Adaptive immunity (T cells and B cells) in rheumatic diseases

AB0010 LILRB3 EXPRESSION ON T CELLS CORRELATES WITH DISEASE ACTIVITY IN RA

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Background: Leukocyte immunoglobulin-like receptors (LILR) participate in the generation of immunological tolerance (1,2). LILRB3 can be expressed on T cells and is an inhibiting receptor (3).
Objectives: We wanted to study LILR expression on T cells in RA compared to SLE and controls.
Methods: Heparinised human blood from blood donors was obtained from the Institute of Transfusion Medicine, Medical School Hannover (Germany). Blood samples from RA (DAS28 <3.2 n=11; DAS28>3.2 n=8) and SLE patients (n=9) were obtained from Outpatients' Clinic of the Department of Rheumatology and Immunology after informed consent. PBMCs were stained with LILRA2 (Biolegend, APC), LILRB3 (Biolegend, PE), CD3 (Biolegend, APC-Cy 7), CD4 (BD, PerCP-Cy5.5), CD8 (Biolegend, V500), CD25 (Biolegend, PE/Cy5), CD28 (Biolegend, Pacific Blue). Results were compared to isotype controls.
Statistical analyses and figures were made with GraphPad Prism, ANOVA and the Mann-Whitney Test.
Results: The percentage of both CD4+ and CD8+ T cells expressing LILRB3 was significantly higher in both inactive as well as active RA compared to controls or SLE (See Fig. 1) (p=0.0397 ANOVA: RA all vs. SLE vs. controls). Within the group of RA patients, the percentage of LILRB3 expressing T cells was highest in active compared to inactive (DAS28<3.2) RA (p=0.0287). LILRA2 was not expressed on T cells.

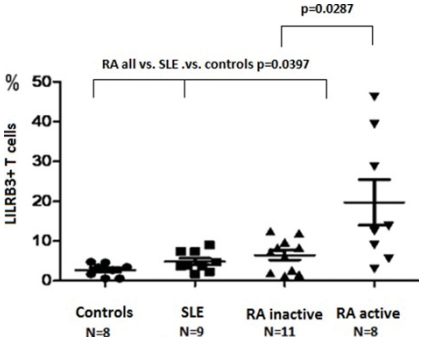

Figure 1

Conclusions: Expression of LILRB3 correlates with disease activity of RA and is decreased after successful treatment with DMARDS or biologicals. Since LILRB3 is an inhibiting receptor the increased expression in active RA may be a counterregulation to reduce disease activity.
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AB0011 KLRG1 AS A MARKER OF CD28 NEGATIVITY IN RHEUMATOID ARTHRITIS, COMPARISON WITH CD57 AND CD45RA

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Background: Our research has shown that patients with RA have higher proportions of peripheral blood CD3+CD8+CD28- Treg cells compared to healthy individuals. CD3+CD8+CD28- Treg cells in patients with RA have lost their ability to suppress lymphocyte proliferation[1]. Thus CD28 negativity may mark senescent T cells. CD57[2] CD45RA[3,4] and killer-cell lectin like receptor G1 (KLRG1)[5] cell surface molecules have been associated with CD8+ T cell activation and senescence. Defining the phenotypic signature of CD8+CD28- Treg cells will help establish their significance in the immunoregulation of RA.
Objectives: To use immunofluorescence and flow cytometry to define the phenotype of CD3+CD8+CD28+/- cells in relation to early RA progression.
Methods: The effector characteristics of peripheral blood CD8 T cells were evaluated by flow cytometry. RA patients with established (n=21) and early disease (n=20) were recruited, and compared to twenty four healthy controls. The mean age of the subjects was 59 (SD=12.5), 25/38 (66%) were female, 27/38 (71%) were anti-CCP positive and 25/38 (66%) rheumatoid factor positive. The mean age for the controls was 43 (SD=11.6), 14/20 (70%) were female.
Results: Confirming our previous work, a significantly higher proportion of CD3+CD8+CD28- cells was observed in RA patients compared to healthy individuals (P=0.03) (Figure 1).
Flow cytometric evaluation of peripheral blood demonstrated a significantly higher expression of CD57, CD45Ra and KLRG1 in CD28- compared to CD28+ T cells.

Table 1. CD57, CD45Ra, KLRG1 and CD28 on CD3+CD8+ T cells

	CD28–	CD28+	P value
CD57+	40	9	<0.0001
CD45Ra+	38	23	0.0026
KLRG1+	36	20	0.0012

A further evaluation of these markers revealed that 69% of the CD8+CD28- cell pool was KLRG1+, in comparison to 66% being CD57+ and 55% KLRG1+CD57+ double positive. This suggests that KLRG1 is a robust and clinically relevant marker of CD28- T cells in RA. Our current research is investigating the significance of KLRG1 and CD8+CD28- cells as prognostic markers in RA.

CD3+CD8+CD28- cells in Controls (n=24) v.s RA patients (n=41) with median.

