



Abstract published in *It. J. Anat. Embryol.* 116: 1 (suppl.) 136, 2011
<http://www.fupress.net/index.php/ijae/article/view/10160>
<http://dx.doi.org/10.13128/IJAE-10160>

Macrophages of the Mucosa-Associated Lymphoid Tissue (MALT) as key elements of the immune response to vitamin D binding protein-macrophage activating factor (GcMAF)

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Introduction (1)

Probiotic yogurt consumption is associated with an increase of CD4 count among people living with HIV/AIDS (J. Clin. Gastroenterol. 44:e201-5, 2010), and the results obtained with the probiotic yogurt are not too dissimilar to those obtained with Anti Retroviral Therapy (ART) (Gut Microbes 1:6, 411-414, 2010).

Encapsulated probiotics with the same probiotic strains, however, proved ineffective in preserving the immune function (Gut Microbes 2:2, 80-85, 2011), thus lending credit to the hypothesis that other factors might be required for full restoration of the immune system in conditions of immunodeficiency.





Introduction (2)

We recently demonstrated that an original milk-derivative containing microorganisms introduced in order to maximize natural GcMAF (Gc-protein-derived Macrophage Activating Factor) production (<http://pag.ias2011.org/EPosterHandler.axd?aid=2401>), stimulated immune system restoration leading to an increase of CD4 count and normalization of CD4/CD8 ratio. In fact, vitamin D binding protein-macrophage activating factor (DBP-MAF, also known as GcMAF) is a powerful stimulator of the immune system that proved effective in the eradication of HIV and cancer (J. Med. Virol. 81:16–26, 2009; Cancer Immunol. Immunother. 57:1007–1016, 2008; Int. J. Cancer: 122, 461–467, 2008; Transl. Oncol. 1, 65–72, 2008).





Introduction (3)

Therefore, we reasoned that the effects reported in (J. Clin. Gastroenterol. 44:e201-5, 2010) could have been due not only to the probiotic content of the yogurt, but also to the endogenous production of natural GcMAF by bacteria that produce enzymes that convert milk Gc-protein into active GcMAF, an event that cannot occur if the same bacteria are administered as encapsulated probiotics.

We hypothesized that this natural Gc-MAF activated the Mucosa-Associated Lymphoid Tissue (MALT) widely diffused in the walls of the entire gastrointestinal tract. In particular, we hypothesized that naturally produced GcMAF activated the MALT that is abundant in the Waldeyer's-Pirogov tonsillar ring (or pharyngeal lymphoid ring).





Materials and Methods

In this observational study, members of the research team, close friends and relatives consumed 125 ml/day of an original probiotic yogurt, prepared in a common home kitchen, for eight weeks. Here we also report preliminary data from two representative subjects (#4, #5) participating in a trial on 21 Chronic Fatigue Syndrome (CFS) patients, conducted for four weeks at the Cheney Clinic, Asheville, North Carolina, USA (August 2011).

Participants in both studies did not modify their usual diet and lifestyle or therapeutic regimen, if present. Blood analyses were performed before and after consumption at the indicated time intervals (three, four and eight weeks). Results of blood analyses performed in Italy are conserved at the Department of Anatomy, Histology and Forensic Medicine of the University of Firenze and are available to physicians within the limits of current regulations on professional secrecy and privacy rules. Results of subject #4 are expressed as %, as communicated by the laboratory.





Results (1)

In subject #1 (male, 55, HIV serostatus, unknown) CD4 count rose from 372 to 853 cells/ μ L in eight weeks.

In subject #2 (female, 35, HIV-negative), CD4 count rose from 857 to 1279 cells/ μ L, and CD4/CD8 ratio rose from 1.1 to 2.0, in just three weeks.

In subject #3 (male, 37, HIV-positive, on combination ART), CD4 count rose from 446 to 478 cells/ μ L in eight weeks and CD4/CD8 ratio rose from 0.8 to 0.85 in eight weeks. CD4 count was the highest recorded since the diagnosis of AIDS.

