Reviewer's report

Title: A sensitive flow cytometric methodology for studying the binding of Leishmania (Leishmania) chagasi with canine peritoneal macrophages.

Version: 1 Date: 8 November 2004

Reviewer: Aiyappa Palecanda

Reviewer's report:

General
The study addresses an important aspect of the in vitro analysis of host cell parasite interaction i.e. identifying a more quantitative and objective assay to analyse these interactions. As the authors rightly point out that using flow cytometry in place of subjective microscopy and hazardous radiochemicals is indeed advantageous.

The manuscript, as is, has several shortcomings as described below.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. The authors state in their abstract that they have shown that flow cytometry could be used to measure interaction of Leishmania to macrophages and simultaneously measure expression of the integrin, CD11b/CD18 involved in this interaction. However, the expression and/or the involvement of CD11b/CD18 on canine peritoneal macrophages in Leishmania binding is not shown. It is discussed that the authors have data (not shown) that CD11b/CD18 is involved in Leishmania binding by canine peritoneal macrophages. This data is required to substantiate the flow cytometry data presented in this manuscript since there is no specific inhibition of binding in the flow cytometry assays presented. Also the interpretation of the flow cytometry data is not substantiated (see below).

2. The fluorescent micrograph (Figure 1B) and the author's text suggest that there is a significant amount of fluorescent debris in the Leishmania prep. The uptake of this fluorescent debris might be most of the macrophage associated fluorescence and not due to macrophage binding of whole parasites. This needs to be clarified i.e. the majority of macrophage associated fluorescence is due to binding of Leishmania and not the phagocytosis of fluorescent debris (for example performing the binding assay in the presence of cytochalasin D).

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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)
1. The photomicrograph (Figure 2) and the text suggest that there is about 50% neutrophils in the peritoneal lavage cell population. The FACs profile of the lavaged cells (Figure 1C) does not corroborate this interpretation. Where are the 50% neutrophils in the FACs plot?

2. The data in Figure 4 and table 1 show that the fluorescence intensity of the macrophages when they are incubated with C5a deficient serum. The reason for this increase in fluorescent intensity has not been addressed. The authors do state that complement is required for efficient uptake of Leishmania by macrophages, but do not discuss the rationale behind using the C5a deficient serum and the observed increase in macrophage fluorescence. It is confusing as to the interpretation of the
presented data.

3. The axis of the FACs plot in figure 1C needs to be labelled (i.e.is the X-axis Forward Scatter ?)

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 whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interest