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Effects of parenteral nutrition on the immune system in patients with head and neck squamous cell carcinoma

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BACKGROUND

The head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. HNSCC is associated with several alterations of the immune system, which ultimately lead to markedly depressed antitumor immunity (Moy, et al. 2017). A complicating factor in this set of patients is malnutrition, due to swallowing impairment related to the tumor site or to treatment sequaelae. Improving patients' nutritional status may restore immunocompetence with consequent recovery of antitumor activity (Jin, et al. 2018). HNSCC patients receive parenteral nutrition (PN) during chemo-radiotherapy, when oral intake is compromised and enteral nutrition is not feasible. PN bags contain mostly lipids that provide calories in a concentrated form and are enriched with essential fatty acids (FAs) such as ω3, ω6 (polyunsaturated fatty acids, PUFA) and ω9.
Dietary lipids, hence also PUFAs, are not immunologically inert. ω3 FAs have been shown to be immunosuppressive as they induce expansion of T regulatory cells (Tregs) while restraining NK cell activity; also they favor the differentiation of Th2 lymphocytes at the expense of the Th1 anti-tumoral subset. On the other hand, ω6 FAs induce lymphoproliferation and production of IFN-γ, IL-17A and TNF-α (Harbige 2003).
Therefore, lipid emulsions administered as PN to patients may also influence the immune response, with different mechanisms and effects (Russell and Wischmeyer 2018) (Wanten and Calder 2007). Clinical guidelines suggest caution in the use of lipid emulsions based on ω6 class FAs in patients with uncontrolled inflammatory responses; these may instead be advisable in patients unable to develop an adequate immune (e.g. anti-tumoral) reaction.

AIM OF THE STUDY

The aim of our project was to investigate the effects of fatty acids on immune responses of patients with advanced HNSCC.

We studied the effects of fatty acids in *in vitro* cultures of PBMCs by measuring expression of activation markers on multiple cell subsets. We also determined the effects of two types of parenteral nutrition (olive oil-based PN and fish oil-based PN) on the immune responses of HNSCC patients through the measurement of cytokine production by innate and adaptive immune cells.

MATERIALS AND METHODS

- We enrolled 9 HNSCC patients and 3 healthy donors
- 6 patients were treated with olive oil-PN (OO)(Olimel, Baxter Healthcare Corporation, 80% olive oil 20% soybean oil) and 3 patients received fish oil-PN (FO) (PeriSmofven, Fresenius Kabi italia, 43% soybean oil, 35% olive oil, 22% fish oil enriched with ω3) for 1 week. Blood was drawn before and after PN administration.
- Cells were stimulated with PMA /ionomycin or LPS
- PBMCs were incubated with alpha-linolenic acid (aLA, 0.01 µM), linoleic acid (LA, 0.01 µM), oleic acid (OA 0.01 µM), palmitic acid (PA 0.01 µM) (Sigma-Aldrich) dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) for 24h.
- Multi-color FACS analysis was performed on Cytoflex (Coulter).
- Data were analyzed using FlowJo software and statistical analysis was performed with Graph Pad Prism's t test.

RESULTS

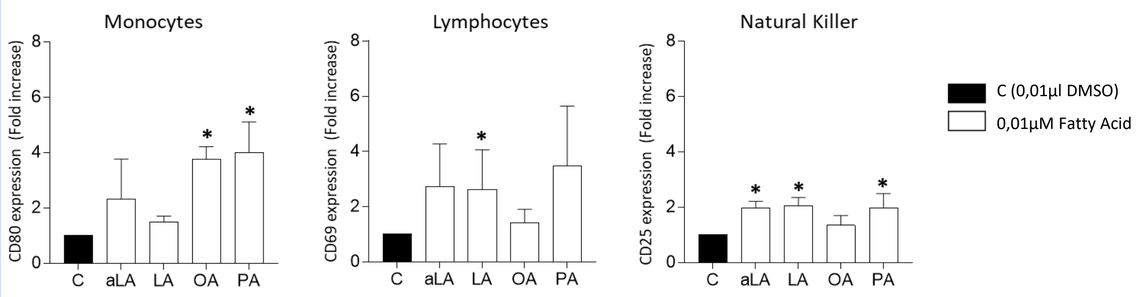

Fig. 1. Immunogenicity of lipid components. Activation markers were evaluated upon lipid challenge on innate and adaptive immune cells. (alA) Alpha-linolenic acid, (LA) linoleic acid, (OA) oleic acid, (PA) palmitic acid. 2way ANOVA multiple comparisons. *p < 0.05.

