Humoral immunity following neural precursor cell administration in naive rodents

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Introduction

Multiple Sclerosis (MS) is a complex disorder mediated by two crucial immune players, B- and T- cells[1]. Despite the plethora of immunoregulatory treatments, interest in remyelination promoting therapies is increased.

Neural Precursor Cells (NPCs) of the Central Nervous System (CNS) are a valuable cell population, that fulfills the characteristics of stem cells, such as multipotency, self-renewal and differentiation[2]. Therefore, studies on NPCs via transplantation in Experimental Autoimmune Encephalomyelitis (EAE), animal model of MS[3], aim to explore their role in the CNS.

NPCs transplantation positive outcomes are exerted by immunomodulation in the periphery or by remyelination through their migration to the lesions[4]. Nevertheless, it can not be ruled out that the existing autoimmune inflammatory microenviroment, may modify NPC function. Specifically, it may lead to NPC antigenic epitope recognition and production of antibodies and/or immune cells.

The purpose of this experimental study is to examine whether NPCs inoculation in naive mice may provoke immune response(s).

Methods

NPCs were isolated and cultured from C57BL/6 neonate mice, using an established protocol[5] and were inoculated subcutaneously in naive C57BL/6 adult mice. MOG_{35-55}-EAE mice were used as a positive control and NAIVE mice as a negative control.

On day-17-Acute phase blood sampling was performed and antisera (NPCs-AS, EAE-AS, NAIVE-AS) were collected. Western Blotting (WB) was performed on normal SC homogenate and on total protein lysate from NPCs in order to investigate the autoantibody presence in the collected antisera.

On days 17 and 50 (Chronic phase) post EAE induction, mice were sacrificed and brain (BR) and spinal cord (SC) studies were performed:

(a) Immunohistochemistry (IHC) for anti-CD3, anti-Mac-3 and anti-B220 (markers of inflammation)
(b) Real-time PCR (qPCR) for CCL2, CCL20 and IL-17RA (genes related with inflammation).

Results

WB results on spinal cord substrate showed 26-28kDa band, which corresponds to MOG, when using the positive control EAE-AS and only one band (25kDa) with NPCs-AS, suggesting a mere presence of immunoreactivity against CNS. On NPCs substrate, when using NPCs-AS, specific bands (100kDa, 60kDa, 50kDa, two bands above 40kDa, 30kDa and 25kDa) were yielded, indicating dominant immunoreactivity. When using the positive control-EAE-AS, on NPCs substrate, four distinct bands (60kDa, two bands above 40kDa and 30kDa) also appeared **(Figure 1)**.

Brain sections of NPCs, EAE and NAIVE mice were stained with anti-CD3, anti-Mac-3 and anti-B220 and revealed elevated expression (cells/mm^2) of all markers in NPCs- and EAE-group during acute and chronic phase respectively;

NPCs:CD3: 111.0±18.84 and 18.73±7.90, Mac-3: 83.69±15.36 and 416.1±73.90, B220: 9.82±5.12. EAE:CD3: 91.66±18.96 and 82.40±32.43, Mac-3: 213.0±39.31 and 221.7±38.27, B220: 4.42±2.05 **(Figure 2).**

qPCR results indicated that CCL2 and CCL20 mRNA expression levels were elevated mainly in the brain of NPCs-inoculated group versus EAE-group; CCL2-Acute:15.55- vs 57.69-fold,$p<0.001$, CCL2-Chronic:28.48- vs 8.46-fold,$p<0.01$; CCL20-Acute:5.59- vs 1.13-fold, $p<0.01$; CCL20-Chronic phase:19.94- vs 1.82-fold, $p<0.001$. IL-17RA mRNA was upregulated in spinal cord of both groups in acute phase (NPCs-group:23.21-fold, EAE-group:35.17-fold) and remained increased only in NPCs-group in chronic phase (38.19-fold vs 2.7-fold) **(Figure 3)**.

