that the ICF is a time-consuming evaluating tool with scarce feasibility and requiring a high skillfulness [6].

In conclusion, the contribution of rehabilitation to the treatment remains a fundamental tool for the recovery of the RA patient. However, specific outcome measures are still awaited to allow the validation of rehabilitative techniques proposed to fight disability and handicaps of a disease such as RA.

The authors have declared no conflicts of interest.

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**Monoclonal antibody HC10 does not bind HLA-G**

Sir, Recently, Raine et al. [1] published a study about measuring the ratio of β2m associated MHC class I and their free heavy chains (FHCs) on human peripheral blood cells and human placental trophoblast cells. The authors used the monoclonal antibodies (mAbs) W6/32, specific for fully assembled MHC class I molecules and the mAb HC10, specific for FHCs. Flow cytometry analysis revealed higher levels of FHCs on leucocytes of a reactive arthritis (ReA) population than in healthy controls. In addition, the authors described a very high relative level of FHCs on placental extravillous trophoblasts (EVTs), suggesting HLA-G to be the main source of FHCs on this cell type. However, the assumption drawn by the authors is misleading, since the employed mAb HC10 is not the appropriate tool to come to such conclusion. It is of particular scientific relevance to comment on this work, since the mAb HC10 binds HLA-B and -C but not to HLA-G.

The used antibody was an integral tool of their study and has an impact on the obtained results. Hence, it is indispensable to clarify the reaction patterns of this mAb in order to avoid misinterpretation of published results and already drawn conclusions. In this context, we demonstrate that HC10 definitely does not react with human HLA-G. We want to refer to some facts about the relevant antibodies (Table 1) and show immunohistochemical data, which might contribute to an adjustment of the results obtained with isolated trophoblasts in their study.

The well-known and extensively used antibody W6/32 binds a conformational dependent epitope on all HLA class I products [2].

**Table 1. Antibodies used for immunohistochemistry (IHC)**

<table>
<thead>
<tr>
<th>mAbs</th>
<th>Specificity</th>
<th>Subclass</th>
<th>IHC</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>4H84</td>
<td>HLA-G</td>
<td>IgG1</td>
<td>1 μg/ml</td>
<td>10</td>
</tr>
<tr>
<td>W6/32</td>
<td>HLA-A,-B,-C,-G,-E,-F + β2m</td>
<td>IgG2a</td>
<td>1:100</td>
<td>2</td>
</tr>
<tr>
<td>HC10</td>
<td>HLA-B,-C</td>
<td>IgG32a</td>
<td>1:25</td>
<td>3</td>
</tr>
<tr>
<td>HCA2</td>
<td>HLA-A,-G</td>
<td>IgG1a</td>
<td>1:25</td>
<td>13</td>
</tr>
</tbody>
</table>

*BD Biosciences Pharmingen.*

*Dako.*

*Kind gift from H.L. Ploegh.*

*Supernatant of hybridoma cells.*

In contrast, mAb HC10 was originally raised against free class I, HLA-B locus heavy chains [3], but was subsequently shown to bind also HLA-C [4, 5]. Another FHC specific mAb, HCA2, raised against HLA-A locus products [6], was not employed by Raine et al. HCA2 recognizes also HLA-G [7], due to an 80% homology between both products and could have served as a good marker in the respective study to measure HLA-G FHCs levels on trophoblasts. In Fig. 1 we show and confirm the abilities of the mAbs W6/32, HCA2, and HC10 to bind to HLA-G produced by extravillous trophoblast cells (EVTs) in human first trimester placenta and JAR-HLA-G, a cell line stable transfected with genomic HLA-G. HC10 does not react with HLA-G but binds HLA-G present in EVT and in the mesenchymal core of the villous chorion (M).