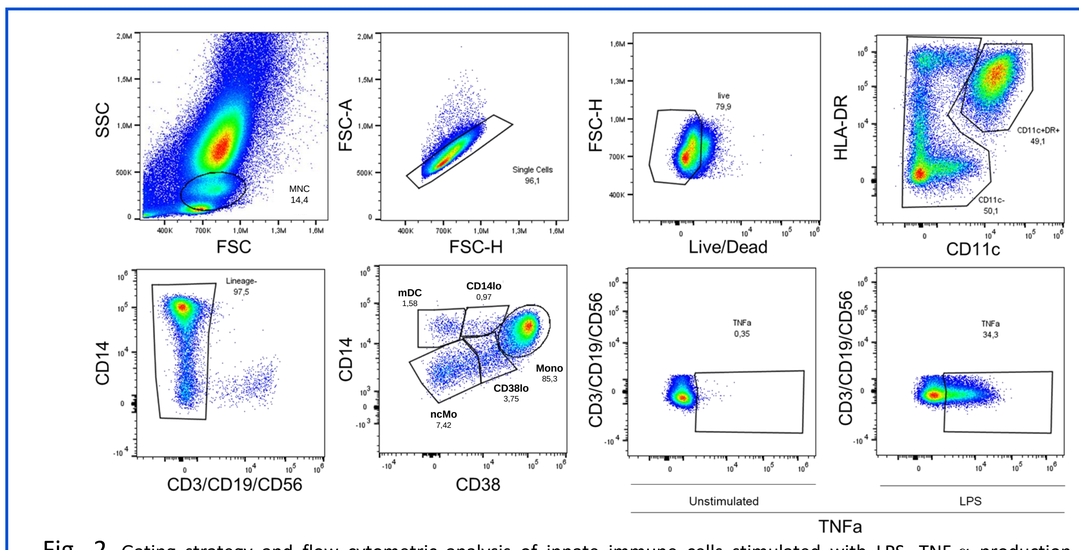

Fig. 2. Gating strategy and flow cytometric analysis of innate immune cells stimulated with LPS. TNF-α production by monocytes is displayed.

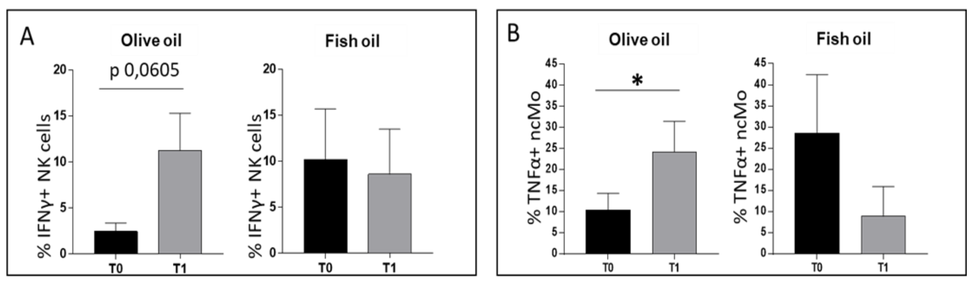

Fig. 3. Innate immune responses in HNSCC patients before and after administration of olive or fish oil PN. A-C IFN-γ and TNF-α production by NK cells (stimulated with PMA and Ionomycin), monocytes, and non-classical monocytes (ncMo) (stimulated with LPS) before (T0) and after (T1) PN treatment. Analysis: T-Student test *p < 0.05

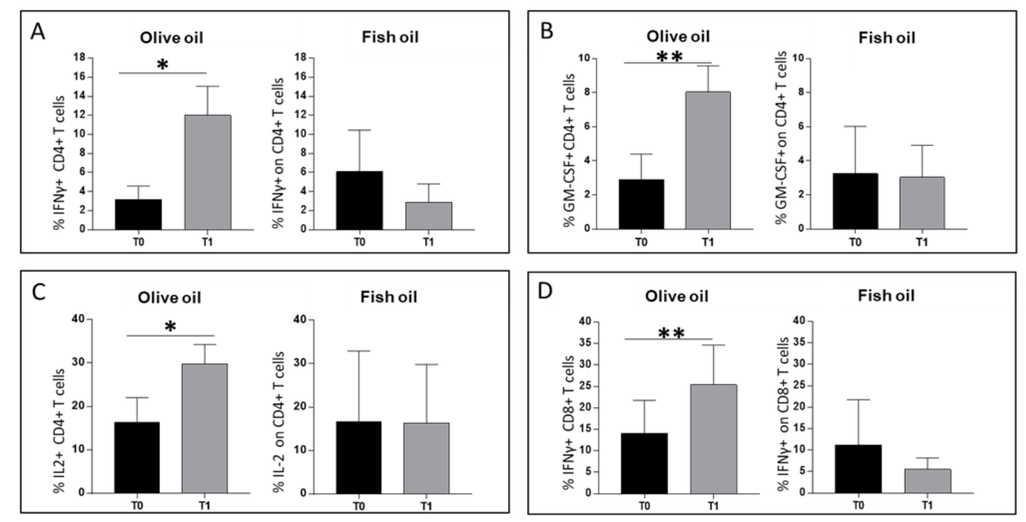

Fig. 4. Adaptive immune responses following stimulation with PMA and Ionomycin in HNSCC patients before (T0) and after (T1) 1 week of treatment with olive oil- and fish oil-based PN. A-C: Fraction of IFNγ-, GM-CSF-, and IL2-producing CD4+ cells. D: Fraction of IFNγ- producing CD8+ cells. Analysis: T-Student test *p < 0.05; **p < 0.01