In subject #4 (female, 52, CFS patient, XMRV-positive, treatment naïve), CD4 count (expressed as %) rose from 44.8 to 60.7, and CD4/CD8 ratio rose from 1.55 to 2.22 in four weeks.

In subject #5 (female, 49, CFS patient, XMRV-positive, treatment naïve), CD4 count rose from 510 to 820 cells/ μ L, and CD4/CD8 ratio rose from 1.93 to 2.16 in four weeks.

To put these increases in perspective, studies have estimated that ART increases the average annual CD4 count by 90 cells/ μ l versus an average decline of 20–50 cells/ μ l/year without treatment (Gut Microbes 1:6, 411–414, 2010).





Results (2)

We observed that the increase in CD4 cells and in CD4/CD8 ratio was accompanied by an increase in Natural Killer (NK) cell count. It is worth of note that the innate immune response, and in particular, NK cells play a central role in determining the quality of the host immune response to infection and NK cells influence the clinical fate of HIV-infected individuals (J. Intern. Med. 265: 29–42, 2008). In fact, NK cells play a major role in HIV controllers, individuals that spontaneously control HIV replication in the absence of ART (Curr. Opin. HIV AIDS 6: 208-13, 2011).

The increase in NK cells after consumption was as follows:

In subject #1 from 165 to 397 cells/ μ L in eight weeks.

In subject #2 from 251 to 340 cells/ μ L in eight weeks.

In subject #3 from 165 to 195 cells/ μ L in four weeks

In subject #4 from 7.13 to 9.50 (% of NK cells in blood, i.e. CD16, CD56+/CD3-) in four weeks.

In subject #5 from 200 to 410 cells/ μ L in four weeks.





Results (3)

In addition to these data reflecting immune system function, in subject #3 (HIV/AIDS), we observed an increase in red blood cells and platelets with normalization of values within eight weeks.

The red blood cell count rose from 4.33 to 4.72×10^6 , and the haematocrit from 39.3 to 42.4% in eight weeks. Haemoglobin rose from 13.4 to 14 g/dL in eight weeks. Platelet count increased from 170.000 to 217.000 in four weeks, and to 285.000 in eight weeks.

Normalization of haematological parameters reflects restoration of bone marrow function, a fundamental issue in HIV/AIDS patients. In fact, thrombocytopenia is observed in about 10-50% HIV patients as one of the first clinical signs of infection (Acta Haematol. 124:13-8, 2010), and anaemia was consistently shown to be a predictor for increased disease progression and decreased survival of patient infected by HIV (Niger J. Med. 15:203-6, 2006).





Results (4)

The trend toward normalization of haematological parameters in subject #3 included decrease of total B lymphocytes (CD19) from 26 to 21.98% (normal value 6-25%) as well as decrease of IgG from 1941 to 1630 (n.v. < 1600) and IgA, from 623 to 599 (n.v. <400) in eight weeks.

Increase in red blood cells, haematocrit, haemoglobin and platelets, however, was not limited to subject #3. The values of subject #1 changed as follows:

	before assumption	after three weeks
Red blood cells, $\times 10^6$	4.55	4.85
Haematocrit, %	42.9	45.9
Haemoglobin, g/dL	14.8	15.6
Platelets	165.000	206.000





Discussion (1)

Taken together, these results indicate that consumption of a probiotic yogurt enriched in natural GcMAF, restore immune system and bone marrow function within weeks, irrespective of HIV or XMRV serostatus and, most important, irrespective of concomitant assumption of ART.

This latter observation marks a clear difference with chemical GcMAF, a substance that proved effective in HIV patients in one study where it was administered without concomitant ART (J. Med. Virol. 81:16–26, 2009).

