To the editor:

The expression of CD10 by apoptotic lymphocytes is preceded by a pronounced externalization of phosphatidylserine

Cutrona et al demonstrated the expression of CD10 on human postthymic T cells when undergoing apoptosis and discussed how, in vivo, this effect may act to limit potential inflammation at the site of the apoptotic cell or aid macrophage recognition. Identification of apoptosis and CD10 in many of Cutrona et al’s experiments was dependent on flow cytometry, using the combined staining of anti CD10 and annexin V. This process has also been observed when resting human tonsillar B cells are activated toward a germinal center phenotype. The resultant cell demonstrates the coexpression of the apoptotic marker annexin V and CD10, as it becomes apoptotic. Recently, we have observed apoptosis using annexin V, in addition to other apoptosis detection systems, using flow cytometry in the lymphocytes of patients treated by extracorporeal photopheresis (ECP). ECP therapy involves the involvement of a patient’s own buffy coat after leukapheresis and exposure to 8 methoxypsoralen and UVA radiation. We were therefore keen to see if the CD10 expression observed in these previous studies was apparent on the apoptotic lymphocytes induced by ECP.

We screened 12 patients, taking samples immediately before commencing ECP treatment and after ECP treatment, immediately prior to the reinfusion of the treated cells. Using a similar flow cytometric testing system, we tested the lymphocytes of the pre-ECP sample ex vivo, whereas the post-ECP lymphocytes were tested after 0 (ex vivo), 6, 24, and 48 hours in an RPMI culture medium. The mean percentage of lymphocytes demonstrating a positive expression of each marker are shown in Table 1.

By replacing the propidium iodide (PI) with anti-CD3, we also assessed the level of CD10 expression on the T cells in the pronouncedly apoptotic 48-hour culture sample. The mean percentages of T lymphocytes demonstrating annexin V and CD10 positivity were 81.39 and 26.21, respectively.

These results are in line with previous investigators, in that apoptotic lymphocytes express CD10. But as Cutrona et al commented, we also observe that the membrane expression of phosphatidylserine residues precedes that of CD10. Our findings also demonstrate that the membrane expression of CD10 is very weak, even at time points in culture where annexin V expression is very strong and conversion to secondary necrosis, as detected by PI uptake, has begun.

Phosphatidylserine (PS) externalization on lymphocytes is a recognition signal for their engulfment by macrophages or Kupffer cells. This process occurs soon after PS expression and while the membrane of the cell remains intact. A loss of membrane integrity occurs at the early stages of secondary necrosis. The presence of PS on the outer membrane of cells is considered not only necessary but also sufficient to trigger recognition. It therefore seems likely that the CD10 expression on the apoptotic lymphocytes in vivo would appear when engulfment or processing by macrophages is imminent or has occurred.

Early macrophage engulfment of apoptotic cells serves to protect the surrounding tissue from the potential inflammatory damage, which may occur by the release of the intracellular contents of a lysed cell. What influence the occurrence of a weak CD10 expression has relatively late in the swift cell processing mechanism of macrophages is unknown, but it is probably minimal.

The role of CD10 as a marker to identify and isolate apoptosing T cells in vitro and ex vivo, as suggested by Cutrona et al, is also questionable, as there are other more specific apoptotic markers that are technically as easy to use and readily available.

Table 1. Mean percentage of lymphocytes demonstrating positive express of markers

<table>
<thead>
<tr>
<th></th>
<th>Before ECP</th>
<th>After ECP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hrs</td>
<td>6 hrs</td>
</tr>
<tr>
<td>Annexin V</td>
<td>6.21</td>
<td>16.93</td>
</tr>
<tr>
<td>Propidium iodide</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>CD10</td>
<td>&lt;1</td>
<td>1.28</td>
</tr>
</tbody>
</table>

References

The expression of CD10 by apoptotic lymphocytes is preceded by a pronounced externalization of phosphatidylserine

John Bladon and Peter Taylor