Inhibition of HIV-1 replication by alloantigen-stimulated supernatants derived from PBMC cultures of healthy blood bank donors and HIV-1–infected individuals

<table>
<thead>
<tr>
<th>Donors</th>
<th>HIV-1BaL</th>
<th>IL-2</th>
<th>IFN-γ</th>
<th>IL-10</th>
<th>IFN-α</th>
<th>RANTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6/12 (0-86%)</td>
<td>4/9 (0-91%)</td>
<td>87 ± 25</td>
<td>172 ± 72</td>
<td>27 ± 11</td>
<td>∼20</td>
</tr>
<tr>
<td>HIV*</td>
<td>5/13 (0-87%)</td>
<td>6/12 (0-99%)</td>
<td>27 ± 15</td>
<td>96 ± 30</td>
<td>12 ± 4</td>
<td>∼20</td>
</tr>
</tbody>
</table>

*Fraction of individuals with supernatants that inhibited HIV-1 replication greater than 50% (range of inhibition of viral replication).

§Median CD4 T-cell counts = 662 cells/µL (range, 195-787).

plain the generation of this activity by alloantigen-stimulated cells from HIV-infected patients, which have been reported to exhibit immune costimulatory defects.8,9

These findings contribute to characterization of the molecular mechanisms and costimulatory requirements for alloantigen-stimulated anti-HIV activity that might be important for the development and application of immune-based strategies against HIV.

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The views expressed herein are those of the authors and do not reflect the official policy of the Department of Defense or other departments of the US government.

References

To the editor:

Correction of the PNH defect by GPI protein transfer: still an open question

Sloand et al1 report that in human red blood cells (RBCs) the paroxysmal nocturnal hemoglobinuria (PNH) defect can be corrected by transfer of glycosylphosphatidylinositol (GPI)-anchored proteins to GPI-deficient cells. It has been demonstrated before by a number of authors that cell-to-cell transfer of GPI-anchored proteins can occur, and I have no doubt that a similar event also takes place between erythrocytes and microvesicles enriched in GPI-anchored proteins. However, in my opinion, it can be questioned whether the experiments performed by Sloand et al1 warrant the conclusions that were drawn.

In Figure 1B the authors show an immunoblot using a monoclonal antibody (mAb) against CD55. This antibody reacts with more than a dozen bands (see lanes 1, 2), making it surprising that the authors are able to identify CD55 among many other bands of similar intensities in lane 3 of the same figure. The antibody apparently reacts with such a large number of proteins that the immunoblot shown is by no means a proof for the presence of CD55 in the sample—a result that the authors rely on in a number of subsequent experiments in the paper. Such a broad reactivity of a monoclonal antibody would prompt me to question its specificity or check the methodology for immunoblotting. Similarly, in Figure 1C the authors show a blot with an mAb against CD59 that reacts with 2 bands of similar intensities. Although in this case the identification of CD59 may not give rise to much criticism, one should be aware that the same antibody is used throughout the paper in flow cytometry experiments, and it is not clear with what it may react in these experiments. The situation is even more worrisome in the case of the mAb against CD55, which is also used in their flow cytometric analyses.

Even more importantly, the authors claim that in order for a transfer of GPI-anchored proteins to RBCs to occur, the GPI anchors must be intact. This conclusion is based solely on their observation that treatment of the material containing the GPI-anchored proteins with bacterial phosphatidylinositol-specific phospholipase C (PI-PLC) abolishes transfer. Interestingly, however, it is well known from the literature that the GPI anchors of human RBC proteins are substituted with a fatty acyl chain on the inositol,2-5 which renders the structures insensitive to cleavage by PI-PLC.2-5 A careful study of the literature would have revealed this fact. Thus, their observation that transfer of proteins to RBCs was decreased after PI-PLC treatment cannot be explained by GPI-anchor hydrolysis, and I wonder what might have caused the altered findings.
Response:

Strong evidence for correction of the PNH defect

In an article recently published in *Blood*¹ we demonstrated that GPI-linked proteins can be passively transferred from cell to cell and incorporated into the membrane as exemplified using decay accelerating factor (DAF) and membrane inhibitor of reactive lysis (MIRL). Both of these GPI-linked proteins are well characterized on a molecular and functional level. Our results are in agreement with other studies in vitro and in vivo, including one by the author of the letter,² who demonstrated that exogenous GPI-linked molecules can incorporate into human erythrocyte membrane.

With regard to Dr Bütkofer’s criticisms of our manuscript, we make the following comments:

1. Different CD55 and CD59 antibodies were used for immunoblotting and for flow cytometry. The antibody used for immunoblotting was selected because it had been previously tested for this application by the commercial provider. Immunoblot results and flow cytometry data are not linked, but rather are confirmatory of each other. Immunoblots were developed using mouse CD55 and CD59 mAb and using the APAAP method (antimouse IgG-AP conjugate and anti-AP IgG-AP conjugate). Multiple bands were obtained for both CD55 and CD59; all immunoblots contained the desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band, however. Immunoblots of microvesicle preparations obtained from patients with PNH lacked the specific bands or desired band.

2. The flow cytometric data was very clear: multiple antibodies expressed them very faintly when equal protein loads were applied to the gel. The two bands obtained for CD59 were 19 and 36 kd molecular weight. 19 kd is the molecular weight of CD59. The 36 kd band corresponds to CD59 dimer, as previously described in the literature.³ ⁴ Indeed, dimeric CD59 is the primary form on cell membranes. Bands obtained for CD55 were of 60 kd, 38 kd, and 55 kd molecular weight. The 60 kd band represents CD55 (DAF-A), while additional forms of CD55 have been described at 55 kd (DAF-B) and 43 kd (precursor form⁵). Immunoblotting was performed on the specimens to document the presence of GPI-linked molecules in the eluate, microvesicles, and high-density lipoprotein (HDL). Flow cytometry was also used, and produced compatible results, with both microvesicle preparations and intact negative cells where an increase in specific fluorescence after experimental transfer was detected.

All data presented in the article provide strong evidence for our conclusions. We appreciate the author’s concerns regarding the necessity to validate the data but believe that they are unfounded, as meticulous attention was given to ensuring that antibodies were specific for CD55 and CD59, and that results using different techniques were congruent. I am pleased that Dr. Bütkofer agrees with us regarding the basic importance of the study concepts as well as the results.

We apologize to Dr Bütkofer for not citing his work, but our manuscript was submitted to *Blood* immediately prior to his publication.

References