Reviewer's report

Title: Supplemental treatment of rheumatoid arthritis with natural milk antibodies against enteromicrobes and their toxins: results of an open-labeled pilot

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Reviewer: Douglas L Schmucker

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Synopsis: This study attempts to assess the efficacy of (RA). The experimental design appears to be based on the evidence that certain probiotic foods, e.g., fermented dairy products, have been shown to be an effective treatment for some gastrointestinal maladies. While the causative agent for rheumatoid arthritis remains unknow, there is some data that indicate a role for certain pathogenic bacteria in the intestine and their toxins. The suggestion is that the natural milk antibodies may ameliorate the deleterious impact that such pathogenic microorganisms may exert on the presentation of RA.

Major compulsory revisions:

(1) As this manuscript describes what amounts to a brief, preliminary study, it seems appropriate that its length should be shortened. Since the differences in responses to the primary variable are barely statistically significant (<.05-.09), the Discussion can be shortened.

(2) The authors should clearly state why they did not measure serum titers of anti-cyclic citrullinated peptide antibodies (CCP) as it is a major rheumatoid factor and an index of the disease. This is particularly important since some of the conclusions are based on clearly subjective clinical assessments by both the subjects and the investigators.

Minor essential revisions:

(1) While this Reviewer recognizes that certain deficiencies in this study cannot be corrected at this time, some concerns may be assuaged by addressing them in the text or muting the interpretation or conclusions. For example, the authors chose to measure serum anti-LPS IgA antibodies, yet serum IgA titers are rarely indicative of this particular isotype response at the primary site, e.g., the gastrointestinal tract or other mucosal surfaces. A more precise measurement would have been to monitor the IgA titers in colonic effluent, e.g., GoLytley-induced intestinal lavage (Taylor et al., Immunology 75, 614, 1992)

(2) The subjects are all aged 59 years or older. It is fairly well established that the intestinal flora in the elderly is more subject to overgrowth by one or more commensual species of bacterial than is the case in the younger population. This may reflect, in part, the documented intestinal immunosenescence in old animals and geriatric pateients. Such shifts in the balance of normal intestinal flora in the elderly may contribute to the exascerbation of RA, yet the authors fail to discuss this issue.
(3) Although probiotic foods have been shown to alleviate certain gastrointestinal disorders, the mechanism responsible have not been resolved. Interestingly, feeding kefir to young adult rats immunized intraduodenally with cholera holotoxin resulted in significantly higher serum anti-cholera toxin IgA titers and in vitro anti-cholera toxin IgA secretion by intestinal lamina propria lymphocytes. However, kefir had no apparent effect on either parameter in senescent rats. (Thoreux et al., Communicating Current Research and Educational Topics and Trends in Applied Microbiology, A. Mendez-Vilas (ed), Formatex, Spain, 2007). Thus, the authors may wish to briefly discuss the possibility that age per se may be a factor in the response to the milk antibodies.

(4) There are several typographical errors and inappropriate syntax in the cur

(5) The authors refer to this as a "quasi-randomized" study (pg. 5). What is meant by this phrase, i.e., how is it not random?

(6) The description of the modified ACR response criteria and the ad hoc evaluation point is confusing to this Reviewer.

(7) The finding that the serum titers of inflammatory cytokines were not affected by the milk antibodies, yet the titer of anti-collagen antibodies increased dramatically requires some explanation, Could this not reflect an increased autoimmune response against synovial cartilage in the "responder" group?

(8) Figures 2 and 3 are very difficult to understand based on the brief figure legends provided. What do the thin lines signify in Figure 3?

(9) Also in Figure 2 it appears that the serum c-reactive protein titer actually begins to increase during both the first and second milk treatments. Furthermore, both the c-reactive protein level and the RBC sed rate begin to decline between the first and second treatments. These seemingly incongruent results require some explanation since they suggest changes in the absence of the milk antibodies.

Discretionary revisions: None

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.

Note: More than five years ago my research laboratory did receive substantial funds from Dannone for pursuing studies on the impact of probiotics on intestinal immunity.