Mixed lymphocyte reactions do not predict severity of graft versus host disease (GVHD) in HLA-DR compatible, sibling bone marrow transplants

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SUMMARY Mixed lymphocyte reactions (MLRs) were measured in 25 HLA-A, B and DR compatible sibling bone marrow transplants. Only four of 25 MLRs were positive and in these the low reactivity was of doubtful clinical importance. There was no correlation between MLR and the subsequent development or severity of graft versus host disease (GVHD). A survey of bone marrow transplant units in the United Kingdom showed that most centres perform HLA-DR typing as well as an assessment of the MLR. Factors other than histocompatibility are important in the pathogenesis of GVHD and the data from this study suggest that conventional MLRs can be omitted in HLA-A, B and DR compatible sibling bone marrow transplants.

The use of histocompatible sibling donors and intensive immunosuppression means that graft rejection is no longer a major problem after allogeneic bone marrow transplantation.¹ Graft versus host disease (GVHD), however, remains a serious complication in 25 to 70% of patients receiving grafts from HLA-A, B, DR identical siblings.²

Minor transplantation antigens may also have a role in GVHD,³ and mixed lymphocyte reactions (MLR) are commonly measured in the belief that they predict this risk. The MLR, however, is a time consuming and expensive investigation, and factors other than histocompatibility may influence the development of GVHD.⁴

This study of HLA-DR compatible sibling bone marrow transplants was therefore carried out to assess the usefulness of the MLR in predicting the development and severity of GVHD.

Material and methods

Allogeneic bone marrow transplant from HLA-A, B and DR compatible siblings was performed on 25 patients with acute myeloblastic leukaemia (n = 8), acute lymphoblastic leukaemia (n = 5), chronic granulocytic leukaemia (n = 9), aplastic anaemia (n = 1), myelofibrosis (n = 1) and myelodysplastic syn-

drome (n = 1). All patients received prophylaxis with cyclosporin A, methotrexate, or T-cell depleted marrow against the development of GVHD, and all blood products were irradiated. The diagnosis and severity of GVHD were graded according to the Seattle grading system.⁵

MIXED LYMPHOCYTE REACTION

One-way MLRs in the GVH direction were performed by a modification of a previously described method.⁶ Peripheral blood lymphocytes were separated on Ficoll/Triosil and were washed and suspended in RPMI 1640. Responder cells from the donor were cultured with stimulatory cells treated with mitomycin C from the recipient. Triplicate cultures of peripheral blood lymphocytes in microtitre plates were performed in RPMI/10% v/v AB serum, and cells from random normal subjects were used as controls. After incubation for six days the dividing cells were labelled with ³H-thymidine for 16 hours. Radioactivity in harvested cells was quantified in a beta-counter and the results expressed as a relative ratio:

$$\frac{\text{dpm (responder + mitomycin-C treated stimulator)}}{(\text{dpm responder}) + (\text{dpm mitomycin-C treated stimulator})}$$

Results

Grade I-IV GVHD occurred in 20 of the 25 patients

Figure 1 MLR relative ratios in various grades of GVHD.

and the figure shows that the MLR results did not correlate with the grade of GVHD. A relative ratio of less than 2 was regarded as showing D-loci compatibility and only four MLRs had a value of more than 2. The greatest MLR ratio was 4.2 and this patient did not develop GVHD. In these assays stimulatory peripheral blood lymphocytes from random unrelated subjects gave a mean relative ratio of 13.0 (range 2.5–47.5).

By contrast, some donor/recipient sex matches seemed to have a strong predictive value for the development of GVHD. All three transplants performed from a female donor to a male recipient developed grade IV GVHD despite having an MLR ratio of less than 1. Other sex match combinations had no apparent effect.

The mean age of patients with grade 0 GVHD was 33 (SEM 3) years compared with 29 (7) years for grade I disease, 28 (3) years for grade II, 29 (3) years for grade III and 31 (4) years for grade IV. In this relatively narrow age range, therefore, age had no significant effect on the severity of GVHD.

A survey was performed of 20 bone marrow transplant centres in the United Kingdom. This showed that 18 performed HLA-A, B/DR typing in addition to an MLR, one performed HLA-A, B/DR typing but no MLR, and one performed HLA-A, B typing and MLR but no DR assessment.

Discussion

In assessing patients for sibling bone marrow transplant, HLA genotypic identity between donor and recipient is usually accepted if the HLA-A, B antigens are identical and there is evidence of compatibility in the HLA-D region. In unusual circumstances, however, non-identical HLA genotypes can exist in siblings who are HLA-A, B and DR compatible. Thus a chromosomal recombination with undetected MHC determinants such as DP would not be detected using routine serotyping. A parent can also be phenotypically homozygous for HLA-A, B and DR antigens but not genotypically identical, giving rise to siblings who are phenotypically HLA identical but genotypically different.⁷ Such uncommon events might not be detected by an additional MLR and their role in GVHD in sibling bone marrow transplants remains unclear.⁸

The conventional MLR detects antigens capable of stimulating alloreactive T lymphocytes and is a composite reaction to multiple subregional lymphocyte activating determinants. At least three subregions have been identified within the HLA-D region of the MHC, and these are designated HLA-DR, DP, and DQ. HLA-DR, and to a lesser extent, DQ can both stimulate the MLR,⁹ but strong linkage disequilibrium exists between DR and DQ genes, so that DR identical subjects are usually DQ identical.⁹ HLA-DR serological typing therefore correlates closely with the results obtained in the MLR. HLA-DP incompatibility does not affect the conventional MLR, though it can be detected by the primed MLR.¹⁰

Siblings who are HLA-A, B and DR compatible should generally be genotypically HLA identical and therefore MLR non-reactive. In these cases an additional MLR should be unnecessary and in practice a reactive MLR is seldom found.¹¹ Our data support this view as 21 of 25 HLA-A, B and DR compatible sibling MLRs were unreactive, and the level reactivity in the remainder was not high enough to be clinically important.

Despite HLA-A, B and DR compatible donors, GVHD remains common after sibling bone marrow transplant.² In these circumstances an MLC is often performed in the belief that it determines minor incompatibilities and more accurately predicts GVHD. Our own survey showed that DR typing and the MLR are both performed in most bone marrow transplant centres in the United Kingdom. Furthermore, our results showed that there was no correlation between the MLR and the subsequent development or severity of GVHD.

In recent years it has become apparent that factors other than MHC compatibility are important in the pathogenesis of GVHD, including recipient's age,

donor/recipient sex match,⁴ and infections.¹² Our own results confirmed a sex match effect but the patients' age range was too narrow to show any age effect.

The role of hitherto unrecognised genetic histocompatibility antigens which are not linked to the MHC loci on chromosome 6 remains unknown. It has been suggested that such histocompatibility differences between HLA-A, B and DR compatible siblings can be detected in the mixed epidermal cell lymphocyte reaction (MECLR) and these results seemed to correlate with GVHD¹³ and clearly warrant further study.

There is, however, little evidence that conventional MLR testing in HLA-A, B and DR compatible siblings provides further useful information regarding the development or the severity of GVHD, or the selection of donors. We therefore suggest that it be omitted in the routine assessment of histocompatibility in sibling bone marrow transplant.

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