科技资料

New Antibacterial Strategies

FRONTIERS OF INFECTIOUS DISEASES

NEW ANTIBACTERIAL STRATEGIES

PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM SPONSORED BY GLAXO RESEARCH, BROCKET HALL, HERTFORDSHIRE 30 JUNE–3 JULY 1990

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Preface

This book provides the record of a meeting held at Brocket Hall, Hertfordshire, England between 30 June and 3 July, 1990. The meeting was the third in an ongoing series on Frontiers of Infectious Diseases and was devoted to New Strategies in Bacterial Infections. The proceedings are published within six months of the meeting so that the material will be up to date and provide readers with the current opinions of leaders in the field of infectious diseases. As in the previous two meetings, which dealt with virology (1988) and parasitology (1989), the focus was an attempt to integrate the latest advances at a molecular level with the important infectious disease entities.

The first presentation by myself addressed the unsolved problems that bacterial diseases present for the 1990s and beyond. Diseases such as meningitis, hospital-acquired pneumonia, sepsis, and diarrhoeal disease illustrate conditions in which there are major problems of mortality and morbidity in both developed and developing countries. Bacterial resistance of staphylococci, enterococci, Enterobacteriaceae, and other pathogens such as *Pseudomonas aeruginosa* and related species will also offer major challenges for the development of new antibacterial agents. Even more complex is how immunomodulators may be utilized in the treatment of bacterial infections, since even though we know much about the activity of these substances, the cost of this approach will far exceed that of current antimicrobial agents.

The first session of the meeting dealt with the problem of Borrelia infections. Lyme disease has been known by different names for many years in Europe, but only within the past decade has it become an illness of note in the United States. Gerold Stanek (Vienna, Austria) presented data on the epidemiology of Borrelia infections in central Europe during the last few years. While it is clear that the disease is increasingly recognized, its real incidence remains uncertain. It is inconceivable that there has been a suggested 1000-fold increase in some areas. Russell Johnson (Minneapolis, USA) reviewed the current knowledge of the structure of the Borrelia organism and the potential for vaccines, pointing out the problems in determining the antimicrobial susceptibility of Borrelia. The complexity of the serologic response to Borrelia was reviewed by Bettina Wilske (Munich, Germany), who referred to the many cross-reacting antigens that confound currently used serological tests. Where diagnosis can be made from the distinct clinical rash, serology is not required, but in the confusing

neurological problems, serum serology may be negative, while the CSF serology, similar to syphilis, may be positive for years.

In the second session, Alf Lindberg (Stockholm, Sweden) provided a superb review of the polysaccharide vaccines needed for the 1990s. He pointed out the value of the conjugate vaccines which utilize not only B-cells but the T-dependent axis. Indeed, the discussions highlighted the great progress made with the Haemophilus conjugate vaccines, which may be of major importance in reducing Haemophilus meningitis, a disease with important morbidity. Myron Levine (Baltimore, USA) reviewed the status of vaccines for *Salmonella typhi*, where major progress has been made in the past decade, and an oral vaccine may now be truly possible. Many of the lessons learned from the Salmonella vaccine hopefully will be applied to other vaccines for enteric infections.

Session three focused on musocal surface interactions. The role of surface fimbriae of *Escherichia coli* in attachment to bladder and kidney as reviewed by Gary Schoolnik (Stanford, USA) has been well known for the past decade. The observations of Catharina Swanborg (Lund, Sweden) that chronically infected bacteriuric patients do not have adhering strains and that blocking p-adherence would not prevent renal scarring raises serious questions about the possibility of vaccination as a strategy to prevent pyelonephritis. Torkel Wadstrom (Lund, Sweden) then provided an excellent review of our knowledge of *E. coli* as a cause of diarrhoea, and the concept that the enteroadherent organisms alter the cytoskeleton of the intestinal cell offers further areas of investigation for this field.

