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Abstract

The possibility of inactivating genes in *Leptospira* allows to envisage a study of their functions. The participation of Toll-like receptors in innate immune responses generated by leptospires and its LPS is under study. In *Borrelia*, the *ospC* gene analysis, when compared to genus phylogeny, allows to differentiate three distinct bacterial populations: invasive, cutaneous and non infectious for vertebrates. In *Yersinia*, analysis of chromosomal determinants and their comparison among different pathogenic species opens a new field of investigation to understand the exceptional pathogenicity of *Y. pestis*.

Annual Report

LEPTOSPIRA

"Toll-like receptors" (TLRs) and leptospiral lipopolysaccharide (LPS). (C. Werts, E. Fournié-Amazouz, M-A. Nahori and I. Saint Girons)

Toll-like receptors (TLRs) are essential components of innate immunity that are able to sense conserved microbial patterns like LPS, lipoproteins, peptidoglycan, bacterial DNA. We showed that TLR2, instead of TLR4 used by LPS from Gram negative bacteria, was required for cell activation by leptospiral LPS (Werts *et al.*, 2001). The peculiar structure of leptospiral lipidA has been deciphered by the group of C.R.H. Raetz (Duke, Durham, USA) (Que *et al.*, in preparation). We are now studying the relationships between the structure of leptospiral lipidA and its biological functions. Preliminary results indicate that despite a lack of endotoxic properties, leptospiral lipidA is able to activate mammalian cells but differs from *Escherichia coli* lipidA in the TLR use. These results may help to understand some of the features of leptospirosis.

Furthermore, we have obtained a clonal derivative strain of *L. interrogans* which had lost its virulence for guinea pig that will be useful for studying virulent determinants.

Targeted inactivation of genes in *Leptospira* saprophytic species. (M. Picardeau, A-P. Tchamedeu-Kameni, H. Bauby, E. Couture-Tosi and I. Saint Girons)

Recently, the development of new genetic tools has facilitated the construction of targeted mutants in the saprophytic species. By using these techniques, we generated a *recA* mutant in *L. biflexa*. The *recA* mutant showed poor growth in liquid and solid media and was considerably more sensitive to DNA-damaging agents such as mitomycin C and UV light than the wild-type strain. In addition, the *recA* - cells showed aberrant nucleoid morphologies. Our data indicate that the *L. biflexa* RecA plays a major role in ensuring cell viability via mechanisms such as DNA repair and, indirectly, active chromosome partitioning. We also reported the characterization of the first auxotroph in *Leptospira* spp. (the *L. meyeri* *trpE* mutant which is auxotroph for tryptophan) and the identification of a second selectable marker, the spectinomycin-resistance cassette, for genetic manipulations of *Leptospira* spp.

CNR and WHO Collaborating Center

See: <http://www.pasteur.fr/sante/clre/cadre/cnr/lepto-index.html>

D. Postic and G. Baranton in collaboration with Institut de Veille Sanitaire (INVS).

A cluster of leptospirosis cases in Rochefort (Charente-Maritime) has been detected by PCR first and further evaluated by serology. The epidemiological study performed by INVS showed that it involved five teenagers who used to swim in canal not allowed to these leisure activities.

D. Postic in collaboration with D. Raoult and P. Brouqui.

A case of leptospirosis in Marseilles has been described. The originality was the unexpected isolation by Marseilles team of a *Leptospira inadai* strain from the CSF of the patient. The pathogenicity of *L. inadai* is controversial.

Consult also: <http://www.pasteur.fr/recherche/Leptospira/LeptospiraF.html>

BORRELIA

See: <http://www.pasteur.fr/recherche/borrelia/Welcome.html>

***Borrelia* and VNTRs** (J. Ferlow, D. Postic, K.L. Smith, Z. Jay, G. Baranton and P. Keim).

With the team of Paul Keim in the USA (Northern Arizona Faculty Flagstaff), 41 strains of Lyme disease associated *Borrelia burgdorferi* sensu lato have been studied by Variable Number of Tandem Repeats (VNTR) markers. It showed that, as expected, 33 of these strains were distributed in 3 clusters corresponding to the 3 pathogenic *Borrelia* species *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*. However, 8 isolates identified as *B. burgdorferi* sensu stricto exhibited a diversity superior to the interspecific one. A further study with a largest number of isolates is planned to determine whether or not these deeply branched isolates correspond to an ancestral population.

OspC* and pathogenicity of *Borrelia (V. Lagal, D. Postic and G. Baranton).

