Presence of immunological markers preceding the onset of rheumatoid arthritis

Mikael Brink

Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt förvar i Sal D, Målpunkt T, våning 9, Norrlands Universitetssjukhus, fredagen den 29 Maj, kl. 09:00.
Avhandlingen kommer att förvaras på svenska.

Fakultetsopponent: Docent, Anca Catrina,
Karolinska Institutet, Stockholm, Sverige.
Abstract
Rheumatoid arthritis (RA) is a chronic inflammatory disease with an unknown aetiology characterized by joint destruction. Both genetic and environmental factors contribute to the disease development with HLA-DRB1* alleles and smoking identified as most important. The disease is characterized by the presence of autoantibodies, originally by rheumatoid factor (RF) and more recently by anti citrullinated protein/peptide antibodies (ACPA) and antibodies against carbamylated peptides (CarP). These autoantibodies are present, not only after the onset of disease, but also prior to the onset of symptoms. The development of RA is a gradual process lasting several years before the onset of any joint symptom, but when and if there is a temporal difference in the development both between and within the different antibody systems is currently unknown. B-cells produce the antibodies, and a subset of B-cells, i.e., B-regulatory (Breg) cells, produces interleukin-10, and thus have the ability to down-regulate pro-inflammatory cytokines. Whether the Breg cells are involved in the pathogenesis of RA is, as yet, unknown.

The aim of this thesis was to increase knowledge of the pathophysiological processes in the development of RA through identification of factors involved. The analyses involved detection of autoantibodies to post-translationally modified peptides/proteins in addition to RF isotypes, cell surface markers on immune cells in asymptomatic individuals, who have an increased risk of developing RA. In a co-analysis of the registers of patients with RA attending the Department of Rheumatology, with the registers from population based screening programmes within the Biobank of Northern Sweden, blood samples collected from individuals prior to the onset of symptoms were identified, as were those from population control subjects. A cohort of pre-symptomatic individuals also donated samples at the time of receiving a diagnosis of RA. First-degree relatives (FDR) of patients with RA were also identified and included for analyses.

The levels of ten different ACPAs, i.e., (fibrinogen (Fib) α563-583(573), Fibs580-600(591), Fibβ62-81a(72), Fibβ62-81b(74), Fibβ36-52, α-enolase (CEP-1), triple helical collagen type II (citC1), filaggrin (Fil)307-324), vimentin (Vim) 2-17, and Vim60-75) were measured using the ImmunoCAP ISAC system (Phadia/ThermoFischer, Uppsala, Sweden) in blood samples from individuals before the onset of symptoms and when diagnosed with RA in comparison with those in population based controls. In a subset of samples, the levels of anti-CarP antibodies were measured using ELISA coated with anti-CarP-FCS, as well as analysis of RF of IgM, IgG and IgA isotype using the EliA assay (Phadia, Uppsala, Sweden). Breg cells were analysed both with and without stimulation ex vivo along with other cell types using flow cytometry in samples from patients with RA, their first degree relatives (FDR) and healthy controls.

In paper I it was shown that levels of ACPA were initially restricted to a few antibodies but disseminated over time to involve additional different antibodies. The levels of antibodies to CEP-1, Fibβ36-52, and filaggrin were significantly increased. In paper II, anti-CarP antibodies were positive in 5-13% of the individuals negative for the various ACPA studied. The presence of anti-CarP antibodies was significantly related to radiological destruction of joints at baseline, at follow-up after 24 months and to the radiological progress between baseline and 24months. In paper III, the relationships between the frequencies of RF isotypes, the ten different ACPA, anti-CCP2 and anti-CarP antibodies before the onset of any symptoms and the presence of certain combinations of antibodies were associated with a very high risk of developing RA. In paper IV Breg cells from patients with RA are functionally impaired and FDR showed a similar pattern by responding less to stimulation ex vivo than cells from healthy controls.

In conclusion, individuals who subsequently develop RA have an increased number and amount of ACPAs, anti-CarP antibodies and RF of IgM, IgG and IgA isotype, several years before symptom onset. Most of the different antibodies analysed remain associated with disease development after adjustments for each separate antibody. In FDRs, Breg cells were functionally altered in that they produce less IL-10 and consequently contribute to a more inflammation-prone status, as in their relatives with RA. These findings contribute to information about the development of RA as well as a given individual’s risk(s) of developing RA and its progression.

Keywords
Rheumatoid arthritis, pre-symptomatic individuals, autoantibodies, anti-citrullinated protein/peptide antibodies, anti-carbamylated protein antibodies, rheumatoid factor, B-regulatory cells, first degree relatives

Language
Swedish

ISBN
978-91-7601-259-8

ISSN
0346-6612

Number of pages
80 + 4 papers