Future Impact of Molecular Biology and Biotechnology on Bacterial and Viral Diseases

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Summary

The advent of recombinant DNA technology has already made a significant impact on various aspects related to the basic understanding of pathogenic mechanisms in infectious diseases, as well as practical applications related to diagnostics and prevention. The present paper discusses recent technological innovations and increased analytical capabilities which promise to have an even more significant impact on the control of viral and bacterial diseases.

Key words: Molecular biology, biotechnology, infectious diseases.

Introduction

The recombinant DNA era, which began in the early 1970s, has undoubtedly had a dramatic impact on the understanding of bacterial and viral diseases at the molecular level. Progress continues at an astonishing pace and much of the advances are now moving from the theoretical to the practical as new knowledge and techniques are being applied to important problems associated with the diagnosis, therapy and prevention of these and other infectious diseases.

The wide repertoire of methods developed for the construction, manipulation and cloning of recombinant DNA molecules has enabled viral and bacterial genes to be studied and expressed in a wide variety of host cell systems. This has resulted in rapid advances related to better understanding of gene structure and function, development of diagnostic DNA probes and recombinant vaccines.

One of the more impressive examples relates to the pertussis toxin (PTX) from *Bordetella pertussis*, the causative organism of whooping cough. In just 4 years since the PTX gene complex was cloned, incisive insights into structure-function relationships of this complex molecule were obtained, resulting in a recombinant vaccine for this important disease[1]. The relatively rapid development of a recombinant vaccine for hepatitis B infection is another case in point. On the diagnostic front, a whole new generation of diagnostic tests are becoming available, based on the polymerase chain reaction (PCR) and DNA probes. PCR enables the specific, more than a millionfold amplification of a target DNA sequence[2] and DNA probes are able to specifically hybridize with target DNA, for example, in tissue specimens directly. The cloning and expression of genes coding for cytokines has also enabled the therapeutic use of these factors in controlling bacterial and viral infections in clinical settings, e.g., in bone marrow transplantation[3].

In relation to future trends, it would be of interest to assess the possible impact of molecular biology and biotechnology on various aspects of bacterial and viral diseases, including basic knowledge related to virulence, genetics and pathogenic mechanisms, as well as the impact on applied aspects related to diagnosis, vaccine development and therapeutics. The remainder of this article will briefly discuss some of the technologies and approaches which may play important roles in the immediate future.

Polymerase chain reaction (PCR)

Since its introduction in 1985, the polymerase chain reaction (PCR)[2] has dramatically altered the way molecular studies are carried out. Its most obvious and direct application is in the diagnosis of various bacterial and viral infections, where specific oligonucleotide primers (short pieces of DNA which are synthesized chemically) have been used to directly amplify target DNA from infectious agents in clinical specimens. Some examples are given in Table I. PCR is particularly valuable where isolation of the causative agent is hazardous, laborious, technically difficult and often unsuccessful, e.g., most viruses, mycoplasmas, mycobacteria, spirochaetes, chlamydiae and rickettsiae (Table I). In addition to its application in diagnostic procedures, PCR has also been applied to other areas, for example, clinical monitoring of hepatitis B virus infection[20] and DNA fingerprinting of bacteria and viruses[21], which are useful in epidemiological studies and in assessing genetic variation and extent of relationships between strains. PCR can also be extremely valuable in studying the natural history of infectious diseases, e.g., assessing the significance of minute amounts of microbial DNA/RNA in healthy individuals, and the mechanisms by which dormant infections are reactivated[22]. The importance of PCR and its future impact cannot be overstated. In the words of Kary Mullis, the inventor of PCR, "It's so widely used by molecular biologists that its future direction is the future of molecular biology itself."[23]

Multiple peptide synthesis

Complimenting the recombinant DNA approach will be the more widespread use of the multiple peptide synthesis techniques which enable the synthesis of hundreds of short peptides on polyethylene pins[24]. In its convenient, 96-pin microtitre plate format, this technique can be used to rapidly screen hundreds of short

Table I
The use of PCR in diagnosis of some infectious diseases

Organism	Reference
Escherichia coli	4
Clostridium difficile	5
Bordetella pertussis	6
Mycobacterium leprae	7
Mycoplasma genitalium	8
Rickettsia ricketsii	9
Chlamydia pneumoniae	10
Chlamydia trachomatis	11
Treponema pallidum	12
Borrelia burgdorferi	13
Rotavirus	14
Cytomegalovirus	15
Dengue viruses	16
HIV	17
Papillomavirus	18
Herpes simplex virus	19

peptides (6 to 12 amino acid residues) for reactivity with selected antisera. In this manner, epitope mapping of even large protein antigens is greatly facilitated. In addition to scanning epitopes which bind to antibodies, cleavage of the synthesized peptides also allows for scanning of T-cell epitopes. This approach has been used, for example, in studying antigenic epitopes of influenza virus[25], dengue-2 virus[26], and *Mycobacterium bovis*[27].

