The different aspects of B cell biology in arthritis.

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Ethical review	Approved WMO
Status	Recruiting
Health condition type	Autoimmune disorders
Study type	Observational invasive

Summary

ID

NL-OMON38525

Source
ToetsingOnline

Brief title
The different aspects of B cell biology in arthritis.

Condition

- Autoimmune disorders

Synonym
Rheumatic diseases

Research involving
Human

Sponsors and support

Primary sponsor: Leids Universitair Medisch Centrum
Source(s) of monetary or material Support: Ministerie van OC&W,Reumafonds,Boehringer Ingelheim,Europese Unie;bedrijven

Intervention

Keyword: Arthritis, B cells, Blood

Outcome measures

Primary outcome

1) Heparinized peripheral blood:

a) different cell types will be isolated from the heparinized/citrate blood tubes after a first isolation step using Ficoll (peripheral blood mononuclear cells) and subsequent purification using beads/cell sorting using standardized protocols.

b) cells will be characterized phenotypically in the absence/presence of different stimuli using cell type-specific stimuli

c) Isolated B cells/total PBMCs will be cultured in our in-vitro culture system to study the effects of CD40-blockade or other compunds on the development of autoantibody-producing B cells in RA

d) cells that are left over, will be stored in liquid nitrogen to enable validation of the results in a follow-up experiment.

2) Citrate peripheral blood:

a) Platelet poor plasma will be stored at -20 degrees and used to determine levels of sCD40L and other cytokines

b) Platelet phenotype will be determined in the absence/presence of platelet agonists (ADP, TRAP)

3) Serum/plasma

a) Serum/plasma will be obtained by centrifugation of the gel-tube and collecting the upper cell-free layer.

b) Serum/plasma will be either used immediately for measurements of different factors, the isolation of autoantibodies or will be stored at -70 degrees for later use.

4) Synovial fluid

a) The cellular component will be isolated using centrifugation and cells will be used in the described in vitro techniques; analyzed by flow cytometry or stored in liquid nitrogen for later use.

b) The non-cellular component will be used to determine autoantibody levels and to isolate autoantibodies for glycosylation analysis.

Serum, plasma, synovial fluid and cells such as B cells, T cells and monocytes that are left over will be stored for max. 15 years.

Secondary outcome

Not applicable

Study description

Background summary

Arthritis, i.e. inflammation of joints, is a common symptom of several autoimmune or auto-inflammatory disorders. The most common forms of arthritis are osteoarthritis (OA) and rheumatoid arthritis (RA). While OA is regarded as primarily degenerative disease, RA is a systemic, chronically-progressive inflammatory disease that specifically targets the synovial tissue of diarthrodial joints. RA is characterized by joint pain, swelling and

destruction of bone and cartilage, leading to functional failure of the joint and impairment of quality of life. This disease afflicts approximately 1 % of the population worldwide.1 The etiology of RA remains unresolved. However, two main observations point to a crucial role for B cells in the pathogenesis of RA. First, the majority of patients harbor autoantibodies that, in part, are highly associated with disease risk, disease severity and progression. 2Second, B cell depleting therapy has proven to be an effective treatment for RA.3 Both aspects, however, are not necessarily related, as the depletion of B cells ameliorates disease while the levels of autoantibodies remain high. Therefore, multiple functional aspects of B cells as mentioned above (i.e.: antibody production, cytokine production, antigen presentation) need to be considered to understand the role of B cells in the pathogenesis of RA. In addition, it is important to study the characteristics of the autoantibodies themselves, as these allow to conclude on characteristics of the B cells that produced the antibodies.

Study objective

This project intends to study the biology of B cells in arthritis. B cells are important components of the human immune system and have several functions. Next to the production of antibodies, B cells can function as efficient antigen presenting cells and as producers of cytokines and of other mediators relevant to the immune system. In order to make use of human material as efficiently as possible, it is intended that the material obtained for this project (see below) will be used to study several aspects of B cell biology in the context of rheumatoid arthritis (to analyze autoreactive B cells and their functional requirements, the interaction with autoreactive T cells, the characteristics of the autoantibodies in serum and those produced in culture, as well as functional aspects of relevant autoantibodies) These data need to be analyzed in the light of clinical characteristics such as disease activity, disease duration, treatment and age and sex of the patient.

Autoantibody-specific B cells:
Several autoantibodies have been described in RA. Among those, anti-citrullinated protein antibodies (ACPA) exhibit the highest specificity for the disease, predict disease onset and severity, and identify a subgroup of patients with distinct genetic background and requirement for aggressive treatment.4 Because of these associations, ACPA-specific B cells and ACPA themselves are the focus of intense research efforts. Recently, we have shown that particular subsets of B cells, called plasmablasts and plasmacells, are capable of producing ACPA and circulate in peripheral blood of patients with RA. 5Preliminary data also indicate that these cells are present in high frequency at the site of inflammation, i.e. the inflamed joint. Therefore, the first part of the project described will focus on further characterization of the phenotype of these cells and on compounds that might be able to inhibit their function.

