable hosts and manipulate them for variety of functions specially the production of their gene products. Today we have several such genetically engineered products available for use. These include insulin, several chemokines/cytokines etc. As *M.leprae* is still not cultivable in any acceptable *in-vitro* medium system one has to depend upon alternative approaches to develop methods of diagnosis and management. Molecular biological technologies have enhanced the capacity to investigate these aspects. What began as a dream 15 years ago[1] has been translated into reality to a great extent.[2] Cloning and sequencing of genes of *M. leprae* have provided significant newer information about specific target sequences and has led to the development of several gene probes/ amplification assays for diagnosis of leprosy.[2] Besides this the cloning and expression of metabolically or immunologically relevant components has several applications which range from preparation and production of immuno- diagnostic or immunoprophylactic reagents to identification of drug sensitivity sites.

Address correspondence to:
Dr. VM Katoch
Deputy director (SG), Central JALMA Institute for Leprosy (ICMR) Taj Ganj, Agra - 282 001.

Advances in cloning of *M. leprae* genes : Last decade (1980s) wintessed a major progress in the development of techniques for cloning of genes of leprosy bacillus. Construction of lambda gt 11[3], cosmid libraries of *M, leprae*[4] and partial library of *M. leprae* using plasmid vectors[5] were important landmarks. Since then various immunologically/biologically relevant genes of *M. leprae* have been expressed in various vector-host systems.[6-24] Improtant achivements are :

(i) Production of recombinant proteins : A large number of *M.leprae* protein antigens have been expressed in lambdagt/ *E.coli* as well as other systems and have been immunologically characterized.[6-19] Some of these antigens have also been explored for their role in immunological responses [6-13,15-21] and have been found to be highly immunodominant for both T and B cell responses.[6,7] Some have species and genus specific epitopes. One protein named as LSRantigen has been shown to have epitopes which are preferentially recognized by ENL cases.[17] Most of these gene products are thought to be potentially useful for generation of immunodiagnostic and or immunoprophylactic reagents but none except for LSR

protein have been adequately explored for clinical application. As most of these artificially expressed proteins belong to heat shock families,these may have limited scope as immunoprophylactic reagents because of fear of auto-immunity. Unlike many other pathogens, these recombinant proteins have not become established as alternate reagents for serology or substitute to lepromin/ leprosin.

(ii) Biologically improtant genes : Recombinant DNA techniques have helped in identification of several new genes related to physiology of the *M.leprae* in terms of its multiplication, interaction with the host, drug action and intracellular survival. These include genes encoding for many important cell surface proteins, drug action related sites (ahpC, katG etc) genes associated with growth (thioredoxin reductase, ori C etc) and other regulatory proteins.[22-24] Practical application of these advances need to be investigated in future.

(iii) Mycobacterium as a host for gene cloning - genetically engineered vaccines :

Experience with cloning of genes in *E.coli* has been a major success but only a few mycobacterial genes have been expressed in *E.coli*. This led to the interest on other expression systems including streptomyces and mycobacteria. Introduction of foreign DNA using a shuttle phagemid has been reported in mycobacteria and replication of *E coli* plasmids in mycobacteria has been observed.[25] Vaccinia as well as mycobacterial vectors have also been described to be useful for cloning and expression of *M.leprae* genes.[26] It is believed that the ability to infect BCG vaccine strain with the shuttle vectors would be important for expression of genes of slow growing mycobacterial recombinant multivaccine vehicle.[26] These are encouraging reports and the impact of these advances on devising newer immunodiagnostic agents or immunoprophylactic agents is being ascertained.

Impact of genetic engineering methods :

While several genetic engineering methods to manipulate and investigate the genes of *M.lepre* have been standardized and applied, we have hardly any new generation of reagent(s) produced by this technology which is available for use. At present the reasons appear to belong to the following categories.

