

Presentation of the funded projects in 2010 for the "Integrated Mechanisms of Inflammation" Programme

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Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title

ATHLO ATHerothrombosis and adventitial tertiary Lymphoid Organs : pathophysiology and clinical potential

Abstract

Atherothrombosis is a chronic inflammatory disease and represents the first cause of death in the world despite improved treatments. Our group has a long-standing track record in atherothrombosis immunobiology. Recently, we and others have reported the formation of tertiary lymphoid organs (TLOs) in chronically inflamed arteries, notably in the adventitia of atherothrombotic segments. The development of TLOs in the adventitia of atherothrombotic arteries is a major finding since this ectopic lymphoid tissue can sustain the local activation and maturation of pathogenic immune effectors, two processes previously thought to be restricted to ontogenetically programmed secondary lymphoid organs (SLOs). This discovery initiates a new paradigm and raises the following questions: i) Does the immune response elicited in adventitial TLOs affect disease evolution? If immune effectors generated in adventitial TLOs are directed against the vascular wall components, lymphoid neogenesis should accelerate destruction of the vessel wall. We will test this hypothesis via experimental approaches aiming at modulating TLO formation. ii) What are the mechanisms leading to the genesis of adventitial TLOs? Based on our data in inflammatory conditions, we hypothesize that the TLO formation recapitulates the developmental program of SLO organogenesis. This requires that lymphoid "inducer" cells interact and activate stromal "organizer" cells. We will identify the cells that take on the role of inducers and organizers in the context of atherothrombosis. We will characterize whether infectious agents and epigenetic modifications can trigger adventitial TLO formation. iii) What are the clinical applications of this new concept? We are currently unable to predict the fate of atherosclerotic arteries. Since lymphoid neogenesis evolves concomitantly with atherothrombotic diseases, we propose that TLO formation could become a prognostic marker. We will therefore set up non-invasive molecular imaging techniques to monitor TLO evolution. In addition, understanding the impact of TLO modulation on the disease could open the way to innovative treatments which

will be tested in pre-clinical models. This project is based on an integrative vision of the inflammatory process underlying atherothrombosis. Since the expertise of the two partners encompasses ontogenetics of lymphoid tissue (G Eberl, Partner 2), and vascular immuno-pathology (A Nicoletti, Partner 1), this project has the potential to lead to a paradigm shift. Furthermore, it has a translational design, including preclinical evaluation of new prognostic and therapeutic options.

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Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title

GEMISA GEnetics, Microbiota, Inflammation and Spondyloarthritis

Abstract

Spondyloarthritis is a prototypical chronic inflammatory disease with prevalence of 0.3% in France. It mainly affects young adults and is characterized by strong genetic association with HLA-B27. Recent data, both genetic and immunologic, point to a critical role of the Th17 pathway in development of the inflammatory process, as well as to an anomaly of the interaction between dendritic cells and CD4+ T lymphocytes. Moreover, the intestinal microbiota appear to be a key player in perennation of the chronic inflammatory response. The overall goal of our project is to achieve a detailed description of the various factors, genetic, immunologic and microbiologic, involved in SpA and to elaborate a pathophysiologic model of their interplay. To this end, we will rely on a genome-wide linkage study we recently completed and that is the largest ever performed in spondyloarthritis. In this study, 154 multiple cases families were genotyped using 250 kSNPs Affymetrix DNACHIPS. Non-parametric linkage analyses allowed us to identify 5 highly significant loci including the MHC on 6p21, the locus SPA2 on 9q previously mapped by our team, and 3 entirely new loci on 6p11-q11, 13q13 and Xqter with LOD scores between 4.38 and 5.94. Building on this discovery, we propose as a first aim to identify genetic variants associated with SpA susceptibility in these linked regions, using family-based design and a dense map of tag-SNPs. This approach will be coupled to an investigation of differentially expressed genes and exons mapping to the best associated regions in the transcriptome of dendritic cells and CD4+ T lymphocytes of patients compared with matched familial healthy controls (aim2). Both aims 1 and 2 will lead to a list of best candidate loci. These will be resequenced to identify causal polymorphisms, accounting for differential gene expression and disease predisposition (aim 3). These findings will be validated by replication studies in independent case-control cohorts. In parallel, we will compare the DNA (metagenomic) and the mRNA (metatranscriptomic) of intestinal microbiota of familial patients and of their healthy siblings, using deep sequencing (aim 4). We will incorporate the above studied

factors in a multivariate model potentially including interaction terms and will hopefully elaborate a consistent and detailed pathophysiologic scenario of SpA pathogenesis (aim 5). Finally, a genetic algorithm for diagnostic prediction will be developed and tested in a dedicated French multicenter cohort, ECHOSPA, and in the GAZEL cohort that is representative of the French population (aim 6).

