Programme Doctoral « Sorbonne Université - Institut Pasteur »

Projet PUTEEN

Deep Analysis of B cell lymphocyte in thrombotic thrombocytopenic PUrpura and pre-clinical study of new anti-CD19 (CD nineTEEN) therapy.

Etude approfondie des lymphocytes B auto-réactifs dans le purpura thrombotique thrombocytopénique et étude pré-clinique de différents traitements anti-CD19.

Context

Thrombotic thrombocytopenic purpura (TTP) is a rare and severe autoimmune disease (AID) mediated by IgG auto-antibodies directed toward ADAMTS13 (A13), a plasmatic protease involved in coagulation. Once diagnosed, the disease required aggressive therapy such as plasmapheresis and immunosuppressive drugs including rituximab, which targets certain B cell lymphocytes and prevents relapses, which still occur in around 50% of cases after the first year (1). Plasma cells and plasmablasts, which secrete pathogenic antibodies, are not eliminated by this biotherapy, and some patients are resistant to rituximab or could relapse many times, so called R/R TTP, requiring more effective treatments (2) adapted to the specific pathogenic cells involved in the disease. Recently, new therapies targeting B lymphocytes via the CD19 membrane surface marker have been successfully used to treat several refractory auto-immune diseases such as lupus (3) with Anti-CD19 CAR-T-Cells, rheumatoid arthritis (4) with blinatumomab, a bi-specific anti-CD3/anti-CD19 antibody and neuromyelitis optic (NMO) with inebilizumab, an anti-CD19 monoclonal antibody (5). By targeting a broader population of B lymphocytes, including plasmablasts, these promising treatments would make possible to suppress the cells that secrete pathogenic antibodies, leading to the prospect of a potential cure, or at least a prolonged remission of the AID, but they are accompanied by hypogammaglobulinemia with a risk of infectious adverse effects.

Aims

Thus, the PUTEEN project aims to address the following questions:

1. What are the characteristics of the B lymphocyte populations involved in the development of TTP, particularly in patients who are refractory to rituximab or with multiple relapses?
2. Does the various anti-CD19 treatments mentioned above can prevent the appearance of or eradicate anti-ADAMTS13 antibodies by eliminating the B lymphocytes that produce them?
3. What is the effect of different anti-CD19 therapy on antibody repertoire in patients with AID, including TTP?

Methods :

Task 1. Isolation and analysis of ADAMTS13-specific B lymphocytes (Months 1-15)

In collaboration with the team of P.Bruhns, we will isolate from peripheral blood mononuclear cells (PBMC, minimum 10 million) of patients with R/R PTT, autoreactive B cell lymphocytes specifically directed against A13 by flow cytometry using a fluorescent recombinant A13 protein produced in the Cordelier's lab (6). The characteristics and properties of these sorted specific single B cell lymphocytes are studied at the phenotypic and transcriptomic levels including a sequencing of the antibody variable genes (VH-VL) using Sanger-based sequencing. Bioinformatic analysis will be performed by the student to develop bioinformatic tools to compare datasets (repertoires) from different patients.

Analysis of specific B lymphocytes derived from PBMC from refractory or multiple relapser patients will be considered in order to validate the presence of the CD19 marker in these patients, and we will repeat the phenotypic and transcriptomic analysis by studying the B lymphocytes located in the patient's bone marrow and lymph nodes. Ethical authorizations (CPP, CNIL,…) and all clinical information corresponding to the patients are monitored by Prof. P. COPPO in National Reference center for TTP at Saint Antoine Hospital, Sorbonne University.

Task 2. Pre-clinical study of anti-CD19 treatments (Months 12-33)

Among the compounds used, we are considering the use of blinatumumab, that drives T lymphocytes, particularly cytotoxic T lymphocytes, closer to targeted B cells that are destroyed. In this way, an effect similar to that of CAR-T-cells is achieved, with much easier and less costly use, while being less toxic (7).

In vitro studies

In this section, B lymphocytes expressing the CD19 surface marker previously identified will first be immortalised and maintained in culture after infection with the EBV virus using techniques commonly used in the laboratory. The production of anti-A13 antibodies in the supernatant will be sought by ELISA in order to validate that these are indeed A13-specific B lymphocytes. This work was already initiated by the Cordeliers Team, using *in vitro* viability and

cytotoxicity tests in the presence of rituximab, blinatumumab or inebelimumab, using another model of specific B lymphocytes (directed against Factor VIII). For experiments involving blinatumumab, the cell lines will be co-cultured with CD8+ T lymphocytes from different donors (allogeneic), lines, or autologous CD8+ T lymphocytes, in the presence of blinatumumab.

Development of a new mouse model and evaluation of the in vivo efficacy of the bi-specific antibody

Due to the lack of homology between human and mouse phenotypic markers for T and B lymphocytes, we will have to use a humanised mouse model of TTP, invalidated for A13, from a NSG immunodeficient mouse strain in order to evaluate the *in vivo* effect of this new therapeutic approach. Patients' PBMCs will then be injected into the mice with immunisation against recombinant human A13 in order to produce anti-A13 antibodies and TTP. The mice will then be treated with blinatumumab or inebelizumab. The disappearance or non-appearance of anti-A13 antibodies will indicate the success of the treatment.

Task 3. Study of antibody repertoires (Months 1-30).

For this part of the work, we will use human proteomics chips (HuprotV4, CDI Labs Baltimore) containing more than 20,000 proteins in order to determine the overall binding profile of antibodies present in the serum of patients with TTP. We will combine this analysis of the natural antibody repertoire with an exhaustive analysis of the repertoire of antibodies directed against pathogenic agents (PEPperCHIP, Pepperprint,).

Our hypothesis is that antibody repertoire profiles will allow us to observe differences between groups of patients. We suspect that refractory patients or multiple relapsers have a more general disturbance of their immune system, with other autoantibodies as well, as has been observed in other diseases (8). Moreover, around 40% of patients with TTP have anti-nuclear antibodies at diagnosis, with no identified specificity, and some develop other AID during their follow-up. It therefore seems reasonable to ask whether the new antiCD19 treatments could have a broader effect on the autoimmune mechanism involved.

In collaboration with the teams of Prof Collongues and Prof De Seze (CHU Strasbourg), we will also analyse the serum of patients with NMO (n=10) before and after treatment with inebilizumab. Finally, in collaboration with the DESCAR-T registry at LYSA, we have recently identified nearly 60 patients with a history of AID, suffering from lymphomas treated with anti-CD19 CAR-T Cells. These rare patients, rarely described in the literature because they were excluded from CAR-T Cell registration clinical trials, have changes in their antibody repertoire induced by this treatment and make it possible to approach the conditions of patients with AID specifically treated with CAR-T Cells. Validation by the Scientific Advisory Board of LYSA and the DESCAR-T registry is ongoing.

Expected results and future prospects:

These experiments are only possible because of the important role of B lymphocytes in PTT, the presence of a soluble, plasma antigen and the identification of specific B lymphocytes. These remarkable properties make it an ideal model for investigating antibody-mediated autoimmune diseases. These results will pave the way for the clinical use of bi-specific antibodies in TTP and other AID.

References:

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