Martin Blaser (Nashville, USA) provided a most compelling rationale for the role of *Helicobacter pyloi* in gastritis and duodenal ulcer. His model of the pathogenesis of the infection with its effect on gastrin secretion and possible ultimate role in gastric malignancy proved most provocative. Although Guido Tytgat (Amsterdam, The Netherlands) provided impressive responses to triple antimicrobial programs, we have yet to solve the problem of recurrence of this disease.

Session four, which centres on the subject of resistance began with Staffan Normark (St Louis, USA) proposing a most interesting new model to explain β-lactamase induction. A peptidoglycan fragment is generated which is transported across the cytoplasmic membrane to interact with ampR. Equally fascinating has been the appearance of new plasmid β-lactamases as discussed by Laurent Gutmann (Paris, France).

Quinolone antimicrobials have caught the interest of scientist and practitioner alike. As Mark Fisher (London, UK) pointed out, our understanding of the interaction of the fluoroquinolones with DNA gyrase and DNA is far from complete. Excellent work by his group has demonstrated that changes in tyrosine-122 of the DNA gyrase of *E. coli* explain resistance in clinical isolates. Analogous changes occur in *Staphylococcus aureus* clinical isolates which have become resistant. How to prevent clinical resistance remains unclear, but a better understanding of the molecular changes associated with resistance may aid us in developing new compounds. The problem of transport in resistance in bacteria was reviewed by Christopher Higgins (Oxford, UK), who pointed out the value of the TonB system as an uptake system for the periplasmic space. Current research is directed at use of catechol compounds to capitalize on accessory channels to introduce antibiotics into bacteria.

In the second plenary lecture, Keith McAdam (London, UK) provided a most up to date look at the important infection, leprosy. Modern advances in immunology and molecular biology offer ways to control the disease and to identify those individuals in whom early treatment may abate the illness.

Paul Tulkens (Brussels, Belgium) and William Craig (Wisconsin, USA) each then presented and provided an up to date discussion of the intracellular location of antibiotics. Tulkens rightly concluded that different locations in the phagocytic cell are needed for different pathogens, after which Irun Cohen (Rehovot, Israel) provided a most provocative article on the 65k dalton heat shock protein and its association with murine autoimmune disease.

Immunomodulators provided the subject for the sixth and final session of the meeting. The interleukins, now well characterized, unfortunately have not so far been manipulated successfully in infectious diseases as the review by Chris Henney (Seattle, USA) showed. We clearly have a long way to go before we can manipulate interleukins to our benefit in disease. In contrast, the colony stimulating factors, as reviewed by Don Metcalf (Melbourne, Australia), have proved extremely useful in a number of clinical situations, though while potential uses are many, the costs may prove prohibitive.

I would like to thank Glaxo Research for providing the funding of these meetings, and in particular, Richard Sykes, Grahaem Brown and Carolyn Bennet of Glaxo, without whose continued help the meeting would not run in the efficient manner that it has over the past three years. Finally, I thank Amanda Ryde of Churchill Livingstone, without whom these proceedings would never have seen the light of day.

H.C.N.
1990

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Plenary Lecture 1

Chairman: S. R. Norrby

1. Bacterial diseases: unsolved problems

H. C. Neu

INTRODUCTION

Although there are many antimicrobial agents available in a large number of different classes to inhibit and kill bacteria, the problems of bacterial infections are not solved. There are two approaches that can be taken to examine problems of bacterial diseases: one organism directed and the other disease oriented. I have chosen to use both approaches.

There are unsolved problems in both diagnosis and therapy for a number of important infections.

MENINGITIS

The organisms causing meningitis in different age groups and clinical settings has been established by investigations over the past several decades (Meningitis Study Group 1987, 1989). Although there has been a shift from *Escherichia coli* to group B streptococci as the major cause of neonatal meningitis in developed nations, there has been minimal decrease in the incidence of neonatal meningitis. Furthermore, even though excellent antimicrobial agents are available, there has been minimal improvement in mortality and morbidity in recent years. The same arguments could be made for childhood meningitis in which *Haemophilus influenzae* and *Streptococcus pneumoniae* are the pathogens. Indeed in childhood meningitis with a decline in mortality there may be an increase in morbidity due to survival of children who would previously have died. Permanent neurological sequelae occur in 10 to 20% of survivors (Klein et al 1986). Among the elderly there is major morbidity and mortality in meningitis due to *Streptococcus pneumoniae*. Do we lack adequate antimicrobial agents? No. Do we lack agents that enter the cerebrospinal fluid or the brain? No. What is the problem? The problem centres around the body's inflammatory response to organisms or even to bacterial cell wall in the spinal fluid. Bacterial cell wall components bind to CNS endothelium and promote the response of leukocytes with the associated interleukin (IL-1) which stimulates tumour necrosis factor (TNF). Autoregulation of cerebral blood flow is lost, and there is resultant brain oedema with ultimate irreversible neurone damage. In effect it would appear

