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Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	Ms_IJBCRR_30998
Title of the Manuscript:	The effect of Phorbol 12-Myristate 13-Acetate on CD11b and CD62-L cell surface expression of neutrophils and monocytes.
Type of the Article	Original Research Article:

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	The article entitled "The effect of Phorbol 12-Myristate 13-Acetate on CD11b and CD62-L cell surface expression of neutrophils and monocytes" is interesting since the authors obtain a high yield in percentage of cells PMNn (77%) and monocytes (65%), using an isolation technique proposed by Lennie et al 1987. The effect of PMA to activate monocytes / neutrophils is effective, as they found an increase in CD11b expression on the cell surface. However, it was not the same for CD62-L expression, since even the unactivated cell had a greater expression (22%), compared to the activated one (17%), which is not well explained by the authors. These percentages are high under the conditions used by the authors (at room temperature 30 min), ie the cells were not cultured in an enriched or supplemented culture medium, or under atmospheric conditions, humidity, oxygen and CO₂. Additionally they have been identified a number of issues that need to be addressed. Abstract The authors mention that their objective of the study was to optimize the method for isolation of leukocytes, however, in the discussion and conclusion they do not mention the result of the optimization of the method. Introduction Authors fail to report references in the text. According to the general guide for authors, references cited within the text should be in square brackets [1] and not with the first author's name.	The aim of the pilot study was to determine the effect of PMA on CD62-L and CD11b cell surface expression of neutrophils and monocytes. Data generated from the studies provided some interesting results, which will provide a sound platform to undertake future studies involving larger cohorts. An explanation is included (highlighted in yellow, lines 256-259) and attempts to explain why cells activated with PMA results in a tendency to decrease their expression in comparison to un-activated (natural) cells. The leukocyte isolation method employed by the authors provides a highly reliable means to investigate leukocyte function. Although it would have been interesting to compare the effects of human blood with those under varying conditions (e.g. cells enriched with supplements and CO₂), this was not possible for the present study due to financial limitations. Abstract With regards to optimising the methods for isolation of leukocytes; this was not an aim of the paper. Therefore, and in agreement with the reviewers comments, the word optimise has been removed from the manuscript.



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	-The introduction is very long Methodology N = 4 is too small which affects the results especially in terms of statistical power. Healthy adult volunteers are not difficult to include in the study. -In paragraph 96, the authors do not mention the amount of peripheral blood obtained from each volunteer. - In paragraph 108, the authors mention that subsequent to the isolation of PMN and monocytes, the cells were re-suspended in Buffer PBS + EDTA, and were they counted, but authors did not determine cell viability -Paragraph 111, the authors did not mention the number of cellular events that were used in the analysis by flow cytometry. -Paragraph 119, the authors do not mention how they obtained the optimal dose of PMA (3.14 nM) - Paragraph 133, according to the indications to the author, the value of P should be reported as P = .05, not p=0.05 Results Table 1 was already mentioned in paragraph 91, so there is a repetition of the information -It is unclear whether the technique described by Lennie et al. serves to separate all leukocytes, or separates the different subpopulations of PMNn, monocytes, lymphocytes etc. in addition the authors, from my point of view, should have used another gradient density separation technique (Böyum 1968) to compare the results obtained. -Figure 3 and 4 it is not clear what the negative control was. -With regard to the expression of CD62L on the surface of neutrophils and monocytes, the authors do not explain why in cells activated with PMA, it presents a	Introduction References have been corrected – please refer to the revised manuscript. With regard to the number of participants recruited for the study (n=4), this was rather disappointing in the whole, as it was intended for more volunteers to be recruited to the study (n=15). The main reasons for the low number recruited were that individuals who were approached did not want to give blood, and there was a limited time restriction to undertake the study. With regards to paragraph 96, the author have acknowledged (highlighted in yellow, lines 99-100) the volume of blood obtained from each volunteer (10ml). With regards to paragraph 108, total cell counts were determined via the Coulter MicroDiff haematological analyser to determine the efficacy of the isolation methods and to help adjust counts to the required concentration prior to analysis. No cell viability studies were undertaken. With regards to paragraph 111, the authors have acknowledged the number of cellular events (5000) analysed by flow cytometer (highlighted in yellow, lines 121-122). With regards to paragraph 119. PMA at a concentration of 3.14nM was employed, as this dose has previously been used by the co-author (Dr SF Hughes) in previous studies [e.g.
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	tendency to decreasing their expression in comparison with inactivated cells, nevertheless that no significant differences were registered. - It might be interesting to include a representative dot plot in Figures 3, 4, 5 and 6. Discussion - Repeated information from paragraphs 204 to 210. - Paragraph 211 is not clear since they only verified one method (Lennie 1987), without comparing with any other method of leukocyte isolation (eg Böyum 1968). They mention that they used the Coulter® MicroDiff18 analyzer to count the blood cells. This is a method that is routinely used in pediatrics, in any clinical laboratory, low volume is used and up to 100 samples can be measured. -The authors mention the many limitations of this research; perhaps a more relevant issue is that the authors could have done a flow cytometry analysis of some chemokine receptors strongly linked to the migration of PMNn and monocytes to sites of inflammation in the immune response.	Hughes et al (2006)]. With regards to paragraph 133, this has now been amended (highlighted in yellow, line 147). Results With regards to Table 1 any repetition of the information has been removed (primarily from the discussion). With regards to the methods for isolating leukocytes, the reviewer proposes the method reported by Böyum 1968. This is a valid point, and something to consider for future investigations. Negative controls (Figures 3-6) were represented by employing PBS as auto-fluorescence (highlighted in yellow, lines 134-135) An explanation is included (highlighted in yellow, lines 256-259) and attempts to explain why cells activated with PMA results in a tendency to decrease their expression in comparison to un-activated (natural) cells. Representative dot plots figures and gating of isolated leukocytes is included in Figure 2. The inclusion of additional representative dot plots has not been included to the revised paper. Discussion Repeated information paragraphs 204-210 have now been removed.
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		With regards to the comment relating to comparing other methods of leukocyte isolation (e.g. Böyum 1968), this has been acknowledged above (results) and something to consider for future investigations. With respect to the Coulter® MicroDiff¹⁸ haematology analyser, provide an automated and reliable method for blood counting. Due to financial constraints, the authors were unable to undertake additional studies involving specific chemokine receptors (e.g. CXCR1 and CXCR2) and other chemotactic agents (e.g. LPS, fMLP) strongly linked to the migration of phagocytic cells to sites of inflammation during the immune response.
<u>Minor</u> REVISION comments	- Only numerate the first page. --The title may not have punctuation. -The authors fail to cite the references are not written according to the guide for authors.	With regard to numeration of the first page, the authors are adhering to the template provided by the journal. The title has been amended, i.e. full stop (.) removed. All references have been amended to comply with the journals format.
<u>Optional/General</u> comments		Thank you to the reviewer for their comments.