Dear Editor of PeerJ,

We are very grateful for the valuable comments raised by the reviewers. These have helped us to clarify the description of the aim of the study and to amend the manuscript according to the reviewers suggestions. The comments from the reviewers have been addressed point by point below. We believe that our manuscript had improved after these changes and we hope that it is now acceptable for publication in PeerJ.

Sincerely yours

Birgitta Sander

MD, PhD, Professor in Pathology

Department of Laboratory Medicine

Division of Pathology

F46

Karolinska Institutet

SE 141 86 Stockholm

Sweden

Reviewer 1

Basic reporting

Accession number to deposited microarray data was not provided.

Answer: The data is reposited in to the Gene Expression Omnibus (GEO) repository at NCBI with the number GSE68055.

References and in-text-citations should be adjusted to journal style according to Instructions for Authors

Answer: We have up-loaded the PeerJ style to Endnote and applied it to the manuscript.

Experimental design

No comments

Validity of the findings

No comments

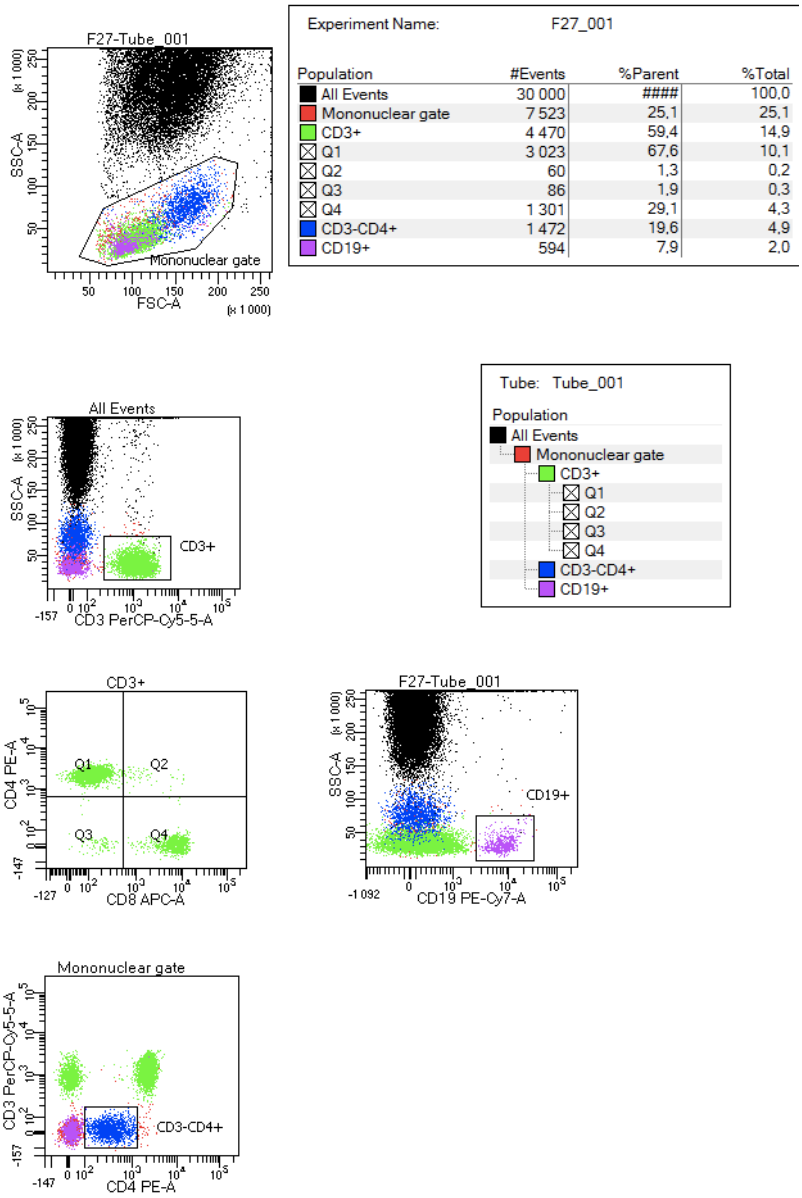
Comments for the author

1. The major concern about the study is a phenotyping of blood monocytes using only CD3 and CD4 markers (although some previous studies used these markers to describe monocytic cell population). In my opinion, CD14 marker is necessary to identify monocytes since CD3-CD4+ phenotype may be observed in other cells (e.g. pDC).

Answer: We agree with the reviewer that DC in blood may express CD4 as was shown by Dzionek A. et al. J. Immunol. 2000 and others. Unfortunately we do not have results for CD14 in our samples. We therefore changed the writing in the manuscript to clarify that the CD3-CD4+ population is a mixture of monocytes and DC. The figure 1 and the figure legend has also been changed so that the markers expressed by cells is indicated.

