Inserm U1011. Team 3.

Thesis projet (supervisor: Prof. D. Staumont-Salle)

Psoriasis and metabolism: a physiopathological link

1. General aim
Several clinical studies have reported a strong association of psoriasis with atherosclerosis, type 2 diabetes (T2D) and metabolic syndrome, together the leading cause of mortality in western world. No physiopathological mechanism accounting for this association has been demonstrated as such a question has never been addressed in experimental models. We aim to decipher, in mouse models of psoriasis, the cellular and molecular mechanisms underlying this association and to correlate the results with those from our parallel investigation in humans.

2. State of the art

Psoriasis:
Psoriasis is an inflammatory skin disease affecting 2-3% of the general population and mainly characterized by keratinocyte hyperplasia leading to erythematous oval plaques with adherent silvery scales. The precise aetiology of psoriasis remains poorly understood and results from a complex interplay between genetics, environment, skin barrier disruption and immune dysfunction.

Genetics:
Several Psoriasis Susceptibility loci (PSORS) have been identified and evidence the importance of both keratinocytes and immune system to physiopathology. Loci with confirmed association include HLA-C, genes involved in IL-23 signalling, two genes that act downstream of TNFα and regulate NF-κB signalling and genes involved in the modulation of Th2 immune responses. Other loci are shared with other autoimmune diseases and involved in regulating T-cell function, in innate host defense, including interferon-mediated antiviral responses, macrophage activation and nuclear factor (NF)-κB signalling. Association of ApoE4 gene polymorphisms to psoriasis and a recent metaanalysis showing an association with cardiometabolic disease genes also points toward a link between metabolism and psoriatic inflammation.

Immune mechanisms:
The immune cells and mechanisms involved in psoriasis have been extensively reviewed. Defining histological features of psoriasis include the presence of neutrophils and significant mononuclear infiltrates in the epidermis. There is marked infiltration of mononuclear leukocytes into the dermis. T cell contribution to the pathology appears essential. Originally considered, as arthritis to which it has been associated, as a classical Th1 pathology with IFNγ and IL-12 production, other Th populations, and their associated cytokines have been shown to play an important role in psoriasis: Th17, Tregs, Th22, γδ T cells and some innate lymphoid cells (ILC), as well as IL-17, -20 to -21, -22, -23, -27, -33 and 36. As a typical inflammatory disease, TNF production is essential to the pathology and one major therapeutic target. Besides lymphocytes, other immune cell populations have been associated to psoriasis. Indeed, Langerhans cell migration is impaired, DC produce increased amounts of TRAIL, macrophages contribute to the pathology and NK cells appear to exacerbate inflammation.

Vasculature:
Psoriasis is characterized by enlarged, tortuous and hyperpermeable blood vessels. Lymphatics enlargement and VEGF appear to play a key role in the development of pathology, at least in some experimental models. Experimental chronic psoriasiform skin inflammation promotes vascular inflammation and thrombosis and psoriatic patients display impaired endothelial function and thicker carotid intima-media.

Metabolism.
- Fatty acid metabolism:
Differential gene expression in skin form patients with psoriasis has been investigated using microarrays. These studies consistently point towards an altered fatty acid metabolism associated with alterations of pathways involved in atherosclerosis signalling. Interestingly, some of the upregulated or downregulated genes have been shown to regulate both skin and metabolic function. Lipocalin 2, FABP5, Alox12B. A similar dual regulatory function has also been observed for GATA3 whose expression also follows such a skin/adipocytes pattern. Of note, altered expression has been found for FADS1 and SCL6A14 who are considered as candidate genes in diabetes and obesity respectively, while SC4MOL deficiency leads to psoriasiform dermatitis due to accumulation of Meiosis-activating (C4-Methy)stersols, which are ligands for nuclear receptors LXRα and β (see below). It has also been shown that oxLDL accumulate in skin form psoriatic patients. Psoriatic patients display a more atherogenic lipoprotein profile, altered HDL composition and decreased HDL efflux capacity compared to controls beyond cardiovascular disease risk factors. Finally, in psoriatic patients proinflammatory IL-1β induces insulin resistance of keratinocytes thus interfering with their differentiation.

