T01.03

TNF polymorphisms in psoriasis: Association of psoriatic arthritis with the promoter polymorphism TNF-857 independent of the PSORS1 risk allele

Burkhardt H.[1], Reich K.[2], Hüffmeier U.[3], Lohmann J.[4], König I.[5], Wendler J.[6], Lascorz J.[3], Reis A.[3]
[1]Abteilung Rheumatologie, Johann Wolfgang Goethe Universität Frankfurt; [2]Dermatologikum Hamburg; [3]Institut für Humangenetik, Universitätsklinikum Erlangen-Nürnberg;
[4]Psoriasisfachklinik Bad Bentheim; [5]Institut für Medizinische Biometrie und Statistik, Universität Lübeck; [6]Rheumatologische Schwerpunktpraxis Erlangen

Objective: Single nucleotide polymorphisms (SNPs) of TNF gene at positions -238 and -308 have earlier been associated with psoriasis vulgaris and psoriatic arthritis (PsA). The strong linkage disequilibrium (LD) at chromosomal region 6p21 – a region known to harbour also other risk factors for psoriasis (PSORS1) than SNPs of TNF gene - renders the interpretation of these findings difficult. Therefore, in this study several SNPs of TNF gene and of its neighbouring LTA gene were analyzed independently and dependently on carrying the PSORS1 risk allele.
Material and methods: SNPs in the promoter of TNF gene (-238G/A, -308G/A, -857C/T, -1031T/C), one of LTA gene (+252A/G), of TNFRSF1A gene (+36A/G) and of TNFRSF1B gene (+676T/G), respectively, were genotyped in 375 Psoriasis-patients, 375 PsA-patients, and 376 controls. The tryptophan-tryptophan-cysteine-cysteine haplotype of the CCHCR1 gene (CCHCR1*WWCC) was used to estimate the PSORS1 risk allele.
Results: Whereas the earlier described strong association of allele TNF*-238A with psoriasis could be confirmed, our study revealed that this association was completely dependent on carrying the PSORS1 risk allele. For PsA, but not psoriasis vulgaris without joint involvement strong association with the allele TNF*-857T was detected (OR=1.956, 95% CI 1.33-2.88; pcorr=0.0025) also in patients negative for the PSORS1 risk allele.
Conclusions: Our results indicate genetic differences between psoriasis vulgaris patients with and without joint manifestation. While the previously reported association between TNF*-238A and psoriasis seems to primarily reflect LD with PSORS1, TNF*-857T may represent a risk factor for PsA independent of PSORS1.

T01.04

UVB and TNF-a induce Ro/SSA52 autoantigen on the cell surface of Non-apoptotic human keratinocytes which leads to enhanced anti-Ro/SSA52 dependent cell cytotoxicity

Hostmann B.[1], Ruppert V.[1], Grossmann P.[1], Hausdorf G.[1], Aderajew W.[1], Johnen C.[2], Bräutigam K.[2], Rosowski M.[3], Radbruch A.[3], Hiepe F.[1]
[1]Klinik für Rheumatologie und Klinische Immunologie, Universitätsmedizin Charité, Berlin; [2]Klinik für Transplantations- und Wiederherstellungschirurgie, Universitätsmedizin Charité; [3]Deutsches Rheumaforschungszentrum (DRFZ)