(a) Felt needs : From the success of public health campaigns of mass usage of multi-drug therapy (MDT) during the last decade it was felt that there is no problem about the diagnosis and treatment of leprosy. This led to thinking that there is no need for the application of these new technologies. As a result there was major decline in the interest in this area. Despite good initial success with the production of a few *M.lepre* proteins, very slow progress has been recently reported. Continued trasmission of leprosy despite mass MDT as indicated by high incidence rates has some sobering effect and it is hoped that the interest may be revived. In the emerging scenario, leprosy cases with low bacillary load are being detcted which are not suitable for production of lepromin of human origin. Further the number of colonies of animals (armadillos/ nude mice) suitable for mass growth of *M.leprae* is dwindling. Thus we will have to depend upon genetic engineering methods for production of *M.leprae* specific components and this makes the technology even more relevant in future.

(b) Technical limitation : Besides the above factors, there are several technical limitations which need to be overcome for optimum use of this technology:

(i) Immunologically/ therapeutically relevant antigens: One of the biggest bottlenecks in the exploitation of recombinant DNA technology has been the inadequate knowledge about the antigens of M.leprae which are important for protective immunity. The antigens which were readily expressed have been dissected off.[6,7] As most of the genetically engineered proteins belonged to heat shock/ stress families, these have not been found attractive either as immunodiagnostic or immunoprophylactic reagents.[27] With definitive idea of antigens of interest one

is almost hunting for the unkown target(s). This technology can help in mass production but can not help in identification of targets. These are to be identified by clinical and immunological investigations. It needs to be emphasized that the search for antigens which are of immunological interest should be vigorously pursued. Only then one can aim exploiting the recombinant technology to maximum.

Most of the currently available sero - assays in leprosy are handicapped as antibodies continued to persist for a long time after disease subsidence due to persistence of several of these antigens. This limits their application for monitoring the disease process. It would be better if sero assays are directed toward some component associated with live bacilli. Mycobacterial anzymes could be attractive antigens for such purposes. For example superoxide dismutases from different mycobacteria including *M.leprae* have been immunologically characterized and divergences have been found.[28] Besides the mycobacterial enzymes, secretory proteins have been considered as important potential targets for immunological purposes.[29,30] Some such secretory proteins which are expected to be better indicators of presence of viable organisms have been identified. These are, however, all in the preliminary stages. The strategies for identification of unique specific fragments/ specific amino acid sequences, their production by recombinant DNA technique and finally their application in measuring the immunological responses need to be further pursued.

(ii) Resistance/ persistence : With the widespread application of MDT, the problem of drug resistance in leprosy appears to be under control. With the knowledge about the genes responsible for drug resistance studies to detect resistance mutants to rifampicin and may be other drugs from the biopsies can be planned using molecular methods.[31] Expression of target enzymes or molecules would help in understanding the mechanism(s) of drug sensitivity/resistance,[31,32] search of new drugs as well as development of new generation drug screening system(s).[33] Similarly molecular approaches may help in understanding the phenomenon of dormancy and persister stage.[34] Differential expression of genes in a active/dormant state and studying the effect of knocking off particular gene can help in understanding its functional role. The recombinant DNA technology has not been adequately exploited for this purpose.

(III) Sequencing of the genome of *M.leprae* : In the initial phase genetic engineering methods helped in generation of information about several important gene sequences of leprosy bacillus. Development of PCR based sequencing and other related methods subsequently have provided newer capabilities to sequence major part or eventually total genome of *M.leprae*. This started as an ambitious project even before *M.tuberculosis* but then the priority shifted to tuberculosis. During 1998, total genome sequence of *M.tuberculosis* has been published but work on *M.leprae* genome has become apparently slow. Neverthless this has been progressing and is perhaps nearing completion. Already significant newer information from this project and other related studies in this direction has become available.[35] The information generated from the genome sequencing and the resultant improved undersrtanding of gene functions will hopefully stimulate the work in this area and we have genetically engineered products of *M.leprae* in the next millenium as replacement to native antigens such as lepromin/leprosin etc of animal/ human origin.

References

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