



EDITORIAL

## Maternal immunity, a way to confer protection against enteropathogenic *Escherichia coli*<sup>☆,☆☆</sup>



### Imunidade materna, uma forma de conferir proteção contra *Escherichia coli* enteropatogênica

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Diarrheal diseases caused by *Escherichia coli* remain as important causes of morbidity and mortality worldwide; children in the developing world suffer the major burden of diarrheal illnesses, while the burden among children and adults in the developed world is more evenly distributed.<sup>1</sup> At least six different *E. coli* pathovars have been associated with diarrheal disease, and each one uses a distinct collection of virulence factors to subvert host cellular functions and potentiate their virulence, resulting in distinct clinical manifestations.<sup>2,3</sup> One of the oldest and better characterized diarrheal pathovars is enteropathogenic *E. coli* (EPEC), which encompass a group of isolates that are a significant cause of persistent watery diarrhea among children worldwide.<sup>4</sup> EPEC isolates belong to a limited number of O:H serotypes, and are known to carry the locus of enterocyte effacement (LEE), a region that encodes several proteins mediating the interaction with the host cells and the formation of a distinct lesion known as attaching and

effacing (A/E).<sup>2</sup> Moreover, EPEC strains may contain a virulence plasmid called EPEC adherence factor (EAF), because it encodes gene products involved in the synthesis and assembly of the bundle-forming type IV pilus (*bfp*), and also lacks the genes encoding for Shiga toxin (*stx*) production.<sup>2</sup> The pEAF plasmid is used to sub-classify these strains into typical EPEC (tEPEC; those that are LEE+/stx–/bfp+) and atypical EPEC (aEPEC; those which are LEE+/stx–/bfp–).<sup>5,6</sup> In contrast, the related pathovar enterohemorrhagic *E. coli* (EHEC) possess the genotype LEE+/stx+/bfp–, and production of *stx* is associated with further systemic complications.<sup>2</sup>

To this day, EPEC continues to be responsible for 5%–10% of pediatric diarrhea in developing countries; however, it is reported that the frequency of EPEC (particularly tEPEC) infections drops with age, and therefore adults rarely experience tEPEC episodes.<sup>5,7</sup> Interestingly, infant acute diarrheal episodes caused by tEPEC have been declining throughout the years in many countries, including Latin America and Europe.<sup>4,8</sup> Moreover, a large study conducted in several countries in Africa and Asia by the Global Enteric Multicenter Study (GEMS) network demonstrated that tEPEC strains were not among the leading pathogens causing acute moderate and severe diarrhea in these geographical regions.<sup>9</sup> However, tEPEC infection appears to be associated with a 2.8-fold increased risk of death among infants aged 0–11 months.<sup>9</sup> In contrast, aEPEC continues to be isolated

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in both developing and developed countries; in some countries, they have surpassed tEPEC infections.<sup>4,10</sup> In some studies from developing countries, it was demonstrated that aEPEC isolates were responsible for 78% of all EPEC cases in children aged less than 5 years.<sup>7</sup> Regardless of whether tEPEC or aEPEC are responsible for the diarrheal episodes or the geographic distribution of the isolates, it is clearly evident that EPEC isolates are still among the most important pathogens causing diarrhea.<sup>5,6,10</sup> Thus, there is a need for a therapeutic intervention to reduce the number of cases worldwide.

