AN OFFICIAL JOURNAL OF THE AMERICAN COLLEGE OF RHEUMATOLOGY

Arthritis & Rheumatism

VOLUME 63, NUMBER 10 (SUPPLEMENT) OCTOBER 2011

ABSTRACT SUPPLEMENT

2011 ANNUAL SCIENTIFIC MEETING

November 4–9, 2011
Chicago, Illinois

AMERICAN COLLEGE OF RHEUMATOLOGY
EDUCATION • TREATMENT • RESEARCH

WILEY-BLACKWELL

multi-color flow cytometry. Mitotracker Green extrusion and the staining of CD19, IgD, CD27, CD38, CD24 as well as CD3/live/dead exclusion allow the identification of B cell subsets defined by the expression of IgD and CD27 and the fine discrimination of naïve and transitional B cells. P-values were calculated using the non-parametric Wilcoxon signed rank test for paired samples. To account for the 8 independent hypotheses tested, a Bonferroni adjusted threshold was used for statistical significance ($p<0.006$).

Results: In these patients, $CD19^+$ B cells remained unaffected after 2 months of treatment with fostamatinib. In addition, the fractions of the four core subsets defined by IgD/CD27 within the B cell population remained relatively stable. However, the early transitional (T1/T2) cells were rapidly depleted within the first month of treatment ($p=0.0029$) and continued to decline over the second month ($p=0.0039$). There was also a significant but lesser magnitude decrease in the late transitional (T3) cells at 2 months ($p=0.02$), with a concomitant increase in the naïve population ($p=0.0059$). The decrease in the T1/T2 population was also observed when absolute cell counts for the B cell subsets were calculated ($p=0.0039$). No association was identified between infections or clinical responses and the degree of transitional B cell depletion.

Conclusion: We demonstrate that short-term use of fostamatinib impairs B lymphocyte development at the transitional B cell stage without affecting mature B cell populations. These results provide mechanistic insights of action of syk inhibition in autoimmune diseases. Further, it suggests that prolonged administration may impair development of mature B cells and ultimately functioning of the humoral immune system. As development of syk inhibitors and other agents targeting molecules downstream of the B cell receptor proceeds, careful monitoring for infectious complications is warranted.

1745

Carbamoylation of Vimentin in Patients with Rheumatoid Arthritis: Identification of a Novel Protein Modification with a Possible Link to Disease Pathogenesis. Holger Bang[1], Karl Egerer[2], Anette Krämer[1], Eugen Feist[2] and Gerd R. Burmester[2]. [1]Orgentec Diagnostika GmbH, Mainz, Germany, [2]Charité - Universitätsmedizin Berlin, Berlin, Germany

Background/Purpose: The pathogenesis of rheumatoid arthritis (RA) is believed to result from a multistep process, in which - based on a particular genetic background—environmental triggers initiate or modify specific immune reactions including anti-citrullinated peptide/protein antibodies (ACPA) generation. Especially, smoking has been identified as an important environmental factor potentially leading to protein modification resulting in an autoimmune response in the setting of a disturbed immune system. The aim of the current study was to investigate the possible role of a newly identified modification process, the carbamoylation of vimentin, in causing autoantibody production and immune responses in patients and animal models.

Methods: Human vimentin was carbamoylated and/or citrullinated by non-enzymatic and enzymatic posttranslational modifications. Corresponding ELISA assays were established and sera from human healthy individuals, RA patients in different stages of disease and with different DMARD treatments and disease controls (systemic autoimmune diseases such as SLE and Sjören′ syndrome) were analyzed. Rabbits were immunized with carbamoylated vimentin and mutated citrullinated vimentin (MCV), and antisera were tested for binding to cyclic citrullinated peptides (CCP), vimentin and its modified analogs. Induction of rheumatoid factor in treated animals was characterized as well. Cell models were used to identify pathways for induction of modified vimentin under inflammatory conditions.

Results: Carbamoylated vimentin (carbVim) shows a > 90% homocitrulline content in double mass spectrometry and sequencing analysis. Rabbits immunized with carbVim produced high affinity antibodies against carbVim and, to a lesser extent, against MCV. These antisera also bound human IgG and to a low extent immunoglobulin from other animal species. In 110 RA patients, sensitivities of 86,8 % and 69,2 % were calculated for the anti-MCV and anti-carbVim assays, respectively. Sera from healthy persons and disease control groups showed a comparable specificity for both assays (anti-MCV 97% and anti-carbVim 91%) and none had anti-vimentin abs. A significant correlation of ACPA positivity lacking rheumatoid factor with presence of anti-carbVim abs was documented in early RA patients (91 %). In cell models, modified vimentin was generated among inflammatory (TNF or IFN) or environment stress (hydrogen peroxide) conditions and cross react with abs from RA-patients or immunized animals.