- Nuclear receptors:
Nuclear receptors are essential transcriptional regulators. Microarray data have also evidenced PPARα as one the key regulator of dysregulated genes of fatty acid metabolism in psoriatic skin. However, we have ruled out PPARα as regulated in a psoriasis-specific manner since we have shown that
its expression is down-regulated in atopic dermatitis. PPARγ and PPARβ/δ have been shown to affect metabolism/vasculature and skin function. In the former case, oral treatment with agonists appears to be rather beneficial in psoriasis (see below). Interestingly, overexpression of the later in mouse skin leads to the development of psoriatic lesions while PPARβ/δ antagonists inhibit the pathology in this model. Finally, LXR expression is also downregulated in skin from psoriatic patients.

- **Epidemiological studies:** Unique among inflammatory dermatosis, psoriasis has been fairly recently associated to cardiometabolic diseases and cardiovascular risk in nearly all clinical and epidemiologic studies in the field. However, no throughout experimental investigation of potential mechanisms has been undertaken although some hypothesis have been formulated. Comorbidities have been reported between psoriasis and several cardiovascular diseases. When patients are affected by psoriasis, risk factor significantly increases for myocardial infarction and atherosclerosis. Psoriasis also increases the risk for various metabolic diseases: obesity, correlated to leptin, resistin, adiponectin and concurrent increased in cytokines concentrations, dyslipidaemia and oxidative stress, non-alcoholic fatty liver disease, insulin-resistance and diabetes as well as metabolic syndrome. Taken together these data strongly suggest that psoriasis strongly enhance the risk of being affected by a cardiometabolic disease.

- **Metabolic interventions:** Treatment with antidiabetic thiazolidinediones, PPARγ agonists, or hypolipidemic Simvastatin are beneficial for psoriasis. Furthermore, weight loss, resulting from diet or gastric bypass, leads to psoriasis improvement or increased sensitivity to therapy. These data suggest that regulation of general metabolism might affect skin function.

### 3. Hypotheses

**An intrinsic dysfunction of psoriatic skin triggers vascular and metabolic alterations:** This hypothesis, that we favour, is drawn from the fact that psoriasis would rather precede than follow metabolic and vascular alterations, as clinical and infra-clinical metabolic dysfunction is frequently detected in patients first diagnosed with psoriasis, while psoriasis is (usually) not detected in patients first diagnosed with type 2 diabetes, obesity, atherosclerosis or metabolic syndrome. In at least one experimental model, skin-specific gene inactivation not only leads to cutaneous pathology but also to prevention of diet-induced obesity.

**Metabolic and/or vascular defects lead to psoriatic inflammation:** This hypothesis arose from the observation that treatment of diabetic patients by PPARγ agonist’s thiazolidinediones also clinically improved psoriasis and from the fact that weight loss in obese patients improves responsiveness to treatment of psoriasis. However, we have no knowledge of an animal model with vascular or metabolic dysfunction that develops psoriasis or psoriasiform skin inflammation.

**Psoriasis and metabolic dysfunction have a common origin:** This hypothesis is mainly supported by immunological findings, in particular about T cell compartment. Both psoriasis on one hand, atherosclerosis, obesity and diabetes on the other hand, have been characterized by their Th1 cytokine secretion profile. Recent associations of both types of pathologies to Th17 response and to IL-33 production as well as to decreased regulatory T cell activity have further substantiated these similarities. However, these immune profiles are also broadly shared by arthritis. Interestingly, common antigenic determinants between Streptococcus pneumoniae antigen and oxLDL (phosphorylcholine moieties) in atherosclerosis as well as between Streptococcus M-protein and keratin in psoriasis have been considered as potential triggers of autoimmune reactions in these pathologies. Innate immune response, TLR2/4 and dendritic cells/macrophages have been shown to participate to both psoriasis and metabolic or vascular diseases. However, these activation pathways are common to a very broad range of diseases. Furthermore, no susceptibility gene only common to the two pathologies has been identified so far. Finally, besides immune system, lipids might provide a link common between psoriasis and metabolism.