In contrast, mAb HC10 was originally raised against free class I, HLA-B locus heavy chains [3], but was subsequently shown to bind also HLA-C [4, 5]. Another FHC specific mAb, HCA2, raised against HLA-A locus products [6], was not employed by Raine et al. HCA2 recognizes also HLA-G [7], due to an 80% homology between both products and could have served as a good marker in the respective study to measure HLA-G FHCs levels on trophoblasts. In Fig. 1 we show and confirm the abilities of the mAbs W6/32, HCA2, and HC10 to bind to HLA-G or not. For that purpose we used the MHC class I negative choriocarcinoma cell line JAR as negative control and JAR cells stable transfected with human genomic HLA-G. HC10 does not react with HLA-G but binds HLA-G present in EVT and in the mesenchymal core of the villous chorion (M).

In conclusion, the contribution of rehabilitation to the treatment remains a fundamental tool for the recovery of the RA patient. However, specific outcome measures are still awaited to allow the validation of rehabilitative techniques proposed to fight disability and handicaps of a disease such as RA.

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of HLA-C [4, 5, 11]. Though former studies have already shown immunohistochemical data obtained with the mAbs W6/32, HCA2 and HC10, we have recapitulated the immunohistochemistry in combination with the highly HLA-G-specific mAb 4H84 and a sensitive detection system. Thus, we were able to show that EVTs are the only cells in human placenta that express HLA-G (Fig. 1; mAb 4H84). None of the MHC class I products is expressed in villous trophoblasts, as shown by staining with mAb W6/32. The strong HLA-G expression in EVTs can also be demonstrated with the mAb HCA2. The mAb HC10 gives only a moderate staining of EVTs, indicating the expression of HLA-C, because about 95% of the expression of HLA-B on these cells was excluded previously [12]. The presence of the β2m complexed classical HLA-A and -B molecules in the mesenchymal core of the villous chorion is best demonstrated with mAb W6/32 (Fig. 1). However, HLA-B and HLA-A can also be detected with the FHC specific mAbs HC10 and HCA2, respectively.

Taken together, our data strongly substantiate that Raine et al. in their study measured not HLA-G but HLA-C FHCs on isolated trophoblasts. Considering this fact, the results and conclusions obtained in that study have to be reconciled.

The authors have declared no conflicts of interest.

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Response to Gauster et al.

Sir, We would like to thank Dr Gauster and his colleagues for their interest in our article and their helpful comments regarding the figure on trophoblast MHC-I. In our article, we aimed to assess the relative levels of MHC-I heavy chains compared with conformed MHC-I on the surface of cells from healthy controls and arthritis patients using the monoclonal antibodies HC10 and W6/32. MHC-I heavy chains, as indicated by HC10 reactivity, have been been implicated in the pathogenesis of spondyloarthropathy [1, 2], and we observed an increase in the relative levels of MHC-I heavy chains for arthritic patients compared with healthy controls.

In Figure 4 of our manuscript we used the same antibodies to examine MHC-I heavy chains on trophoblast, as it has been suggested that recognition of FHC on this tissue could contribute to maternal immunoregulation during pregnancy [3]. We again used HC10, in order to allow comparison with results from peripheral blood, and because this antibody had previously been shown to immunoprecipitate both HLA-C and HLA-G [4]. The findings of Gauster and colleagues show that the reactivity of HC10 with HLA-C is not sufficient to stain cells by immunohistochemistry. If a similar situation exists for flow cytometry, it indicates that the particularly high levels of MHC-I heavy chains we observed on trophoblast are, in fact, an underestimate. Thus, in addition to the HLA-G structures previously described [5–7], we can now conclude that trophoblast HLA-C appears to be present at unusually high levels as heavy chains. If trophoblast MHC-I heavy chain levels are, as Dr Gauster’s findings suggest, even higher than our original estimate, it will be important to address their functional relevance. MHC-I heavy chains have been shown to act as a ligand for inhibitory LILR receptors, which are found on maternal decidual macrophages [8, 9], and may therefore suppress maternal macrophage activity. We believe that recognition of MHC-I heavy chains by LILR will prove to be an exciting field in the immunology of pregnancy.

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