Autoantibody characteristics:
As described above, multiple autoantibody reactivity*s have been described in sera of patients with RA. ACPA, the most specific and potentially clinically most relevant reactivity, comprise a group of polyclonal antibodies targeting citrullinated proteins. Recent studies by our group and others have demonstrated that ACPA have distinct properties that indicate that ACPA producing B cells are different from *conventional* B cells. Specifically, we found that ACPA carry distinct glycans which are absent from the majority of other antibodies.6-8 As glycans play a fundamental role in modulating pro- or anti-inflammatory effector functions of antibodies, studying ACPA-specific glycosylation is crucial for understanding ACPA pathogenicity and the biology of the underlying B cell response. Therefore, another, second part of the project described will focus on the characteristics of autoantibodies in RA with a specific focus on ACPA. This also includes studies on the functional consequences of these autoantibody characteristics, e.g. for the interaction with other immune cells, with relation to the pathogenesis of RA.

Development/Generation of autoreactive B cells:
Another research focus is to understand the pathogenesis of RA and more specifically the mechanisms by which individuals develop ACPA. In the context of RA, it is still unclear why, how and where autoreactive B cells develop. B cells require help from T cells to develop into antibody producing plasmacells. Therefore, also the interaction between helper T cells and B cells from RA patients will be studied. The most important genetic risk factor for the development of ACPA positive RA is the HLA class II locus. HLA class II molecules present peptides to CD4+ T-cells and it is proposed that the HLA class II association with ACPA positive RA is explained by (autoreactive) CD4+ T-cells providing help to citrulline-specific B-cells. We are currently studying different implicated (autoreactive) CD4+ T-cells populations and there role in ACPA positive RA.

Study design

Our in-vitro culture system offers the possibility to assess the influence of therapeutic and/or immunomodulatory agents on the generation of autoantibody-producing B cells. The amount of autoantibody positive culture wells as well as the mean titer of autoantibodies produced serve as readouts. Changes in the ratio between autoantibodies and total IgG can serve as indicator for effects specifically affecting autoantibody production. We propose to add different inhibitory compounds relevant to B cell function in grading concentrations to our cultures, e.g. an anti-CD40 monoclonal antibody or a JAK/STAT-inhibitor. In addition, different B cell subsets will be isolated to high purity by FACS sorting and stimulated separately with or without such compounds in order to assess the individual effects of these compounds on autoantibody producing B cells. In all cultures, we will measure the production of total IgG as control.

Furthermore, relevant phenotypic markers will be examined on various B cell subsets using flow cytometry to determine which subset of B cells is potentially affected by individual compounds and to determine if there is a correlation between the phenotype of B cells and autoantibody titers. In addition, platelet poor plasma will be collected to specifically determine soluble CD40 ligand (sCD40L) levels in correlation to autoantibody titers. As a beneficial side effect of the preparation of platelet poor plasma, a pure population of platelets will be obtained. As activated platelets (CD62P+) are the main circulating resource of sCD40L, platelet phenotype will be determined using flow cytometry (expression of CD62P, soluble and membrane bound CD40L, platelet-platelets aggregates). Furthermore, platelets will be activated in-vitro using platelet agonists (TRAP, ADP) to prompt sCD40L production and the interaction between platelets and B cells will be studied.
With regard to autoantibody characteristics, we will study the glycosylation autoantibodies in detail, with a specific focus on the Fab region of ACPA. The predominant function of the Fab region of an antibody is the binding of its cognate antigen. Therefore, glycans located in the variable region of IgG Fab are likely involved in increasing or decreasing affinity for antigen binding and could serve as a potential new biomarker in rheumatoid arthritis. To this end, we will determine the structure and precise localization of ACPA Fab-linked glycans and we will investigate the biological relevance of ACPA Fab glycosylation for RA pathogenesis. These studies will also involve in-vitro culture of B cells isolated from these compartments for the stimulation of ACPA production as described before and subsequent glycosylation analysis.

In relation to the presence of autoreactive CD4+ T cells we are now able to stably produce HLA class II tetramers that can be used to quantify and characterize specific populations of antigen-specific T-cells. This enables us to track vinculin-DERAA specific T-cells and other autoreactive T-cells in the peripheral blood and the synovial fluid of patients. We will follow these T-cells during disease progression and determine if there is a correlation between these specific T cells and ACPA production. Furthermore, these specific tetramers enable us to isolate autoreactive T cells from patients and to study their T-cell repertoire and capacity to activate B-cells. A biased TCR repertoire could provide us with a new therapeutic strategy by specifically targeting only those immune cells contributing to the pathogenesis of ACPA positive RA.

Study burden and risks

Blood sampling will occur at the *Prikpost* B4 at the LUMC. Therefore, the risks of this study is limited to a minimal bruise due to the blood collection. The aspiration of the synovial fluid will be performed by a rheumatologist only when he/she decides that this is required for optimal medical care. The participants do not benefit from this study but could lead to improved future therapeutic care.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

All RA and OA patients older than 18 years that have the ability to understand the patient information form and have signed the written informed consent form.
RA patients: RA as diagnosed by a rheumatologist, biologic naïve patients, DAS44 score >1.6 to ensure active disease .
OA patients: OA as diagnosed by a rheumatologist.

Exclusion criteria

Individuals who fail to meet the inclusion criteria.

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	17-01-2014
Enrollment:	250
Type:	Actual

Ethics review

Approved WMO

Date:	28-11-2013
Application type:	First submission
Review commission:	METC Leiden-Den Haag-Delft (Leiden) metc-ldd@lumc.nl

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL45684.058.13