Author's response to reviews

Title: Regulation and Splicing of Scavenger Receptor Class B Type I in Human Macrophages and Atherosclerotic Plaques.

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Version: 2 Date: 12 July 2005

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Comments to reviewers

David Rhains Reviewer:

**Major Compulsory Revisions**

The authors should discuss (and not only mention) their findings on mmLDL increasing SR-BI expression in macrophages in relationship with other studies (i.e. Hirano (cited) and Han et al. J Biol Chem 2001 276:16567-72) that show a reduction of SR-BI expression with OxLDL, also considering different types and degrees of LDL oxidation.

Answer: The literature regarding the effects of oxLDL on human monocyte-derived macrophages is conflicting and we agree that this merits further discussion. The majority of data presented by Han et al have been generated using the Raw264.7 murine macrophage cell line. The data presented on human macrophages indicate differences in SR-BI expression in response to oxLDL depending on macrophage differentiation. Han et al only present data from one experiment and it is unclear if the experiment has been repeated.

Hirano et al present data showing that oxLDL increases SR-BI expression, however, it is unclear of how many different donors that were analyzed and the differentiation status of the analyzed macrophages.

A more detailed discussion section on the results obtained by Han et al and Hirano et al and discussions on the potential causes of the observed differences has been included in the manuscript (Page 13, Section 3; see below).

Previous studies have shown disparate effects of highly oxidized LDL (oxLDL) on SR-BI expression in human monocyte-derived macrophages. Hirano et al reported an increased expression of SR-BI during macrophage differentiation and that oxLDL induced SR-BI mRNA and protein expression after 24 h of treatment [26]. In contrast, Han et al reported that 10 days differentiated macrophages displayed a decreased SR-BI expression in response to 16 h oxLDL treatment [27]. However, less differentiated macrophages (3 days differentiation) responded to 16 h of oxLDL treatment with increased SR-BI expression [27]. This indicates that the differentiation status of the macrophages and the time of oxLDL treatment influences SR-BI expression. In this study we show that SR-BI expression in macrophages is increased in response to 24 h of mmLDL treatment. Differences in chemical properties of mmLDL compared to oxLDL can affect macrophage SR-BI expression. It is unclear which of these *in vitro* foam cell formation models that best resembles foam cell formation *in vivo*.

**Discussion and review of the litterature are overall skinny and do not integrate recent advances obtained from mouse models on the role of macrophage SR-BI in atherosclerosis.**

Answer: The discussion has been extended and includes a section on the *in vivo* studies of macrophage SR-BI expression and atherosclerosis (Page 13, section 1).
The key role of SR-BI in the development of atherosclerosis has been shown in genetically modified mice models. In particular, hepatic overexpression of SR-BI reduces atherosclerotic lesion formation [9], which is attributed to the role of SR-BI in the uptake of HDL-cholesterol in the liver. This is supported by the finding that SR-BI disruption increases plaque formation [26]. Genetically modified mice models have also been used to investigate the role of macrophage SR-BI expression in the development of atherosclerosis. Bone marrow transplantation studies have shown that SR-BI disruption in macrophages augment the development of advanced atherosclerotic plaques [10, 27]. This effect is attributed to SR-BI role in cholesterol efflux. However, a recent study by Van Eck et al shows that the development of early atherosclerotic plaque (fatty streaks) is reduced in macrophages lacking SR-BI expression [28]. This effect could be related to the scavenger receptor function of SR-BI by enhancing uptake of oxidized LDL or VLDL [28]. This indicates that macrophage SR-BI probably plays multiple roles in the development of atherosclerosis.

**Minor Essential Revisions**

1) Figures 4AB and 5 (some details) do not reproduce clearly (streaky bands, arrows and letters) when printed and has to be adjusted for higher resolution. By comparison, figure 6 is crystal-clear.
Answer: The figures 4 and 5 have been redone in Tiff/JPG format instead of PPT to ensure high resolution.

2) Missing word "donors" in line 2 of legend to figure 2.
Answer: This has been corrected (New figure legend Fig1, Page 23)

**Discretionary Revisions**

In figure legends, "normalized" or "standardized" would be preferable to "related".
Answer: This has been altered to normalized (Figure legend 1-2, Page 23).

**Kathleen M Botham Reviewer:**

**Minor Essential Revisions**

1. The macrophages used were obtained from healthy subjects, or those with atherosclerotic lesions. It is not clear however, which type of macrophages were used for the experiments on the effects of hypoxia and mmLDL, and for the investigation of the expression of SR-BI. I assume these were from healthy donors, but this should be indicated in the Abstract, methods/results and appropriate.
Answer: The macrophages from atherosclerotic subjects and healthy subjects are in vitro differentiated macrophages from blood (buffy coats). The same type of macrophages was used for the hypoxia and mmLDL experiments, using blood from healthy volunteers (blood donors). This has been clarified at several places in the manuscript as indicated below.
Abstract: human monocyte-derived macrophages from healthy subjects (Page 2, Line 13)
Methods: atherosclerotic subjects or healthy control subjects in the macrophage INTERGENE study or healthy volunteers. (Page 7, Line 8)
Methods: from healthy volunteers or from subjects in the macrophage INTERGENE study (Page 8, Line 4)
Methods: from healthy volunteers (Page 8, line 6)
Methods: from healthy volunteers (Page 9, line 6)
Results: Human macrophages from 13 healthy volunteers were exposed to hypoxia for 24 h (Page 10, Line 7).
Results: Human macrophages from 4 healthy volunteers were treated with mmLDL (50 μg protein/ml) for 24 h (Page 10, Line 13)

Figure legend (Fig1): different healthy voluntary donors

Figure legends.
2. The size of the products for SR-BI and SR-BII should be given in the Method section (p8).
Answer: The sentence “The amplification is predicted to generate a 476 bp fragment for the SR-BI transcript and a 347 bp fragment for the SR-BII transcript” has been added in the Method section (Page 10, Line 13).

3. There is no Figure 1A (referred to in para 1).
Answer: This is a mistake. The correct reference should be figure 3A and the text has been revised accordingly (Page 9, Line 5).

4. As Figure 1 contains only two values, it could be deleted and the figures given in the text.
Answer: Figure 1 has been removed from the manuscript and all the following figures renamed accordingly. The data presented in figure 1 have been included in the text as the sentence “The expression of macrophage SR-BI was decreased from 1.26±0.17 arbitrary units to 0.73±0.10 arbitrary units (mean±SEM; p<0.005) after hypoxia exposure as determined by real-time RT-PCR analysis” (Page 10, Line 8)

5. Legend to Figure 2, line 1, the word 'donors' seems to be missing
Answer: This has been corrected (New figure legend 1, Page 23, Line 3)