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Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title	GROVI GPCR regulation of vascular integrity
Abstract	<p>Swelling is a hallmark of inflammation and a reflection of plasma and cellular efflux from blood vessels at sites where the vascular lining is rendered permeable. Although important for extravasation of immune cells recruited to fend off invading pathogens, excessive and/or prolonged opening of endothelial junctions can become a destructive component of exuberant and sometimes uncalled for inflammatory responses. Dissecting the biological mechanisms that regulate vascular integrity may both help understand why its equilibrium is lost during inflammation and facilitate targeted manipulation of vascular integrity. We study G protein-coupled receptor (GPCR) families that regulate endothelial and epithelial barrier function in response to serine proteases and bioactive sphingolipids, both of which are abundant at sites of inflammation. Through this proposal, we seek to better understand the capacity of these receptors to regulate cellular interactions, the mechanism by which they exert these effects, and - most importantly - when and why they are engaged to modulate the vascular barrier in physiological and pathological processes. To do so, we will combine cell biology techniques, mouse genetics and mouse models of vascular development and local and systemic vascular inflammation. By parallel studies of developmental biology and adult disease, we are hopeful that lessons learnt from how a signaling system helps direct the development of the vascular system will refine our approach to addressing how it is re-engaged during inflammatory challenge in adult life.</p>
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Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title	IgA-ToIDC TOLEROGENIC DENDRITIC CELLS INDUCED BY SECRETORY IgA : ANTI-INFLAMMATORY THERAPEUTIC APPROACHES
Abstract	<p>Treatments of autoimmune diseases are often inefficient and they have not been improved since the last two decades despite of new biotherapy such as anti-CD20 or anti-TNF mAb. Conventional treatment is based on corticosteroids associated with immunosuppressive agents, which is frequently associated with unresponsiveness and important side effects. Thus, the development of new immunoregulatory molecules, that are able to prevent autoimmunity development, is of crucial importance in medicine. In this proposal, we will investigate the efficacy of secretory IgA (SIgA) to prevent the activation of the immune system by modulating the maturation of dendritic cells (DCs). SIgA-conditioned DCs are anti-inflammatory as they produce large amounts of IL-10 and induce regulatory T cells. Using mouse models, we have observed that SIgA-conditioned DCs can prevent organ-specific autoimmune diseases such as type 1 diabetes and experimental autoimmune encephalomyelitis. Therefore, we propose to characterize molecular mechanisms that inhibit DC maturation and promote their IL-10 production. We would like to determine the cellular mechanisms that prevent the development of autoimmune diseases using various mouse models of organ-specific and systemic autoimmune pathologies. Our project corresponds to a first proof of concept for the utilization of SIgA-conditioned DCs to prevent and treat autoimmune diseases.</p>
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Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title

IMPRO-Fprau Immuno-modulatory effects mediated by peptides derived from a specific protein secreted by *Faecalibacterium prausnitzii*, a commensal bacterium involved in Crohn's disease.

Abstract

Crohn's disease (CD) is a chronic inflammatory bowel disease characterized by chronic inflammation and focal transmural intestinal mucosa whose pathophysiology remains unknown. It involves complex mechanisms of cross talk between the immune response of genetically predisposed subjects and some products of intestinal microbiota. We have shown recently that: i) at the ileal a low rate of *Faecalibacterium prausnitzii* (commensal bacteria belonging to the phylum Firmicutes) level was predictive of postoperative recurrence of CD and ii) that this bacterium possessed powerful anti-inflammatory properties both on epithelial cells models and in a mouse model of inflammatory colitis. Our group has evidenced that the anti-inflammatory *F. prausnitzii* was related to one (or several) molecules present in the culture supernatant, by blocking NF- κ B activity and secretion of IL-8 and by stimulating the secretion of IL-10. Comparative analysis of the supernatant of *F. prausnitzii* and its culture medium by mass spectrometry revealed 7 peptides in supernatant fractions reproducing the immunomodulatory effect of total supernatant in a cell system. The characterization of these peptides showed that they were all issued from a single protein of approximately 15kD synthesized by *F. prausnitzii* and whose function and structure remain unknown. We hypothesize that the observed immunomodulatory effect of supernatant of *F. prausnitzii* is mediated by these peptides / protein. Since our preliminary results are consistent with this hypothesis, we propose to develop a project to decipher the mechanisms of immunomodulatory effects brought by these peptides and protein. The skills of 3 laboratories (ER7 Seksik UPMC, INRA MICALIS Langella, UMRS7203 Lavielle) will be joined to conduct this work using complementary approaches in microbiology, cell biology, biochemistry and chemistry. Our program is divided in several tasks: Task 1 devoted to the production of the protein in heterologous and genetically modified bacteria (*L. lactis*) and validation of its immunomodulatory effects on

