EDITORIAL REVIEW

Virulence factors of urinary pathogens

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Introduction

Despite the widespread use of antibiotics urinary tract infections (UTI) remain among the most common bacterial infections in the human population. About 5% of adult females are likely to have a UTI at any moment in time, while more than 50% of all women will experience a UTI at some stage in their lives [1]. Because individual women can alternate between symptomatic and asymptomatic episodes of infection it seems likely that the bacteria associated with overt infections are more pathogenic than their covert counterparts.

The observation that most cases of UTI are caused by strains of *Escherichia coli* with a restricted range of O-serotypes [2-4], and that the pattern of these O-serotypes does not correspond to the distribution for *Esch. coli* strains in the normal faecal flora [5], supports the idea that urinary pathogens possess definite determinants of virulence. Furthermore, carriage of genes coding for haemolysin synthesis [3, 6], resistance to the cidal effect of serum [7] and the ability to cause mannose-resistant haemagglutination (MRHA) of human erythrocytes [3, 6] are all associated with the O-serotypes which predominate in urinary *Esch. coli* isolates. Such markers of virulence appear to be particularly common among bacterial strains isolated from patients with infections of the upper urinary tract [2], and in particular, the MRHA adhesions designated P fimbriae have been shown to occur with a frequency of between 81% [8] and 94% [9] in subcultured pyelonephritic strains of *Esch. coli*.

A great deal of attention has been paid in the last decade to the identification and characterization of potential bacterial virulence determinants. The ultimate goal of defining the nature of the pathogenic mechanisms which operate in the urinary tract must be to enable a suitable prophylactic measure, such as a specific vaccine, to be developed for use in high-risk groups. There are, however, a number of pitfalls which hinder progress. Firstly, there is the problem that bacteria adapt very rapidly to growth in different environments so that characteristics expressed on subculture are not necessarily expressed *in vivo*, and vice versa. Secondly, many traits tend to be linked genetically and it is frequently very difficult to distinguish between properties which make a direct contribution to virulence and those which are simply co-expressed with true virulence determinants. Finally, there is the semantic question as to what is actually meant by the term 'virulence'.

In this Editorial we examine each of these problems and we review current knowledge on the pathogenesis of both cystitis and the renal scarring which characterizes chronic pyelonephritis.

Concept of bacterial virulence

The establishment and progression of a bacterial infection can involve a number of different stages. These include: (a) bacterial adherence to, and colonization of, mucosal surfaces; (b) growth in tissues; (c) erosion of host defences; (d) production of tissue damage. Different bacteria possess various capabilities at each of these stages. It is clear, for example, that bacterial growth *per se* does not necessarily cause tissue damage and may not even elicit symptoms. Asymptomatic bacteriuria (ABU) is well known, and covert infections in the kidney have been labelled acquiescent renal infections [10].

Bacterial attributes cannot be considered in isolation but must always be related to known host factors. Opportunistic pathogens, which take advantage of compromised host defences, and virulent pathogens, which produce infection in the
normal host, must represent two extremes of a spectrum rather than two separate entities.

This certainly appears to be the case in UTI since a spectrum in the distribution of known virulence markers is evident among urinary isolates of *Esch. coli*, the frequency decreasing in the order pyelonephritis strains > cystitis strains > ABU strains [2, 9, 11]. However, the situation is more complex than appears at first sight. It is known that renal scarring occurs most commonly in patients with a history of both UTI and vesico-ureteric reflux [12, 13], but a recent study has revealed that strains of *Esch. coli* isolated from children with acute pyelonephritis in the absence of reflux possess a significantly higher frequency of virulence markers than strains from pyelonephritic children with reflux [14]. Hence reflux must enable poor invaders to reach and colonize the renal parenchyma, and yet these bacteria are more capable of provoking tissue damage than those which would be regarded as being more virulent by standard criteria. It is apparent, therefore, that the virulence determinants associated with the production of tissue injury must be quite different from those associated with tissue invasion, bacterial growth and bacterial survival. This is an important point to consider when attempting to evaluate the role of any candidate virulence factor in the initiation of any type of infection.