Kits, automation and other technical trends

Many recombinant DNA procedures are now available in convenient, easy-to-use, commercially available kits, thus making many procedures readily available to most researchers. This includes plasmid/DNA/RNA preparation and purification, cloning, PCR, expression systems, sequencing, probe labelling, multiple peptide synthesis, etc. Non-radioactive labelling techniques are also becoming more popular and may mean easier acceptance of many, new generation diagnostic assays based on probe or PCR technology. The analysis of large DNA molecules has also received a boost with the development of pulsed field gel electrophoresis (PFGE) and its variants which are capable of separating large DNA molecules up to 10 megabase pairs[28]. In contrast, conventional electrophoresis is limited to sizes smaller than 50 kilobases. The ability of PFGE to generate unique patterns of large DNA fragments means that the technique is very useful for studies related to genetic mapping and molecular epidemiology. The synthesis of short oligonucleotides for various purposes (e.g., probes, PCR primers) can now be conveniently and economically done on automated synthesizers.

Another clear trend is increased automation in techniques which are tedious, repetitive and labour-intensive, such as DNA sequencing. As a direct result of the technical challenges and demands of the Human Genome Project, automated DNA sequencers have been developed and are now commercially available and capable of sequencing up to 20,000 bases per day. It is envisaged that, by the year 2001, these machines will be able to generate half a million to 1 million bases of sequence per day[29]. Similar automated systems are also available for peptide sequencing. To improve accuracy and efficiency, much of the automated sequencing technologies will be closely coupled to sophisticated, computer-assisted *image analysis* technology, which will handle 1-dimensional and 2-dimensional gels, blots, RFLPs, PFGE restriction mapping and DNA sequencing[29].

Antisense strategies

There is also considerable interest in the potential of what is commonly referred to as 'antisense' strategies. This basically refers to the use of artificially synthesized oligonucleotides (the 'antisense' agent) that binds to target DNA or RNA (the 'sense' message, e.g., mRNA) inside a cell, thus inhibiting gene expression at the level of transcription, or block protein synthesis at the level of translation. Antisense oligonucleotides could thus be used as drugs for treating viral diseases by inhibiting the synthesis of viral proteins, and the first *in vivo* demonstration of this potential was described recently where a genetically engineered antisense sequence was used to block the replication of Moloney murine leukaemia virus in mice[30]. Despite its promise, however, substantial questions remain about the related problems of specificity, stability, delivery, efficacy and toxicity[31].

Engineered antibodies and production by microbes

In relation to the use of monoclonal antibodies as diagnostic or therapeutic agents in bacterial and viral infections, the recent development of techniques to produce monoclonal antibodies in microbes, such as *Escherichia coli*, may prove to be an important technological advance[32]. The use of antibodies in toxin-mediated bacterial infections (e.g., septic shock) is one important application. In addition to the advantages of fast growth and increased yields, it will also be possible to carry out genetic shuffling ('engineering') of antibody domains to create chimaeric molecules with new combinations of binding and effector functions, humanised antibodies[33] and novel antigen-binding functions[32].

Cytokines in infection control

Advances in molecular biology and biotechnology have meant that recombinant cytokines are now available in pure form and significant quantities. The cytokines are a large group of protein factors which play a central role in the development of anti-microbial immunity. They are produced by a wide variety of cell types and the most important cytokines are the interferons (e.g., IFN-gamma), interleukins (e.g., IL-1, IL-2, IL-4, IL-6), haemopoietic growth factors (granulocyte colony-stimulating factor, G-CSF; macrophage colony-stimulating factor, M-CSF; granulocyte macrophage colony-stimulating factor, GM-CSF) and tumour necrosis factor (TNF). Cytokines have been shown to play an important role in protective immunity against a variety of infectious agents, including bacteria and viruses[34]. In addition, GM-CSF has been used to control infections in patients with non-myeloid malignancies treated with high-dose chemotherapy and autologous bone marrow transplantation[35]. In this situation, GM-CSF accelerated neutrophil recovery reduced the number of febrile days, the number of days required for isolation in reverse-barrier nursing and the number of days of parenteral antibiotic therapy. GM-CSF has also been used to successfully reduce bacterial and viral infection in other conditions such as aplastic anaemia, myelodysplastic syndrome and AIDS[36].