cellular and animal models, task 2 devoted to the synthesis of peptides from this protein, and to their screening on a cell model regarding their immunomodulatory effects and validation of peptides of interest in animal models of colitis; Task 3 devoted to the structure- function of proteins and peptides to find out their sites of action and cellular partners; Task 4 designed to describe the signaling pathways of inflammation involved in the immunomodulatory effects; Task 5 devoted to a clinical study aiming to detect peptides and proteins from *F. prausnitzii* in the stools of patients with CD. The first two tasks will confirm the role of immunomodulatory peptides / protein specific for *F. prausnitzii* and provide sufficient amount of molecules to achieve the task 3. Task 3 together with task 4 will unravel the molecular mechanisms involved in this immunomodulatory effect. Finally Task 5, will explore in humans the value of these peptide / protein from *F. prausnitzii* as new tools potentially usefull in prognostic of the disease. All together, it is an original and consistent research program focusing on bio-active molecules from a commensal bacterium involved in the pathogenesis of chronic inflammatory bowel disease. This project could lead to the development of new approaches in the prevention and control of inflammation in CD.

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Reference

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Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title

INFLAFER Mechanisms of anemia of inflammation: cross-talk between inflammation and iron metabolism

Abstract

During inflammation, release of storage iron by macrophages is inhibited by a mechanism that has evolved to protect organisms against microbial infection. Excessive hepcidin production in the liver and/or the spleen of patients with inflammatory diseases contributes to this iron sequestration, but the exact molecular pathways that mediate hepcidin overproduction are not yet known. Hepcidin promotes the internalization and degradation of ferroportin, the only known cellular exporter of elemental iron, and thus inhibits the export of iron out of storage cells. Secondly, inflammation downregulates ferroportin gene expression by a mechanism that is not understood either. This paradoxical state, where there is abundant storage iron but low red blood cell production, leads to anemia of inflammation, also known as anemia of chronic disease. Understanding how inflammation induces hepcidin synthesis and represses ferroportin mRNA is crucial because anemia of inflammation is found in a very high percentage of patients suffering from infections, malignancies and auto-immune disorders and can add substantially to the morbidity of the underlying disease. It is often a predictor of adverse outcome. Our proposal is aimed at better understanding the molecular pathways that mediate macrophage iron sequestration in inflammation in order to pinpoint molecular targets for the treatment of this anemia in those situations when the underlying disease cannot be reversed. We will take advantage of the Bmp6 KO mice in which the iron-sensing pathway leading to hepcidin production is entirely blunted by the lack of Bmp6. These mice keep their capacity to upregulate hepcidin in response to an inflammatory challenge and will allow us to investigate the cross-talk between inflammation and iron metabolism in vivo. Noticeably, this cross-talk occurs in several complex tissues, in particular intestine, spleen, and liver, and the regulation of hepcidin expression by inflammation involves interactions between tissues and also between differing cell types within the same tissue. These interactions will be much better modelled with in vivo approaches in Bmp6 KO mice than with

in vitro experiments on cells cultured out of their physiological context. Our workprogramme is subdivided into four tasks. Briefly, we will precisely establish the evolution in time of different parameters following an LPS challenge in Bmp6 KO mice: cytokine profiles, iron parameters, levels of gene expression of molecules classically involved in BMP or TGF- β signaling, levels of phosphorylation of Smad effectors, and mRNA and protein expression of different molecules involved in iron metabolism, particularly ferroportin. We will then dissect the different steps of the inflammatory pathway that leads to hepcidin synthesis, particularly the switch-off of the iron-regulatory pathway through down-regulation of hemojuvelin and ferroportin, the activation of the BMP signaling cascade by a yet unknown ligand that we will characterize, and the influence of vitamin A on the IL-6/STAT3 activation pathway. At each step, we will test potential targets for the development of new treatments of the anemia of inflammation. The two partners involved in this project have complementary expertise on mouse genetics, gene expression studies, including large-scale studies with DNA microarrays (partner 1), on macrophages and, more generally, on protein investigations (partner 2). They both have a very good knowledge of iron metabolism and have recently demonstrated the importance of Bmp6 in the regulation of iron metabolism. Their joined efforts will be essential to decrypt the mechanisms leading to anemia of inflammation.

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Reference

ANR-10-MIDI-004

Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title

iNKT-SAP Analysis of the SLAM-R/SAP signaling pathways in the mechanisms regulating inflammation through IL-17 production: implication of iNKT and conventional T cells.

