HLA RESTRICTED KILLING OF MALE TARGETS BY MULTITRANSFUSED FEMALES IN THE CELL MEDIATED LYMPHOLYSIS TEST

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INTRODUCTION

T-cell-mediated cytotoxicity directed against minor histocompatibility antigens has been found in rodents and in man. We report here, in vitro studies in which we have shown that the gene products of the major histocompatibility complex are involved in the recognition of minor histocompatibility antigens, such as H-Y, by cell mediated lympholysis.

SUMMARY

A group of 14 aplastic anaemia patients was studied, who had received multiple blood transfusions. Three female patients showed HLA-restricted H-Y killing. In two cases lysis was directed only to HLA-A2 positive male target cells and in the third case the killing was directed not only against HLA-A2, but also against HLA-B7 male target cells. Using a monolayer absorption technique, it was possible to absorb the specific effector cells using monolayers derived from HLA-A2 male individuals. Monolayers obtained from females and non-HLA-A2 males were ineffective. Furthermore it was demonstrated with the third patient, that effector cells directed to either HLA-A2 or -B7 could be independently absorbed.

The level of the HLA-restricted H-Y killing declined with time. However, it was possible to reactivate it by in vitro stimulation with lymphocytes from an HLA-A, -B, -C identical, but HLA-D different male donor.

H-Y incompatibility could be of importance in kidney transplants.
plantation since a significant difference was found in graft survival between HLA-A2 positive females, receiving HLA-A2 positive male kidneys, and non-HLA-A2 females receiving non-HLA-A2 male kidneys.

MATERIALS AND METHODS

The cell-mediated-lympholysis (CML) assay has been previously described in detail (1). The immune absorption technique used in this study was demonstrated during the 12th International Leucocyte Culture Conference and described in detail elsewhere (2).

RESULTS

1. Specific absorption of effector cells

After in vitro sensitization, the responder cells of one of the female aplastic anaemia patients reacted specifically against HLA-A2 males in a CML assay (3). These effector cells were absorbed on different monolayers and the non-adherent cells were removed and tested on a panel of unrelated target cells.

Figure 1 shows, that unabsorbed effector cells were able to lyse HLA-A2 positive male target cells giving 82% kill at an effector to target ratio of 50:1. At 10:1 the lysis was still 48%. When the effector cells were absorbed respectively on an HLA-A2 negative male monolayer, an HLA-A2 negative or an HLA-A2 positive female monolayer, we were not able to deplete the clone of cytotoxic cells. However, when absorbed on an HLA-A2 positive male monolayer we were able to reduce the specific cytotoxicity from 82% down to 29%. This implies that considerable reduction had occurred when one took into account that the percentage lysis of the unabsorbed target cell was 48% at ratio of 10:1.

In a second part of this equipment we investigated whether it was possible to specifically absorb one clone of the cytotoxic cells and leave the other intact, thus demonstrating the clonal expression of specific killer cells. For this we used the cells of the female patient who was able to show cytotoxic activity against two independent specificities, namely HLA-A2 and HLA-B7 male target cells.

Figure 2 shows, that unabsorbed effector cells lysed an HLA-A2 positive, HLA-B7 positive male target cell to a high level giving 84% at an effector to target ratio of 50:1. At 10:1 68% lysis was obtained. When absorbed on an HLA-A2 negative, HLA-B7
Fig. 1. Sensitized cells of one of the female patients before and after absorption on different monolayers. Phenotype of the patient ϕ: HLA-A2, B12, w40; Cw3, -. Monolayers (Petri-dishes treated with Poly-L-Lysine) were made of fresh peripheral blood lymphocytes. The non-adherent cells were removed after 1 hour at room temperature. The effector cells were then overlaid and incubated for 1 hour at 37°C and the non-adherent cells were removed and tested on several target cells.

Monolayers: χ = Non-HLA-A2 male. o = Non-HLA-A2 female. o = HLA-A2 female. Δ = HLA-A2 male.