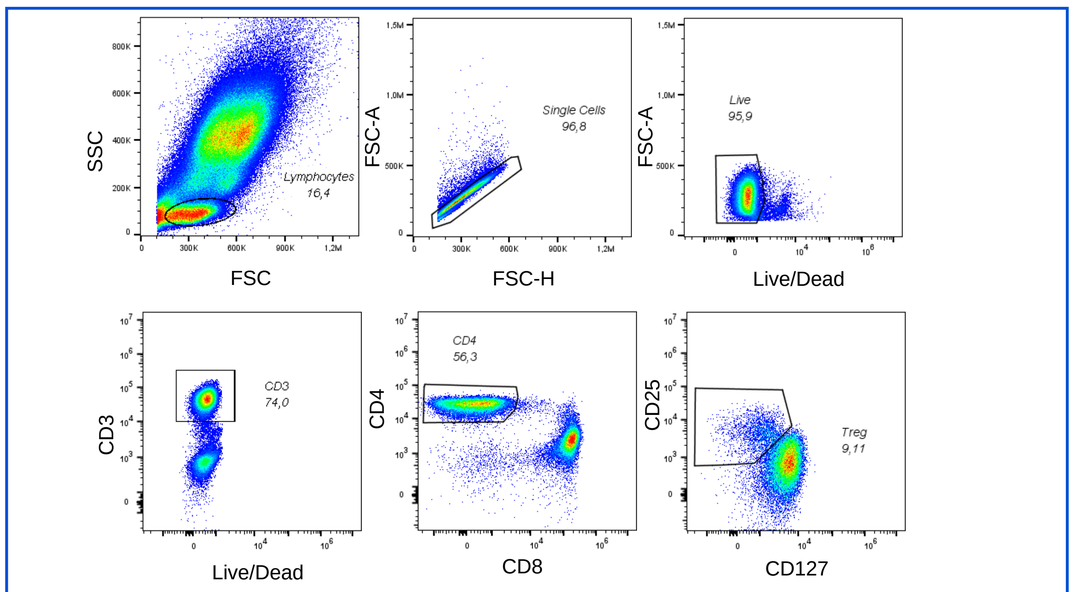

Fig. 5. Gating strategy and flow cytometric analysis of Treg cells.

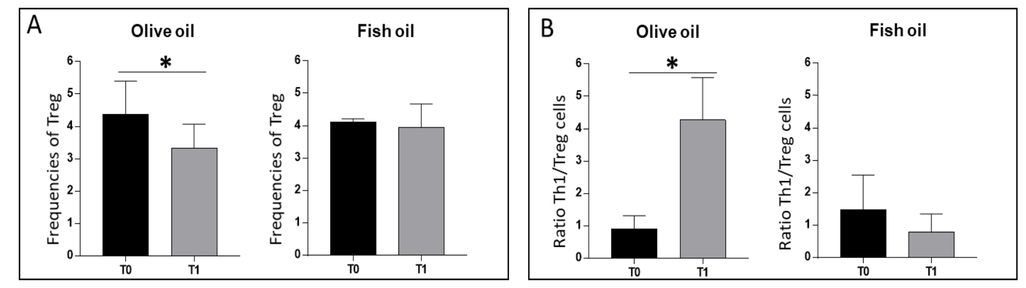

Fig. 6A. Treg cell frequencies before (T0) and after (T1) 1 week PN treatment. Fig. 6B. Th1/Treg ratio before (T0) and after (T1) PN treatment. Analysis: T-Student test *p < 0.05.

CONCLUSIONS

✓ FAs may modulate the activation of immune cell subsets, and different cells respond to distinct lipid molecules (Fig. 1).

✓ The figures 3 and 4 show that indeed the composition of the bags for PN may influence the quality of the immune response. One week of OO-based PN was sufficient to affect the functional response of innate and adaptive immune cells, inducing a significant increase in the release of pro-inflammatory cytokines; FO-enriched formulations conversely determined a reduction in the frequency of cytokine-producing cells. This suggests that OO-based PN fosters and reestablishes protective anti-tumor responses mediated by IFN-γ, TNF-α, IL-2 and GM-CSF.

✓ We find that patients treated with OO show a decrease in the frequency of Tregs cells after 1 week of treatment, while in patients treated with FO enriched PN Tregs frequency is unchanged (Fig.6A). Interestingly, the Th1/Treg ratio, which indicates the tendency of the immune system to carry out an active proinflammatory response (Fig.6B), shows that in patients treated with OO there is a clear imbalance in favor of the Th1 population, while in patients treated with FO the ratio is inverted in favor of Tregs.

In conclusion, these data point to a positive effect on the antitumoral immune response induced by PN with oils enriched in ω6 fatty acids. It is indeed possible to foresee that, in the near future, the individualized choice of specific nutrients could allow the patient to deal with chemotherapy/ immunotherapy/ radiotherapy/ surgery with less toxicity, but also to obtain an advantage of response and therefore of survival. Further properly sized similar studies in different cancer types are warranted.

This work was partially supported by Baxter Healthcare Corporation

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