It is worth of note that consumption of GcMAF-enriched probiotic yogurt was associated with a significant improvement in overall health conditions, if compromised. For example, subject #3 noticed regularization of intestinal function and disappearance of the chronic diarrhoea that afflicted him since beginning ART as well as normalization of body temperature that tended to rise after minor exertion. We attribute the improvement of these symptoms to decrease of the pro-inflammatory state that is often associated with chronic HIV infection and/or ART. This hypothesis is corroborated by the observed decrease in gamma-globulins (from 24.3 to 23.9%) together with the trend toward normalization of the haematological parameters reported above. It is also interesting to notice that subject #3 reported disappearance of gum bleeding while brushing his teeth, a phenomenon that we attribute to increased platelet number.





Discussion (2)

Subject #5 reported her impressions as follows:

“I am pleased to describe my experience with MAF314 as it has transformed my life. Having had CFIDS for approximately seven years with no informed treatment, I became seriously ill this winter until by spring I was lying down or in bed asleep almost every day for several months. From August to March I had been in a very stressful leadership position until becoming so ill that I was dismissed just two weeks after finding Doctor Cheney and finally having a solid diagnosis and treatment plan. I spent the rest of winter and spring barely functioning and sometimes sleeping or lying down 20 hours or more a day. I did not want to live anymore and cried privately through my oldest child's high school graduation in June (2011) because I was without hope that even my children and loving husband were reason enough to live in this agony. MAF314 changed all of that. I began treatment on August 2, 2011 for the 28-day trial, and within a few days could tell my system was changing. After only a couple of weeks, I had been given my life back, with a will to live and a delight in life again. I spend about 9 hours in bed or lying down now. If I take a nap, I am restored. If I overdo activity, I may be knocked down again for a day, but will be back to functioning in 24 hours or so if the relapse has been a serious one (this has only happened once so far). I love to eat, where I did not care about food or anything else normal for many months and even years. I take no antidepressants since starting MAF314 after years of trying to stop taking them.”





Discussion (3)

I laugh and play with my children and plan and dream and plant flowers and cook. I was dead and now I am alive! MAF314 has given me significant restoration and tremendous hope that I'll sleep well again in the future, be without constant pain, and participate fully again in my husband and children's lives."

These clinical observations are consistent with the preliminary results recorded in 21 CFS patients, adopting a response criteria with patient global perception given the dominant but not exclusive weight. 76.2% (16/21) responded and 23.8% (5/21) did not respond and 2 of these worsened. Of the responders 10 of 16 or 62.5% or almost 2 out of 3 of the responders had a significant response with at least one or more symptoms either significantly improving (a "4") or was abolished (a "5"), among the seven primary CFS symptoms. Global scores ranged from a low of "3" to a high of "28" (maximum score would be 35 = no symptoms at all = cure). Lymphocyte enumeration data is still being analyzed but there was a notable tendency to normalize the CD4/CD8 ratio to a common mean (2.18) of the group. In addition, the most recent preliminary results on 8 CFS patients participating in the trial, indicate that serum Nagalase levels decreased in 7 out of 8 CFS patients, with 3 patients reaching normal levels after four weeks of probiotic yogurt consumption.





Discussion (4)

These results are consistent with those reported by Yamamoto et al. demonstrating that decrease of Nagalase was associated with eradication of HIV or cancer following chemical GcMAF treatment (J. Med. Virol. 81:16–26, 2009; Cancer Immunol. Immunother. 57:1007–1016, 2008; Int. J. Cancer: 122, 461–467, 2008; Transl. Oncol. 1, 65–72, 2008).

We hypothesize that the observed clinical and laboratory results can be attributed to the combination of re-establishment of a healthy gut microbiome associated with direct stimulation of the immune system by naturally produced GcMAF, a task that cannot be accomplished by administration of encapsulated probiotics or chemically produced GcMAF alone.

Although it may be too early to define this type of natural immunotherapy “a cure for AIDS” (<http://hivskeptical.wordpress.com/2011/07/18/a-cure-for-aids/>) or for any other disease, we foresee that this approach will become common in the management of chronic diseases ranging from HIV infection to CFS and cancer.



Acknowledgements: R. Karpinski for inspiring discussion and editing this manuscript.