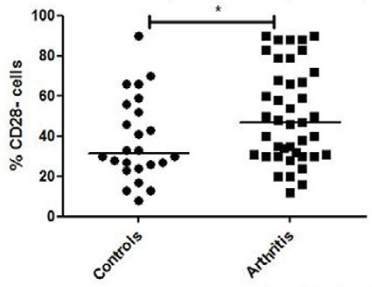

Figure 1: Graph showing the percentage of CD3+CD8+CD28- in RA patients and healthy controls.

Conclusions: CD3+CD8+CD28- cells are enriched in the peripheral blood of RA patients. KLRG1 expression is increased in line with CD57 and CD45Ra in CD8+CD28- cells, and will be used to evaluate the functional significance of these cells in relation to their activation status and potential senescence in the immunoregulation of RA pathogenesis.
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AB0012 IN THE ELDERLY ACPA-NEGATIVE RA IS MORE PREVALENT THAN ACPA-POSITIVE RA WHILE THE COMPOSITION OF THE ACPA-RESPONSE APPEARS IDENTICAL

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Background: Rheumatoid arthritis (RA) consists of two syndromes, one autoantibody-positive and one autoantibody-negative. This multi-cohort study

assessed the age of onset in relation to the presence of autoantibodies. The association with characteristics of the anti-citrullinated protein antibodies (ACPA)-response was also explored.
Objectives: 1) determine the association between age of RA-onset and the presence of ACPA, rheumatoid factor (RF) and anti-carbamylated protein (anti-CarP) antibodies, 2) study if age of onset was associated with characteristics of the ACPA-response, 3) substantiate previously reported associations between age of onset and clinical characteristics.
Methods: 3,321 1987-positive RA-patients included in the Leiden-EAC, BARFOT, ESPOIR, Umeå and Lund cohorts were studied at presentation on age of onset and the presence of ACPA, RF and anti-CarP antibodies. Logistic regression analyses were performed; effect sizes were summarized in inverse-weighted meta-analyses. Within ACPA-positive RA, ACPA-level was studied in all cohorts; ACPA-isotypes, ACPA-fine-specificity and ACPA-avidity index and clinical characteristics were studied in the Leiden-EAC.
Results: From the age of fifty onwards, the proportion of ACPA-negative RA-patients increased in Dutch, Swedish and French cohorts. Similar observations were done for RF and anti-CarP. The composition of the ACPA-response did not change with increasing age of onset with respect to titer, isotype distribution, fine specificity and avidity index. With increasing age of onset RA-patients smoked less often, had higher acute phase reactants and more often a sub(acute) symptom onset.
Conclusions: Data of five cohorts revealed that with higher age of onset ACPA-negative RA is more frequent than ACPA-positive RA, while characteristics of ACPA-positive RA as judged by the composition of the ACPA-response appeared not age-dependent. Although more biologic studies are needed to characterize the pathogenesis of ACPA-negative polyarthritis at older age, the present data can promote personalized treatment decisions in ACPA-negative patients in daily practice.
Disclosure of Interest: None declared
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AB0013 IS THERE IMMUNE DISREGULATION IN NON-SJÖGREN SICCA SYNDROME? A STUDY OF BLOOD LYMPHOCYTE SUBPOPULATIONS

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Background: A large number of patients with sicca syndrome not fulfilling Sjögren's syndrome (SS) classification criteria, present manifestations of autoimmunity, like arthritis, Raynaud's, rash or hematologic disturbances, and have anti-nuclear antibodies, lacking however more specific antibodies. The designation Undifferentiated Connective Tissue Disease was coined to refer to those patients, and some will eventually progress to a definite disease, of which SS would be a likely candidate. Immune cell disturbances could be progression markerr, since diseases like pSS have distinct lymphocyte profiles.
Objectives: We aim to study the circulating lymphocyte subsets in non-Sjögren sicca patients (n-SS), and compare them with pSS and healthy controls.
Methods: We included 65 n-SS patients, 53 pSS patients (2002 AECG criteria) and 22 healthy controls. Lymphocyte subsets were characterized by flow cytometry, including follicular and regulatory T cells and naïve, mature, memory, plasmablasts and regulatory B cells. Statistical analysis was performed with GraphPad, and significance was considered for $p<0.05$
Results: Comparing to controls, n-SS patients had lower counts of T cells (p=0.016), with lower CD4 (p=0.0028), however that difference was not as pronounced as between SS and controls. n-SS patients had higher percentages of CD4 (p=0.0005) and lower CD8 percentages (p=0.0009) than pSS. Additionally, there was a decrease in absolute counts of Tregs (p=0.0028) in n-SS patients compared to controls, which was less pronounced than the comparison between SS and controls (p=0.0008). Th17 cells were decreased in SS compared to controls (p=0.0005), but not in-SS patients. Compared with controls, both n-SS and SS patients presented decreased absolute count (p=0.0001 and p<0,0001, respectively) of $CXCR5^+$ Tfh cells, with no differences between n-SS and SS patients. However, higher levels of $IL21^+CD4$ T cells and Tfh1 cells were found comparing SS patients with both controls (p=0,0209 and p=0,0092 respectively) and n-SS patients (p=0,0051 and 0,0028 respectively).
Absolute counts of memory, unswitched and switched memory cells in n-SS patients present intermediate levels between controls with significantly higher levels, and SS patients with significantly lower levels. Accordingly, using the Bm1–5 classification, we have found decreased Bm1 (p=0.004), eBm5 (Abs, p=0.0273) and Bm5 cells (Abs, p=0.0444) in n-SS patients compared to controls. Though not significant, there was an increase in eBm5 (Abs, p=0.063) and Bm5 cells (Abs, p=0.05) in n-SS compared to SS patients. Again, $CD24^+CD27^+$ Bregs were also decreased in n-SS patients compared to controls (p=0.036), but increased in n-SS compared to SS patients (p=0,0007).
Conclusions: Our data showed that n-SS patients present immune disregulation, represented by alterations in the B cell compartment but also in Tfh subset, known to modulate the humoral immune response. Although less pronounced, these modifications resemble the ones found in SS patients. Wether n-SS is a stage in the evolution to SS remains to be clarified. The identification of a characteristic disregulation of the immune system in n-SS could be usefull for diagnostic and prognostic purposes.
Disclosure of Interest: None declared
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AB0014 DIFFERENTIAL EFFECTS OF A SINGLE DOSE OF CYCLOPHOSPHAMIDE ON CIRCULATING CELL SUBPOPULATIONS IN PATIENTS WITH VASCULITIDES AND AUTOIMMUNE SYSTEMIC DISEASES

