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Title: Sleepiness influences cytotoxic lymphocytes independent of respiratory events

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Body: Introduction: Obstructive sleep apnoeas have been shown to stimulate the immune system. However, the relevance of sleepiness is less clear. We investigated the influence of objective sleepiness (OS) on the expression of the cytotoxic proteins granzyme-B (GrB) and perforin (P) in peripheral blood lymphocytes (PBL). Methods: 43 participants performed polysomnography followed by the pupillary sleepiness test (PST®). PBL were stained for CD8, gamma delta cells (gd cells), natural killer cells (NK), P and GrB and analysed by flow cytometry. Results are shown as means (+/- SEM). Results: 29 probands were not sleepy (NS) (pupillary unrest index=PUI< 6,8). 14 patients were sleepy (PUI> 9,8). Results were not statistically significantly different for: age, BMI, AHI, ODI or oxygen saturation < 90%. In NS we found a higher percentage of P in CD8+ 36,3 +/- 3,0 vs. 27,3 +/- 4,4 and gd cells 39,1 +/- 3,5 vs. 27,3 +/- 5,0, but results failed to reach statistical significance (p=0,07 and 0,06, respectively). Results for GrB are listed below.

Percentage of Gr-B+ cells

	% of GrB+ cells	CD8GrB/CD8	gdGrB/gd	NKGrB/NK
PUI <6,8	24.8 (1.8)	46.3 (14.9)	42.3 (3.6)	80.1 (3.3)
PUI >9,8	19.9 (1.3)	35.5 (4.5)	23.4 (3.9)	82.2 (3.8)

GrB+ cells (+/-SE). Total percentage of GrB+ cells (p: 0.04) and GrB+ gd cells (p<0.001) were significantly lower in sleepy subjects

Discussion: In these preliminary results we show that OS influences cytotoxic PBL independently of the respiratory events. The PST uses the balance between sympathetic and vagal activity on the pupil. These

results could indicate that GrB and P are increased in presence of daytime sympathetic activation.
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