

## POST-DOCTORAL POSITION AVAILABLE

**A 3-year post-doctoral position starting in October 2010 is available in the INSERM laboratory U819, at the Institut Pasteur, Paris, France.**

### Proposed project

#### *CXCL12 and tissue regeneration : Mechanisms of action and therapeutic potential*

The chemokine CXCL12 is unique among chemokines by the pleiotropic roles that it plays in embryo and adult life. The exceptional conservation of CXCL12 structure & function in mammals announces the essential roles played by this singular chemokine. To this regard, the contribution of CXCL12/CXCR4 chemokine axis in the recruitment of stem cells from bone marrow and non bone marrow origins is of paramount importance, occupying a predominant hierarchic role in the orchestration of tissue regeneration. Indeed, CXCL12 regulates with unchallenged capacity both the tissue homing and survival of circulating tissue-specific progenitors, hematopoietic- and bone marrow stem cells, as well as triggers inflammatory cells infiltration. The involvement or the contribution of CXCL12 to stem-cell-based tissue repair has been investigated in several models of ischemia or tissue injury among which, cardiac or limb ischemia, liver or renal injury.

Beyond the binding to its cognate receptor CXCR4, the interaction of chemokines with the glycanic moiety of proteoglycans, in particular with heparan sulfates (HS), is supposed to enable the formation of chemokine gradients which determine the orientated migration and tissue recruitment of circulating cells. In a former project supported by the ANR, we investigated the structural and functional characterization of the interactions of the CXCL12 chemokine with HS and we identified the novel CXCL12 isoform as the paradigm of proteins with haptotactic capacity *in vivo*. It is the purpose of the proposed project to pursue and implement the investigation of this important mechanism in both homeostasis and pathological situations where the contribution of CXCL12 is hierarchical and critically involved in the successful return to homeostasis. To this aim we have developed an animal model where the *Cxcl12* gene has been selectively mutated (knock-in) in the critical binding sites that disable CXCL12-HS interactions (*Cxcl12* HS<sup>-/-</sup> mice) without affecting the agonist capacity of CXCL12 isoforms on CXCR.

The project will focus on one hand, on the investigation of tissue homeostasis during development and adult life in *Cxcl12* HS<sup>-/-</sup> animals, to provide a detailed histological analysis in *Cxcl12* HS<sup>-/-</sup> embryo and adult animals and characterize the trafficking of leukocytes between blood and primary lymphoid organs. This animal model is already available and relies in a number of preliminary results probing the existence of an original phenotype characterised by a deregulated bone marrow homing and leukocyte trafficking. On the other hand, we will assess the role played by CXCL12/HS interactions in the physiopathology of tissue repair and analyze the therapeutic potential of CXCL12 isoforms differing by their capacity to interact with HS proteoglycans. For this purpose we will use, three different models of tissue regeneration including skeletal muscular repair following notexin injury; vessel growth and remodeling after hindlimb ischemia and woundhealing and will analyze the kinetic of the spontaneous tissue reparation in these settings models of tissue regeneration in the *Cxcl12* HS<sup>-/-</sup> animal model. Finally, we will analyze the therapeutic potential of CXCL12 and in particular this of CXCL12 $\gamma$ , which binds with unsurpassed affinity to HS proteoglycans and is the paradigm of haptotactic proteins. Overall, our project aim to investigate the paradigm of chemokine HS-immobilisation and gradient formation during pathological situations associated with tissue regeneration and we believe that the proposed physiopathological settings are ideally suited for investigating the postulate that CXCL12 plays a hierarchical and non redundant role in the orchestration of tissue repair.

The project will be conducted under the supervision of Fernando Arenzana-Seisdedos, head of the INSERM U819 unit and Viral Pathogenesis laboratory at the Institut Pasteur in Paris. The project is supported by a 2010-grant for the Agence National pour la Recherche 2010 (ANR-

BLANC/Chemrepair) and relies in the collaboration with a two other laboratories in Paris specialised in muscular and vascular regeeration.