that effective antibiotics cause rapid destruction of bacteria with an inflammatory burst and associated brain tissue damage. Ideally an antimicrobial agent would penetrate the blood-brain barrier rapidly, halt proliferation of bacteria, kill the bacteria, but not release surface components which activate IL-1 and TNF. Choramphenicol immediately comes to mind as an agent, but chloramphenicol is bactericidal and lytic to the CNS pathogens.

Clinical trials of dexamethasone for treatment of meningitis in children and adults have reported a reduction in neurological sequelae for pneumococcal meningitis. There was a 13% mortality rate in patients receiving dexamethasone with antibiotics versus 41% for patients who received ampicillin plus chloramphenicol alone (Girgis et al 1989). A similar study of treatment of meningitis by Lebel et al (1988) showed an improvement in hearing loss and neurological sequelae only in children with *Haemophilus influenzae* meningitis. These studies cannot be translated to all forms of meningitis. Unfortunately, there is only a small time period that agents that blunt the host's response to bacteria can be administered to obtain optimal effects. But it would seem that to make significant progress in the treatment of bacterial meningitis attention will have to switch from the antibacterial agents which have been the focus of the late 1970s and early 1980s to agents modifying the inflammatory response. It is important to realize that mortality and morbidity in a disease which requires that patients have rapid access to healthcare will not change in many developing and even developed countries. Patients who are not seen or diagnosed until well into the course of meningitis will do poorly. The economic incentive to improve morbidity is great since neurological sequelae are extremely costly in the long term to the health care system.

HOSPITAL-ACQUIRED PNEUMONIA

Nosocomial pneumonia is the second most common cause of hospital-acquired infection (Horan et al 1986), and the leading cause of death from nosocomial infection (Gross et al 1980). Patients who are at highest risk for nosocomial pneumonia are the ventilated patients, some 7 to 10-fold above non-ventilated hospitalized patients (Horan et al 1986, Celis et al 1988). We know that colonization of the oropharynx with Gram-negative bacilli or staphylococci is necessary before pneumonia develops, and we know that many factors contribute to colonization of the oropharynx with Gram-negative bacteria. Gross et al (1980) reported that 60% of fatal nosocomial infections were due to pneumonia, and a more recent study by Craven et al (1986) showed that of 233 mechanically ventilated patients there was a 55% fatality rate for patients with pneumonia compared with 25% for patients without pneumonia.

We know that most cases of nosocomial pneumonia result from aspiration of bacteria from the oropharynx or the stomach into the tracheobronchial tree. We know that oropharyngeal colonization with bacteria has been associated with increasing severity of illness, longer duration of hospitalization, use of antibiotics, advanced age, intubation, and many other factors. None of these factors will be changed in the coming years. Indeed, there will be more elderly patients and those patients who are hospitalized will be more severely ill as more and more attempts are

made to treat less severe illness in the community thereby decreasing costs. Attempts to decrease the incidence of pneumonia in intubated patients by maintaining the pH in the stomach at higher levels by avoiding H_2 blockers have met with variable success since even in patients who did not receive H_2 blockers, 41% developed pneumonia (Daschner et al 1988). Also, a study by Driks et al (1987) showed a very low incidence of pneumonia in patients who received a H_2 blocker alone. Thus it seems likely, irrespective of what is ultimately found for H_2 blockers, that there will be many patients who develop pneumonia in the hospital.

