Objectives To determine Rap1 activation and its correlation with oxidative stress in T cells from healthy controls compared to peripheral blood (PB) and SF T cells isolated from patients with RA.

Methods T cells from healthy donors and PB and SF from RA patients were isolated and 5 min after stimulation with either CD3 antibodies or PMA+ionomycin, whole cell lysates were prepared in Ral lysis buffer. GTP-bound Rap1 was precipitated using the bacterial expressed fusion protein GST-RalGDS and detected by ECL Western blotting. In whole cell lysates, total Rap1, RapGAP and Spa1 were detected by ECL western blotting as well.

Results Our data show that in T cells isolated from healthy controls, Rap1 activation was sensitive to redox balance alterations. Upregulation of intracellular GSH by incubation with 10 mM NAC for 48 h resulted in diminished Rap1 activation, while depletion of GSH by pre-incubation with 200mM BSO resulted in increased Rap1 activation. We also observed that treatment of T cells with H₂O₂ led to the rapid activation of Rap1.

Despite an environment of oxidative stress in the inflamed joints of RA patients, we found that in SF T cells Rap1 was present in its inactive GDP-bound state. Furthermore, while the PB T cells of RA patients could be normally activated, Rap1 remained inactive in SF T cells upon stimulation. The defective Rap1 activation was not due to increased levels of the Rap1 GTPase activating proteins RapGAP or spa1. While restoration of the intracellular redox balance seems to reverse the hypo responsiveness of the SF T cells, this had no effect on the defective rap1 activation.

Conclusion

- Rap1 activation is defective in SF T cells from RA patients, and cannot be restored by the replenishment of GSH with NAC.
- This defective activation is not due to increased levels of the Rap1 GTPase activating proteins RapGAP or Spa1.

THU0022 KIR3DL1 (NKB1) AND CD94 EXPRESSION ON PERIPHERAL BLOOD T CELLS, NK CELLS AND NKT CELLS IN BEHCET'S DISEASE

¹G Saruhan Direskeneli, ¹FA Uyar, ²A Cefle, ²S Celebi Onder, ²M Inanc, ²L Ocal, ²A Gul. ¹Department of Physiology, ²Department of Internal Medicine, Division of Rheumatology, Istanbul Faculty of Medicine, University of Istanbul, Istanbul, Turkey

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Background Behcet's disease (BD) is strongly associated with HLA-B51. However, the role of HLA-B51 in the pathogenesis still remains to be elucidated. We have recently identified a weak association of HLA-B*2702 with BD. HLA-B51 and B*2702 shares one of the 5 Bw4 motifs at positions 77–83, which interacts with a group of recently described receptors on mainly NK cells, but also CD8⁺ and gamma-delta T cells. Interaction of HLA-B molecules with one of these receptors, killer immunoglobulin-like receptor (KIR) on NK, NKT, CD8⁺ and/or gamma/delta T cells may be one of the pathogenic mechanisms involved in BD.

Objectives We aimed to investigate the KIR3DL1 and CD94 (a C-type lectin receptor which mainly interacts with HLA-E) expression in BD.

Methods The study group consisted of 51 patients, who fulfil the International Study Group Criteria, and 32 HLA-Bw4 motif matched healthy controls. All patients and controls were already HLA-typed, and they were selected and grouped according to

the presence and number of the shared Bw4 epitope. All of the patients were receiving treatment and their disease activity was under control. Expression patterns of CD3, CD16, CD56, CD45, CD14, KIR3DL1 (NKB1) and CD94 in peripheral blood leukocytes were determined with fluorescent-labelled monoclonal antibodies using flow cytometry.

Results The percentage of CD3⁺ cells was found to be increased in BD patients, but there was no significant difference in NK (CD56⁺/CD16⁺, CD3⁻/CD56⁺) and NKT (CD3⁺/CD56⁺) cells between BD patients and controls. The distribution of KIR3DL1 receptor did not show any significant difference in patients, however we observed a significant increase of CD94 expression in T cells, NK and NKT cells. Subgroup analysis in patients carrying 1, 2 or no copies of the shared Bw4 motif also did not reveal any effect on the expression of KIR3DL1 and CD94.

Conclusion We did not observe any difference in the expression of KIR3DL1 receptors in this group of BD patients compared with healthy HLA-Bw4 motif matched controls. Increased CD94 expression in peripheral blood T cells, NK and NKT cells of BD patients needs to be confirmed and analysed further.

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THU0023 ANTIGEN INDEPENDENT GENERATION OF IMMUNOMODULATORY TH2 CELLS IS REGULATED BY MAP KINASE ACTIVATION AND IL-4

¹A Skapenko, ²PE Lipsky, ¹JR Kalden, ¹H Schulze-Koops. ¹Department of Internal Medicine III, University Erlangen-Nuremberg, Erlangen, Germany; ²NIAMS, NIH, Bethesda, USA

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Background Evidence suggests that human autoimmune diseases might be driven by pro-inflammatory Th1 cells whereas immuno-modulatory Th2 cells are rarely found. The mechanisms regulating Th2 cell differentiation from resting human T cells, however, are not completely characterised.

Objectives To delineate the mechanisms controlling Th2 differentiation, we defined optimal Th2 inducing conditions in human naive and memory CD4^{pos} T cells *in vitro* and investigated activation of intracellular signalling pathways and their individual role in Th2 differentiation.

Methods A cell culture system was employed that permitted Th2 cell differentiation after short term priming. The phenotype of freshly isolated and primed T cells was determined by cytometric analysis of intracellular cytokines. Activation of intracellular signalling pathways was assessed by western blot analysis or kinase activity assays.

Results In the absence of TCR ligation, stimulation of CD28 induced Th2 differentiation from purified CD4^{pos} peripheral blood memory but not from cord blood naive T cells. Co-stimulation via CD28 and the TCR, on the other hand, enhanced Th2 cell differentiation from naive T cells but suppressed it from memory T cells. Comparison of naive and memory T cell responses to CD28 stimulation, therefore, revealed critical signals facilitating Th2 cell differentiation. CD28 engagement initiated IL-4 gene transcription and activation of p38 MAP kinase in memory T cells but not in naive cells. By contrast, PI3-kinase and JNK/SAPK pathways were activated in both subsets. Whereas IL-4 had a synergistic effect with anti-CD28 on the generation of Th2 cells in memory T cells, it was not sufficient to complement CD28 signals in naive T cells. T cell differentiation in the presence of specific inhibitors to IL-4, the MAP