Primo-colonizing genotoxic *Escherichia coli* : a threat to intestinal stem cells ?

Emilie Cloup[a,b], Delphine Payros[a,c,d,e,f], Gabriel Cuevas-Ramos[a,b,c,d,e], Frédéric Taieb[a,c,d,e], Thomas Secher[a,c,d,e], Patricia Martin[a,c,d,e], Isabelle Raymond-Letron[b], Claude Petit[a,b,c,d,e], Maiwenn Olier[a,c,d,e,f], Eric Oswald, [a,c,d,e,g], Jean-Philippe Nougayrède[a,c,d,e]

[a] Inserm, UMR1043, Toulouse, France.
[b] Ecole Nationale Vétérinaire de Toulouse, Toulouse France.
[c] INRA, USC 1360, Toulouse, France.
[d] CNRS, UMR5282, Toulouse, France.
[e] Université de Toulouse, UPS, Toulouse, France.
[f] Neuro-gastroentérologie & Nutrition, UMR Toxalim INRA/ENVT 1331, Toulouse, France.
[g] CHU Toulouse, Hôpital Purpan, Service de bactériologie-Hygiène, Toulouse, France.

Abstract : *Escherichia coli* (*E. coli*) is one of the first commensal bacterium that colonizes the intestinal tract of newborns and persists in adults as a long-term colonizer. Among this species, some strains of *E. coli* produce a genotoxin named colibactin. This bacterial toxin induces DNA double-strand breaks, chromosomal instability and genetic mutations in mammalian cells. Therefore, we have examined the consequences of the neonatal colonization by these genotoxic *E. coli*. Following oral inoculation, we observed *E. coli* bacteria mainly in their niche, the colic mucus gel layer, and some bacteria interacting with upper epithelial cells. Although direct interaction is required for genotoxic effect in vitro, we found γH2AX$^+$ cells in the basal crypt region, especially at day 8. Although DNA damage cannot be seen any more at adulthood, abnormal mitosis figures persisted and the renewal of the epithelium was enhanced. Thus, the perinatal period is a critical period when the intestinal epithelium is directly exposed to genotoxic primo-colonizer bacteria that could leave a persistent footprint in intestinal stem cells.

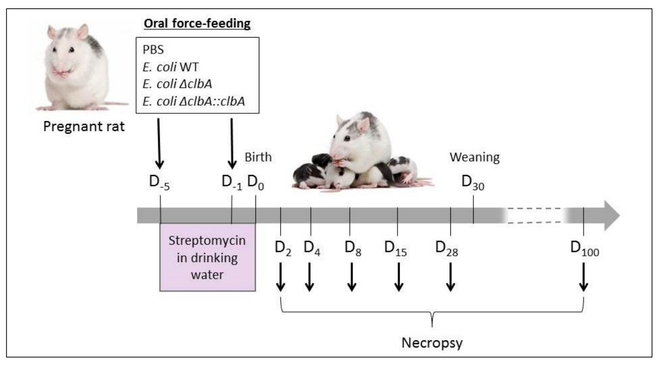
Colibactin-producing *Escherichia coli* strains can damage DNA in intestinal epithelial cells of unweaned newborn mice

At birth, commensal *E. coli* colonize rapidly the neonatal gut. Among this pleiomorphic species, we observe in industrialized countries a newly dominant phylogenetic group, and especially in infants, the B2 group. These B2 *E. coli* frequently produce a genotoxin named **colibactin**. This toxin can trigger **DNA double-stranded breaks** and chromosomal instability in mammalian cells.

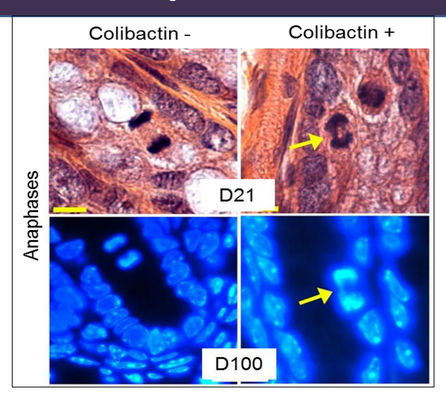
Here, we demonstrated an **increased reporter *cII* gene mutant frequency** in infected Big Blue fibroblasts.