Abstract

This proposal aims at a better understanding of the cellular and molecular mechanisms of inflammatory responses involving iNKT cells and SLAM-R/SAP signaling pathways. Inflammation is a complex process mediated by both innate and acquired immune responses and is essential to protect the host from harmful stimuli such as pathogens, damaged cells or irritants. However, when uncontrolled it becomes pathological. For this reason, inflammation is normally closely regulated by the cross-talk between innate and acquired immune responses. Among the regulatory T cell populations and factors influencing this process we can mention invariant Natural Killer T (iNKT) cells, IL-17 and the SLAM (signaling lymphocytic activation molecule) family receptors (SLAM-R) and SAP (SLAM-associated protein) dependent pathways. The latter play a pivotal role in the control of several immune responses implicating T, B and NK cells. Moreover, SAP is critically needed for the development of iNKT cells. These cells represent a particular lineage of T lymphocytes capable to promptly and massively produce Th1, Th2 and Th17 cytokines such as IFN- γ , IL-4 and IL-17. By these means, they bridge innate and acquired immunity and regulate the outcome of numerous inflammatory immune responses. Consequently, these cells are considered as a "swiss-army knife" of the immune system and should be tightly controlled. In this context, it was already reported that SAP is critically needed for the development of iNKT cells and our recent findings demonstrate that SAP signaling can also regulate the IL-17-producing capacities of these cells. Since this cytokine plays a pivotal role in inflammation, we could hypothesize that SLAM-R/SAP signaling pathways can influence inflammatory responses by controlling IL-17 production. In the present project, we propose to dissect this new mechanism capable to influence inflammatory responses. For this, we will perform in vitro assays to determine the molecular mechanisms implicated, use experimental mice models of lung inflammation, and finally analyze human cells from health

donors and from SAP-deficient patients. Results obtained will highlight potentially new mechanisms influencing the pathophysiology of some inflammatory diseases and propose new therapeutic trials. This project cannot be achieved without the expertise of the three teams who accepted to join forces to take up this challenge: Partner 1 (coord): Maria Leite-de-Moraes et collaborateurs, CNRS UMR8147, Hôpital Necker, Paris Partner 2: Sylvain Latour et collaborateurs, INSERM 768, Hôpital Necker, Paris Partner 3: Gérard Eberl et collaborateurs, Institut Pasteur, Paris.

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Reference

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Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title

iSa Systems Biology of Immunoreceptor Signaling in Allergic Inflammation

Abstract

Allergy is a disorder of the immune system that occurs in response to a variety of normally harmless substances known as allergens. Most allergens are environmental. They include pollens, animal hair, dust mites, moulds, and venoms from stinging insects. Other allergens are mostly food and medications. Allergies are initiated by the activation of mast cells by anti-allergen immunoglobulin E (IgE) antibodies, resulting in an acute inflammatory response. Such acute responses often evolve into chronic inflammation that result in progressive tissue lesions. Allergic manifestations affect primarily the respiratory tract (rhinitis, asthma) and the skin (atopic dermatitis, eczema). Others are systemic (anaphylaxis). They can be benign or severe. Anaphylaxis is a hyperacute life-threatening reaction, and it takes only minutes for a mild allergic reaction to escalate to anaphylaxis. Mast cell activation is a pivotal event in the initiation of inflammatory reactions associated with allergic disorders. It is triggered by the aggregation of high-affinity IgE receptors (FcεRI), on the mast cell surface. FcεRI aggregation is induced by the binding of a multivalent allergen to FcεRI-bound IgE antibodies. Mast cell activation is a complex process relying on multiple layers of tightly controlled intracellular signaling molecules, which form an intricate and dynamic network. To understand and predict the behaviour of this signaling network it is crucial to study it as a complete system and not only its isolated parts. The iSa (iSa stands for immunoreceptor signaling in allergy) project, is such a comprehensive approach of immunoreceptor signaling in allergy through a multidisciplinary effort. The iSa project specifically aims to understand at systems level, how information is generated and propagates through the FcεRI signaling network with high temporal and spatial resolution. The iSa project will develop new analytical and mathematical tools to generate and integrate high-density quantitative data describing mast cell activation. Genetics, proteomics, high-resolution 3D imaging, and modeling will be applied to obtain a holistic model of the FcεRI signaling network. By combining complementary state-of-the-art expertises, the iSa project

will allow the identification of critical decision nodes within the FcεRI signaling network that are likely to be highly vulnerable to pharmacological interventions and thereby can constitute new drug targets in the prevention and/or the treatment of allergic inflammation.