The diagnosis of pneumonia in the hospitalized patient in the intensive care unit (ICU) is not a simple matter, and in spite of extensive study, it is often impossible to separate pneumonia of a bacterial aetiology from an acute respiratory distress lung picture.

The difficulty in making a diagnosis of pneumonia in ICUs and the frequency with which it occurs has caused a resurgence of interest in antibiotic prophylaxis in ICUs. Early studies of aerosolization of antibiotics such as polymyxin B into the trachea reduced pneumonia due to *Pseudomonas aeruginosa* but resulted in increased Gram-positive pneumonia and resistant Gram-negative bacilli (Feeley et al 1975). More recently both systemic and local antibiotic prophylaxis have been used in ICUs to prevent colonization of the oropharynx and gastrointestinal tract (Stoutenbeck et al 1986; Ledingham et al 1988). These studies have reported a major reduction in Gram-negative pneumonia compared with placebo groups, but have not resulted in a major reduction of the time patients spend in ICUs or in fatality rates. Although antimicrobial resistance is said not to have occurred in these studies, the time of observation has been short, and it is likely that it will be a problem.

Hospital-acquired pneumonia thus remains an unsolved problem in spite of the reduction in infection due to the mechanical devices and other equipment used on intubated patients. Preventive measures that decrease oropharyngeal colonization, methods to diagnose pneumonia more rapidly, and better methods to deliver antimicrobial agents to all parts of the lung are needed.

CYSTIC FIBROSIS

Respiratory infection in cystic fibrosis patients is another problem which has not been solved by the introduction of new antimicrobial agents. Each new antimicrobial agent with activity against *Pseudomonas aeruginosa* has been used since the late 1960s with the development of carbenicillin. Ureido-penicillins such as azlocillin and piperacillin, cephalosporins such as ceftazidime, the monobactam aztreonam, and the carbapenem imipenem have been used to treat acute bacterial respiratory exacerbations in these patients. However, with use, all of the aforementioned agents, aminoglycosides, the newly introduced quinolones, such as ciprofloxacin, become ineffective due to resistance of the *Pseudomonas* (Scully et al 1987). It has been impossible to clear the tracheobronchial tree of *Pseudomonas* in those patients who routinely have 10^8 to 10^{10} CFU per ml of sputum. Clinical studies have clarified that antibiotics do make a difference in the course of the cystic fibrosis patient. Thus, it is imperative to continue to find new antibacterial agents for these patients. This is no small task since the patients are not a major part of the population, and if drugs

are used to treat these patients early in the development of the drug, it can cast a shadow over a compound because of resistance development. It is necessary to develop novel ways to administer antimicrobial agents to the cystic fibrosis patient, and it is important to learn better ways to overcome *Pseudomonas aeruginosa*, about which I will comment more subsequently.

SEPSIS

In the past two years, there has been an enormous advance in our understanding of the pathogenic processes which occur during septicaemia. The organisms that cause sepsis and the sources of the bacteria have been delineated so that it is possible to suspect the aetiologic agent with a high degree of accuracy. We have excellent antibacterial agents which inhibit many, if not most of both community and hospital-acquired organisms. Methods to decrease bacteraemia have been developed by improved hospital infection control procedures. The haemodynamics and pathophysiological changes which occur in the heart, lung, and kidney have been described. We realize that there is activation of the kinin cascades, and that IL-1 and TNF are produced and that cardiac contractility diminishes and capillaries in the lung become excessively permeable. Nonetheless, there is still a significant mortality due to sepsis, and the question remains how can this be altered?

Studies utilizing polyclonal, and more recently monoclonal, antibodies of either murine or human origin have shown a protective value in selected septic patients. More studies are needed to establish the efficacy of this treatment before it becomes a general procedure for suspected sepsis. The costs of antibody administration will be significant, and it is necessary to delineate specifically those patients in whom such therapy is unequivocably beneficial.

Equally complex is how to adjust the production of IL-1 and TNF in the septic patient. Can specific antibodies be made to modulate finely the host's response to sepsis? Are there situations in which it would be reasonable to modify the production and activity of these cytokines knowing that there is the potential for sepsis? Could agents be developed that could be administered before surgery which by modulating cytokine activity would cause a major reduction in septic shock?