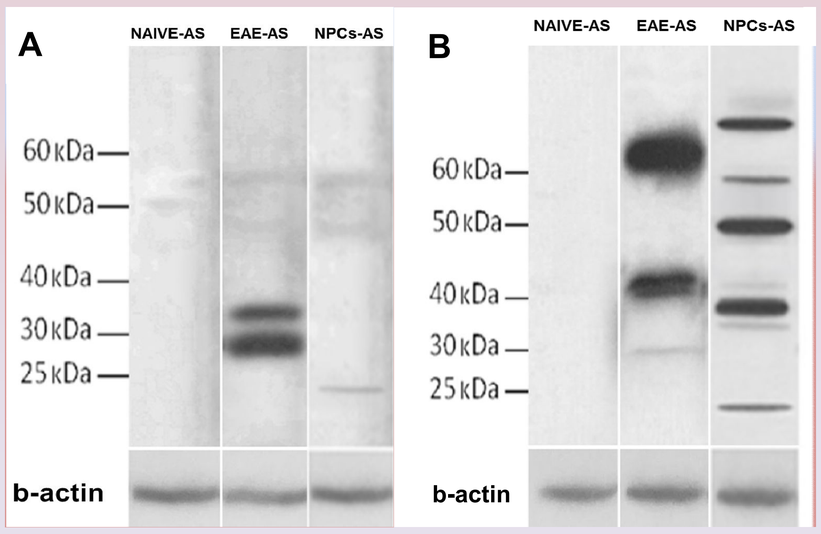

Figure 1. Western blot on SC (A) and NPCs (B) lysate. EAE-AS elicits a distinct humoral response against MOG (26-28kDa) on SC (positive control), while NPCs-AS recognized a single band. NPCs-AS and EAE-AS yielded definite bands on NPCs substrate, showing robust immunoreactivity.

