AHCC Induces *in vitro* Functional Activity of PBMC-derived Mononuclear Cells from Subjects with Recurring Herpes Simplex Type 1 and 2 Infections

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Previously we showed that subjects with recurrent HSV-1 & 2 infections secrete lower levels of interleukin (IL)-12 and interferon (IFN)-α and γ, suggesting that such individuals could benefit from immunostimulatory therapy. AHCC, registered as Immunomax® in Ukraine, represents a polymolecular compound from mycelia culture extract with high content of α-1,4-glucans. AHCC can potentially modify immune-inflammatory responses *in vivo* and is used in Japan in cancers and viral infections. We aimed to characterize the effect of AHCC *in vitro* on the ability of PBMC-derived mononuclear cells (PBMC-MNC) and $CD56^+$ natural killer (NK) cells from individuals with recurrent HSV-1 & 2 to: (1) proliferate; (2) produce nitric oxide (NO); (3) function in an oxygen-dependent way; (4) produce IL-2, IL-4, IL-10, IL-12 as well as IFN-α & γ, and transforming growth factor beta (TGF-β); (5) express perforin by $CD56^+$ cells. After the standard ficoll-verographin density-gradient isolation, 1.5×10^6 cells/ml of PBMC-MNC were incubated either in culture medium (control group; spontaneous production) or in presence of 0.64 mg of AHCC as a stimulant (experimental group; induced production). We show that, compared to a medium alone group, the AHCC-treated PBMC-MNC exhibited significantly higher proliferative activity (0.038 ± 0.002 vs 0.136 ± 0.001 UOD), oxygen-dependent functional activity (0.117 ± 0.008 vs 0.151 ± 0.01 UOD) as well as the secretion of NO (46.4 ± 5.1 vs 141.8 ± 6.1 pg/ml), IL-2 (35.9 ± 4.6 vs 75.2 ± 7.5 pg/ml), IL-12 (29.3 ± 0.9 vs 77.4 ± 3.8 pg/ml), IFN-α (16.4 ± 1.5 vs 53.0 ± 3.3 pg/ml), IFN-γ (44.2 ± 3.2 vs 92.1 ± 5.4 pg/ml), and TGF-β (27.5 ± 2.3 vs 64.1 ± 3.7 pg/ml), with $p<0.001$, but not IL-4 or IL-10. Further, in presence of AHCC, the expression of perforin by $CD56^+$ NK cells was also significantly higher, compared to their controls (66.9 ± 2 vs 74.1 ± 3%). Collectively, our results clearly indicate the potential of AHCC to be recommended as an alternative treatment for individuals with frequently recurring HSV infections, but in remission, in order to promote immune rehabilitation and decrease HSV recurrence frequency.