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Mammary pathogenic *Escherichia coli*

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Pathogenic *Escherichia coli* can be classified into several pathotypes, and it is believed that each pathotype carries one or more specific gene repertoire (or virulence factors combination) that distinguishes them from non-pathogenic *E. coli* strains and from other pathotypes. In contrast to this notion, it was proposed that this is not the case for *E. coli* mastitis, a common disease in farm animals and that any given *E. coli* isolate can cause this disease, even strains that are considered non-pathogenic. In this review we will re-examine this latter concept and recent advances in the study *E. coli* mastitis.

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Introduction

The natural habitat of *Escherichia coli* is mammalian intestine and most *E. coli* strains are harmless. Some strains, however, acquired genes that convert them into pathogens. The genomic DNA sequence of about 10 *E. coli* strains is available now, and genome comparison indicates that while each isolate contains around 4000–5000 genes or even more, only ~3000 genes are shared by the different strains (our unpublished genomes comparisons). This indicates that *E. coli* comprises extremely diverse group of organisms. This diversity is also reflected by the traditional *E. coli* typing method, which is based on genetic diversity between the *fliC* gene encoding flagellin (H antigen), the genes involved in biosynthesis of the polysaccharide side chain of the LPS (O antigen), and in the synthesis of the capsular polysaccharide (K antigen). More than 180O, 60H, and 80K antigens have been proposed for *E. coli* [1]. It is reasonable to assume that both commensals and pathogenic strains harbor specific colonization or virulence genes that allow them to interact with the host and better colonize it. In the case of commensals, however, this interaction is not associated with the disease.

Pathogenic *E. coli*

The pathogenic strains of *E. coli* can be classified into different pathotypes, where each pathotype causes a distinct disease [2]. All the pathotypes can be divided into two large groups: the intestinal pathogenic *E. coli* and the extraintestinal pathogenic *E. coli*. The intestinal pathogenic *E. coli* not only colonize the human intestine they also cause diseases ranging from mild diarrhea to severe colitis and dysentery. The extraintestinal pathogenic *E. coli* reside in the intestine asymptomatically but can cause severe infection upon reaching extraintestinal niches such as the urinary tract or blood stream.

The intestinal pathogenic *E. coli*

The enteric pathogenic *E. coli* consists of several pathotypes, including enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), and diffusely adherent *E. coli* (DAEC) [2]. In addition, the involvement of adherent invasive *E. coli* (AIEC) strains in Crohn's disease was also implicated [3]. Each of these pathotypes consists of a collection of closely related organisms that carry similar virulence genes. For instance, all the ETEC isolates produce and secrete similar heat-labile and/or heat-stable toxins and express similar pili that function as a colonization factor. Similarly, all the EPEC isolates contain a chromosomal region, the locus of enterocyte effacement, encoding a type III secretion system, which is essential for virulence [2].

Extraintestinal pathogenic *E. coli*

The most common extraintestinal pathotype is uropathogenic *E. coli* (UPEC), which causes urinary tract infections. Other important pathotype of this group is the meningitis/sepsis associated *E. coli* (MNEC). Interestingly, genomic comparison, as well as functional studies, indicates that different UPEC isolates can carry different combination of virulence factors without having an essential virulence gene that is common to all of them [4]. Thus, it is believed that several specific alternative combinations of virulence genes can lead to similar outcome: urinary tract infection and inflammation.

Serotypes and epidemiological studies

Epidemiological studies indicate that most isolates of each of the above pathotypes consist of only limited number of typical serotypes [1]. This, together with other evidences indicates a high degree of clonality within each

pathotype [2]. It cannot be excluded, however, that the specific structure of some of these antigens (O, H and K) provides some advantage to the pathogen.

E. coli mastitis

Mastitis, an inflammatory response of the mammary tissue to invading bacteria, is a common disease in breast-feeding women and dairy animals. In dairy animals mastitis is a worldwide problem, leading to multibillion-dollar economic losses [5], and *E. coli* is a leading cause of acute mastitis in dairy animals [6,7]. However, epidemiological studies could not demonstrate that specific *E. coli* serotypes are involved in mastitis, nor that these *E. coli* strains share a common set of virulence factors [8–13]. These observations lead to the paradigm that in this case virulence is based not on specific virulence factors but on bacterial associated molecular patterns (BAMPS) that are detected by the innate immune system and shared by all *E. coli* bacteria such as LPS, flagellin, CpG-DNA.

