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HLA allotype expressivity in transplantation

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In this issue of *Blood*, Petersdorf et al observe more adverse outcomes in hematopoietic stem cell transplantation with unrelated donors when the patient's mismatched HLA-C allele has a high level of expression.[1]

The authors imputed the expression levels of each HLA-C allele present in each of the 1975 patients and in their corresponding donors according to previously published quantitative measurements of median fluorescence intensity coefficients[2] of the common HLA-C alleles. Differential cell surface expression levels of all common HLA-C allotypes in $CD3^+$ cells have been demonstrated by flow cytometry analyses using the monoclonal antibody DT9[2]; this antibody reacts only with HLA-C and HLA-E proteins, with the latter being expressed at low levels in $CD3^+$ cells.[3] Therefore, the predicted expression level of each HLA-C allele can be assigned as a continuous variable. The examination of the effects of HLA-C allotypes on outcomes of HIV-infected individuals showed that higher HLA-C expression levels directly correlated with better control of HIV viral load, slower progression to HIV-AIDS, increased likelihood of cytotoxic T-lymphocyte responses, and frequency of viral escape mutation individuals.[2] These observations underscore a functional role for HLA-C expression levels in modulating the strength of immune responses.

In the present study, Petersdorf et al assess the clinical significance of the level of HLA-C expression in a large international population of patients and unrelated transplant donors with a single mismatch in HLA-C. They observed that increasing the expression level of the patient's nonshared HLA-C allele was significantly associated with increased risks of acute graft-versus-host disease (GVHD), nonrelapse mortality, and mortality; expression levels of the patient's HLA-C mismatched allele did not associate with disease relapse. Less notorious was the effect of the increasing expression level of the donor's nonshared HLA-C. The authors postulate that the strong effect of patients' HLA-C expression level on GVHD risk likely results from enhanced graft-versus-host recognition of highly expressed patient allotypes by the donor graft.

Petersdorf et al evaluate the effects of expression levels in which the mismatched HLA-C alleles included or did not include amino acid differences at residues 116 and 77/80; these residues reside in the F pocket and influence the size, shape, and charge of the peptide-binding groove and the carboxy-terminal peptide anchor.[3,4] It is generally believed that mismatches in alleles that differ in their peptide binding elicit allo-recognition.[4] The groupings of mismatches according to differences in these residues was examined, following previous studies that demonstrated that HLA-C-mismatched transplants involving differences at these residues have higher risks for acute GVHD and mortality than HLA-matched transplants.[5] The authors observed that increasing the expression level among HLA-C mismatches with differences at residues 116 or 77/80 was associated with increased nonrelapse mortality. The levels of expression did not show distinct outcomes in patients with allele mismatches with no differences at residues 116 or 77/80. It appears that the immunogenicity of HLA-C mismatches may be determined by both the location of the amino acid differences and the level of expression, defining whether a particular patient's mismatch is either a good or poor graft-versus-host target.

In addition, the effect of expression levels was examined in groups of transplants stratified according to allele (mismatches involving structurally related alleles differing only by a few amino acid substitutions) or antigen level mismatches (mismatches involving alleles differing by many amino acid substitutions). The group of allele level mismatches included a high representation of common low-expression C*07 and C*03 mismatches, and therefore, the effect of levels of expression could not be examined in this group. The mismatch in the alleles C*03:03/C*03:04 is a well-tolerated high-frequency mismatch.[6] These alleles differ only by a single amino acid replacement at residue 91 that is not a contact site with peptides and therefore may have equivalent peptide presentation properties; the inconsequential effect of this mismatch in transplant outcomes may be a reflection of their functional similarities. The authors of the current study speculate that this permissive mismatch may have resulted from the low expression of the C*03 alleles; these hypotheses are not mutually exclusive. In a previous study,[6] the transplants with C-allele level mismatches other than C*03:03/C*03:04 predominantly included the low-expression mismatch C*07:01/C*07:02; this mismatched group presented similar outcomes to those observed in transplants with mismatches at other HLA loci. Additional studies comparing the effects of the patient's HLA-C-mismatched allele and their corresponding expression levels with mismatches at other loci are needed to delineate criteria for optimization of donor selection. Because the patient's HLA-C alleles and their corresponding expression levels are unmodifiable factors, the criteria for prioritization of mismatched donors when no fully matched donor is available will require taking into account the potential impact of the specific mismatches present with each potential donor, as well as the level of expression the patient's HLA alleles that may potentially be recognized as graft-versus-host targets.

The findings of the current study underscore the importance of assessing the expression levels HLA mismatches and evaluating their potential risks for adverse outcomes; the observations regarding low expression are consistent with observations[7] indicating lower or no impact in survival of isolated DQ and DP mismatches; the allelic protein variants encoded by these are expressed at low levels on the cell surface of antigen-presenting cells. As indicated in a previous study,[8] the patient's low-expression mismatched allele may have a weak individual

effect on outcome; however, the combination of multiple mismatches including low- and high-expression alleles may result in adverse outcomes.[8] The occurrence of low-expression alleles has been demonstrated for alleles of HLA-A[9]; the genomic mutations determining low expression can now be assessed in both patient and donor by the application of novel next-generation sequencing-based methodologies for HLA typing.[10] The study by Petersdorf et al provides insights into the immunogenicity of individual HLA allele mismatches; this study opens new avenues of investigation and has practical implications for optimal donor selection and at the same time expanding the pool of acceptable unrelated donors.

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