Studies on *ospC* gene coding for a *Borrelia* outer surface membrane major protein showed that *ospC* groups determined by SSCP PCR

corresponding to "invasive" phenotype were more numerous than previously expected. In addition, their prevalence seems to be geographically distributed in Europe. Indeed the diversity is associated with antibodies selective pressure leading to many serotypes. However their geographical distribution is poorly understood. In fact in the USA, in a given area, the whole diversity is present.

CNR *Borrelia* (D. Postic, E. Ferquel and C. Pérez-Eid). <http://www.pasteur.fr/sante/clre/cadrecnr/borelia-index.html>

Lyme disease, the most common vector born disease in Europe as in North America, is our main research activity. We focus our work on two goals: the vector and the transmitted bacteria and human incidence of the disease in France.

In Europe, *I. ricinus*, the vector of Lyme disease, transmits the three species responsible for human cases of the *B. burgdorferi* complex. Each of the three species of *Borrelia* seems to be associated with specific clinical manifestations depending on the tropism of the *Borrelia* species for a specific organ. It is therefore of interest to know the respective geographical distribution of each *Borrelia* species. Such a study needs to be done in the vector and not in humans since (1) the identification of the *Borrelia* species is easier in the vector; (2) few biological specimens are available in humans; (3) the organ which is most likely to allow the isolation of the *Borrelia* is not known in human and finally (4) it is very difficult to conclude on the geographical origin of a *Borrelia* isolated in humans. In 2002, we collected ticks on the field in Brittany, the most occidental area of France and Europe, and in the centre of France (Department of Allier). Collects are done using a method allowing statistic analysis of the results according a methodology previously adjusted by our team, to evaluate their density. The infectious status of ticks for *Borrelia* was evaluated by using PCR technique.

The incidence of Lyme disease in France is presently unknown, the results from older studies varying from 16.4 to 40 cases for 100 000 inhabitants. Our goal is to obtain more accurate data in order to be able to better know the incidence and to detect possible differences in the incidence and in the symptoms of the disease in different geographical areas. For this purpose, we are performing a retrospective study based on serological analysis requests from the Pasteur-Cerba laboratory (one of the two major medical analysis laboratories in France) and a prospective study in 2002, with a net of physicians (from private practice and hospital) working as generalist or in different specialties, and biologists.

YERSINIA (Head: Elisabeth Carniel)

The genus *Yersinia* is composed of 3 species pathogenic for humans: the enteropathogens, *Y. pseudotuberculosis* and *Y. enterocolitica*, and the plague agent, *Y. pestis*.

The main fields of activity of the *Yersinia* laboratory are:

- The characterization of a pathogenicity island whose presence confers to the host bacterium the ability to cause systemic infections in humans and to be lethal in mice;
- The molecular bases for the exceptional pathogenicity of *Y. pestis*. Sequencing of the genome of *Y. pseudotuberculosis*, a bacterium genetically almost identical to *Y. pestis* but of much lower pathogenicity, has been completed recently. A comparative genomics approach is now undertaken;
- The relations between *Y. pestis* and its insect vector, the flea;
- The physiopathology of *Yersinia* infection;
- The evolution of *Y. pestis* since its recent emergence from *Y. pseudotuberculosis*;
- The resistance of pathogenic *Yersinia* to antibiotics and the evaluation of new treatments;
- Public health (French Reference Centre and WHO Collaborating Centre for *Yersinia*).

The works published in 2002 dealt with:

Comparison of the effects of deferiprone versus deferoxamine on growth and virulence of *Yersinia enterocolitica*. (B. Lesic, J. Foulon, and E. Carniel).

Deferoxamine, a drug used to treat patients with iron overload, has the capacity to promote systemic *Y. enterocolitica* infections in humans. The aim of this study was to determine whether deferiprone, the only orally active alternative treatment, has the same potential. When *Y. enterocolitica* IP864 was grown in an iron-poor chemically defined medium, addition of deferoxamine promoted its growth, while various concentrations of deferiprone did not display this activity. Similarly, on iron-poor agar plates, various *Y. enterocolitica* strains were able to grow around paper disks impregnated with deferoxamine in a dose-dependent manner, while no growth was observed around the deferiprone disks. In a mouse experimental model of infection, the 50% lethal dose (LD50) of strain IP864 was decreased by more than 5 log units in mice pretreated with deferoxamine, while a deferiprone pretreatment did not affect it. Therefore, in contrast to deferoxamine, deferiprone does not enhance growth of pathogenic *Y. enterocolitica* *in vitro* and does not have the potential to promote *Y. enterocolitica* septicemia in a mouse model of infection. Deferiprone may thus represent a useful alternative iron-chelation therapy during invasive *Y. enterocolitica* infections.

Palindromic unit-independent transposition of IS1397 in *Yersinia pestis*. (E. Carniel -Collaborators: C. Wilde, S. Bachelier, M. Hofnung and J-M. Clément).