A putative new pathotype: mammary pathogenic *E. coli* (MPEC)

In contrast to the notion that bacterial virulence factors are not involved in *E. coli* mastitis, large variations are observed among field cases of *E. coli* mastitis in dairy animals, and the disease can vary from a mild, self-curing to a fatal septic condition. Some field strains (e.g. P4) are highly virulent, and upon experimental infection consistently cause severe septic mastitis in cow [14], while others consistently cause mild mastitis and/or latency in the chronically infected dairy animals [15–18]. These differences are traditionally attributed to the host immune response and genetic makeup, the 'cow factors' rather than variations in the bacterial virulence factors [19]. However, given the similarity between the highly inbred dairy cow and the extreme diversity among *E. coli* isolates, an alternative explanation is that, like in the case of other extraintestinal pathogenic *E. coli*, different strains carry different set of virulence genes, resulting in the differences in the caused disease. Therefore, *E. coli* that causes mastitis might form a new putative pathotype, mammary pathogenic *E. coli* (MPEC). It can be alternatively grouped together with the other extraintestinal pathogenic *E. coli* (ExPEC), given that the traditional distinction between uropathogenic *E. coli*, newborn meningitis-causing *E. coli*, sepsis-causing *E. coli*, and avian pathogenic *E. coli* has nowadays exceedingly been replaced by the more general ExPEC pathotype. Accordingly, MPECs cause infection upon reaching niches found within the mammary gland (including the stratified epithelia lining the luminal walls of the teat canal and cistern, gland cistern, milk tubules, and the specialized alveolar epithelium) taking advantage of fitness traits they already have and that may also contribute to efficient colonization of their primary habitat, the gut. MPEC might make use of the same pathogenesis scheme used by other extraintestinal pathogenic *E. coli* that includes invasion, immune evasion, replication, and damage characterized by necrosis and apoptosis [20] of mammary alveolar epithelial cells. There are, however, many publications on the absence of known virulence-associated genes of ExPEC and intestinal pathogenic *E. coli* among *E. coli* mastitis isolates. This indicates that there may be different ways to cause mastitis.

The mouse model

Some of the symptoms seen in cow *E coli* mastitis could be mirrored using a mouse model (Figure 1a and b) [21]. However, some MPEC show host specificity. For example, the P4 strain is highly virulent in cow, but it causes mild, self-curing disease in certain mouse strains and latent infection in others [22•], suggesting host specificity of some MPEC strains. Nevertheless, as with other pathogen, the mouse model is a powerful tool in dissecting the molecular and genetic basis for the infection process.

Natural history of MPEC infection

Upon contamination of the teat skin with MPEC, they are required to invade the teat canal and milk ducts system, to adhere to lining epithelium not to be flushed by the flowing milk, to replicate in the milk, and overcome non-specific humoral innate antimicrobials encountered in milk and on the epithelial surfaces [23]. The required initial inoculum is very small and as little as 50 cfu can elicit an innate immune response and disease [24], which correlates in severity with intramammary bacterial growth rate [25,26]. Both alveolar macrophages and epithelial cells were shown *in vitro* to respond to microbes by producing inflammatory mediators and modulators [23,27] (Figure 2). However, activation of mammary alveolar macrophages is sufficient for recruitment of additional macrophages and to elicit neutrophil recruitment into the alveolar space, which is IL1β dependent. The efficacy and speed of neutrophil recruitment is one of the main predictors of the outcome of mammary infection. A swift response results in rapid clearance of infection and relatively mild clinical signs [28,29]. Interestingly, although present in the mammary alveolar, dendritic cells remained inert and non-responsive to LPS or MPEC infection [22•].

Bacterial phenotypes/factors that are, or might be, associated with mammary infection

Biofilm formation

Using both cow infection and the mouse model, large microbial biofilms as well as planktonic organisms can be seen adherent to mammary alveolar epithelial cells after challenge with MPEC strain P4 (unpublished results and Figure 1c). However, a large variation in biofilm *in vitro* formation was observed among field strains of bovine mastitis, and its significance in the pathogenesis is currently unknown (Shpigel NY, unpublished results).