Introduction

In any endeavour of enquiry, certain questions take longer to answer than others, some remain unanswered, and the answers to some simply raise new questions. In this context, it is of interest to compare the present position with those of 1984 and 1988, when the first attempts at drawing up a list of unanswered questions about Clostridium difficile were made.\textsuperscript{1,2}

Neonatal resistance to infection\textsuperscript{3}

The resistance to disease exhibited by neonates and infants, even though high levels of toxins A and B may be present in the gut, remains unexplained. A number of hypotheses can be proposed, including the possibility that there are few or no receptors in the gut for the toxins. Maturation of the receptors may include microbial degradation of glycoproteins and exposure of internal motifs. This may be age-dependent and may parallel the development of a normal gut flora. Also unknown are the age at which this early life-protective mechanism is lost and the age at which colonization resistance takes over in a protective role. Work in the hamster model indicates that the two mechanisms may overlap for a period, but that colonization resistance becomes the sole protective mechanism at approximately day 25.

It is unlikely that breast-feeding and any substances in breast milk contribute much to protection, as there is no indication of increased infection and disease in formula-fed infants. The possibility that there is no protection in the newborn also has to be considered. Excretion of C. difficile is generally sporadic, and excretion of toxin follows a similar pattern but is less frequent. It is possible that there is occasional mild diarrhoea which goes unrecorded.

Spectrum of disease\textsuperscript{3,4}

Not all adults excreting toxigenic C. difficile have disease and not all of those who have antibiotic-associated diarrhoea progress to pseudomembranous colitis. This is even true among the elderly, which is the age group at highest risk. Obviously, this spectrum of outcome following infection may be related to differences in bacterial virulence. That not all strains of C. difficile are equally virulent in the hamster model of disease is well established and this also appears to be true in humans. There is some evidence that certain strains of C. difficile within the same hospital are more frequently associated with severe disease than others, but strong evidence is still awaited.

The virulence factor or factors which may contribute to differing virulence have also yet to be fully delineated. There is evidence that those strains which are more virulent in the hamster model produce more toxin A in vivo and tend to be more proteolytic in vitro, but this distinction for all enzymes and toxins measured is not true in every case. This is true also of putative colonization factor antigens. The exact nature of the putative adhesins described is unknown, and whether or not the flagella or pili function as adhesins remains to be determined.

It is likely that host factors also contribute to the spectrum of disease or asymptomatic excretion. However, there is little evidence for this. Conclusive evidence for a protective association between levels of serum antibodies to toxin A and disease severity is lacking. Further, no similar association has been shown between concentrations of copro-antibodies and disease severity. However, there is some evidence that colonic tissue from some individuals is resistant to the measurable effects of toxin A in vitro. The implication is that some individuals may be less susceptible to disease, but the mechanisms of this protection is unknown.

It could also be the case that some individuals are highly susceptible to disease, and that such individuals succumb to frequent relapses. Certainly, the relatively high incidence of symptomatic recurrences argues against the development of a protective immune response in many patients.

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A ge-related susceptibility in adults\(^3\)

There has been a lot of evidence for a long time that patients on antibiotics have a higher risk of infection if they are more than 60 years old. This difference is unlikely to be related to characteristics of the pathogen, unless we are to suppose that highly virulent strains prefer elderly hosts! It is possible that the elderly are more at risk because they are subjected to higher-risk procedures, are on antibiotics more frequently and spend more time hospitalized and in a high-risk environment. Equally, the higher incidence in the elderly could be directly related to age-associated changes. There is some evidence, for example, that the gut flora of the elderly has a less effective colonization resistance capability. The implication of this is that a smaller effect of antibiotics may be needed to induce susceptibility to colonization by **C. difficile** than is the case for younger patients.

What is the mechanism of colonization resistance?\(^3\)

It is not known which components of the gut flora are essential for excluding **C. difficile**. It is likely that, in different people, a number of different complex groups of organisms are responsible for natural colonization resistance. The indication from in-vitro mixed culture work is that the anaerobic component of the faecal flora can prevent growth of **C. difficile**, whereas the facultative component alone cannot. A gain, the mechanism(s) by which this growth inhibition is effected remains unknown.