Palindromic units (PUs) are intergenic repeated sequences scattered over the chromosomes of *Escherichia coli* and several other enterobacteria. In the latter, IS1397, an *E. coli* insertion sequence specific to PUs, transposes into PUs with sequences close to the *E. coli* consensus. Reasons for this insertion specificity can relate to either a direct recognition of the target (by its sequence or its structure) by the transposase or an interaction between a specific host protein and the PU target DNA sequence. In this study, we showed that for *Yersinia pestis*, a species deprived of PUs, IS1397 can transpose onto its chromosome, with transpositional hot spots. Our results are in favour of a direct recognition of target DNA by IS1397 transposase.

High-frequency conjugative transfer of antibiotic resistance genes to *Yersinia pestis* in the flea midgut. (M.L. Rosso, E. Carniel - Collaborators: J. Hinnebusch and T.G. Schwan).

The acquisition of foreign DNA by horizontal transfer from unrelated organisms is a major source of variation leading to new strains of bacterial pathogens. The extent to which this occurs varies widely, due in part to lifestyle factors that determine exposure to potential donors. *Yersinia pestis*, the plague bacillus, infects normally sterile sites in its mammalian host, but forms dense aggregates in the non-sterile digestive tract of its flea vector to produce a transmissible infection. We showed in this study that unrelated co-infecting bacteria in the flea midgut are readily incorporated into these aggregates and that this close physical contact leads to high-frequency conjugative genetic exchange. Transfer of an antibiotic resistance plasmid from an *Escherichia coli* donor to *Y. pestis* occurred in the flea midgut at a frequency of 10³ after only 3 days of co-infection, and after 4 weeks 95% of co-infected fleas contained an average of 10³ antibiotic-resistant *Y. pestis* transconjugants. Thus, transit in its arthropod vector exposes *Y. pestis* to favourable conditions for efficient genetic exchange with microbial flora of the flea gut. Horizontal gene transfer in the flea may be the source of antibiotic-resistant *Y. pestis*

strains recently isolated from plague patients in Madagascar.

Analysis of the NKT cells-containing inflammatory lesions induced by *Yersinia pseudotuberculosis* glycolipids. (F. Guinet, E. Carniel - Collaborators: C. Ronet, M. Mempel, M. Huerre and G. Gachelin).

Valpha14-expressing NKT (invNKT) cells are a population of non-conventional T lymphocytes (TL) that bridge mammalian innate and adaptive immunity. Their role in infectious diseases and inflammatory processes is still largely understood. A previous report has shown that an acute granulomatous-like reaction can be elicited by sub-cutaneous injection of *Mycobacterium tuberculosis* glycolipids in mice, and that recruitment of invNKT cells at the injection site is instrumental in this process. The mouse response to enterobacterium *Yersinia pseudotuberculosis* glycolipids extracts during the first week post injection was investigated. The cellular reaction is an acute inflammatory infiltrate where TL are abundant from early times on. InvNKT cells are present in the lesions, detectable as early as day 1 post injection. They compose all of the Valpha14-expressing TL, although conventional T cells expressing non-Valpha14 chains can be detected. The reaction is strictly dependent on ester-linked fatty acids as mild alkaline treatment of the extract prior to injection results in the absence of analysable lesions. Thus, glycolipids from *Yersinia* induce inflammatory lesions comparable to those induced by mycobacteria glycolipids, in spite of the totally different cell wall composition in the two genera.

National Reference Laboratory and WHO Collaborating Centre for *Yersinia* (L. Martin, F. Guinet and E. Carniel)

See the *Yersinia* National Reference Centre web site:

(<http://www.pasteur.fr/sante/clre/cadreocr/yersinia-index.html>)

Comparative genomics of *Y. pestis* and *Y. pseudotuberculosis* . (V. Chenal, D. Dacheux, A. Derbise and E. Carniel - Collaborators: E. Garcia, P. Chain and C. Médigue.

Comparison of the genomes of *Y. pestis* and *Y. pseudotuberculosis* (a bacterium genetically closely related to *Y. pestis* but of much lower virulence) might be a highly useful tool for identifying genes responsible for the extraordinary pathogenicity of *Y. pestis* . Sequencing of the genome of *Y. pseudotuberculosis* has been recently completed at the Lawrence Livermore National Laboratory (US), in collaboration with our laboratory. Comparative genomics analyses are now in progress.

Keywords: Spirochetes, Leptospira, Borrelia, TLR, lipid A, genetics, allelic exchange, epidemiology, phylogeny, VNTR, OspC, Yersinia, genomics, virulence

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