Control		Gamma-irradiation		Bleomycin		*pks+ E. coli*	
Mean MF	SD	Mean MF	SD	Mean MF	SD	Mean MF	SD
3,6	1,7	8,2*	1,5	8,8*	1,4	10,2**	2,0

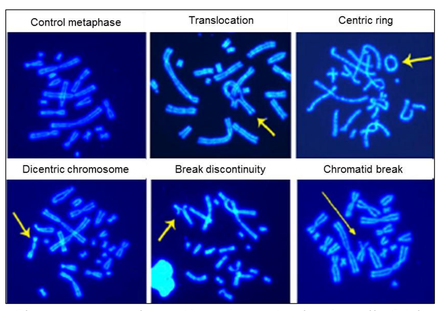
Reporter *cII* gene-mutant frequencies (MF x 10^{-5} mutations per *cII* locus) were determined in Big Blue fibroblasts infected with genotoxic *E. coli*, using the λ Select-*cII* Mutation Detection System for Big Blue Rodents. Mean frequencies were obtained after 3 independent experiments and were analyzed by a one-way ANOVA followed with a Dunnett's post-test (*p < 0,05, ** p < 0,01 vs. control).



A rat model mimicking the natural transmission of *E. coli* from mothers to newborns.



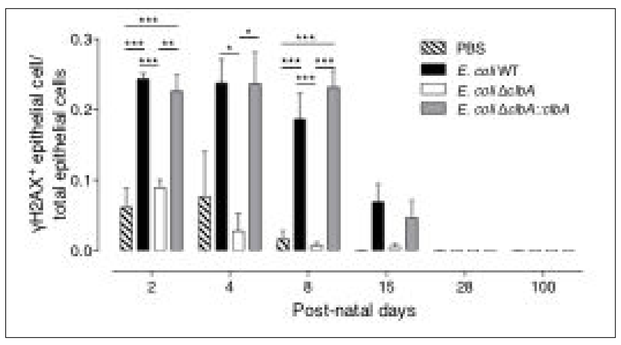
Colibactin exposition can induce anaphase bridges in intestinal epithelial cells



Chromosome aberrations in metaphasic cells 24 h after infection with *pks+ E. coli.* The chromosomes were labeled with DAPI after metaphase spreading.

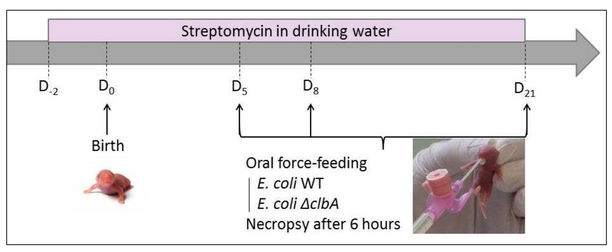
Quantification of γH2AX-positive epithelial cells. Groups of 5-10 rats were analyzed. Mean ± SEM are shown. * P≤0.05, ** P≤0.01 and *** P≤0.001

Besides, in a rat model mimicking the natural transmission of *E. coli* from mothers to newborns, we showed that these genotoxic bacteria alters the intestinal epithelium both during the neonatal period, by activating the DNA-damage response (γH2AX), and into adulthood, with signs of genotoxic damage including **anaphase bridges**, crypt fission, and **increased intestinal proliferation** and cellular renewal.

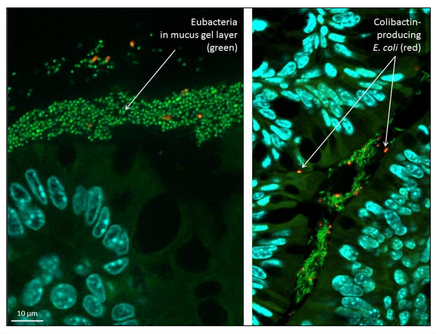


Neonatal colonization with *pks+ E. coli* damage basal crypt region

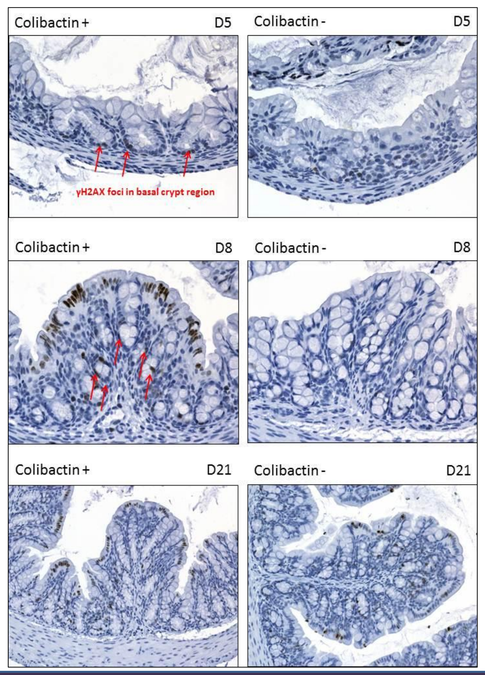
We examined the consequences of the neonatal colonization by these genotoxic *E. coli*. A colibactin-producing *E. coli* strain, or its non-toxic isogenic mutant, was administrated by oral route to newborn mice. Gut tissues were collected 6 hours after exposure to localize bacteria by FISH and DNA-damaged γH2AX$^+$ intestinal cells by IHC. Some *E. coli* can quit their niche, the colic mucus gel layer, and then **interact with epithelial cells** from both the surface epithelium and the upper crypt region.



