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Paper Poster Session II

Host factors and pathogenesis

The phagocyte functional impairment in treated multiple sclerosis patients

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Objectives. Although an infectious pathogen may not be causative in multiple sclerosis (MS), it may still influence the disease course by enhancing the neurological progression, developing new symptoms and increasing the acute attack risk in relapsing-remitting (RR) MS. Phagocytes may pass information to other players of the innate/adaptative immunity, as they are able to release mediators that attract other phagocytes from other sites, contributing to central nervous system inflammation. Since any alteration in MS patient innate immune system might provide clues to understanding the early steps of the MS pathogenetic process, this interdisciplinary study, involving neurologists, immunologists and microbiologists, was aimed to evaluate different aspects of neutrophil functions from MS patients either untreated or treated with immunosuppressive or immunomodulatory drugs.

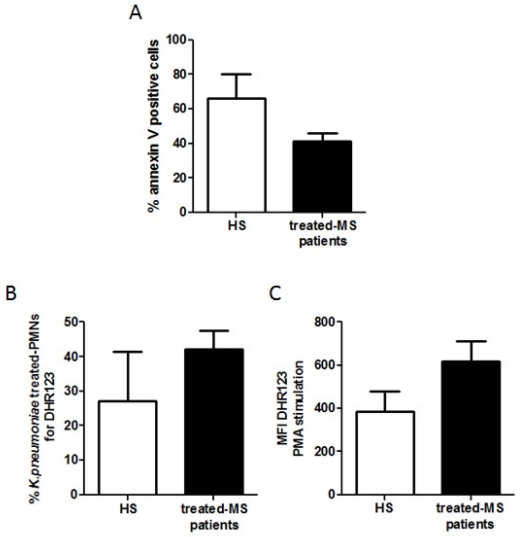
Methods. Fifty RR-MS patients (with different therapy, disease duration and clinical classification) and 25 healthy subjects (HSs) were enrolled in the study. *In vitro* polymorphonuclear cell (PMN) functional activity was determined by testing, in the presence of *Klebsiella pneumoniae* or *Candida albicans*, phagocytosis and intracellular killing activity, cytokine release profile, apoptosis and reactive oxygen species (ROS) production. Pre-treatment assays on HS-PMNs with either immunosuppressive or immunomodulatory drugs were also conducted. Statistical analysis was performed using the Graphpad Prism version 6.00 by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. *P*-values inferior to 0.05 were considered significant.

Results. PMNs collected from MS patients displayed a reduced intracellular killing activity, expressed as Survival Index, if compared with HS-PMNs. This reduction, statistically significant in treated MS patients, with either immunosuppressive or immunomodulatory drugs (Table 1), was related to the treatment itself as confirmed by pre-treatment assays, where drug pre-treated HS-PMNs showed a PMN reduced bacterial/fungal intracellular killing with values significantly lower than those registered with not pre-treated HS-PMNs. A gradual increased level of pro-inflammatory cytokines was detected, even if statistically significant differences were not observed between MS patients and HSs. In addition, PMNs were analyzed for survival and ROS production: treated-MS patients showed a decrease in spontaneous apoptosis and a higher oxidative burst in response to *K. pneumoniae* and phorbol myristate acetate (PMA) respect to HS neutrophils (Figure 1), but no statistically significant. A reduced phagocytosis activity ($p<0.01$) was registered for treated MS-PMNs if compared to HSs ones.

Table 1. PMN intracellular killing activity (%) against pathogens in RR-MS patients and HSs.

SURVIVAL INDEX (mean ± SD)					
	Healthy subjects	Untreated RR-MS patients	Treated RR-MS patients		Statistical analysis
			Immunosuppressive treatment	Immunomodulatory treatment	Tukey's multiple comparison test
	A	B	C	D	
	Klebsiella pneumoniae				
30'	1.48±0.09 (52%)	1.50±0.09 (50%)	1.83±0.30 (17%)	1.78±0.18 (22%)	p<0.0001 A vs. C p=0.0001 A vs. D and B vs. C p<0.05 B vs. D
60'	1.75±0.14 (25%)	1.84±0.20 (16%)	>2 (0%)	1.99±0.03 (1%)	p<0.0001 A vs. C p=0.001 A vs. D p<0.05 B vs. C and B vs. D
90'	>2 (0%)	>2 (0%)	>2 (0%)	>2 (0%)	n.s.
	Candida albicans				
30'	1.56±0.12 (44%)	1.69±0.04 (31%)	1.88±0.10 (12%)	1.90±0.20 (10%)	p<0.0001 A vs. C and A vs.D p<0.01 B vs. D p<0.05 B vs. C
60'	1.54±0.12 (46%)	1.64±0.09 (36%)	1.87±0.13 (13%)	1.87±0.20 (13%)	p=0.0001 A vs. D p<0.01 A vs. C p<0.05 B vs. C and B vs. D
90'	1.52±0.12 (48%)	1.57±0.13 (43%)	1.86±0.07 (14%)	1.81±0.26 (19%)	p<0.01 A vs. C and A vs.D p<0.05 B vs. C and B vs. D

Figure 1. **A**) Percentage of apoptotic cells (annexin V positive) in HS and treated-MS patients. PMNs were separated and cultured for 2 hours in RPMI + 10% FBS before annexin staining. **B**) Oxidative burst of PMNs from HS and treated-MS patients stimulated with *K. pneumoniae* (MOI 10) or PMA (5μM) (**C**) for 30 min and evaluated for DHR123 fluorescence in comparison to unstimulated cells by FACS analysis.



Conclusions. Since PMNs are the immune system's key defenders against microbial infection, the evaluation of their primary functions could be a clue for the complete understanding of MS patient response to infection and disease exacerbation. From our results, that complete the project supported by ESCMID Research Grant 2012, an impairment in MS-PMN functions was detected with a reduced PMN intracellular killing activity, not dependent to cytokine release pattern, ROS production and apoptosis, but probably related to a decreased phagocytosis towards microorganisms.