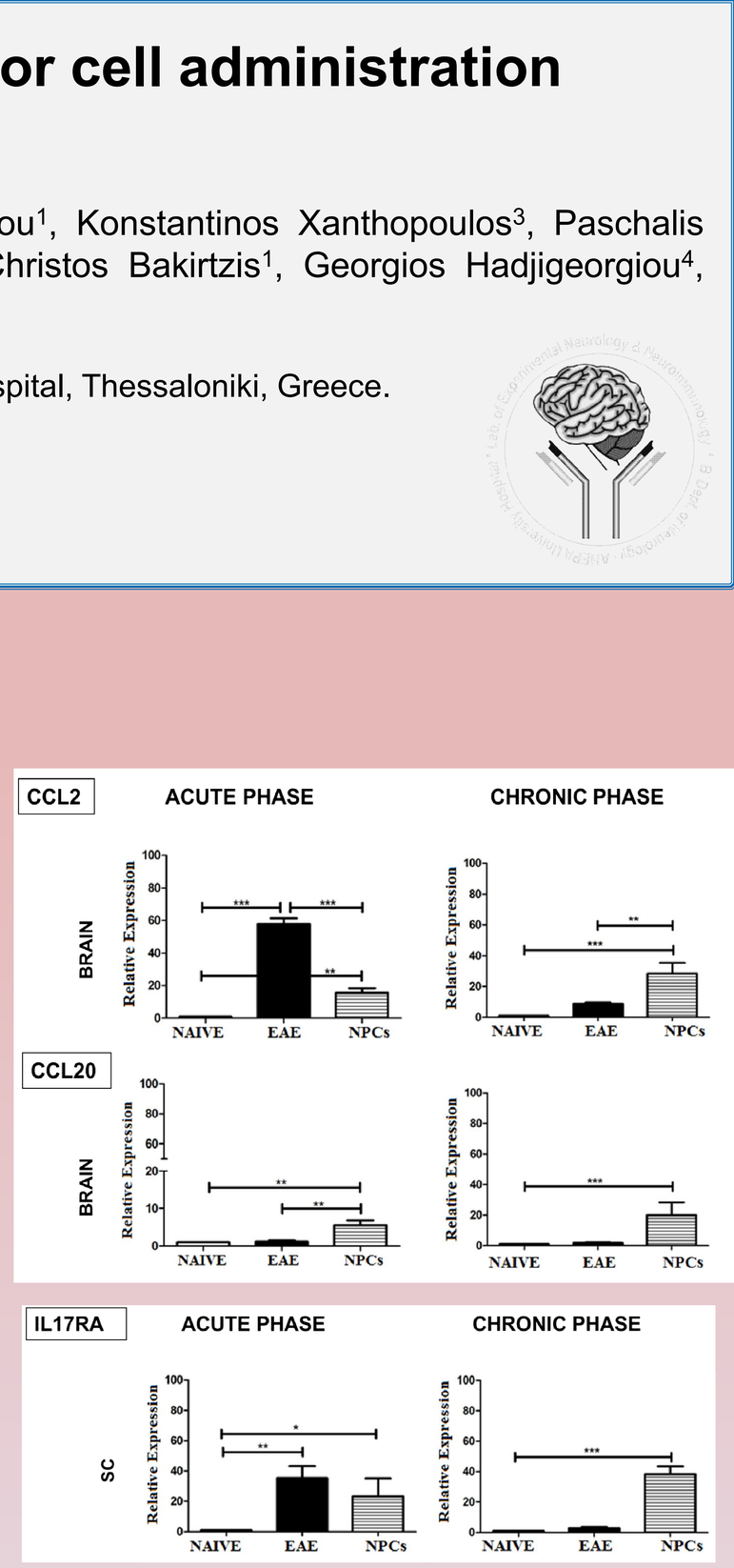

Figure 3. qPCR study of Brain and SC from NAIVE, EAE and NPCs animals. The charts represent relative mRNA expression of CCL2, CCL20 and IL-17RA in the acute and chronic phase. Error bars indicate SEM (*$p<0.05$, **$p<0.01$*** $p<0.001$).

Conclusions

Cellular and humoral immune responses after naive mice immunization with NPCs provide evidence of their antigenic capability, thus raising questions about their exact involvement in autoimmunity. Cross-talk between NPCs and CNS immune mechanisms warrants further investigation, before their use as cell therapy for neurodegenerative diseases. Our findings and further studies may contribute to a better understanding of NPCs immune role.

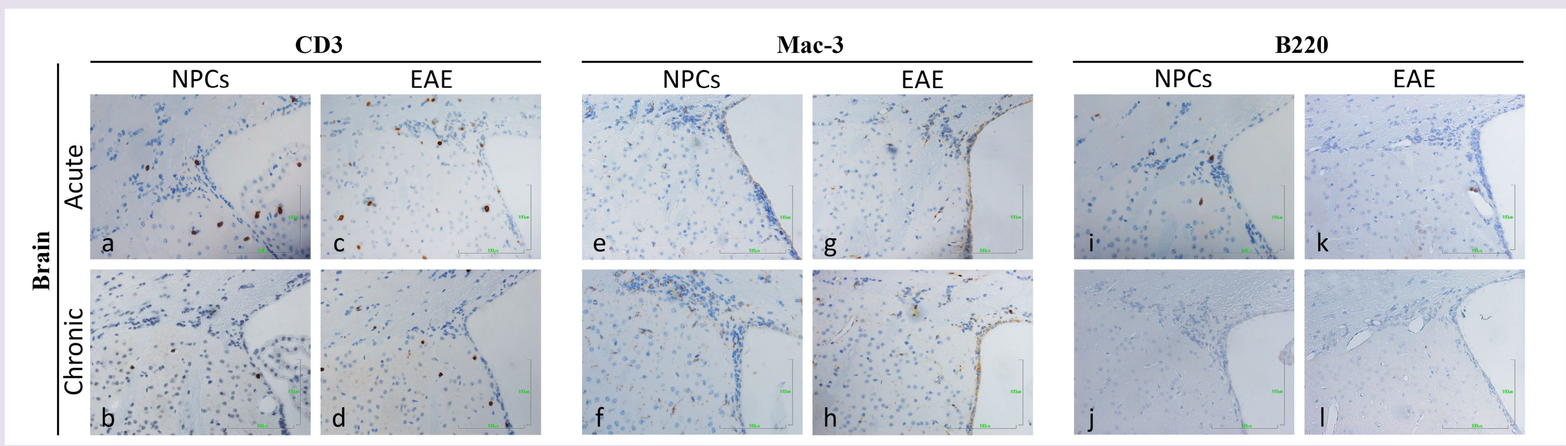

Figure 2. Representative brain sections from Periventricular and Subventricular zone of NPCs and EAE animals from Acute and Chronic phase stained with anti-CD3 (**a-b,c-d**), anti-Mac-3 (**e-f, g-h**) and anti-B220 (**i-j, k-l**). IHC signal with DAB staining. Nuclei are labeled (blue) with Hematoxylin. Scale 40X.

Literature

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