**Potential virulence factors**

**Bacterial adhesion**

The adhesion of bacteria to mucosal surfaces is thought to be an important first stage in the establishment of infection at mucosal surfaces. Accordingly, a great deal of attention has been paid to defining the adhesion mechanisms which operate in the urinary tract, but conflicting viewpoints have emerged and the problem is far from being resolved. The most popular hypothesis has been that bacterial fimbriae with MRHA activity bind to carbohydrate receptors on epithelial cell surfaces. Indeed, bacteria with MRHA adhesions (such as P fimbriae) have been shown to bind to isolated human uroepithelial cells but not to uromucoid [15, 16], and this adhesion may be blocked by synthetic receptor analogues [17-19].

However, the phenotypic expression of fimbriae is well known to vary enormously under different conditions of bacterial growth [20], and we [21-23] and others [24] have found that the great majority of urinary pathogens do not possess fimbriae when they are growing exponentially in urine in vivo. These observations suggest that fimbriae are unlikely to be important mediators of adhesion in the bladder, but do not preclude a pathogenic role for fimbriae once bacteria have gained access to the renal parenchyma, whose growth conditions are likely to be quite different from those prevailing in the bladder urine.

An alternative hypothesis is that bacterial adhesion to the bladder mucosa is mediated by the polysaccharide or glycoprotein polymers that constitute the bacterial glycocalyx. This term covers acidic polysaccharide capsules and slime layers as well as more diffuse polysaccharide structures which may be expressed in vivo but which may be lost on subculture [24-26]. Glycocalyces may provide a protected environment for the growth of microcolonies and may also anchor microcolonies to the bladder wall. Glycocalyx-mediated adhesion to uroepithelial cells has been demonstrated both in vitro [27] and in vivo [27, 28], although the precise nature of the interaction between the bacterial glycocalyx and the bladder mucin layer (both of which are hydrophilic) is not clear. It may be that the two polysaccharide surfaces are brought together by physicochemical forces such as van der Waals' forces, and that they resist forces of separation if they are both wetted by the intervening water layer [29].

**Iron-sequestering mechanisms**

All micro-organisms require iron as a nutrient for growth, and the availability of iron is the usual rate-limiting factor for microbial growth in vivo [30, 31]. Because of this most pathogens have evolved elaborate mechanisms for iron-sequestration. Strains of *Esch. coli* can produce two iron-binding siderophores, enterochelin and aerobactin, the latter being coded for by Col V plasmids. Both have a higher affinity for iron than the human proteins transferrin and lactoferrin and consequently they are capable of sequestering protein-bound iron [32, 33]. Their synthesis is associated with the production of a number of novel outer-membrane proteins which appear to function as receptors for siderophore-iron complexes or for auxiliary iron-uptake systems [34, 35].

The enterochelin iron-uptake system is particularly efficient but is energetically expensive because the ferric-enterochelin complexes are hydrolysed by an esterase on the bacterial surface so that the siderophore is not re-usable [36]. In contrast, aerobactin is re-usable, and although it appears to be kinetically inferior to enterochelin for sequestering iron from transferrin there is evidence that it may be an important component in the virulence of invasive strains of *Esch. coli* [37].
An alternative method for pathogens to obtain iron is to haemolyse erythrocytes, by means of a haemolysin, to release haem [36, 38]. Avirulent, non-haemolytic strains of Esch. coli may be rendered nephropathogenic in experimental models by co-administration with low molecular weight iron complexes [39], haemoglobin [40] or purified haemolysin [40], indicating a potentially important role for haemolysin in the establishment of pyelonephritis. This is supported by the observation that the genotype for haemolysin synthesis occurs more commonly among pyelonephritic isolates [2]. Furthermore, the close association between the genetic determinants for haemolysin synthesis and those coding for MRHA activity [3, 6, 41] may explain the high prevalence of the latter property among strains of Esch. coli isolated from patients with symptomatic UTI [42, 43], particularly acute pyelonephritis [8, 9]. Hence, MRHA activity might simply be a marker of virulence, whereas haemolysin synthesis is a prime candidate as a true virulence factor. Such a distinction between virulence markers and virulence factors is not always obvious, but is in an important one to be made in all studies relating to bacterial pathogenicity.

