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## Abstract

The focus of our research is on

I *The Infections of the nervous system* in particular the entry of neurotropic enteroviruses in the organism and poliovirus persistent infection in the mouse spinal cord. A new project concerns the mechanisms of propagation of prion infectivity from the periphery to the central nervous system.

II *Development, communication/signalling and regeneration of the nervous system*

We focus on the the development and regeneration of *myelin-forming cells* from neural stem cells, in particular on the molecular controls of migration and differentiation of oligodendrocytes as well as the role of *Récepteur tyrosine phosphatases* in the function of neural cells. Finally we investigate cell communication mediated by *connexins in gap junctions* in neural progenitors and neuronal networks as well as the molecular mechanisms underlying diseases caused by connexin mutations.

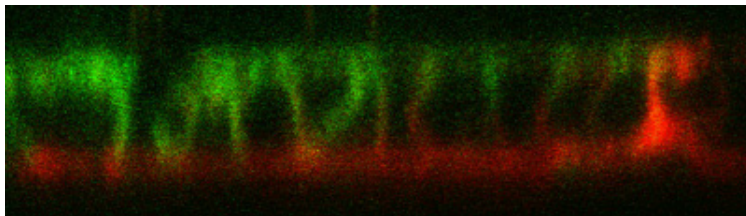
## Annual Report

### I. Infections of the nervous system

#### A Poliovirus infection

##### Poliovirus transcytosis through M-like cells (F. Colbère et al.)

During the digestive phase of infection, poliovirus (PV) is found in the oropharynx and the intestine. It has been proposed that PV enters the organism by crossing M cells that are scattered in the epithelial sheet covering lymphoid follicles of Peyer's patches. However, PV translocation through M cells has never been demonstrated. A model of M-like cells has been previously developed using monolayers of polarized Caco-2 enterocytes co-cultured with lymphocytes isolated from Peyer's patches (Kerneis et al. Science, 1997, 277, 949). In this model, lymphoepithelial interactions trigger the appearance of epithelial cells having morphological and functional characteristics of M cells.



We demonstrate efficient, temperature-dependent PV transcytosis in Caco-2 cell monolayers containing M-like cells. This experimental evidence is consistent with M cells serving as gateways allowing PV access to the basal face of enterocytes, the underlying immune follicle cells, and PV transport toward mesenteric lymph nodes.

##### Characterization of the Poliovirus 147S particle: New insights into poliovirus uncoating (F. Colbère et al.)

A Sabin 1 strain poliovirus (PV) mutant, S1(2Y-11), carrying a Tyr at amino acid position VP2142 and an Ile at position VP1160, can establish persistent infections in HEp-2c cells. This mutant forms atypical 147S particles upon interaction at 0°C with either cells expressing PV receptor (PVR) CD155, or PVR-IgG2a, a chimeric molecule consisting of an extracellular moiety of PVR and the hinge and Fc portion of a mouse IgG2a. Upon interaction with PVR at 37°C, S1(2Y-11), like the parental strain, forms both 135S A particles and 80S empty capsids. At 0°C, surprisingly, at a concentration equal to or greater than 5 nM, PVR-IgG2a induced both the extrusion of VP4 from the capsid of S1(2Y-11) and the formation of 80S particles. The same transitions were observed at 0°C with the parental strain Sabin 1 at 40 nM PVR-IgG2a. Thus, the formation of 80S particles and VP4 extrusion, considered as one of the steps of PV uncoating, can be temperature-independent at high PVR concentration. This implies that structural changes of the PV capsid occurred following adsorption at low temperature.

##### Poliovirus as a model for studying virus-nerve cell interactions : apoptosis and persistence (B. Blondel and T. Couderc).

Neurotropic viruses can persist in the central nervous system following the acute phase of infection and induce new pathologies several years after the initial infection. Poliovirus is currently one of the best-characterized neurotropic viruses. Patients having recovered from acute poliomyelitis developed after several decades of clinical stability a new disease, called post-polio syndrome, characterized notably by slowly progressive muscle weakness and atrophy. One hypothesis to explain this syndrome could be poliovirus persistence in the central nervous system, possibly associated with an immunopathological process.

We have previously developed a mouse model susceptible to poliovirus infection and we have shown that poliovirus can persist in the central nervous system after the onset of paralysis throughout the life of animals. We have also shown that the poliovirus persistence could be due, at least in part, to an inhibition of viral genome synthesis in the central nervous system. During the acute phase of poliomyelitis, we have demonstrated that poliovirus kills motoneurons by an apoptotic process.

We have recently developed a model of mixed mouse primary nerve cell cultures to study the molecular mechanisms of poliovirus-induced apoptosis in nerve cells. We have shown that PV-induced apoptosis involved both activation of initiator caspases and mitochondrial dysfunctions. Moreover, the interactions of poliovirus with its cellular receptor (CD155) could modulate PV-induced apoptosis and this modulation could play a role in poliovirus persistence.

