Author's response to reviews

Title: Immunologic Testing of Xeno-Derived Osteochondral Grafts Using Peripheral Blood Mononuclear Cells from Healthy Human Donors

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Version: 2 Date: 13 April 2005

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April 13, 2005

Dear Editor:

We would like to thank the reviewers for their thoughtful comments. We will address their points one by one.

Reviewer: Benichou

Point 1 – “The authors should comment on the purity of monocyte preparations”.

The purity was greater than 92% for each separation. This statement is in the Methods section, page 6 of the manuscript.

Point 2 – “Are purified monocytes used in each assay?”.

The answer is yes. This has been added to the legends of each figure.

Point 3 – “The authors should elaborate on the possible effect of photooxidation of the innate immunity in this model. The role of reactive oxygen species may be evoked”.

We thank the reviewer for this comment. To comply with this criticism, we have added the following statement to the Discussion section: “Previous work has established that photooxidized cartilage is accepted by the host better than untreated cartilage. It is likely that the oxidation process chemically modifies the protein components in such a way that these structures stimulate cells of the innate immune system to induce, for example, prostaglandins. Frequently, sterile inflammation of this sort enhances wound repair and therefore enhances the vascularization and integration of the grafted tissue, rather than rejection. Because the clinical acceptance of these grafts is common we assume that such reactions are also induced in this model”.

Reviewer Mankin

Point 1 – “Monocytes are not really a part of the host tissue in the joint…it is the chondrocyte that is likely to have a response and since they are considerably different in the reaction to immune challenge than monocytes it seems reasonable to use them as part of the study…and in fact compare the monocyte and chondrocyte responses”.

The reviewer correctly states that monocytes are not really part of the host tissue in the joint, and that chondrocytes are integral parts of the response cascade. We have included this notion in our discussion by adding the following statements. “Monocytes are not constitutive parts of the joint, however these cells are rapidly recruited to the joint after tissue injury, including surgical implantation of grafts. Unlike chondrocytes that are not professional antigen presenting cells, monocytes can phagocytose, process and
present graft antigens and initiate an immune response. This initial phase was evaluated in this study. Once the T cells are primed, they can interact with chondrocytes and other non-professional antigen presenting cells in the joint to exert the effector arm of the rejection reaction.

Point 2 – “The preparation of the monocytes may have diminished the likelihood that they would respond to xenografts and despite the difference in the control and the photo-oxidized material there is some concern regarding the validity of the model.”

The reviewer is correct, any type in vitro experimentation can affect biological systems. There is no way around this limitation to experimental work. However, the ready acceptance of such grafts in vivo by the recipients animal species tested so far (goats, sheep, and rabbits) suggests that the conclusions drawn in vitro also apply to the in vivo situation. We added a paragraph to the Discussion section to elaborate on this point. “The conclusions reached in this study were drawn using in vitro experiments. The isolation of monocytes and their tissue culture plates might affect the functionality of these cells and modify them relative to their in vivo activation state. These constraints apply to any in vitro work. However, the purpose of this study was to establish whether monitoring the activity of such cells in vitro would provide insights on additional activation of them by stimulation with compounds such as the tissue culture material that was used. It is clearly shown that beyond whatever activation occurs in tissue culture, the graft materials tested induce a variable level of activation. Because activation of such cells of the innate immune system is key to their immunological activity, this study shows that sensitive monitoring of monocytes activity in response to such materials can be done in vitro.”

Point 3 – “Photo-oxidation kills the cells in the graft and presumably that is reasonable as defined by use of valve grafts. There is some concern however for cartilage grafts which are probably very dependent on some degree of cell survival for any kind of functional result. Were the cells in the untreated grafts still viable? Is this the reason for the marked change in the macrophage response?”

As indicated on page 5 of the Methods section, all osteochondral grafts used as antigen in the study, whether untreated or treated, were frozen at -80°C, ground to a fine powder averaging 2-5 μm and subsequently lyophilized. Thus, there were no viable cells in any of the antigen materials. The marked change in the macrophage response was thus determined to be the result of the processing steps.

Point 4 – “Have the authors considered using porcine grafts in ovine or bovine cartilage to assess the response of the cartilage to the immunologically foreign material?”.

Preliminary studies have already been conducted using photooxidized bovine osteochondral grafts in the joints of sheep and rabbits, with encouraging results. These studies were histological studies and are discussed in the Background section of the manuscript. In the future, we hope to evaluate the immunological response when a photooxidized implant is placed in the joint. The following statement was added to the
Conclusions section: “This will be followed by evaluation of the implant and immune reactions when a photooxidized implant is placed in the joint environment”.

Sincerely,

Vincent J. Hetherington, DPM