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986207 k€

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Reference

ANR-10-MIDI-006

Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title

MigreFlame Macrophage tissue infiltration and inflammation : identification of inhibitors of 3D-migration

Abstract

Macrophage tissue infiltration is associated with the worsening of several pathologies such as chronic inflammatory diseases, atherosclerosis and cancers. Thus, specific inhibition of macrophage tissue infiltration has been proposed as an anti-inflammatory strategy. The challenge is now to identify pharmacological targets. The extracellular environment encountered by migrating macrophages is organised in three-dimensions (3D) but the mechanisms controlling macrophage 3D migration have been poorly studied. Recently, team 1 showed that: i) the tyrosine kinase Hck, specially expressed in phagocytes, is involved in 3D macrophage migration; ii) macrophages use a mesenchymal or an amoeboid 3D migration modes; and team 3 showed that differentially polarized M1 (inflammatory) and M2 (immunomodulatory) macrophages have been associated with distinct pathologies. Our proposal encompasses 4 objectives, that are first, to progress with the proof of concept that Hck is a pharmacological target; second, to identify Hck inhibitors; third, to decipher the molecular mechanisms implicated in human macrophage 3D-migration in order to propose new pharmacological targets; and finally to determine the impact of macrophage M1/M2 polarization on their 3D migration capacities. It will require the concerted action of team 1 working on macrophage migration and Hck (I. Maridonneau-Parini, CNRS UMR5089 Toulouse), team 2 specialized in small molecules high throughput screening (B. Déprez, Inserm U761 Lille), team 3 specialized in macrophage polarization and transcriptomic analyses (JL. Mège, CNRS UMR 6236 Marseille) and the biotech company Ambiotis (Toulouse), a facility specialized in animal models of inflammation. To further validate Hck as a pharmacological target for the inhibition of macrophage tissue infiltration, we will compare in wt and hck^{-/-} mice macrophage recruitment in tissues during acute (peritonitis) or chronic (colitis) inflammations and experimental solid tumour development, and also the evolution and/or resolution phases of the diseases. To identify Hck inhibitors, a high throughput

screening will be done using a drug design and pharmacophore strategy. The specificity of these molecules towards Hck and their effects on cellular processes controlled by Hck, such as 3D-migration in vitro, will be determined. To identify the molecular mechanisms involved in 3D macrophage migration and propose new pharmacological targets in addition to Hck, a differential transcriptomic approach will compare the gene expression profiles between human macrophages migrating in 2D and 3D and between the mesenchymal and amoeboid modes. The role of candidate genes specifically involved in 3D migration will be assessed by si/shRNA extinction and/or the use of appropriate knock-out mice. To characterize the role of M1/M2 polarization on 3D macrophage migration, several subpopulations of murine and human macrophages either differentiated in vitro or obtained from mouse tissues or patients will be used in 3D-migration assays. In conclusion, the expected results will establish whether Hck is a target for future anti-inflammatory therapies, identify Hck pharmacological inhibitors, propose new candidates potentially involved in 3D macrophage migration, and thus contribute to a main challenge in the field of anti-inflammatory drug research. They will also shed light on several open questions such as the molecular mechanisms controlling macrophage tissue infiltration and the impact of macrophage polarization on this process.

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Reference

ANR-10-MIDI-013

Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title

PATHIMMUN Pathogens teach us about novel antiinflammatory strategies

Abstract

Our project is based upon the original concept that bacterial pathogens, under strong selective pressure in order to avoid the inflammatory immune reactions of their infected host and to achieve efficient colonization and invasion, have accumulated, during their long co-evolution with higher primates, a variety of genes encoding very efficient « anti-immunity » mechanisms. Enteroinvasive bacterial pathogens such as Shigella, Salmonella and Yersinia which recapitulate all possible steps of interaction (i.e. intracellular, extracellular) with the intestinal epithelium, and resident/recruited immune cells of the lamina propria, harbor a rich array of “anti-immunity” effectors that are injected into the target cell cytoplasm via a dedicated type III secretory apparatus (TTSS). Altogether these effectors represent a “gold mine” of strategies - thus of ideas - to manipulate and control the arms of the immune system that cooperate to eradicate invading microorganisms. Whether innate or adaptive, host immune mechanisms converge in eliciting cells (i.e. monocytes, dendritic cells, Th1 and Th17 T-lymphocytes) producing high amounts of pro-inflammatory cytokines and chemokines that recruit and activate phagocytes at the cost of massive tissue damage. For pathogens, survival indeed requires that they can inactivate key signaling cascades of this inflammatory reaction both in epithelial and immune cells. The ongoing molecular and cellular analysis of the mode of action of these “anti-immunity” effectors reveals that they are in general enzymes able to carry out modifications of original functions of host proteins that represent – or belong to – check points in pro-inflammatory cascades (i.e. I- κ B, IKK, ERK/P38, etc...). Enzymatic activities vary from ubiquitin ligases to deubiquitinases, kinases to phosphatases or phosphothreonine lyases, methylases, etc... It appears that this variety of enzymes is largely dedicated to the dampening of the NF- κ B and MAPKinases pathways, than one may consider “expected” therefore less prone to provide original targets for the development of novel anti-inflammatory drugs. Based on this rationale, we decided to select another TTSS-injected effector, IpgD, that stands out as a