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Background: High-dose cyclophosphamide (CFA) is used for treatment of patients with severe manifestations of systemic autoimmune diseases. The knowledge about the effect of CFA on circulating cell populations in these diseases is still limited.
Objectives: To identify the effect of a single dose of CFA on circulating cell populations in patients with vasculitides and autoimmune systemic diseases.
Methods: We immunophenotyped T lymphocytes (CD3, CD4, CD8, CD25, CD127, HLA-DR), B lymphocytes (CD19, CD20, CD27), NK cells (CD3, CD16, CD56, CD69), neutrophils (CD15, CD11b, CD16, CD54, CD62L, CD64), and monocytes (CD11b, CD14, CD16, CD64, HLA-DR) using 6-color cytometer BD FACSCanto II (Becton Dickinson) in peripheral blood of patients with vasculitides (n=6) and systemic disorders of connective tissue (n=6). From each patient, we obtained paired samples before and one month after a single CFA dose. Statistical tests were performed using GraphPad Prism (GraphPad Software, Inc). *P*-value <0.05 was considered as significant.
Results: Single dose of CFA resulted in increase of percentage of CD8+ T lymphocytes (*P*=0.002), leading to marked decrease of CD4+/CD8+ ratio (*P*=0.009). Except three patients, overall percentage of neutrophils decreased after the treatment (*P*=0.01). Although CFA pulse did not influence the percentage of NK cells, the percentage of NK cells carrying stimulatory receptor CD69 increased after CFA (*P*=0.037). Similarly, CFA enhanced the percentage of co-stimulatory molecule CD27 on B lymphocytes (*P*=0.048). Among subsets of monocytes, CFA treatment increased percentage of CD14-CD16+ monocytes (*P*=0.006) and increased expression (MFI) of Fc fragment CD64 (*P*=0.02). Moreover, increase of HLA-DR was observed on CD14-CD16+ monocytes (*P*=0.037). The investigations whether the changes in immune cell subpopulations in treated patients have prognostic potential are ongoing.
Conclusions: Single dose of CFA resulted in increase of CD8+ T lymphocytes, activation of NK, B lymphocytes and monocytes as well as in some patients decrease in neutrophil counts. Further investigation of selected markers may lead to identification of new prognostic markers and predict the effectiveness of the treatment.
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AB0015 CAPTURE OF IGA IMMUNE COMPLEXES AND ENRICHMENT IN IGA IG GENE EXPRESSION BOTH SUGGEST A ROLE FOR FCRL4+ B CELLS IN THE LINK BETWEEN MUCOSAL AND JOINT INFLAMMATION

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Background: Increasing evidence points to the autoimmune process of rheumatoid arthritis (RA) originating at mucosal surfaces. Previous work from our group described a subset of B cells in the synovium and synovial fluid (SF) of RA patients which can be distinguished by their expression of the Fc-like receptor 4 (FcRL4) and elevated expression of RANKL, indicating a unique pathogenic function[1,2]. B cells expressing FcRL4 were originally described as a distinct memory B cell subset in human tonsils where they accumulate in the epithelium[3]. We have recently shown that they are enriched in cells recognizing citrullinated autoantigens (Amara, K. et al. under revision). Recent *in vitro* work suggested that FcRL4 is a low affinity receptor for aggregated IgA[4].
Objectives: 1) To investigate the interaction of RA synovial fluid FCRL4+ B cells with IgA
2) To examine the distribution of Ig classes by flow cytometry and PCR
Methods: SF mononuclear cells were isolated, labelled for FcRL4, IgA and CD19 and analysed by flow cytometry. In experiments identifying IgA B cell receptors, SF mononuclear cells were briefly incubated in an acidic buffer to remove surface receptor bound antibodies before staining. Heat-aggregated purified human IgA