Ideally, the most effective way to protect infants from EPEC infection is by antibodies acquisition through active or passive immunization. Several studies have strongly suggested that breastfeeding might be able to protect infants against EPEC due to the presence of antibodies directed to specific EPEC virulence factors.<sup>11–15</sup> There have been several reports of antibodies against various secreted or surface-located EPEC virulence factors, including those encoded on the LEE pathogenicity island or the virulence pEAF plasmid, which can bind these factors and potentially block bacterial colonization.<sup>2,5,11–15</sup> From those immunogenic proteins, a key virulence factor is intimin, the main EPEC and EHEC membrane-located adhesin, which is encoded by the *eae* gene within the LEE pathogenicity island.<sup>2</sup> Several different types of intimins have been reported based on the polymorphisms within their C-terminal region (known as Int280) and the most frequently found are the  $\alpha$ ,  $\beta$ , and  $\gamma$  types.<sup>2,15</sup> The distribution of these intimin proteins is associated with different EPEC and EHEC evolutionary lineages. Intimin  $\alpha$  is found within the EPEC lineage known as EPEC-1; intimin  $\beta$  is distributed among human and animal pathogens, including EPEC-2, EHEC-2, and *Citrobacter rodentium*; whereas intimin  $\gamma$  is associated with EHEC O157:H7 and aEPEC strains.<sup>8</sup> It has been previously shown that human immunoglobulins (e.g., secretory IgA) from breast milk can inhibit EPEC localized adherence to cultured cells *in vitro* and that these immunoglobulins are also able to recognize different intimin proteins.<sup>11–15</sup>

Epidemiological studies in Brazil<sup>16</sup> have elucidated a quite unique map for the distribution of EPEC pathovars, confirming the prevalence of these pathogenic *E. coli* isolates and reinforcing the fact that aEPEC strains have replaced tEPEC as the most prevalent EPEC subclass associated with human disease in this country.<sup>17</sup> Interestingly, early acquisition of antibodies against intimin by healthy Brazilian children living in endemic areas has been demonstrated,<sup>14</sup> suggesting that those antibodies might be responsible for protection against the pathogen. Moreover, it has been found that serum IgG and secretory IgA antibodies are reactive against conserved and variable regions of the intimins  $\alpha$ ,  $\beta$ , and  $\gamma$ ; the cross-reactivity among anti-intimin antibodies has also been demonstrated.<sup>15</sup> These studies suggest a correlation between the concentration of the antibodies in both serum and colostrum and the probable protection elicited in children receiving these antibodies *via* breastfeeding. Furthermore, the cross-reactivity of the antibodies with different intimins suggest that it is possible to protect against different A/E-producing *E. coli* (e.g., EPEC or EHEC).<sup>15</sup>

In a recent study published in the *Jornal de Pediatria*, Altman et al. investigated the transfer of maternal anti-intimin antibodies from healthy Brazilian mothers to their newborns through the placenta and colostrum, and found a variability in the concentrations of these antibodies.<sup>18</sup> The antibodies displayed differential reactivity to different types of intimins as well as to the different (conserved and variable) regions of the intimins  $\alpha$ ,  $\beta$ , and  $\gamma$ . Interestingly, the investigators found that IgG serum antibodies, reactive to all the intimin subtypes, were transferred to the newborns, and that the antibody concentrations varied depending of the structural region of the intimin protein tested (conserved vs. variable).<sup>18</sup> This study has significant implications for the development of a potential treatment because in addition of demonstrating that anti-intimin antibodies can be transferred from mothers to newborns through the placenta, reinforcing the importance of passive immune protection against pathogenic *E. coli*, it opens the door for the development of other immunization approaches to effectively combat these pathogens.

Because EPEC and other A/E-producing *E. coli* require multiple virulence factors to cause disease, in addition to the intimin protein, it might be feasible to propose the development of a vaccine that targets multiple EPEC/EHEC antigenic epitopes and which is administered to expecting mothers. If maternal antibodies are generated against different *E. coli* antigens and can be transferred to newborns, then this type of vaccine should be the next priority to combat pediatric diarrheal disease.<sup>10</sup> In the past, EPEC has also been the target for development of cattle immune milk.<sup>19</sup> Initial efforts using milk immunoglobulin concentrate containing antibodies to different EPEC strains, prepared by hyper-immunization of pregnant cows, and used to treat infants with gastroenteritis, resulted in over 80% of children testing negative in their stools for different EPEC isolates.<sup>19</sup> This approach, as well as the study by Altman et al.,<sup>18</sup> provide evidence that treatment with specific antibodies found in milk should be further explored as an effective way to eliminate or prevent EPEC colonization of the intestine.

## Conflicts of interest

The author declares no conflicts of interest.

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