Resistance to host defences

When pathogens gain access to host tissue they are faced with two major lethal mechanisms: direct killing by serum, mediated by the membrane attack complex or complement [44], and intracellular killing after ingestion by phagocytic cells. Because complement components are important for phagocyte chemotaxis and for opsonization of bacteria before phagocytosis, as well as for direct anti-bacterial activity, it is evident that the capacity to prevent complement activation is a particularly important attribute for successful pathogens. Lipopolysaccharide (LPS) of Gram-negative bacteria can readily activate the alternative pathway by providing a surface which favours the formation of the C3 convertase [45], while the lipid A portion of LPS can activate the classical pathway [46]. These properties are lost if the lipid A and core polysaccharide moieties of the LPS are masked by O-specific (smooth) polysaccharides or by capsular polysaccharides [47]. Inhibition of the alternative pathway appears to be largely dependent on the presence of sialic acids [48] among the bacterial surface polysaccharides, these greatly increasing the binding affinity of the inhibitor B1H for C3b [50], thereby preventing formation of the C3 convertase.

Some capsular polysaccharides have another protective effect for pathogens in that they can also inhibit opsonization with specific antibody. The terminal sialic acid residues of K1 and K5 capsules, for instance, are poorly immunogenic because they are chemically identical to host gangliosides or glycoproteins [49, 50]. Furthermore, many O and K antigens will prevent direct association between bacteria and phagocytic leucocytes owing to their hydrophilic nature [51]. It is clear, therefore, that surface polysaccharides can have an enormous influence on the interaction between bacteria and host cells and hence also on the outcome of infection. As with other candidate virulence factors the frequency (and amount) of K antigens has been shown to be significantly higher among strains of Esch. coli isolated from patients with pyelonephritis than among isolates from infections of the lower urinary tract or from normal faeces [52].

In the early 1970s an association was reported between K antigen content and resistance to complement-mediated killing [53, 54]. Some doubt has since been expressed as to whether or not K antigens are directly responsible for such resistance [44, 45], but molecular cloning of the genes coding for K1 indicates that this antigen does have a protective effect [56, 57]. However, the outer-membrane proteins coded for by the increased survival in serum (iss) gene, located on Col V plasmids [58], and the tra T gene, located on certain antibiotic resistance plasmids [59-61], may well be more important in conferring serum resistance to Esch. coli. The precise mechanism of action of these proteins is unknown although they appear to interfere with the action of the complement membrane attack complex and not its formation [62]. The fact that Col V plasmids possess the potential to confer an advantage on invading micro-organisms by two distinct mechanisms [37, 58] is particularly interesting, but the importance of this (and other virulence determinants) in infections in man is still far from clear [36].

Pathogenesis of bacterial cystitis

Bacteria can readily gain access to the bladder after colonization of the periurethral area [63] and they can multiply rapidly in urine because this is a highly nutritious medium [1]. The bladder mucosal surface, unlike the serosal surface, is not protected by complement although other serum-derived proteins in urine may play an opsonic role and so facilitate phagocytosis by neutrophils released into the bladder lumen in the course of symptomatic cystitis [64]. Other defence
mechanisms in the bladder include secretory IgA and IgG [65] and the bladder surface mucin layer [66, 67], all of which may serve to prevent, or at least reduce, bacterial colonization. However, the most important antibacterial mechanism appears to be hydrokinetic, that is, wash-out after regular voiding [1]. It is because of this that the hypothesis arose that bacterial adhesion to uroepithelium must be an important virulence factor for urinary pathogens [68], and why the debate about the mechanism of such adhesion, referred to earlier, is so pertinent.

Scanning electron microscopy has revealed that bacteria grow on the bladder wall in microcolonies [69], and also that the most dense colonization occurs at sites where the bladder mucin layer has been disrupted [70]. Colonization may be followed by penetration of the epithelium [23, 71], induction of an inflammatory response [23] and desquamation of the surface epithelial cells. The latter process may represent a defence mechanism whereby large numbers of invading bacteria are removed from the bladder [71], but it may also be largely responsible for the symptomatology associated with cystitis by allowing toxic mediators in the urine to penetrate to pain receptors in the lamina propria [23]. The difference between bacteria which cause symptomatic infections and those which do not is unknown but it could be at any of a number of stages: adhesion to the bladder mucosa, penetration of the epithelium, desquamation of surface cells or chemotactic attraction of neutrophils. The answer to this question would be the key towards solving the problem of recurrent bacterial cystitis.

