Reviewer's report

Title: Effects of Mycophenolate Mofetil on Key Pattern of Coronary Restenosis: a Cascade of in vitro and ex vivo Models

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Reviewer: William Huckle

Reviewer's report:

General
This manuscript describes studies of the effects of MMF, an esterified form of mycophenolic acid, on 3 models of arterial cell responsiveness: endothelial cells (EC) or smooth muscle cells (SMC) stimulated with TNF in static culture; these same cell types in a co-cultured leukocyte attack model; and an ex vivo porcine coronary artery injury model. The authors report that MMF potentiates TNF-stimulated ICAM mRNA expression but inhibits ICAM protein expression in HCAEC cultures and that MMF inhibits SMC proliferation in the co-culture model. No significant effects of MMF on BrdU index or neointimal hyperplasia were noted in the ex vivo model. To their credit, the authors have employed multiple models, of increasing complexity, to probe relevant effects of MMF. However several features of their report make a complete appraisal of its merit difficult.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. There is no description of the type of statistical analysis used, although p-values are reported throughout. In fig. 3A, the magnitude of the error bars shown would suggest that there is no significant difference in adhesion with or without MMF at any of the time points, yet in the text on p. 10 significance is claimed for times 1, 2, and 6 hrs. In fig. 5A, the variance in BrdU labeling appears to indicate that none of the measurements in tissues derived from ballooned arteries are different from the non-ballooned control. With respect to experimental design, important controls are missing. For example, in Figs. 1, 2 and 3 there are no results shown for treatment with MMF alone; this is especially important for the interpretation of the potentiating effects of MMF on TNF responses.

2. Page 7: How were cells recovered from adherent culture preliminary to flow cytometry? Was trypsin used? Also, since results of flow cytometry are given in terms of mean fluorescence as opposed to % positive cells, what advantage does this analysis have over immunoblotting?

3. Page 9: Although the methods section stated that northern blots were controlled by measuring GAPDH expression, there is no evidence in the results that this normalization was performed.

4. A strength of this manuscript is the authorsâ€™ effort to use concentrations of inhibitor (in this case MMF) in vitro that are relevant to those associated with effects in vivo. Thus 50 ug/ml MMF was often used, but a complete rationale for the choice of this concentration was not given. Where does hydrolysis of MMF to MFA occur, in the plasma or intracellularly? The introduction (p. 12) seems to indicate that plasma levels of MFA have been measured to gauge the absorption of MMF; is this correct? Can the authors be confident that conversion to MFA has taken place in all of their experimental systems, such that the absence of an effect can be distinguished from the absence of the inhibitor? Could the efficiency of this conversion vary according to cell type?

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

There are numerous errors of grammar and syntax in the text. Also, the figure legends and figure labeling need to be made more informative.
Discretionary Revisions (which the author can choose to ignore)

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article of importance in its field

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

**Statistical review:** Yes

**Declaration of competing interests:**

I declare that I have no competing interests.