2. Please provide example of flow cytometry gating for mononuclear cell phenotyping

Answer: The gating strategy for the CD3-CD4+ cell population is enclosed for the reviewer below. If the reviewer would like us to include this figure as a supplementary file this is possible.



3. In the Results section authors describe that no significant differences were found for total CD3+ T cells, CD4+ T cells, CD8+ T cells, B cells and monocytes. Please provide p-values for each cell type.

Answer: P-values (paired t-test) were as follows: CD3+ p=0,47; CD3+CD4+ p=0,25; CD3+CD8+ p=0,11; CD19+ p=0,13; CD3-CD4+ p=0,11. These p-values have now been added to the figure legend.

4. Please provide the data about expression levels of CB1 receptor on different cell types or whole PBMCs before and after treatment with rimonabant.

Answer: The mean and standardeviation of CB1 expression in PBMC before treatment was 12,5 +/- 3,24 and after treatment 10,79 +/- 2,43. This information is now included in the results section line 151.

5. In the Methods section it is written that both CD16 and CD56 abs were labeled with PE. Since NK cells were identified as CD16+CD56+

double-positive cells it is unclear how authors differentiated between these markers.

Answer: It is correct that these 2 antibodies were combined and therefore detected CD16+ cells, CD56+ cells and CD16+CD56+ double positive cells. These cells did however not express CD3. The reviewer is correct that the phrasing is unclear and we apologize for this. We have now changed the phrasing to cells expressing CD16 and/or CD56 were this was previously unclearly stated i.e. in the figure and figure legend.

6. Authors use term "Antagonist" for rimonabant in the Abstract and "Inverse agonist" in other sections. The difference exists between these terms. Please define what is more proper in case of rimonabant.

Answer: We apologize for this inconsistency. Rimonabant (SR141716A) is selective antagonist of CB1 that behave as an inverse agonist in some bioassays where it produces opposite effects than those produced by agonists for CB1 (see for instance a very good review on this topic by Pertwee RG in Life Sciences 2005). Many publications use the term "antagonist" for rimonabant including those cited in our manuscript. For the sake of simplicity we have now changed the phrasing to "antagonist" throughout the manuscript.

7. The text of the manuscript has to be checked for typing mistakes

Answer: This was also a comment from reviewer 2. We apologize and have carefully checked and corrected for language and typing errors.

Reviewer 2

Basic reporting

In this work "Influence of rimonabant treatment on peripheral blood mononuclear cells; flow cytometry analysis and gene expression profiling" by Almestrand et al., the authors investigated possible effects on peripheral blood mononuclear cells (PBMC) in patients treated with rimonabant. Then, they evaluated leukocyte subsets by 6 color flow cytometry in eight patients before and at treatment with rimonabant for 4 week and whole-transcript gene expression profiling in PBMC before and at 4 weeks of rimonabant treatment. They evidence no significant changes of monocytes, B cells, total T cells or T cell subsets in PBMC during treatment with rimonabant, but only a small but significant increase in NK cells after rimonabant therapy. Finally, they observed by gene expression analysis, changes in expression of genes associated with innate immunity, cell death and metabolism. It is not clear how the aim of the study is connected with the background. The author highlight in the background how rimonabant can exert anti-proliferative effects in malignant lymphoma cells, on activated normal lymphocytes in vitro, two systems that are not " normal ", while in another study performed on a "normal "system, non-activated PBMC, the anti-proliferative effects are

not evident. In their study, they recruited obese patients treated with rimonabant and isolate the blood of these patients to see differences in leukocyte subsets, the authors should add a link between obesity and immune cells to better correlate the aim of the study with the background.

Answer: Rimonabant is a selective CB1 antagonist, formerly registered for treatment of obesity but withdrawn due to psychiatric side effects. We agree with the reviewer that CB1 is not highly expressed on normal blood leukocytes. CB1 is however highly expressed on certain malignant lymphomas and since previous studies have demonstrated that rimonabant inhibits lymphoma cell proliferation, rimonabant could be attractive as a novel therapeutic agent for lymphoma treatment, especially if resting lymphocytes are spared. The aim of this study was to investigate effects on peripheral blood cells in patients treated with this CB1 antagonist to investigate how previous studies on isolated cells in vitro (with no effect on resting lymphocytes but anti-proliferative effects on activated lymphocytes) translated to an in vivo situation. The only patients receiving rimonabant were obese patients but the main focus of the study is not obesity but effects on leukocytes in rimonabant treated subjects. This in now clarified in the introduction section in the manuscript.