Toxins A and B\(^3,4\)

A number of intriguing questions remain regarding the toxins of **C. difficile**. The evidence, from purified toxin use in animal models, that toxin A is more important than toxin B in the disease process, was questioned by the observations that a natural toxin A-negative, toxin B-positive strain caused full pathology in hamsters and that filtrates of the strain caused fluid accumulation in rabbit ileal loops. Furthermore, such strains have now been identified in the faeces of symptomatic patients in the U.K. Evidently, much is still to be learnt about the roles of these toxins or natural variants of them. The possibility remains that there are other enterotoxin-like proteins produced by **C. difficile**. There have been a number of reports of such toxins, but they have not been truly substantiated. Further evidence against the original conclusions on the relative roles of these two toxins was recently provided by the observed effects of toxin B on human gut tissue. However, the ideal control for this would have been to measure the effects on hamster caecal tissue in the same way, as there is experimental evidence of a lack of activity of toxin B alone in the intact caecum in vivo.

Questions still remain about the identity of the receptors for the toxins. There is good evidence that the Lewis X, Y and I blood group antigens can act as receptors for toxin A, but the extent to which they act as functional receptors in the gut is unknown, and there is as yet no idea of the nature of receptors for toxin B. The receptors on cells in tissue culture also remain unknown for either toxin.

A major recent breakthrough has been delineation of the cellular mechanism of action of the toxins. Both toxins glycosylate Rho family proteins. However, this finding also raises a fundamental question: “if the cellular mechanisms of action of the two toxins are identical, why are the biological end-effects in the whole organism so different?” Further, some of the biochemical changes within cells in tissue culture also differ. It is likely that toxin A has other effects on cells in addition to its ability to glycosylate Rho. The identity of factors that regulate expression of the toxins and the tertiary structure of both toxins still remain unresolved, but evidence is likely to be forthcoming over the next 5 years.

Patient issues\(^5-8\)

There are a number of unanswered questions relating to patient management and treatment and the routes of transmission of infection. At the number of reported cases of **C. difficile** diarrhoea continues to escalate, we are clearly recognizing more cases and/or failing to control this nosocomial infection. It is likely that both of these factors are involved. The pressure on use of hospital beds is such that optimal infection-control precautions cannot be taken and this trend must be addressed. We need to devise effective strategies to control antimicrobial prescribing, particularly in patient groups at high risk of **C. difficile** infection. Although antibiotics have been labelled as low and high risk in terms of their propensity to induce **C. difficile** infection, supportive data are scant. It is also unclear whether all such risk can be attributed to antibiotic-mediated effects on colonization resistance alone. For example, can antibiotics directly affect the virulence of **C. difficile**?

Using an antibiotic to treat an antibiotic-mediated disease is, arguably, not the most sensible approach. Antibiotics can lead to persistent disruption of gut flora, and types of therapy that can speed the reattainment of a normal flora would be preferable. To this end, biotherapeutic and immunological approaches to treatment will be explored further. The optimum approach to the treatment of cases of relapse/reinfection and, in particular, a means of overcoming the continued susceptibility to **C. difficile** infection need to be determined. We also require data on the effectiveness of disinfectants/sporicides for removing **C. difficile** from the environment.
C. difficile infections of the gut

Consensus on the optimal specimen selection criteria and diagnostic methods for C. difficile detection is required. Only by such an approach can we be confident that one centre’s experience is applicable to that of another. One intriguing question is whether or not prompt laboratory diagnosis and immediate treatment with antibiotics exacerbates the situation in some patients. It is known that, in many cases, the condition resolves if the inciting antibiotics can be withdrawn. Immediately after treatment, the patient may be more susceptible to colonization and expression of virulence by C. difficile than before the original infection. A relapse at this point may lead to more severe disease.

Conclusions

There is still a great deal to be learnt about C. difficile. Progress will inevitably be slow, particularly in view of the current lack of molecular tools that can be applied to the analysis of this pathogen. The major question that has been answered in the pathogenesis of the disease is the nature of the molecular mechanism of action that leads to disruption of the cell cytoskeleton. It is, however, somewhat salutary to realize that most of the questions first posed 14 years ago, and again 10 years ago, remain unanswered.

References