The area of sepsis is one for interface of antimicrobial agents and immune modulators if further progress is to be made in reducing the mortality of sepsis. Antimicrobial agents, if active against the infecting organism, result in very similar cure rates. The number of organisms resistant to all agents currently available or under study is quite small. Thus the problem of this bacterial infection is primarily one of altering the host so that defences do not run amok and kill the patient.

Recently it has become evident that colony-stimulating factors, such as GMCSF and GCSF, are important in providing adequate re-establishment of phagocytic cells that are critical to prevention of serious bacterial infection which occurs during neutropenia. How these agents are to be optimally used has not been established. It is not clear that deleterious effects will not occur in some situations. Can the agents be used in a way that will not result in proliferation of undesired clones of cells in the patients with malignancy? Sepsis in the neutropenic patient remains a serious problem; albeit to a lesser extent due to aerobic Gram-negative bacteria, but

aggressive chemotherapy directed against the malignancies and the use of oral agents of the quinolone class to sterilize the intestine has caused the appearance of Gram-positive species such as the viridans streptococci. This has been particularly evident in the bone marrow transplant patients. Thus new bacterial pathogens are likely to appear in the markedly immunocompromised patient. Strategies for the use of antimicrobial agents in these populations will undergo continued change.

GASTROINTESTINAL PATHOGENS

The role of *Helicobacter pylori*, formerly *Campylobacter pylori*, in gastritis is being more and more firmly established (Blaser 1987, Rauws et al 1988, Chamberlain & Peura 1990). Challenge of humans with *Helicobacter* produces gastritis (Marshall et al 1985, Morris & Nicholson 1987). Recently it has been shown that there are intrafamilial clustering of *Helicobacter* infections (Drumm et al 1990). It is now possible to culture *Helicobacter* easily, to detect serum antibodies to the organism, and even to detect it rapidly by the (^{13}C) urea breath test (Booth et al 1989, Goodwin et al 1989, Graham et al 1988, Evans et al 1989). Unfortunately, so far no serotyping system exists to characterize *Helicobacter pylori* isolates well in order to understand better the epidemiology and transfer of the organism.

Medical therapy has been associated with clearing *Helicobacter pylori* from the gastrointestinal tract and with healing of inflammation in patients with duodenal ulcers (Rauws et al 1988, Chamberlain & Peura 1990). However, relapse is common, and there has been the development of resistance to antibiotics such as the quinolones. It is clear that agents more effective against *Helicobacter* are needed. The fact that bisumuth subcitrate combined with amoxycillin has been more effective than bismuth alone makes it unclear if the effect of the antibiotic is on the organism or on other commensals which potentiate the *Helicobacter*. Long-term studies with more careful analysis of whether there is recurrence, reinfection, or both, occur is necessary if progress in this disease is to be achieved.

Intestinal infections are a major problem in the developing world, and with an increase in world travel a problem seen in the developed nations as well. Antimicrobial approaches to diarrhoeal disease have met with success, but there has been the development of major resistance that is plasmid-mediated in *Shigella*, *Salmonella* and other enteric pathogens (Murray 1986). Systemic disease due to *Salmonella typhi*, a pathogen unique to man, is still a problem in many parts of the world. Vaccination logically seems a way to approach the problem of bacterial enteric infections, and there has been extensive work in the field of vaccines against typhoid, *Shigella* and cholera. Unfortunately problems remain. The cholera-inactivated oral vaccines have been well tolerated and protect older children and adults for at least three years. The inactivated vaccines require multiple doses and do not protect the very young children who are most likely to suffer the serious effects of fluid loss (Clemens et al 1990). Ideally, an attenuated strain of vaccine will ultimately be possible that will not cause side-effects as did the early recombinant vaccines. Currently clinical trials are in progress to see the effect of the CVD 103 single-dose vaccine in 2 to 4-year-old children.

Shigella in many parts of the world have become increasingly resistant to