phosphatidylinositol phosphatase hydrolyzing PI(4,5)P2 in PI5P. We have recently shown that in experimental models of Shigella infection, IpgD is the effector that expresses the strongest anti-inflammatory effect. Our preliminary evidence indicate that through the loss of PI(4,5)P2, or rather the acquisition of the largely unknown PI5P, IpgD interferes very efficiently with various cellular mechanisms that altogether contribute to strong "anti-immunity" functions, particularly anti-inflammatory. Altered mechanisms may be provisionally classified into three categories which will support the four lines of our project: (i) the regulation of danger signals by hemichannel closing that regulates the pro-inflammatory release of ATP induced by invaded epithelial cells; (ii) the efficient control of Ca²⁺ fluxes induced by bacterial invasion signals, inhibition of these fluxes participating in the regulation of hemichannel opening; (iii) the regulation of EGF receptor activation and recycling whose function may not be essential to regulate inflammation, but will serve as a blue print to study how IpgD affects the function and surface exposure of key immune receptors; (iv) the control of immune cell migration via modifications of the dynamics of the cytoskeleton of immune cells. From this very basic dissection of the anti-inflammatory mode of action of IpgD, we wish to identify possible original targets for novel anti-inflammatory strategies, and to develop robust dedicated readouts that we will ultimately propose to interested company partners to identify new type/families of anti-inflammatory drugs. We will actively seek industrial partnership in the course of this project.

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Reference

ANR-10-MIDI-007

Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title

PPAR α Role PGE $_2$ Relationship PPAR-PGE $_2$ in chronic inflammation : an opportunity for preventing atherothrombosis ?

Abstract

Chronic inflammation of the arterial wall triggered by lipid deposition and their oxidation induces atherosclerosis. Atherothrombosis, the ultimate cause of most of cardiovascular events, is the occurrence of thrombosis at the surface of atherosclerotic plaques. Antiplatelet treatments inhibit thrombosis and myocardial infarction; however their efficiency is limited by their inhibiting effect on hemostasis and the risk of bleeding. We previously showed that prostaglandin E $_2$ (PGE $_2$) is produced in atherosclerotic plaques and aggravates atherothrombosis without impacting hemostasis. However, the pathway controlling the PGE $_2$ production in atherosclerotic plaques is not described. PPARs are nuclear receptors involved in mechanisms controlling action of other eicosanoids ; however their interaction with PGE $_2$ is not clear. Clinical studies suggest that PPAR γ activation with rosiglitazone increases the risk of cardiovascular events; however these data are controversial. Our global objective is to determine whether a functional pathway PPAR γ /PGE $_2$ is functional in atherosclerotic plaques, and whether this pathway would allow modulating the PGE $_2$ production in order to prevent atherothrombosis. In our first aim, we will test pharmacologically and in vitro whether one PPAR can control PGE $_2$ production in macrophages. Our preliminary results showed that rosiglitazone, a PPAR γ activator, increased the PGE $_2$ production. We will test its specificity by repeating the same experiment in presence of a PPAR γ inhibitor. To confirm this approach, we will transfect macrophages with siRNA in order to inhibit PPAR γ expression in macrophages. In our second objective, we will test whether the PPAR γ /PGE $_2$ pathway is significant in vivo, in atherosclerotic plaques. We will measure the expression of PPARs in mature/old murine plaques; our preliminary data showed that expression of PPAR γ is largely predominant (over other PPARs). Mice will be given rosiglitazone in order to detect its impact on the PGE $_2$ production in plaques. We will confirm our results using a conditional and inducible line of mice knocked-out for PPAR γ , because PPAR γ interferes with

the plaque development. Our third objective is aimed at exploring the role of PGE2 in plaque, since it might be either pro- or anti-inflammatory. We will examine whether PGE2 can “reprogram” macrophages so that they respond to inflammatory stimuli by producing anti-inflammatory mediators, such as LXA4. We will then assess the results in vivo by crossing mPGES-1 and ApoE-deficient mice. The double KO will be unable to produce PGE2, and the measure of their LXA4 production will indicate whether PGE2 has any role in its production. In the last aim, we will test whether manipulating the PPARg/PGE2 pathway impacts atherothrombosis. If PGE2 has a resolutive role, inhibiting its production might render plaques unstable. We will thus study the effect of rosiglitazone on plaque vulnerability by histology and scanning electronic microscopy. We will finally study the rosiglitazone effect on atherothrombosis, using a model that we set up in our lab. Our research program will establish whether PPARg controls the PGE2 production in atherosclerotic plaques, whether PGE2 is pro-inflammatory or resolutive, and will examine the rosiglitazone effects on the plaque (stability, thrombogenicity). The latter point is important in regard of the controversy about the cardiovascular effects of rosiglitazone, a molecule largely used in the treatment of mellitus diabetes. Our results might open new avenues in the field of atherothrombosis prevention.