Autoantibodies directed to the intracellular antigen Ro/SSA are strongly associated with photosensitive skin manifestations in lupus erythematosus suggesting their direct involvement in the pathogenesis. This is supported by the deposition of anti-Ro/SSA in the skin and by the damage of keratinocytes via ADCC and complement dependent lysis. It has been assumed that the pathology is caused by antibody binding to Ro/SSA that is translocated on the surface of apoptotic keratinocytes induced by different stress factors. Since a direct access of antibodies to autoantigens in the apoptotic blebs seems to be restricted, we assume a Ro/SSA surface expression which is independent on apoptosis.
The surface expression of Ro/SSA52 on cultured viable keratinocytes was analyzed by FACS and confocal laser scanning microscopy using purified anti-Ro/SSA52-IgG and a detection technique using fluorescent liposomes before and after UVB irradiation and TNFa treatment. The functional relevance of the membrane expression was verified by antibody-dependent cytotoxicity assay (ADCC). UVB exposure and TNFa led to surface expression of Ro/SSA52 in non-apoptotic keratinocytes, but not in annexin V positive cells. The cell surface staining was inhibited by Ro/SSA52 proteins.The Ro/SSA52 membrane expression of was associated with an enhancement of the PBMC-mediated, anti-Ro/SSA52-dependent cytotoxicity in keratinocytes.
This is the first clear description that Ro/SSA52 appear on the surface of viable keratinocytes after exposure to UVB or TNFa. The surface expression pattern of the Ro/SSA52 on viable keratinocytes suggests that an apoptosis-independent mechanism may contribute to the development of autoantibody-mediated photosensitive skin manifestations in lupus and to the enhancement of autoimmune responses.

T01.05

Circulating CD22+CD20-CD19+ Cells At Baseline May Predict The Clinical Response To Rituximab In Patients With Active RA.

Owczarczyk K.[1], Passon D.[1], Roehrs T.[1], Jung N.[1], Taubert D.[1], Valet G.[2], Rubbert A.[1]
[1]University Hospital Cologne, Cologne; [2]Max-Planck Institute of Biochemistry, Munich-Martinsried

Background: Full B cell depletion (BCD) as determined by conventional flow cytometry is achieved in nearly all patients with RA undergoing rituximab (RTX) treatment. B cell persistence as detected by minimal residual disease flow cytometry (MRD-FACS) has recently been linked to an unfavourable response to RTX therapy. Since RTX is highly effective in depleting CD20+ B cells it is feasible that the remaining cells are mainly of CD20dim/negative phenotype, possibly with circulating plasma cell precursor (CPCP) phenotype.
Objectives: We used high sensitivity flow cytometry to define various B cell subsets at baseline prior to treatment with RTX in order to determine whether baseline characteristics of B cell phenotype may predict clinical outcome. BCD (defined as < 5 residual B cells/ul blood) at week 16 was assessed in all patients.
Material and methods: Baseline B cell characteristics were analyzed in n=26 patients with active RA who received 2x 1g RTX on Days 1 and 15 and completed at least 6 months of follow-up. Flow cytometric analysis based on 200-500,000 events was performed at baseline, week 4, 16 and 24. Clinical response was evaluated using DAS28 and EULAR scores. The CLASSIF1 nonparametric data sieving algorithm was used to determine the most discriminatory data pattern of 27 baseline characteristics1.
Results: 19 of 26 patients were classified as RTX (S)ensitive (DAS28 improvement ≥1.2 i.e. good-to-moderate EULAR response) and 7 as RTX (R)esistant. Of the 26 patients analyzed, all but one achieved an effective BCD. No statistically significant differences between the S and R cohort were observed with regard to baseline WBC, lymphocyte, CD19, CD20, CD27 frequency and absolute counts, ESR, CRP, RF positivity, comedication, disease duration, patient age and total IgG. Interestingly, both the absolute number and frequency of CD22+CD20-CD19+cells at baseline were significantly higher in the RTX resistant group ($p<0.0025$ and $p<0.01$ respectively) and inversely correlated to DAS28 change at week 16 (Pearson coefficient $r=-0.52$ $p<0.01$ and $r=-0.42$ $p<0.05$, respectively). Using CLASSIF1 algorithm we could show that the quantification of this B cell subset provided predictive values for both therapy S and R subjects of 90.5% and 100%, respectively.
In linear analysis, the frequency of CD22+ cells at week 16 was significantly higher in the R group ($p<0.025$).
Conclusions: In summary, our data suggest that patients who do not respond to RTX are characterized by a higher number of CD22+CD20-CD19+ B cells at baseline. Based on these results we believe that further trials are warranted to determine the predictive value of this B cell subset in regard to the clinical outcome of RTX therapy.