Future Research Areas and Applications

Some attempt could perhaps be made to identify future areas of research in bacterial and viral diseases which are going to heavily rely upon continued application of the techniques of molecular biology and biotechnology:

1. An attempt to understand how bacterial resistance to antibiotics develops and spreads at the molecular level. This, in turn, may result in fundamentally new approaches to the design of antibiotics.

2. As more and more recombinant cytokines become available for clinical use, these factors may see more widespread use in controlling bacterial and viral infections in a variety of clinical settings. Antisense strategies, engineered antibodies and gene therapy approaches will be tested for a variety of infections.

3. As more convenient cloning, PCR, and automated sequencing techniques become available, sequence data on a large number of bacterial and viral genes will enable further advances in:

    a. understanding of structure-function relationships as a result of mutation studies in selected genes followed by the re-introduction of altered genes into host systems to elucidate biological effects *in vivo*;

    b. the design of extensive sets of PCR primers for diagnostic tests to detect bacterial and viral DNA in clinical and environmental specimens;

    c. more precise epitope mapping using the multiple pin peptide synthesis approach. This could be followed by the synthesis of milligram quantities of epitope-specific synthetic peptide antigens to be used in vaccine formulations or diagnostic assays.

4. Research will continue to improve the ability to construct genetically engineered recombinant viral vaccines, based on vaccinia virus[37] or other viral and bacterial vectors.

5. Development of novel anti-bacterial and anti-viral drugs based on interference with gene functions.

Unexpected spinoffs

As molecular biology continues to be applied to various aspects of bacterial and viral diseases, unexpected spinoffs in other areas are also a distinct possibility. For example, research into the molecular structure and function of the alpha toxin from *Staphylococcus aureus*, with its well-known ability to induce pore-formation on cells and cause hemolysis, could open the way to new medicines, chemical sieves and high-tech microporous materials[38].

Implications for Malaysia

The rapid development of practical applications of molecular biology and biotechnology has important implications for health care and health research in Malaysia. The advances in diagnostic methods, vaccine design, molecular epidemiology and therapeutic alternatives clearly implies that better control of bacterial and viral diseases should be attainable. The benefits would also extend, ultimately, to other important, non-infectious diseases such as genetic diseases, cancer, autoimmune diseases and coronary heart disease. However, for the country to fully benefit from these revolutionary advances, the level of awareness of the concepts and applications of molecular biology among health care personnel needs to be improved. Such awareness needs to be improved among the scientists engaged in research, laboratory personnel involved in diagnostic work, public health workers and medical personnel directly involved in patient care.

It could be attained, for example, through training courses and seminars devoted to clinically relevant aspects of molecular biology and biotechnology. The relevant scientific societies and the Academy of Medicine should play a central role in this regard. In the long run, an appreciation of these latest advances would no doubt result in better health for the population.