Experimental design

It is not surprising that there are not differences in the frequency of monocytes, B cells, total T cells, or T cell subsets in PBMC, because the CB1 is not primarily expressed in these cells. The author also state in the last sentences “to investigate which gene were differentially expressed during CB1 receptor blockade” but the author do not prove that rimonabant actually acts via CB1 in this system. To this aim, they should use an antagonist to show that the regulation of the expression of those genes was regulated by the CB1 or in alternative, a method to silence the CB1 receptor in these cells?

Answer: We cannot formally prove that rimonabant has blocked the CB1 receptor in the peripheral blood cells in the patients in vivo. Our aim was not to show to which extent the CB1 receptor was blocked by rimonabant but to analyze how rimonabant treatment of patients, at the clinically recommended dosage, influenced levels of blood leukocytes and their gene expression profiles. There are, to our knowledge, no other CB1 antagonists in clinical use and rimonabant is withdrawn from the market. Therefore additional studies in patients cannot currently be performed. The effects of rimonabant on various leukocyte populations in vitro has previously been investigated by us and others (as cited in the manuscript) but there are, to our best knowledge, very little information on how cells are affected in patients treated with this drug and therefore we performed this study. To silence CB1 in primary lymphocytes is problematic since primary lymphocytes are difficult to transfect even if polyclonally activated in vitro and we feel that this is outside the aim of

the present study. We do agree with the referee that the phrase "CB1 receptor blockade" in the introduction is not accurate and we have changed the phrasing to "To investigate which genes were differentially expressed in PBMC during rimonabant treatment we used oligonucleotide arrays to compare gene expression profiles in PBMC before and during treatment." (line 56 in the revised manuscript).

Concerning the increment they observe in the NK cells after the treatment with rimonabant is it significant?

Answer: Yes it is of borderline significance p=0,049, as written at line 139 in the manuscript and in the legend to Figure 1.

Can they exclude that the patients have contracted any infections that could alter the immune cell asset and so compromise their results?

Answer: The patients reported no infectious symptoms during the study but subclinical, undetected infections cannot be ruled out. We do however find it very unlikely that all investigated subjects contracted subclinical infections that would bias the results.

Validity of the findings

The authors highlight some genes that are regulated by rimonabant treatment, they should provide more information about these genes, however the gene LILRA1 is not reported in the table, please explain.

Answer: Considering LILRA we are grateful for finding this mistake in our manuscript. It is a typing error and we refer to LILRA2 as listed in the Table 2. This has now been changed in the manuscript text, line 145. We also provide more information on the genes listed in the result section of the manuscript as requested by the reviewer.

The authors list CD177 among the up regulated genes but the fold change is -1,58 , it is not very much up-regulated, please explain.

Answer: As we describe in the Materials and Methods section we selected significantly changed genes and with an unadjusted p-value of <0,001, a False Discovery Rate (FDR) <0,1 and a fold-change equal or greater than >1,5 for up regulated genes and equal or less than <-1,5 for down regulated genes. The fold change 1,5 is an arbitrary cut-off used in many studies.

Comments for the author

In the conclusion, the authors mark differences between their study on blood isolated from obese patients and treated with rimonabant, with cell death induced by rimonabant in malignat lymphoma cells, these are different systems, what is the connection?

Answer: We apologize if the rationale of the study is not well explained. Many lymphomas are difficult to treat with current therapies. We have previously

found that lymphoma cells are susceptible to rimonabant. If there are limited effects on non-malignant lymphocytes in patients this would further strengthen the hypothesis that rimonabant could be investigated as a novel and selective lymphoma treatment since it has few side effects besides reducing appetite and, in some patients depressions. These side effects might be accepted in the situation of cancer treatment. The only patients treated with rimonabant were obese subjects and therefore effects on PBMC could only be investigated in this patient group. We have now clarified the rationale of the study in the introduction (line 36-37) of the revised manuscript.

Finally, they conclude their study, (see conclusion), suggesting further investigation of CB1 targeting in lymphoma treatment, but I do not see how their study can support this conclusion.

Answer: This study alone can not support this conclusion but if combined with previous published studies from our group and others (as it is written in the conclusion) this is a very attractive suggestion for the future. In order to clarify this further we have revised the writing of the concluding paragraph.

Minor revision

There are several grammar errors throughout the manuscript, as example:Material and methods, Flow cytometry, line 80 " The phenotypes of cells…was" it should be " the phenotype".Results, line 141 " It has previously has been shown" please revise this sentence.

Answer: We apologize for the grammar errors and have carefully checked and corrected the language.