### 4. Practical goals

**a.** Investigate whether psoriasis due overexpression of the nuclear receptor PPARβ/δ in keratinocytes in mice also leads to metabolic alterations as this gene is a likely candidate for skin-expressed, psoriasis-associated metabolic modulator. **b.** Monitor the effect of psoriatic condition on the development of diet-induced obesity and conversely assess the impact of obesity on psoriasis development. **c.** Evaluate the contribution of psoriasis to the development of atherosclerosis and vice versa. **d.** Identify the molecular dysregulation(s) in psoriatic skin accounting for such effects on metabolism and/or vasculature. **e.** Determine whether mice developing keratinocyte- and T-cell- or vascular-dependent psoriasis display basal alterations of metabolism and/or vascular function. **f.** If such alterations are evidenced, identify new psoriasis-regulated genes accounting for these effects.

### 5. Methods and procedures
Task 1. Analyze metabolic, vascular and immune functions in psoriasis induced by keratinocyte-specific PPARβ/δ overexpression.

Sub-task 1.1. Generate a psoriatic mouse line with keratinocyte-specific PPARβ/δ overexpression.

On the basis of published data on psoriasis development in transgenic mice with keratinocyte-specific PPARβ/δ overexpression and on the key role of this factor as a metabolic regulator, we chose PPARβ/δ as the likeliest psoriasis-regulated gene (PRG) able to regulate metabolism. We will generate, on a C57BL6/J background, a transgenic line with keratinocyte-specific PPARβ/δ overexpression by crossing female transgenic mice, available in the laboratory, carrying a strong ubiquitous GAG promoter separated from downstream PPARβ/δ cDNA by a lox-flanked stop cassette with available male K14-Cre transgenic mice (to avoid maternal imprinting effects on K14 promoter).

Sub-task 1.2. Characterize psoriasis and investigate the presence of metabolic, vascular and immune alterations in K14-PPARβ/δ Tg mice.

Psoriasis expected to develop in K14-PPARβ/δ Tg mice spontaneously or upon topical application of a PPARβ/δ agonist, GW501516 will be characterized by histology, including laser capture microdissection followed by microarray analysis, immunohistochemistry, flow cytometry and measurement of tissue and circulating inflammatory parameters. Using in vivo imaging, functional metabolic assays and histological techniques we will investigate for alterations of metabolism, vascular and immune functions and correlate these alterations with the cutaneous phenotype and differential gene expression.

- Skin pathology: Lesional and non lesional tissue will processed for cryosections and for resin-embedding for laser microdissection and histology and immunohistochemistry respectively. Keratinocytes, dermis, inflammatory foci (when present) and dermal vessels (if possible) will be obtained by microdissection for microarray analyses. Differential gene expression in these fractions would be correlated to the observed metabolic and/or vascular changes.

- Immune cells - Inflammatory markers: Circulating immune cells will be analysed over the course of disease development using an haematological analyser (blood formula) and by flow cytometry for immune cell types that have been associated to psoriasis development. Presence of an inflammatory syndrome will be investigated by the determination of sedimentation velocity and measurement CRP, TNFα, IL-1β, IL-6. Lymph nodes draining inflamed and non-inflamed skin, thymus, spleen and adipose tissue will only be analysed if metabolic alterations are evidenced. If we identify a subpopulation whose abundance or phenotype is modified by psoriatic conditions, it would be purified by cell sorting and analysed using microarrays. Differential gene expression would be correlated to the metabolic or vascular phenotype.