Fig. 2. Sensitized cells of the third patient before and after absorption on different monolayers. Phenotype of this patient ϕ: HLA-A2, B7; B7, 15; Cw3, -. Monolayers: χ = Non-HLA-A2, Non-HLA-B7 male. o = HLA-A2 male. o = HLA-B7 male. Δ = HLA-A2, HLA-B7 male.

negative male monolayer no reduction in cytotoxicity occurred. However, absorption on an HLA-A2 positive male cells monolayer reduced lysis from 84% down to 52%. The remaining cytotoxic activity was directed against the HLA-B7 positive cells. The reverse occurred when an HLA-B7 positive male monolayer was used (figure 2). It was evident, that the most effective reduction in cytotoxicity was obtained after absorption on a monolayer carrying both specificities. Cytotoxic activity was reduced to 24%, which was more than a 100 fold reduction when one
took into account the high level of lysis of the unabsorbed effector cells at a ratio of 10:1.

In figure 3 the reduction of lysis in CML after absorption on different monolayers is expressed as a percentage of the lysis with unabsorbed cells. It shows that when the effector cells were absorbed on an HLA-A2 positive male monolayer and tested on an HLA-A2 positive male target cell, the reduction in lysis was 81%. In agreement herewith a reduction in lysis of 68% was found after absorption on an HLA-B7 positive male monolayer and tested on an HLA-B7 positive male target cell. Of great interest was the observation that a small but significant reduction (29%) in lysis of B7 positive cells after absorption on A2 monolayers and similarly a small reduction (30%) occurred in the killing of A2 positive cells after absorption on B7 monolayers. There was no clear explanation of this phenomenon but the possibility existed that it was attributable to clones of killer cells which recognized antigens that were shared by both A2 and B7 positive target cells. No reduction at all has been found using non relevant male or female monolayers.


We were able to test one of the aplastic anaemia patients
74 weeks after her last in vivo immunization. Figure 4 shows, that effector cells of this particular patient gave a percent lysis against her HLA identical male sibling of 68% at week 32. A consistent decline in the killing occurred until at week 74 a percent lysis slightly above background level was reached. Nevertheless it was possible to restimulate the H-Y specific killing by in vitro stimulation using lymphocytes from an HLA-A, -B, -C identical, but HLA-D different unrelated male donor; effector cells were formed, which again had specificity for HLA-A2 and maleness (figure 4).

3. Possible relevance for renal transplantation

Cadaveric renal allograft survival data, collected under the auspices of Eurotransplant, were examined at two years after transplantation for the influence of sex and HLA. It was found, that there was a difference of borderline significance, between renal graft survival in HLA-A2 positive females, receiving HLA-A2 positive male kidneys versus non-HLA-A2 females receiving non-HLA-A2 male grafts. This difference was only found in the leucocyte antibody positive group (table 1).
TABLE I. Two year actuarial cadaveric renal graft survival in Eurotransplant patients: sex and HLA

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<thead>
<tr>
<th>Leucocyte antibody positive group</th>
<th>Leucocyte antibody negative group</th>
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<tr>
<td>( \sigma^A 2 + \varphi^A 2 )</td>
<td>( \sigma^A 2 + \varphi^A 2 )</td>
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<tr>
<td>38%</td>
<td>58%</td>
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<td>( N = 48 \times )</td>
<td>( N = 50 )</td>
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<tr>
<td>58%</td>
<td>61%</td>
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<tr>
<td>( N = 53 )</td>
<td>( N = 53 )</td>
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\( \times \) at risk

CONCLUSIONS

The technique of immune absorption gave us the opportunity to test whether we could absorb specifically one clone of specific killer cells. We demonstrated, that HLA-restricted anti H-Y is clonally expressed. This implies either that anti-self-HLA is clonally expressed or anti-altered-self-HLA is clonally expressed. Furthermore, it is possible to reactivate the memory for the specific H-Y killing in vitro. In one case this was demonstrated three years after the last blood transfusion. Finally: male allografts from HLA-A2 positive donors in HLA-A2 positive female recipients might survive significantly shorter.

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REFERENCES