Conclusion: Environmental triggers such as smoking together with inflammatory conditions involving destructive enzymes may result in carbamoylation of vimentin with a possible impact on its function as stress fiber, cell homing or adhesion protein. Anti-homocitrulline responses develop in the early stages of RA, where carbamoylated vimentin represents a major target of autoantibodies in RA patients. These results also suggest a critical reevaluation of the polyclonal immune response of RA patients, so far described as exclusively citrulline specific and may provide diagnostic options for the early detection of patients even before clinical onset of RA.

1746

Early Rheumatoid Arthritis (ERA) Has Lower Levels of Plasmablasts and Memory B Cells Compared to Long-Standing Rheumatoid Arthritis (LSRA) and Responds to Conventional Therapy with a Normalization of B Cell Subsets Abnormalities. Anna Laura Fedele, Barbara Tolusso, Silvia L. Bosello, Silvia Canestri, Elisa Gremese and Gianfranco Ferraccioli. Division of Rheumatology, Catholic University of the Sacred Heart, Rome, Italy

Background/Purpose: B cells have been shown to be important players in RA chronicity and B cell depletion has been shown to be effective in RF seropositive patients. The aim of the study was to evaluate B cells subpopulations distribution in ERA and LSRA patients and their possible association with clinical or immunological data at baseline or with response to conventional therapy (ERA).

Methods: 53 ERA (88.7% females; mean age 53.2±15.1 years; 62% anti-CCP positive) and 49 LSRA, along with 30 healthy controls were studied. Baseline clinical and immunological characteristics and inflammatory status were assessed. Peripheral blood samples were analyzed by flow cytometry for the distribution of circulating B cell subsets by staining with surface markers CD19, CD45, CD38, CD27 and IgD and intracellular marker ZAP70. Plasma levels of IL-6 and BAFF were also determined with ELISAs. 22 ERA patients were followed for 6 months: they were treated with MTX (n=14) and MTX + TNF blockers (n=8).

Results: ERA patients showed an higher percentage of Bm2+Bm2' cells (48.8±20.9%) compared to LSRA patients (33.8±17.8%, *p=0.001*) and a lower percentage of eBm5 (10.0±7.3%) compared to LSRA (14.1±8.6%, *p=0.004*) and controls (16.0±7.1%, *p<0.001*). The percentage of CD19+/IgD-CD27- cells (7.5±4.8%) and CD19+/CD38+CD27+ cells (3.0±4.4%) was lower in ERA compared to LRSA (13.7±7.8%, *p<0.001*; 8.2±5.2%, *p<0.001*, respectively) and controls (16.2±9.1%, *p<0.001*; 8.0±3.5%, *p<0.001*, respectively). The percentage of CD19+/ZAP70+ cells (6.0±7.1%) was higher in ERA patients compared with controls (2.2±1.4%, *p=0.01*), while no difference was seen between ERA and LSRA. There were no differences in the distribution of B cell subpopulations between patients RF and anti-CCP positive and negative. ERA patients with baseline high DAS (>3.7) showed an higher percentage of CD19+/CD27+CD38+ cells compared to subjects with moderate DAS (*p=0.01*). The percentage of CD19+/ZAP70+ cells was higher in ERA patients with baseline plasma levels of BAFF>780 pg/ml (mean±2 SD of controls) (8.0±8.5%) compared to patients with levels of BAFF <780 pg/ml (3.0±2.3%, *p=0.08*). Plasma levels of BAFF correlated positively with percentage of CD19+/ZAP70+ cells (r=0.41, *p=0.002*) and of CD19+/IgD-CD27- cells (r=0.26, *p=0.05*). In ERA patients followed for 6 months, we observed DAS falling from 3.4 to 1.6 (*p<0.001*), an increased percentage of Bm1 cells (T0=21.4±17.8% vs T6=32.5±19.2%, *p=0.005*) and a fall in the percentage of eBm5 (T0=9.9±3.8% vs T6=6.5±4.7%, *p=0.01*) and of CD19+/ZAP70+ cells (T0=6.7±5.6% vs T6=3.8±3.1%, *p=0.02*), irrespectively of the type of therapy administered.

Conclusion: ERA differs from LSRA for higher levels of naive preswitch B cells, lower levels of memory B cells and plasmablasts. After six months of conventional treatment, a fall of memory B cells and ZAP70+ B cells and an increase of naïve B cells were observed. These results suggest that changing the inflammatory milieu in the early phases of the disease, leads to substantial changes in B cell subsets. Further studies are needed to define the molecular events linked to these effects. Early RA represents the best opportunity to normalize the immunological setting.