Neonatal colonization in a mouse model by forced-feeding at 5, 8 and 21 days after birth.



Paraffin colon sections were stained for Eubacteria with the universal FISH probe EUB338 labelled with FITC (green) and for *E. coli* with specific EC1531probe conjugated with Cy3 (red). (Scale bars, 10 µm.).



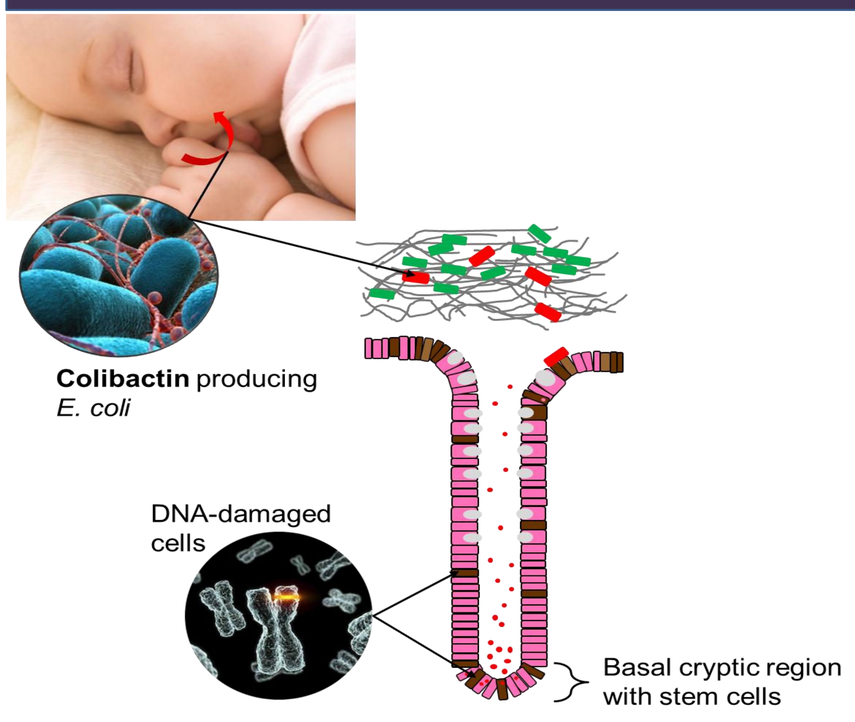
Although direct interaction is required for genotoxic effect *in vitro*, we can find γH2AX$^+$ cells in the **basal crypt region**, especially before weaning.

Percentage of γH2AX$^+$ cells in surface epithelium, glandular epithelium and lamina propria. For each group, 8 microscopic fields were observed (x 400) at least in order to count more than 200 cells.

	Surface epithelium		Crypt epithelium		Lamina propria	
% γH2AX positives cells	WT	ΔclbA	WT	ΔclbA	WT	ΔclbA
Day 5	0,4	0,9	13,7	3,9	2,7	1,9
Day 8	43,1	0,0	24,5	9,8	16,9	1,0
Day 21	39,2	0,8	0,9	0,6	0,9	0,0

Paraffin colon sections were stained for γH2AX (brown, red arrows) and counterstained with hematoxylin.

Conclusions and perspectives



The perinatal period is a critical time window during which the intestinal epithelium is directly exposed to genotoxic primo-colonizer bacteria. The primo-colonization by colibactin-producing strains might result in a persistent footprint in intestinal stem cells. Before weaning, we observed many γH2AX$^+$ cells in the basal crypt compartment. In consequence, both the genome and cellular homeostasis can be compromised.

- Use a physiological model mimicking the natural colonization of the gut of newborn mice from the maternal microbiota.
- Identify the cells harboring DNA-damage (by immunostaining (IHC, IHF, FISH)
- Characterize the spectrum of mutations induced by colibactin (mode of action?)
- Localize and quantify the activation of programmed cell death (apoptosis) with TUNNEL and Caspase stainings.
- Examine long-term effect on epithelium homeostasis : gut organoïds cultured from transient infected ISCs.