Finally, mice surviving paralytic poliomyelitis represent a relevant animal model to study processes leading to regeneration of paralyzed muscle following virus-induced motoneuron death.

**B Prion diseases** Another focus of the unit is the *understanding of prion disease propagation* such as occurred in Creutzfeldt-Jakob cases surging after the spongiform bovine encephalitis epidemic and those resulting from growth hormone contamination (Francoise Lazarini et al). Using the murine model, we study among the immune cells the targets of this infectious agent, in particular dendritic

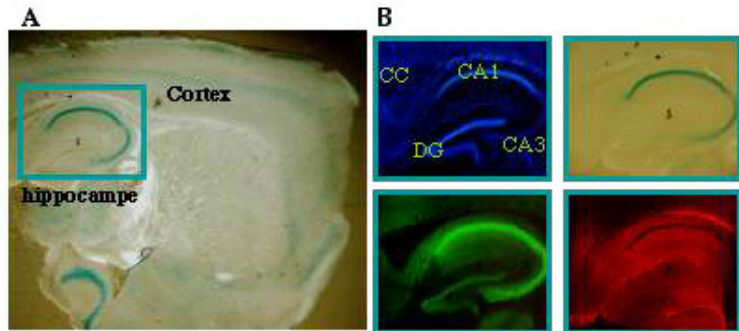
cells and macrophages. We are also exploring whether repeated low doses of infectious prions propagating from the periphery to the CNS can eventually induce this deadly disease.

## II. Development, communication/signalling and regeneration of the nervous system

### *The development and regeneration of myelin-forming cells from neural stem cells*

is a subject of direct relevance to Multiple Sclerosis (MS) as we are progressing in the design of remyelinating therapies (Monique Dubois-Dalcq et al). Together with our collaborators of our EC network, we investigate the role of molecules permissive for neural cell migration such as the polysialylated form of NCAM, alpha chemokines and integrins. We use genetic engineering and small agonists to enhance the migration and myelinating properties of neural precursors which are grafted in myelin mutants and experimental allergic encephalitis (EAE), an animal model for MS.

Research by Harroch et al is focused on the biological function and signaling pathways regulated by **receptor tyrosine phosphatase, RPTPs, and their role in neurodegenerative disease** and regeneration. In view of this, we have generated mice deficient in RPTP beta and gamma, two RPTPs structurally homologous but expressed in different cell types. Indeed, while RPTP $\beta$  is primarily expressed in glial cells, we detected RPTP $\gamma$  in neurons. These mutated animals allow us to study the function of RPTP in vivo. For example, we showed the implication of RPTP $\beta$  in epilepsy since RPTP $\beta$ -deficient mice develop seizures. In a similar way, RPTP $\beta$  deficient mice can be used to study multiple sclerosis; RPTP $\beta$ -deficient oligodendrocytes are more fragile and more susceptible to death compared to control oligodendrocytes. To cure such lesions, we study the role of RPTP in stem cells (neurospheres) and their capability to repair spinal cord lesions. Finally, we would like to determine signaling pathways regulated by RPTPs and in particular the one involved in these neurodegenerative disease.



**Connexin channels** participate in the regulation of signaling between developing and differentiated cell types. The recent discovery of human genetic diseases associated with mutations in six connexin genes and the study of knockout mice have validated the view that this form of intercellular signaling fulfills a crucial role in coordinating several aspects of tissue homeostasis. Understanding in detail how these channels gate offers the potential to develop specific drugs to deal with connexin-based disorders. To address this issue, we propose to take advantage of the naturally occurring connexin mutations to gain insight into the regulatory mechanisms of channel gating and further develop a related project on the specific role of connexins in the central nervous system (CNS), a largely unexplored area.

Dr Bruzzone is presently on sabbatical leave at the University of Heidelberg; investigating with Dr Monyer the molecular basis of oscillatory behavior of nerve cells and the organisation of neuronal networks

**Legend of Fig. 1 :** Polarized enterocytes. Detection of the actin of the brush border (in red, apical face) and of CD155, the human poliovirus receptor (in green, basolateral face). Confocal microscopy ( Centre d'Imagerie Dynamique) was used to detect the fluorescence (K. Labadie).

### **Legend of Fig. 2 Neuronal expression of RPTP $\gamma$**

Identification of LacZ expressing cells in brain sections of RPTP $\gamma$ <sup>+/-</sup> mice.

**A.** Saggital section of adult mouse brain stained with X-gal.

**B.** Immunocytochemistry of saggital section labelled with Hoechst, LacZ, NeuN and GFAP in the hippocampus area.

**Keywords :** Poliovirus, persistent infection, apoptosis, neuron, prion diseases, neural stem cells, oligodendrocytes, receptor tyrosine phosphatase, RPTP beta, remyelination, cell communication, connexins diseases, gap junctions

## Publications

> [Publications of the unit on Pasteur's references database](#)

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