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Cluster label

Alsace Biovalley (ex Innovations thérapeutiques)

Integrated Mechanisms of Inflammation (MI2) programme

YEAR 2010

Project title

PurPID Purinergic receptors signaling : toward the identification of therapeutic targets in inflammatory lung disease

Abstract

Inflammation is a fundamental immune mechanism of defense to infection or to non infectious tissue or cellular damage which allows eliminating pathogen or repairing damaged tissue allowing recovering normal conditions. Nevertheless, non regulated inflammation often leads to abnormal situation where the inflammatory response induces or worsens the pathology in infectious, auto-inflammatory and auto-immune diseases. Lung inflammation plays an important role in pulmonary diseases such as asthma, COPD and lung fibrosis with infiltrated immune cells which produce immune mediators such as chemokines and cytokines and induces the adaptive immune response leading to tissue destruction or abnormal repair. Recently important cellular and molecular mechanisms of inflammatory processes were put in light in particular by the discovery of the NLRP3 inflammasome which activation allows the maturation and secretion of interleukin-1beta (IL-1beta), an essential inflammatory cytokine (Martinon et al., 2009). NLRP3 inflammasome was discovered studying orphan auto-inflammatory diseases which were all due to different mutations in the NLRP3 receptor leading to constitutive activation of the NLRP3 inflammasome and excessive production of IL-1beta. NLRP3 inflammasome activation, IL-1beta production and secretion are the cornerstone of many inflammatory diseases and in particular of lung inflammatory disease (Martinon and Tschopp, 2004; McGonagle et al., 2007). We have shown the essential role of IL-1beta and NLRP3 activation in inflammation upon lung injury (Gasse et al., 2007; Gasse et al., 2009). A better understanding of the molecular and cellular mechanisms of pulmonary inflammation leading to fibrosis is absolutely required to identify new therapeutic targets in the fibrotic pathology without any efficient therapy. We propose to study the mechanisms of inflammation upon lung injury at different levels: at the organism level with studies in mice, at the tissue level with studies on patient biopsies, at the cellular level with studies with murine and human cells from

experimental models or from patients and finally at the molecular level with the analysis of signaling protein partners of cellular receptors involved in the inflammatory responses. The aims of this project are to determine whether extracellular ATP is a danger signal released upon lung injury, alerting the immune system and leading to IL-1 β -mediated lung inflammation and fibrosis. This program will focus on the role of purinergic signaling and particularly on P2X7, P2X4 receptors and pannexin-1 in ATP driven inflammation. The relationship between purinergic signaling and inflammation will be analyzed at the organism level with studies in mice, at the cellular level with murine and human primary or established cell lines and finally down to the molecular level with the identification of P2XR signaling complex involved in inflammation through a proteomic approach. Intracellular pathways linking P2XR to inflammatory phenotypes will be established through the combination of molecular, cellular and integrated models. In parallel, further characterization of ATP as endogenous danger signal induced and/or released by dying cells will be address in clinical material obtained from patients with interstitial pulmonary fibrosis (IPF). Analysis of the expression of P2XR and inflammatory associated molecules in the lungs of patients with interstitial pulmonary fibrosis will be performed. Ultimately, this program should not only allow to validate purinergic receptors as potential therapeutic target in the treatment of lung inflammation, but also to identify new potential signaling targets downstream of these receptors.

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Project title

TLR9 in inflammation TLR9 intracellular trafficking and signaling in inflammation models

Abstract

The innate immune system provides the first barrier against pathogens. It has evolved to recognise a broad range of pathogens and discriminate between self and non-self. Toll-like receptors (TLRs) are the crucial sensors of pathogen associated molecular pattern or PAMPS. TLRs sense a wide variety of PAMPS from lipids, to proteins and nucleic acids. Intracellular Toll-like receptors 3 (TLR3), 7 (TLR7) and 9 (TLR9) localise in endosomes and recognize respectively double, single stranded RNA and nucleotides from viruses and bacteria. This interaction induces their conformational changes resulting in the production of proinflammatory cytokines and up regulation of cell surface molecules of immune cells like dendritic cells (DCs) which are essential in linking innate and adaptative immune response. Macrophages and epithelial cells (ECs), major host cells involved in pathogen recognition and eradication also express TLR9. In the absence of stimulation, TLR9 is retained in the endoplasmic reticulum (ER) together with another ER resident protein, UNC93B. Upon stimulation with CpG DNA, it relocates to the endo-lysosomal compartment, allowing the recruitment of the adaptor molecule, MyD88, and thereafter, activates its signaling pathway that leads to the production of pro-inflammatory cytokines and cell surface expression of molecules such as CD40, CD80 and CD86 in DCs. It has recently been shown that mouse TLR9 is non functional until it is subjected to proteolytic cleavage in the endosomes. Indeed, upon stimulation, full length TLR9 is cleaved into a C-terminal -fragment and this processing is highly dependent on a cysteine protease named Asparagine Endopeptidase (AEP). A recruitment and a boost in AEP activity, which was induced shortly after TLR9 stimulation, was shown to promote TLR9 cleavage and correlated with an increased acidification in endosomes and lysosomes. These results have demonstrated that TLR9 requires a proteolytic cleavage for its signaling and identified a key endocytic protease playing a critical role in this process. Probably, other endosomal TLRs, in particular TLR7, are subjected to similar proteolytic maturation but it remains to be fully investigated. TLR9 recognize DNA from

