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NK Cells Expansion Model from PBMC Led to a Decrease of CD4+ and an Increase of CD8+ and CD25+CD127- T-Reg Lymphocytes in Patients with Ovarian Neoplasia

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Abstract : T-reg lymphocytes are important for the control of peripheral tolerance. They control the adaptive immune system and prevent autoimmunity through its suppressive action on CD4+ and CD8+ lymphocytes. The suppressive action also includes B lymphocytes, dendritic cells, monocytes/macrophages and recently, studies have shown that T-reg are also able to inhibit NK cells, therefore they exert their control of the immune response from innate to adaptive response. Most tumors express self-ligands, therefore it is believed that T-reg cells induce tolerance of the immune system, hindering the development of successful immunotherapies. T-reg cells have been linked to the suppression mechanisms of the immune response against tumors, including ovarian cancer. The goal of this study was to disclose the sub-population of the expanded CD3+ lymphocytes reported by previous studies, using the long-term culture model designed by Carlens et al 2001, to generate effector cell suspensions enriched with cytotoxic CD3-CD56+ NK cells, from PBMC of ovarian neoplasia patients. Methods and Results: Blood was collected from 12 patients with ovarian neoplasia after signed consent: 7 benign (Bng) and 5 malignant (Mlg). Mononuclear cells were separated by Ficoll-Paque gradient. Long-term culture was conducted by a 21 day culturing process with SCGM CellGro medium supplemented with anti-CD3 (10ng/ml, first 5 days), IL-2 (1000UI/ml) and FBS (10%). After 21 days of expansion, there was an increase in the population of CD3+ lymphocytes in the benign and malignant group. Within CD3+ population, there was a significant decrease in the population of CD4+ lymphocytes in the benign (median Bgn D-0=73.68%, D-21=21.05%) ($p<0.05$) and malignant (median Mlg D-0=64.00%, D-21=11.97%) ($p < 0.01$) group. Inversely, after 21 days of expansion, there was an increase in the population of CD8+ lymphocytes within the CD3+ population in the benign (median Bgn D-0=16.80%, D-21=38.56%) and malignant (median Mlg D-0=27.12%, D-21=72.58%) group. However, this increase was only significant on the malignant group ($p<0.01$). Within the CD3+CD4+ population, there was a significant increase ($p < 0.05$) in the population of T-reg lymphocytes in the benign (median Bgn D-0=9.84%, D-21=39.47%) and malignant (median Mlg D-0=3.56%, D-21=16.18%) group. Statistical analysis inter groups was performed by Kruskal-Wallis test and intra groups by Mann Whitney test. Conclusion: The CD4+ and CD8+ sub-population of CD3+ lymphocytes shifts with the culturing process. This might be due to the process of the immune system to produce a cytotoxic response. At the same time, T-reg lymphocytes increased within the CD4+ population, suggesting a modulation of the immune response towards cells of the immune system. The expansion of the T-reg population can hinder an immune response against cancer. Therefore, an immunotherapy using this expansion procedure should aim to halt the expansion of T-reg or its immunosuppresion capability.

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