1747

Identification of Autoantibody Profiles by Monitoring Autoantibody Biomarkers in Rheumatoid Arthritis with Microarray Surface Plasmon Resonance Imaging. Joyce J.B.C. Van Beers[1], Angelique M.C. Segbers-Lokate[1], Wilma T.M. Vree Egberts[1], Richard B.M. Schasfoort[2] and Ger J.M. Pruijn[1]. [1]Radboud University Nijmegen, Nijmegen, Netherlands, [2]University of Twente and IBIS Technologies BV, Enschede, Netherlands

Background/Purpose: Autoantibodies against citrullinated proteins (ACPA) are specifically found in approximately 75% of rheumatoid arthritis (RA) patients. Citrullination is the post-translational conversion

of peptidylarginine into peptidylcitrulline, which is catalyzed by peptidylarginine deiminase (PAD) in a calcium-dependent manner. Several citrullinated antigens have been identified in the inflamed joints of RA patients. These include fibrinogen, alpha-enolase, vimentin and collagen type II. Accumulating evidence suggests a role of citrullinated proteins and ACPA in the pathophysiology of RA. The results of many studies indicate that the ACPA response is highly heterogeneous with diverse patterns of reactivity to distinct citrullinated epitopes. This study aimed to identify clinically meaningful ACPA profiles in RA patients using a microarray containing different citrullinated peptides in combination with surface plasmon resonance imaging (*i*SPR).

Methods: Several pairs of synthetic peptides (citrullinated and the corresponding non-citrullinated control) derived from known ACPA targets (e.g. fibrinogen, alpha-enolase, vimentin) as well as peptides isolated from synthetic citrullinated peptide libraries were used to generate ACPA target arrays. ACPA in RA patient sera were monitored by *i*SPR, which allows the simultaneous detection of autoantibody-peptide interactions in real-time. The present study was started using a 24-spot microarray and currently peptides are spotted using a continuous flow microspotter resulting in a 48-spot microarray.

Results: Using the 24-spot microarray, a total of 94 RA and 46 control sera were analyzed. The results confirmed the heterogeneous nature of ACPA in RA sera. RA patients displayed different patterns of co-occurrence of autoantibodies directed to distinct citrullinated peptides. The recognition of one peptide was very specific for RA and was observed in 62% of the anti-CCP2 positive RA patients. No reactivity was observed in the anti-CCP2 negative patients and a weak reactivity (2%) was observed in control patients. Another peptide showed reactivity in 68% of the RA patients, both anti-CCP2 positive (74%) as anti-CCP2 negative (54%) patients, whereas patients with other autoimmune diseases showed far less reactivity (13%).The median number of citrullinated peptides recognized by anti-CCP2 positive RA patients (4) was significantly higher than that of anti-CCP2 negative RA patients (1) and disease controls (1). The use of continuous flow microspotting instead of non-contact spotting is being optimized to increase the array size and to improve the quality and reproducibility of the microarrays.

Conclusion: Using microarray-iSPR we have shown that RA sera recognize various citrullinated peptides more frequently than other autoimmune disease sera. Sera from different RA patients frequently recognize different citrullinated peptides. Our data are consistent with the existence of different ACPA profiles that may have diagnostic and/or prognostic value. Microarray-*i*SPR represents a suitable system for multiplex autoantibody monitoring and allows the identification ACPA profiles.

1748

Development of a High-Throughput, Multiplex Assay for Profiling the Autoantibody Fine Specificity in Rheumatoid Arthritis. Xiaoyan Zhao[1], P. Scott Eastman[1], Ferhan Qureshi[1], William C. Manning[1], William Robinson[2] and Lyndal K. Hesterberg[1]. [1]Crescendo Bioscience, Inc., South San Francisco, CA, [2]Stanford Univ School of Med, Stanford, CA

Background/Purpose: Rheumatoid Arthritis (RA) is an aggressive autoimmune disease that progressively destroys affected joints with frequent systemic complications. Production of autoantibodies, especially those against citrullinated proteins and peptides, is a hallmark of RA. The causal relationship between the development of autoantibodies and radiographic joint damage to this point remains unclear. To better understand this relationship, we developed autoantibody assays in a multiplex format against a panel of common and novel RA epitopes and applied the assays for profiling the fine specificity of these autoantibodies in RA.

Methods: Individual serum samples from 35 RA patients with different disease activities were evaluated for the presence of autoantibodies. Anti-cyclic citrullinated peptide (CCP) reactivity and rheumatoid factor (RF) status were assessed with commercial kits from Euro Diagnostica (CCP 2) and TheraTest Labs (RF). The reactivity of a panel of peptides derived from multiple proteins including both well known and novel antigens was also evaluated. Nine peptides were printed in a 3×3 grid on the bottom of a 96-well plate (Quansys Bioscience) and probed with RA patient samples. HRP-conjugated secondary antibody against human IgG, IgM or IgA was used to measure autoantibodies to specific peptides in a chemiluminescent format.

