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Title of the research project :

Study of the role of the CD47 immune receptor in uveitis

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Doctorate School

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1 PhD student, Year of first inscription 2021

Study of the role of the CD47 immune receptor in uveitis

Rationale for research

General and scientific interest

Uveitis are frequent inflammatory eye diseases, acute or chronic, mainly of autoimmune origin but which can also be infectious. Some uveitis can also be iatrogenic, such as those recently caused by some anti-VEGFs that treat age-related macular degeneration (AMD). Uveitis are a major cause of reduced visual acuity and blindness throughout the world, and their management therefore represents a major public health issue (1). The main therapeutic weapons available are corticosteroids, immunosuppressant therapy, despite which some patients progress to blindness. The CD47 receptor is a widely expressed cellular receptor well known for its immunoregulatory functions. CD47 acts as a self-recognition, "don't eat me" signal, which allows to inactivate mononuclear phagocytes (2) *via* its ligand SIRPα. In this context, the CD47-SIRPα interaction appears as a checkpoint of phagocytosis. Immunotherapies targeting CD47 are currently under development for the treatment of cancers and could be used for uveitis (3). CD47 also interacts via other ligands such as thrombospondin-1 (TSP-1), SIRPγ and some integrins (4). This receptor therefore has different activities depending on its ligand and the cell type which expresses it: modulation of phagocytosis, transmigration of neutrophils and activation of dendritic cells, T cells and B cells. In particular, our team has shown that the binding between TSP-1 and CD47 expressed by mononuclear phagocytes potentiates their death induced by FAS-Ligand (5) and contributes to the elimination of pro-inflammatory phagocytes.

Thus, studies allowing a better understanding of the pathophysiological mechanisms of uveitis as well as the identification of new therapeutic targets are essential to improve the treatment of uveitis patients.

Scientific and medical context:

In the experimental autoimmune uveitis (EAU) model, tissue damage is associated with the presence of autoreactive T-cells against retinal antigens, with secondary infiltration of pro-inflammatory macrophages that are largely involved in the tissue destruction found in this pathology. We recently showed in the laboratory that activation of CD47 by agonists reduced the infiltration of subretinal immune cells found in our models of AMD (6) (atrophic and exudative AMD). Indeed, AMD is characterized by the presence of mononuclear phagocytes in the subretinal space, which play a major role in the pathophysiology of the disease. The use of CD47 agonists, currently under development in our laboratory, could therefore constitute a new treatment for patients with uveitis, by eliminating pro-inflammatory macrophages. On the other hand, a deleterious role of CD47 has been shown in an autoimmune model of multiple sclerosis (7); this receptor is believed to be involved in the activation of self-reactive T lymphocytes that initiate the pathology. We therefore performed EAU experiments in wild-type (WT) mice and CD47-deficient (CD47ko) mice and observed that CD47ko mice were resistant to the induction of EAU (Figure 1). These results suggest that the activation of CD47 could contribute to the development of uveitis via T lymphocytes, in this context CD47 antagonists would be beneficial.

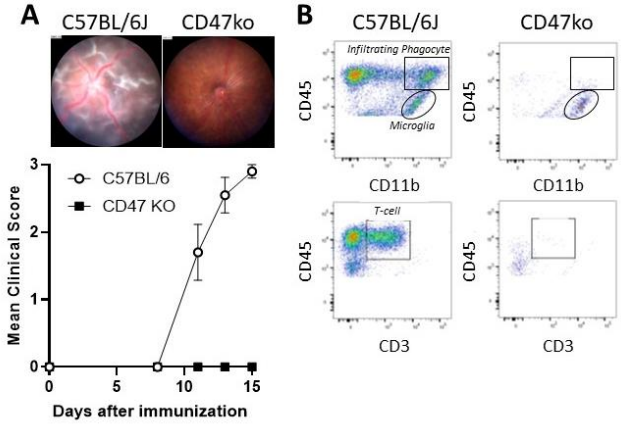

Figure 1: CD47ko mice are resistant to EAU.

A) Fundus showing intraocular inflammation after induction of EAU by retinal antigen IRBP in C57BL/6J and CD47ko mice and graph showing the course of clinical scores during EAU.

