in all or a particular subset of SSc patients. In contrast, IL-17 itself was not detected in the majority of SSc patients. Discussion: The combination of IL-17, IFNγ and TGFβ levels in CD45Ro and CD45Ra cells from SSc patients is useful to distinguish between lSSc, ldSSc or edSSc. Blocking Th17 inducing cytokines such as IL-6 and IL-23 may provide a useful tool to intervene in the progression of SSc.

doi:10.1016/j.clim.2009.03.368

F.117. Are HLA-E Restricted CD8+ T Cells Functionally Altered in Multiple Sclerosis?

Kim Pannemans[1], An Goris[2], Rita Dobosi[2], Benedicte Dubois[2], Bart Van Wijmeersch[1], P. Stinissen[1], Niels Hellings[1]. [1]Hasselt University and Transnationale Universiteit Limburg, Diepenbeek, Belgium; [2]University Hospital Gasthuisberg, Leuven, Belgium

Qa-1 restricted CD8+ regulatory T cells suppress autoreactive CD4+ T cells in the animal model for multiple sclerosis (MS). This study aims at elucidating the involvement of CD8+ T cells restricted by HLA-E, the human variant of Qa-1, in immunoregulatory alterations in MS. In a first part, an MS association study of HLA-E polymorphisms was performed. The HLA-E gene harbors two polymorphic sites, codon 107 (HLA-E*0101 versus HLA-E*0103) and codon 77 (HLA-E*010301 versus HLA-E*010302). These polymorphisms were studied in 1078 HC and 832 MS patients. Plink option analysis showed a significant higher presence of HLA-E*010301 and lower frequency of HLA-E*0101 in MS patients ($p = 0.04$). Conditioning on DRB1*1501 revealed that this HLA-E association actually reflected the effect of DRB1*1501 on the development of MS. Although no genetic association was found, functional alterations in HLA-E restricted CD8+ T cells could still be involved in the dysbalanced autoimmune responses observed in MS patients. Therefore, HLA-E restricted CD8+ T cells are characterised at the level of their phenotype and regulatory potential. First, the phenotype of both NKG2C+ and NKG2A+CD8+ T cells was studied using flow cytometry. The frequency of NKG2A+CD8+ T cells was not significantly different between HC ($n = 10$, 6.9 ± 3.2 %) and MS patients ($n = 10$, 8.7 ± 3.2 %). The same could be concluded for NKG2C+CD8+ T cells (HC: 3.82 ± 2.1 %, MS: 3.32 ± 1.0 %). The percentage of CD28+ T cells within both the NKG2C+ and NKG2A+CD8+ T cell fraction is declined in MS patients compared to HC. The functional consequence of this observation is currently investigated in coculture experiments with autoreactive T cells and these CD8+ subsets. Samples will be subdivided according to their HLA-E genotype. This study could lead to new insights in the pathogenesis of MS.

doi:10.1016/j.clim.2009.03.369

F.118. Association of Impaired NK Cell Activity with Imbalance in CD3-CD16+ Bright and Dim Subset Distribution and Receptor Expression in Metastatic Melanoma Patients

Gordana Konjevic[2], Katarina Mirjačić Martinović[1], Ana Vuletic[1], Nada Babovic[1]. [1]Institute for Oncology and Radiology of Serbia, Belgrade, Serbia; [2]School of Medicine, University of Belgrade, Beograde, Serbia

As we show that NK cell activity in metastatic melanoma (MM) patients prior to therapy is impaired the aim of this study was to investigate the expression of a set of NK cell receptors and activation markers on the two functionally distinct, regulatory CD16dim and cytotoxic CD16bright NK subsets. PBL of MM and controls were analyzed for NK cell activity, percentage of CD3-CD16 dim and bright subsets, expression of CD107a, NKG2D, CD161, CD158a and CD158b on NK cell and intracellular production of IFN-γ, as well as the expression, after *in vitro* 4 h co-culture of PBL with tumor cell lines, of CD161 and CD158a on these subsets by Flow cytometry. We show that MM patients not only have significantly decreased NK cell activity, NK cell CD107a expression and IFN-γ production, but also a significantly larger CD16dim and smaller cytotoxic CD16bright NK subset. Furthermore, after co-culture with target tumor cell lines, K562 and FemX, the percentage of CD3-CD16bright, contrary to CD3-CD16dim NK cells significantly decreased, as well as CD161 and CD158a on CD3-CD16bright NK subset. Also, in support of suppressed NK cells is our novel finding of decreased NK cell IFN-γ production in MM patients, that can affect their cytotoxicity, as reflected in CD107a decrease. The novel results for NK subsets showing how secondary NK cell contact with tumor cells changes CD161 and CD158a expression supports the tumor-related nature of this phenomenon. Therefore, we report an unfavorable tip of the balance towards the less mature, CD16bright158alow NK cell phenotype in MM patients.

doi:10.1016/j.clim.2009.03.370

F.119. FTY720+Sirolimus Increase Skin Allograft Survival via Cell Phenotype Changes

Camila Lopes[1], Flavia Rosin[1], José Cordeiro[2], Valquiria Bueno[1]. [1]UNIFESP Federal University of São Paulo, São Paulo, Brazil; [2]FAMERP São José do Rio Preto Medical School, São José do Rio Preto, Brazil

FTY720(FTY) prevents lymphocyte(Ly) egress from lymph nodes(LN) and thymus whereas Sirolimus(SRL) prevents T cell activation. Skin allograft recipient mice were either non-treated(Tx) or treated with SRL or FTY or FTY+SRL(1 mg/Kg/day).FTY+SRL had no rejection vs Tx:9.8 ± 0.8/SRL:15.0 ± 0.5/FTY:14.8 ± 0.9 days,$p < 0.0005$. On day +5, FTY+SRL had lower counts of splenocytes vs Tx and SRL[Tx:62.0(18.5)/SRL:34.4(10.2)/FTY:30.0(8.7)/FTY+SRL:24.6(6.8)x106, $p < 0.05$];thymocytes (THY) vs Tx and FTY[Tx:43.2(7.8)/SRL:26.3(40.3)/FTY:40.0(6.9)/FTY+SRL:25.2(6.5)x106, $p < 0.005$];CD4+ cells in spleen (SPL) vs Tx and SRL[Tx:15.9(5.2)/SRL:9.0(2.6)/FTY:5.5(3.4)/FTY+SRL:4.3(1.1)x106, $p < 0.005$],in LN vs Tx [Tx:0.6(0.2)/SRL:0.6(0.1)/FTY:0.3(0.2)/FTY+SRL:0.1(0.1)x106,$p < 0.05$], in peripheral blood (PB) vs others[Tx:22.4(13.9)/SRL:25.1(4.4)/FTY:2.2(1.6)/FTY+SRL:1.1(0.4)%,$p < 0.05$;CD8+ cells in SPL vs Tx[Tx:13.0(3.7)/SRL:5.9(1.5)/FTY:4.8(3.6)/FTY+SRL:5.4(1.0)x106, $p < 0.005$],in LN vs others[Tx:0.6(0.5)/SRL:04(0.5)/FTY:0.5(0.2)/FTY+SRL:0.3(0.2)x106,$p < 0.05$] and in PB vs SRL [Tx:15.8(4.0)/SRL:17.9(4.1)/FTY:1.8(0.3)/FTY+SRL:6.5