Results: The levels of anti-CCP antibodies ranged from >5,000 to below the cutoff (<25 arbitrary units) for CCP and from ~600 to 3 units for RF-IgA, suggesting a range of disease. Not surprisingly, levels of autoantibodies, when detected by anti-human IgG, reflected anti-CCP levels, predominantly to citrullinated fibrinogen and citrullinated filaggrin peptides. However, a wide range of anti-IgM/A responses were observed regardless of whether anti-CCP levels were high, intermediate or low. Interestingly, when anti-human IgA or IgM was used as detection, CCP negative and low CCP RA subjects frequently demonstrated strong positive reactivity to epitopes derived from citrullinated apoliporotein, citrullinated biglycan, native histone and/or native fibromodulin. While several of the IgM/A peptide reactivities overlapped with the IgG, in many instances the IgM and/or the IgA profiles demonstrated unique response patterns.

Conclusion: A high-throughput, multiplex assay has been developed to investigate the fine specificity of autoantibody reactivity against a broad variety of RA antigens including both citrullinated and native peptides. While anti-human IgG profiles reflected CCP levels, diverse levels of response were observed with anti-IgM and/or anti-IgA, including at low and intermediate CCP levels. Most studies to date have employed the CCP 2 assay, which uses a mixture of peptides, with mixed results. We observed that profiles of individual peptides may be different with different immunoglobulin isotypes. Thus evaluation of individual peptides in the context of immunoglobulin subtypes may provide insight into disease progression.

1749

Anti-hnRNP Autoantibodies Detected in Inflammatory Rheumatic Diseases in Use to Close the Sensitivity gap left by Rheumatoid Factor and Anti CCP in Early Rheumatoid Arthritis. Bianka Marklein[1], Zoltan Konthur[2], Gerd-Rüdiger Burmester[3] and Karl Skriner[1]. [1]Charité - Universitätsmedizin Berlin, Berlin, Germany, [2]Max Planck Institute for Molecular Genetics, Berlin, Germany, [3]Charité - University Medicine Berlin, Berlin, Germany

Background/Purpose: Autoantigens are produced in bacteria ex-pressed purified using His-tag and Cm-sepharose under native and denaturated conditions. Bacterially expressed recombinant hnRNPs proteins were used in Elisa for confirming the data obtained by macroarray and immunoblotting. Anti-hnRNP-A/B and hnRNP D proteins were detected in a newly developed Elisa with patient sera and sera from animal model of SLE and RA

Methods: Autoantigens are produced in bacteria ex-pressed purified using His-tag and Cm-sepharose under native and denaturated conditions. Bacterially expressed recombinant hnRNPs proteins were used in Elisa for confirming the data obtained by macroarray and immunoblotting. Anti-hnRNP-A/B and hnRNP D proteins were detected in a newly developed Elisa with patient sera and sera from animal model of SLE and RA.

Results: Using a combination of three hnRNPs A2/D/DL, 76% of RA, 84% of SLE sera and 87% SKG mice which spontaneously develop chronic autoimmune arthritis, can be detected. Moreover hnRNP A2/A3/D/DL identified epitopes as well as identified citrullinated peptides (deduced citrullinated peptides thereof) were used to identify patterns associated with disease severity. No crossreactivity could be detected between the affinity purified anti-hnRNPDL and the highly related hnRNPD. A unique sequenz only found in hnRNP DL between aminoacid 81–120 was identified as indistinguishable for autoantibody binding. With citrullinated peptides out of a mutated form of hnRNPA3 and hnRNPA2/D/DL, 94 % out of 130 early RA sera can be identified but only in <10% of osteoarthritis and healthy control patients. With a combination of citrullinated forms of three hnRNPs (A2, D, DL) 98 % of the sera were tested positive in a cohort of 92 early RA patients (<12month). The hnRNP auoantibody response is dependent on Mod88, Tir8 and both TLR 7 and TLR9 costimulation tested with sera from TLR7, TLR9 deficient and double-deficient mice with an MRL-lpr/lpr background.

Conclusion: The hnRNP antibody response is Myd88, Toll 7 and 9 dependent generated. A combination of hnRNPs (A3/A2/DL/D) can be used to predict disease severity and partially close the sensitivity gap left by rheumatoid factor and anti CCP antibodies in early RA patients.

1750

Substantial Influence of Rheumatoid Factor Positivity On the Peripheral Memory B Cells and Its Modulation by TNF Inhibition In Rheumatoid Arthritis. Petra Roll[1], Khalid Muhammad[1], Mathias Schumann[1], Stefan Kleinert[2] and Hans-Peter Tony[1]. [1]University of Würzburg, Würzburg, Germany, [2]Rheumatology, University of Würzburg, Würzburg, Germany

Background/Purpose: The role of B cells has been appreciated with the advent of B cell targeted therapies in patients with rheumatoid arthritis. However, alterations of peripheral B cell subsets have been described also under TNF inhibition. In this study, we focused on the influence of