B) Analysis by flow cytometry of the retina showing infiltration of phagocytes and T lymphocytes in WT and CD47ko mice during EAU.

Therefore, CD47 antagonists may inhibit the activation of autoreactive T cells in the early stages of uveitis, while CD47 agonists may eliminate phagocytes infiltrating the retina at later stages of the disease.
During uveitis, the CD47 pathway may be altered. For example, TNFα or HIF-1 has been shown to increase the expression of CD47 (8,9). Thus, the expression of CD47 and its ligands (SIRPα, SIRPγ, TSP-1) could be increased during uveitis. Identifying these changes would help identify pathways potentially involved in uveitis and indicate pathways to inhibit or activate.
The expression of CD47 and its partners and their roles, have never been investigated in uveitis. The study of the involvement of CD47 in uveitis would determine whether this receptor is a relevant therapeutic target in the treatment of uveitis in order to control the inflammatory response.

Goals:
Our objectives are:

- In the preclinical model of uveitis, to study the effects of agonists or antagonists of CD47 as a potential treatment of uveitis by intraocular or systemic administration and at different stages of the disease.
- In patients with uveitis, to study the expression of CD47 and its ligands in immune cells found in blood sample from patients with uveitis and healthy controls.

Methodology:

Aim 1: In preclinical model of uveitis, our working hypothesis is that the modulation of CD47 activation could be beneficial in autoimmune uveitis, to demonstrate this we will test the effect of inhibition or activation of this receptor during EAU.
EAU will be induced in mice by immunization with a retinal antigen, the protein interphotoreceptor retinoid-binding protein (IRBP). We will compare the development of EAU after treatment *via* intraocular or intraperitoneal administration and at different stages of the disease (early or more advanced stages of the pathology).
The effect of these molecules will be evaluated using:

- Examination of clinical signs by fundus
- Analysis of retinal lesions by optical coherence tomography (OCT) or histology
- Study of the immune response in the retina by flow cytometry

This project has obtained the authorization of the ministry for the experiments using animals for scientific purposes number APAFIS # 13487-2018020911259174.

Aim 2: In patients with uveitis, our working hypothesis is that the CD47 pathway plays a role in the intraocular inflammation of patients with non-infectious uveitis. We want to determine if the expression of the proteins involved in this pathway is modified during the pathology. We will therefore determine the expression of CD47 and its ligands (SIRPα, SIRPγ, TSP-1) by flow cytometry in immune cells present in blood samples from patients with uveitis and compare them to a group of healthy controls. In order to demonstrate this, we wish to use the biobank being set up in the ophthalmology unit of Pitié-Salpêtrière (CPP request in progress) in partnership with the CHNO 15/20 which collects blood samples from patients with uveitis and healthy controls.

Expected results
In the preclinical model of uveitis, we will determine whether agonists or antagonists of CD47 have a therapeutic effect depending on the stage of the pathology *via* a reduction in inflammation in animals receiving the treatment (agonist or antagonist).

In patients with uveitis, we will determine whether CD47-dependent pathways are deregulated during the pathology.

Feasibility

The Vision Institute is dedicated to research on ocular physiology and pathophysiology. The present study will be carried out in the laboratory of Dr Sennlaub under the responsibility of Dr Delarasse who has extensive experience in the field of fundamental research in neuroinflammation. The team has a hundred publications to its credit and the quality of their work is well established, as they have acquired international renown in the role of inflammation and ocular diseases. The team "Inflammation, Degeneration and Vascular Remodeling in Retinal Pathologies" of the Institute of Vision has developed all the tools and experimental protocols necessary for the realization of this project, in particular the protocol to induce EAU and evaluation of clinical signs by fundus, analysis of retinal lesions by histology and OCT, and characterization of immune cells infiltrating the retina by flow cytometry. The feasibility of the project is therefore assured from the point of view of the working environment.

Conclusion

The demonstration of the role played by CD47 in the mouse model of autoimmune uveitis (EAU) as well as the efficacy of agonists or antagonists of CD47 in this preclinical model would provide the proof of concept that CD47 is a relevant therapeutic target for the treatment of uveitis. This would pave the way for the development of a new therapeutic strategy in non-infectious uveitis with the aim of improving the vision of patients with uveitis and reducing their progression to blindness.

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