Pathogenesis of renal scarring

The scarring which characterizes chronic pyelonephritis is usually initiated in infancy after episodes of acute pyelonephritis [72]. However, bacterial growth per se does not result in scar formation [10] and nor is persistent infection necessary [73, 74]. As discussed previously, bacterial traits such as MRHA activity and haemolysin synthesis cannot be directly involved in scar formation since these characteristics are less common among bacteria isolated from patients with vesico-ureteric reflux (the high-risk group) than among those from patients without reflux [14]. Other virulence determinants, as yet to be identified, must therefore endow nephropathogens with the potential to produce scarring.

Perhaps the most relevant information to have emerged within the last decade is that an inflammatory response is an essential prerequisite for scarring [73–76]. Indeed, infiltration of leucocytes into infected kidneys is associated with destruction of tubular basement membrane and tubular epithelia [77], and the most severe renal damage occurs at sites where the inflammatory response is greatest [76]. These observations indicate that tissue damage and scar formation are likely to be mediated by cytotoxic agents released from inflammatory cells after stimulation by bacteria or bacterial products. This hypothesis is further supported by the observation that neutrophils appearing in exudates in rats with experimental acute pyelonephritis are cytotoxic for cultured renal epithelial cells [78].

The outcome of the interaction between pathogens and host cells must be largely dependent on the structures expressed on the bacterial surface in vivo. In the presence of serum opsonins bacteria are likely to be readily phagocytosed and killed by neutrophils, but if the bacteria evade recognition by complement and the immune system, as discussed above, then the inflammatory defence system could be overwhelmed. Bacteria expressing the common mannose-sensitive (MS; type I) fimbriae are well known to adhere strongly to the surface membranes of phagocytic cells [79–83], and also to stimulate respiratory burst activity [83–86] and degranulation [83]. Although the attachment mediated by MS fimbriae can result in phagocytosis [87, 88] this is not always the case [85, 89]. We have observed recently that the pattern of degranulation stimulated in human neutrophils by MS-fimbriate Esch. coli is unique in that proteins are released extracellularly from all three major types of granule [89]. Proteinases from the azurophil and specific granules are known to be capable of degrading connective tissue [90, 91], and the oxygen metabolites which are released concurrently may also cause damage to host cell membranes [92–95] and, in addition, may even potentiate the activity of the granule enzymes by inactivating anti-proteinases [96]. It is apparent, therefore, that expression of MS fimbriae, in conjunction with anti-phagocytic surface polysaccharides, could endow nephropathogens with the ability to provoke extracellular release of large quantities of cytotoxic mediators from inflammatory leucocytes. MS-fimbriate Esch. coli cause severe renal scarring in an experimental model [89], but it is not known at present whether or not MS fimbriae are expressed by pathogens in the kidney parenchyma during the course of ascending pyelonephritis in humans. Identification of bacterial surface structures in vivo is a difficult task but it could greatly increase our knowledge concerning the precise mechanisms involved in renal scarring.
Conclusion

It is apparent that the mechanisms of bacterial pathogenicity in any type of infection, including UTI, are multi-factorial, and that different virulence factors can contribute to pathogenicity at different stages during the course of infection. It is also evident that some genetic traits which do not directly contribute to bacterial virulence may be closely linked to traits which do, and it is not always easy to distinguish between such virulence markers and virulence factors. Yet another major difficulty is the enormous variation in phenotypic expression of bacterial surface structures which can occur in different environments for growth. Despite these problems there has been a tremendous increase within the last decade in our knowledge of the delicate balance that can exist between bacterial virulence determinants and host defence mechanisms. Relatively minor changes on either the bacterial side or the host side can alter this balance, and hence can determine the outcome of infection. It is important, therefore, to gain an even more complete understanding of pathogenic mechanisms if we are to swing the balance more firmly in favour of the host.

References