various parasites, bacteria, viruses and is likely to play an important role in many inflammatory diseases. Indeed, TLR9 deficient mice have been recently described to be more susceptible to infection with *Leishmania major*, a parasite infecting millions of people worldwide. DCs lacking TLR9 failed to be activated by *L. major* since the DNA of this parasite is a TLR9 ligand. Furthermore, *L. major* infected TLR9 deficient DCs were unable to stimulate CD4+ T cells. In addition, natural DNA repetitive extragenic sequences from *P. aeruginosa*, a major cause of mortality in pulmonary infections particularly in patients with cystic fibrosis (CF), have been shown to strongly stimulate TLR9. Signaling through TLR9 appears important in *P. aeruginosa* keratitis, and silencing TLR9 signaling reduces inflammation but likely contributes to decreased bacterial killing in the cornea. Our preliminary data suggest that, DCs lacking AEP activity infected by *L. major* like TLR9 deficient cells are unable to respond and that lung alveolar macrophages deficient for AEP are unable to kill *P. aeruginosa*. This bacterium induces high mortality in mouse model of CF. Thus, TLR9 appears to be critical for *L. major* and *P. aeruginosa* inflammation. We propose to study the role of accessory molecules and proteases, in particular AEP, in the trafficking of TLR7 and 9 and their relevance for the onset of innate and adaptive immune responses in *L. major* and *P. aeruginosa*.

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Integrated Mechanisms of Inflammation (MI2) programme

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Project title

ZebraFlam Signals and cells of inflammatory response monitored live in an entire vertebrate, the zebrafish

Abstract

The zebrafish (*Danio rerio*), a classical model of developmental biology, is also highly suited to the study of host-pathogen interactions. Zebrafish larvae are small, transparent and tolerate extended anaesthesia, making them highly amenable to non-invasive in vivo imaging. In addition, their genetic manipulation is easy, and they display a potent innate immune system involving cell types and cytokines similar to those of mammals. Therefore, zebrafish constitute an excellent system to study the spatio-temporal dynamics of inflammatory responses. We have already developed fluorescence-based systems to visualize viral infections in zebrafish in vivo. In addition, we have generated a transgenic zebrafish line reporting the production of the main interferon accountable for innate antiviral response. The combination of these tools made it possible to monitor in real time the dissemination of a viral infection in a whole vertebrate organism, and to simultaneously follow the advance of the host response, at a resolution where individual cells can be viewed. This project is aiming to extend this approach to the analysis of the inflammatory response induced under various circumstances of infection. In addition to viruses, we will include in our analysis the natural zebrafish bacterial pathogen *Mycobacterium marinum*, a bacterium very close relative of *M. tuberculosis*, causing in zebrafish a disease that recapitulates many features of the physiopathology of human tuberculosis. Mutants of *M. marinum* with deficiencies in the synthesis of several cell wall-associated components or of factors involved in intracellular survival will be generated by reverse genetics. The contribution of these molecules to the inflammatory response will be established. Various components of the host response - leukocyte recruitment, phagocytosis, granuloma formation, cytokine production - will be followed by live microscopic imaging. Furthermore, we will analyze the effect of entry route of bacteria on the host response - in parallel with a study of bacteria entering naturally - and inflammatory reactions induced by biofilms or during DNA vaccination. This work will require the generation

of new transgenic zebrafish lines based on fluorescent reporters. In addition to IFN-expressing cells, these lines will allow us to detect and follow in real-time cells producing TNF α and IL10; and also, the cells that respond to these three inflammatory cytokines. IL8-secreting cells will also be detected with this approach. We will also develop tools to manipulate the inflammatory response, using gain- or loss- of function approaches of cytokines or their receptors, and specific depletion of the main populations of innate leukocytes. To complement our live imaging studies, we will perform a global analysis of transcriptome variations occurring during the early phases of viral and M. marinum infections, distinguishing the part due to response to IFNs, TNF α or IL10. From these analyses we will deduce and propose a model of the gene network of the zebrafish inflammatory response. Because of its integrated approach, this project will attain a further level in the description of inflammatory responses, and probably unearth unexpected phenomena. These results will be widely applicable to the understanding of inflammation in humans, and will have potential applications for anti-inflammatory drug screening or rational adjuvant design.

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