Serum IgG antibodies against Simian Virus 40 are hampered by high levels of sHLA-G in patients affected by inflammatory neurological diseases, as multiple sclerosis.

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Background: Many investigators detected simian virus 40 (SV40) footprints in human brain tumors and neurologic diseases. Recently, an association between multiple sclerosis (MS) and SV40 has been reported[1]. Interestingly, SV40 interacts with Human Leukocyte Antigen (HLA) class I molecules for cell entry[2]. HLA class I antigens, in particular non-classical HLA-G molecules, characterized by an immune-regulatory function, are involved in MS disease, and the levels of these molecules are modified according with the disease status[3]. We evaluated SV40-antibodies and soluble sHLA-G and the association between SV40-prevalence and sHLA-G levels in MS patients.

Materials and Methods: We analyzed SV40-antibody and soluble sHLA-G in serum samples from Italian patients affected by MS, other inflammatory diseases (OIND), non-inflammatory neurological diseases (NIND) and healthy subjects (HS). ELISA tests were used for SV40-antibodies and sHLA-G detection in serum samples. Two indirect ELISAs, against two distinct SV40 viral capsid protein (VP) epitopes, named B and C, were employed to test serum samples.

Results: The presence of SV40 antibodies was observed in 6% of patients affected by MS (N=4/63) and in 10% of OIND (N=8/77), suggestive of a lower prevalence in respect to NIND (15%, N=9/59) and HS (22%, N=18/83) subjects (**Table 1**). The mean OD of sera (VPs B+C ± Std Error) in MS (0.20±0.01) and OIND (0.21±0.02) were lower than that in HS (0.41±0.03) and in NIND (0.35±0.024) subjects (Anova and Newman-Keuls Comparison test; $p<0.0001$) (**Figure 1a**). All MS serum samples were positive for sHLA-G (63/63: 100%), whereas sHLA-G molecules were detected in 58% (45/77) of OIND, 51% (20/59) of NIND and 41% (35/83) of HS ($p<0.0001$; Fisher's exact test) (**Table 1**). MS patients are characterized by higher sHLA-G serum levels (13.9±0.9ng/ml) in comparison with OIND (56.7±0.8ng/ml), NIND (2.9±0.4ng/ml) and HS (2.6±0.7ng/ml) subjects (**Figure 1b**). Interestingly, we observed an inverse correlation between SV40 antibody prevalence and sHLA-G serum levels in MS patients.

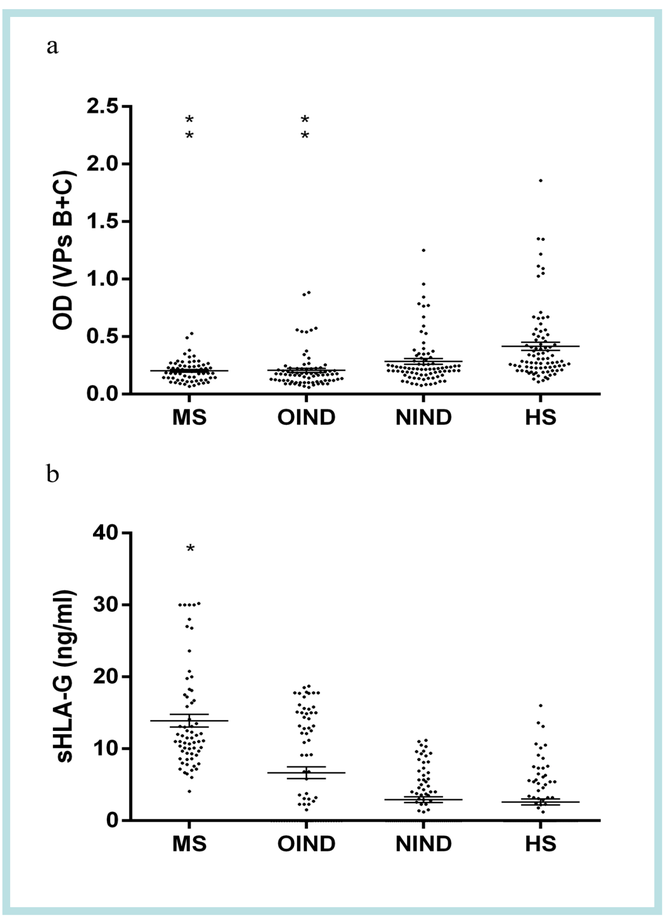
Discussion: The data obtained showed a low prevalence of SV40 antibodies in MS patients. These results seems to be due to a generalized status of inability to counteract SV40 infection via antibody production. We hypothesize that SV40 immune-inhibitory direct effect and the presence of high levels of the immune-inhibitory HLA-G molecules could co-operate in impairing B lymphocyte activation towards SV40 specific peptides.

Table 1. SV40 and sHLA-G results in MS, OIND, NIND and HS subjects.

	SV40 Ab titre OD(mean±SE)[a]	SV40 Ab+ (%)[b]	sHLA-G ng/ml (mean±SE)[a]	sHLA-G+ (%)[b]	>Cut off sHLA-G (>15ng/ml)[b]
MS (63)	0.20±0.01**	6*	13.9±0.9ng/ml**	100**	30***
OIND (77)	0.21±0.02**	10*	6.7±0.8ng/ml	58	29***
NIND (59)	0.35±0.024	15	2.9±0.4ng/ml	51	0
HS (83)	0.41±0.03	22	2.6±0.7ng/ml	41	2

p values: *p<0.05; **p<<0.0001; ***p<0.001
[a]: Anova and Newman-Keuls Comparison test; [b]: Fisher exact test

Figure 1. SV40 antibodies titres and sHLA-G levels in serum samples from from MS, OIND, NIND and HS. (a) SV40 antibodies titres are presented as values of optical density (OD) readings at λ 405 nm of serum samples diluted at 1:20, detected in indirect ELISA. In scatter dot plotting, each plot represents the dispersion of OD values to a mean level indicated by the line inside the scatter with Standard Error Mean (SEM) for each group of subjects analyzed. (b) sHLA-G levels are presented as ng/ml in serum samples diluted at 1:2, detected in indirect ELISA. In scatter dot plotting, each plot represents the dispersion of sHLA-G values to a mean level indicated by the line inside the scatter with Standard Error Mean (SEM) for each group of subjects analyzed. Statistical analysis was performed using Anova and Newman-Keuls Comparison test. (**p <0.0001; *p <0.001).



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