- Vascular function: Alterations of vascular function will be monitored over the course of skin disease development. Arterial pressure will be monitored by tail cuff method. Presence of vascular constrictions or other morphological abnormalities will be monitored by Doppler echography and Magnetic Resonance Imaging. Aortas will be collected and analysed by Q-PCR for genes involved in vascular function.

- Metabolism: Body weight and food intake will be measured. Plasma lipid concentrations will be determined. Cholesterol and triglyceride will be measured in the various lipoprotein fractions separated by gel filtration chromatography. Glycaemia and insulinemia will be measured. Glucose homeostasis will be evaluated in glucose tolerance tests (IPGTT/OGTT) with a follow-up of glucose and insulin after glucose loading, and insulin tolerance tests with a follow-up of glucose after insulin loading. Insulinic clamps, will only be performed if other metabolic measurements show significant differences between psoriatic animals and controls. Liver, adipose tissues, pancreas and muscle will be collected. In vivo metabolic phenotype will be completed by expression profiling of genes involved in lipid and/or glucose homeostasis in various tissues Microarray analyses will be performed in only if biochemical parameters reveal significant changes.

Sub-task 1.3. Assess the impact of diet-induced obesity on skin and metabolic disease development in psoriatic K14-PPARβ/δ Tg mice.

In order to determine whether metabolic or vascular dysfunction affects the development of psoriasis or if psoriatic inflammation worsens metabolic or vascular diseases, the effect of obesity, developed upon feeding with a high fat diet, on skin, metabolism, inflammatory markers, immune and vascular functions will be monitored and differential gene expression analysed as presented in sub-task 1.2.

Sub-task 1.4 Generate and characterize skin pathology and metabolism and vascular function in psoriatic K14-PPARβ/δ Tg mice developing atherosclerosis.

A similar approach as described for sub-task 1.3 will be undertaken to assess the impact of atherosclerosis developed upon crossing the animals with LDL receptor-deficient animals and feeding with a Western diet. Animals will be monitored as described for sub-task 1.2 for skin disease, metabolic and immune alterations. Aortic lesion development (lipid, collagen and macrophage content, apoptotic and necrotic markers expression, angiogenesis and calcification) and recruitment of inflammatory cells and immune cell types, in particular lymphocytes, at the lesion sites will be evaluated.

Task 2. Identify new psoriasis-regulated genes regulating metabolic function in 2 models of psoriasis.

In order to identify new putative PRG(s), we will investigate for metabolic alterations in two (fairly) well characterized psoriasis models with a phenotype considered among the closest to human pathology.
K5.Stat3C mice express a constitutively activated form of STAT3 in keratinocytes and display psoriasis-like lesions with associated VEGF regulation as found in human psoriasis. Development of psoriasis requires T cells. Global transcriptomic analyses of skin is available for this line. K14-VEGF mice overexpress VEGF in keratinocytes and display a wide range of alterations found in human psoriasis including rete ridges, papillomatosis, epidermal T cell infiltration and microabscesses as well as enlarged dermal blood vessels. Animals, on a C57BL6/J background, will be submitted or not to a high fat diet as described in sub-task 1.3 and metabolic and vascular parameters as well as immune and inflammatory markers will be measured as sub-tasks 1.2. Would metabolic alterations be evidenced, skin compartment would be analysed in order to identify (psoriasis-regulated) genes whose expression changes correlate with and likely cause these metabolic dysfunction. Of note, transcriptomic analyses would also be (re)performed on K5.Stat3C mice as we expect them to be more sensitive upon selective microdissection of skin subcompartments.