References

1. Burnette WN. The advent of recombinant pertussis vaccines. Biotechnol 1990;8 : 1002-5.
2. Mullis KB, Faloona FA. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods Enzymol 1987;155 : 335-50.
3. Pang T. Cytokines - An overview. Med J Malaysia 1992;47 : 1-2.
4. Olive DM. Detection of enterotoxigenic *Escherichia coli* after polymerase chain reaction amplification with a thermostable DNA polymerase. J Clin Microbiol 1989;27 : 261-6.
5. Kato N, Ou CY, Kato H *et al.* Identification of toxigenic *Clostridium difficile* by the polymerase chain reaction. J Clin Microbiol 1991;29 : 33-7.
6. Houard S, Hackel C, Herzog A, Bollen A. Specific identification of *Bordetella pertussis* by the polymerase chain reaction. Res Microbiol 1989;140 : 477-87.
7. Woods SA, Cole ST. A rapid method for the detection of potentially viable *Mycobacterium leprae* in human biopsies: a novel application of PCR. FEMS Microbiol Lett 1989;65 : 305-10.
8. Jensen JS, Uldum SA, Sondegard-Andersen J, Vuust J, Lind K. Polymerase chain reaction for detection of *Mycoplasma genitalium* in clinical samples. J Clin Microbiol 1991;29 : 46-50.
9. Tzianabos T, Anderson BE, McDade JE. Detection of *Rickettsia rickesii* DNA in clinical specimens by using polymerase chain reaction technology. J Clin Microbiol 1989;27 : 2866-8.
10. Campbell LA, Melgosa MP, Hamilton DJ, Kuo CC, Grayston JT. Detection of *Chlamydia pneumoniae* by polymerase chain reaction. J Clin Microbiol 1992;30 : 434-9.
11. Griffais R, Thibon M. Detection of *Chlamydia trachomatis* by the polymerase chain reaction. Res Microbiol 1989;140 : 139-45.
12. Wicher K, Noordhoek GT, Abruscato F, Wicher V. Detection of *Treponema pallidum* in early syphilis by DNA amplification. J Clin Microbiol 1992;30 : 497-500.
13. Malloy DC, Nauman RK, Paxton H. Detection of *Borrelia burgdorferi* using the polymerase chain reaction. J Clin Microbiol 1990;28 : 1089-93.
14. Gouvea V, Glass RI, Woods P *et al.* Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol 1990;28 : 276-81.
15. Zipeto D, Revello MG, Silini E *et al.* Development and clinical significance of a diagnostic assay based on the polymerase chain reaction for detection of human cytomegalovirus DNA in blood samples from immunocompromised patients. J Clin Microbiol 1992;30 : 527-30.
16. Deubel V, Laille M, Hugnot JP *et al.* Identification of dengue sequences by genomic amplification: Rapid diagnosis of dengue virus serotypes in peripheral blood. J Virol Meth 1990;30 : 41-54.
17. Guatelli J, Gineras TR, Richman DD. Nucleic acid amplification *in vitro*: Detection of sequences with low copy numbers and application to diagnosis of human immunodeficiency virus type 1 infection. Clin Microbiol Revs 1989;2 : 217-26.
18. Young LS, Bevan IS, Johnson MA *et al.* The polymerase chain reaction: a new epidemiological tool for investigating cervical human papillomavirus infection. British Med J 1989;298 : 14-8.
19. Cao M, Xiao X, Egbert B, Darragh TM, Yen TSB. Rapid detection of cutaneous herpes simplex virus infection with the polymerase chain reaction. J Invest Dermatol 1989;92 : 391-2.
20. Larzul D, Guigue F, Sninsky JJ, Mack DH, Brechot C, Guesdon JL. Detection of hepatitis B virus sequences in serum by using *in vitro* enzymatic amplification. J Virol Methods 1988;20 : 227-33.
21. Caetano-Amolles G, Bassam BJ, Greshoff PM. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Biotechnology 1991;9 : 553-8.
22. Peter JB. The polymerase chain reaction: Amplifying our options. Revs Infect Dis 1991;13 : 166-71.
23. Liversidge A. Interview with Kary Mullis. Omni 1992;14 : 69-92.

24. Geysen HM, Rodda SJ, Mason TM, Tribbick G, Schoofs PG. Strategiesfor epitope analysis using peptide synthesis. J Immunol Methods 1987;102 : 259-74.

25. Schoofs PG, Geysen HM, Jackson DC, Brown LE, Tang XL, White DO. Epitopes of an influenza viral peptide recognised by antibody at single amino acid resolution. J Immunol 1987;140 : 611-6.

26. Aaskov JG, Geysen HM, Mason TJ. Serologically defined linear epitopes in the envelope protein of dengue 2 (Jamaica strain 1409). Arch Virol 1989;105 : 209-21.

27. Radford AJ, Wood PR, Bilman-Jacobe H, Geysen HM, Mason TJ, Tribbick G. Epitope mapping of *Mycobacterium bovis* secretory protein MPB70 using overlapping peptide analysis. J Gen Microbiol 1990;136 : 265-72.

28. Lai E, Birren BW, Clark SM, Simon MI, Hood L. Pulsed field gel electrophoresis. Biotechniques 1989;7 : 34-42.

29. Hodgson J. Molecular biology in 2001. Biotechnology 1990;8 : 190-4.

30. Anonymous. Genetic Engrg Biotechnol Monitor 1991;36 : 27.

31. Ratner M. Waiting for antisense to deliver. Biotechnol 1991;9 : 410-2.

32. Hodgson J. Making monoclonals in microbes. Biotechnology 1991;9 : 421-5.

33. Carter P, Kelley RF, Rodrigues ML. High level *Escherichia coli* expression and production of a bivalent humanized antibody fragment. Biotechnology 1992;10 : 163-7.

34. Scott P, Kaufmann SHE. The role of T-cell subsets and cytokines in the regulation of infection. Immunol Today 1991;12 : 346-8.

35. Sheridan WP, Wolf M, Lusk J *et al.* GM-CSF and neutrophil recovery after high dose chemotherapy and autologous bone marrow transplantation. Lancet 1989;2 : 891-8.

36. Stern AC, Jones TC. Use of human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) in infectious diseases. In: Scarffe JH (ed). Breakthrough in Cytokine therapy - An overview of GM-CSF. Royal Society of Medicine, 1991 : 101-6.

37. Hruby D. Vaccinia virus vectors: New strategies for producing recombinant vaccines. Clin Microbiol Revs 1990;3 : 153-70.

38. Amato I. Molecular design gets into a hole. Science 1992; 255 : 684.