**Experience and skills of the candidate.**

The applicant is expected to be highly motivated and enthusiastic and to work independently. The candidate will be in charge, as a priority, of the immunological aspects of the program and subsequently, of the exploration of the regenerative capacities of the muscle in the mutant animals. A strong background in immunology and confirmed expertise animal models are requested. Experience in cellular biology and microscopy imaging techniques will be appreciated and positively evaluated. Salary: 2300 euros/month.

**Applications should be addressed to**

Dr. Fernando Arenzana-Seisdedos  
INSERM U818, Institut Pasteur  
28, rue Dr. Roux,  
Paris 75724 cedex 15.

e-mail address: <mailto:farenzan@pasteur.fr>  
telephone: 33 145688263  
fax: 33 145688941

## ANNEXE.

### Research work of the laboratory in the field of the project.

#### Biology of chemokine & chemoreceptors

We provided the original evidence that the chemokine SDF-1/CXCL12 is the natural ligand of the GPCR CXCR4, demonstrated the capacity of CXCL12 to prevent entry of HIV viruses with tropism for CXCR4 (X4 HIV) (Oberlin et al., 1996. Nature) and that internalization of chemokine receptors induced by chemokines is an essential inhibitory mechanism that prevents HIV-entry (Amara et al., 1997, J Exp Med). We also provided the original proof of concept that CCR5 ligands virtually devoid of agonistic capacity but retaining the capacity to promote receptor internalization, now experimented in animal models, can still prevent entry of CCR5-tropic viruses (R5 HIV) (Arenzana-Seisdedos et al., 1996, Science). We have characterized the orphan receptor RDC-1 CXCR7 as being a receptor for CXCL12 that does not couple G-proteins (Balabanian et al. 2006 Blood; Lagane et al., 2008 Blood). We isolated and characterized the promoter regions of both CXCR4 and CXCL12 (Caruz et al. 1998 FEBS Lett.; Garcia-Moruja et al, 2005 J. Biol. Mol; Ponomaryov T, J Clin Invest. 2000) provided the first mapping of tissue expression of CXCL12 and performed structure/function relationships studies (Crump et al., 1997. Embo J). We defined the domains, the mechanisms and kinetics of the binding of CXCL12 to CXCR4 and to HS proteoglycans, and investigated the proteolytic mechanisms of CXCL12 inactivation (Amara et al., 1999. J Biol Chem; Valenzuela-Fernandez, 2001.; J Biol Chem; Valenzuela-Fernandez, 2002. J Biol Chem) and characterized the expression of CXCL12 in tissues (Coulomb-LHerminé et al., 1999 PNAS; Pablos et al., 1999 Am J Pathol; Franco et al. *Anat Rec (Hoboken)* 2009). We recently characterized a new isoform of *Cxcl12*, CXCL12 $\gamma$ , which binds HS with the highest affinity ever reported for any HS-binding protein and displays the highest biological activity *in vivo* among CXCL12 isoforms. (Rueda et al., PLoS ONE, 2008; Laguri et al., PLoS ONE, 2007). We have contributed in collaboration with H. Lortat-Jacob (ISB, Grenoble) and F. Baleux (IP), to the development of the first CD4-HS-mimetic glycopeptide with the capacity to prevent the virus-cell fusion and infection of both CXCR4 and CCR5 tropic HIV ( Baleux et al., Nature Biol. Chem. 2009).

#### Physiopathology of CXCR4-CXCL12 cell signaling

We characterized the pathogenic mechanisms of the WHIM syndrome, which associates an increased susceptibility to Human Papilloma Virus, bacterial Infections, hypogammaglobulinemia and retention of mature neutrophils in BM (myelokatexis), (Balabanian et al. 2005, Blood; Lagane et al., 2007. Blood) and revealed that HPV-infection associates with an aberrant and massive expression of CXCL12 in epithelial cells of HPV-induced warts and condylomas (Balabanian et al., 2005., Blood). We have identified new forms of WHIM syndrome not associated to CXCR4 anomalies but implying downstream effector proteins (Balabanian et al., J Clin Invest, 2007). We have engineered a mice model of WHIM which displays the immuno-hematological anomalies identified in humans. Finally, we have recently demonstrated that an abnormal trafficking of CXCR4 is associated to the idiopathic CD4 lymphopenia as a pathogenic mechanisms (Scott-Alagra et al, Blood 2010).