7. Team's activity in the field
In addition to her affiliation to Team 3, Inserm U1011, D. Staumont-Salle, PU-PH, is affiliated to the dermatology department at University Hospital in Lille and has access to a large cohort of patients with psoriasis or atopic dermatitis. She will bring her clinical expertise in skin physiopathology and histology. Team 3 (Inserm U1011) is coordinated by D. Dombrowicz, PhD. His experience in the field of immunoinflammation, including skin inflammation, as well as his expertise in flow cytometry and cell sorting will complement the core expertise of Inserm U1011 in cardio-metabolic diseases and the techniques available at Inserm U1011 and on the platforms it has access to. In particular, Prof. A. Muhr-Tailleux is in charge of the biochemistry and metabolic phenotyping platform at Inserm U1011. The proposed project is funded in part by grants from the Fondation pour la recherche médicale (FRM) and Fondation de France (FDF). One post-doctoral fellow and one study engineer are working full time on the project and will participate to the daily supervision of the PhD student.

Preliminary results.
In order to decipher the link between psoriasis and metabolism in humans, in parallel of the present application, we have set-up a clinical study (funded by FRM -Human Physiopathology-) aiming to perform an extensive phenotypic characterization of psoriatic patients that are metabolically healthy (P) or display a metabolic syndrome (PM). In addition to normal (healthy) control individual, patients with atopic dermatitis, an inflammatory skin disease with no link with metabolism have been included in the study. In addition to metabolic parameters, a detailed immunophenotyping of blood mononuclear cells and granulocytes has been performed. Furthermore, besides identification of the complete set of immune cells by immunohistochemistry, whole transcriptome microarray analyses has been performed after laser capture microdissection of epidermis and dermis in lesional and non lesional skin. A first set of 6 patients per group has now been analysed. Besides the expected increased percentage of CD14+CD16+ intermediate monocytes, compared to P patients, PM patients display increased percentage of Th17 and Th22 effector CD4+ and of effector CD8+ T lymphocytes and decreased percentage of IL-10+Foxp3+ regulatory T cells as well as of plasma cells. An increase of NK cells and neutrophil percentage was also found. Importantly, compared to P patients, in lesional (but not in non-lesional) epidermis from PM patients, various genes involved in glucidic were upregulated at least 2 fold and, in lesional (but not in non-lesional) dermis, 2 factors involved in negative regulation of insulin signalling were upregulated at least 2 fold. While only obtained so far on a limited number of patients, these results however, on one hand, demonstrate the technical feasibility of the approach proposed in the present application, including the key step of whole transcriptome analysis on microdissected samples, and, on the other hand, strongly suggest that, at the molecular level, selective local dysregulation of (metabolic) pathways in psoriatic lesional skin might represent a key step leading to systemic immune and metabolic alterations. Of note, our ongoing study in humans differs significantly from previously published studies as it investigates the difference between psoriatic patients with and without metabolic syndrome and it simultaneously analyzes metabolic, immune (30 markers) and skin parameters (immunohistochemistry and whole transcriptome).

7. Novelty
Despite the fact that several clinical studies have reported a strong association with vascular and metabolic disease, no physiopathological mechanism accounting for this association has been experimentally demonstrated so far. Thus, the proposed approach to experimentally investigate, at the molecular level, the impact of psoriasis on metabolism using 3 models where skin pathology is caused by a molecularly characterized factor is entirely original.

8. Schedule and deliverables

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<th>Tasks</th>
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9. Shared Facilities

In addition to its fully equipped laboratory space, Team 3 Inserm U1011 has full access to the state of the unit's internal platforms for histology including laser capture microdissection, biochemistry and metabolic parameters, molecular biology (Q-PCR) and bioinformatics as well as platforms from EGID, Institut Pasteur Campus, University Hospital Campus, all of which we belong to, for microarrays, high-throughput sequencing, imaging and animal facility equipped with metabolic cages and X-ray, MRI and echography imaging systems for fat mass measurements and assessment of vascular alterations. A new EGID immunophenotyping platform has flow cytometry material with analysis of up to 18 parameters and sorting with 6 ways, 18 parameters and as acquired a mass cytometer.

10. Bibliography


