

HLA-A AND -B NULL ALLELES IN 6,528 SUBJECTS RESIDENT IN WALES



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Introduction

Parallel HLA typing by serological and DNA-based methods has identified several HLA-A, -B and -C alleles that cause poor or non-expression of the corresponding HLA specificity.

Consequently, expression variants (EVs) [low (L) or null (N) alleles], are normally missed by serological methods but detected, although not usually differentiated, by routine 'low resolution' DNA-based typing techniques, such as PCR-SSP.

Clinical relevance of EVs

When HLA matching for transplantation a patient who possesses a null allele that is not expressed could simply be considered as 'homozygous' for the other allele.

However, this may not be appropriate for EVs that produce low levels of mRNA and accompanying low level of HLA protein on the cell surface.

Thus, the reduced level of protein expressed by A*2402L (A*2402102L) is sufficient to stimulate an alloreactive T cell response (Magor et al. (1997) *Journal of Immunology* **158**, 5242). Also clinical evidence suggests that ignoring this allele in unrelated bone marrow transplantation can result in severe graft-versus-host disease (Zanone-Ramseier et al. (1999) *Transplantation* **67**, 1336).

Accordingly, it has been suggested that determination of EVs by molecular methods and assessment of their mRNA expression should be included in the unrelated stem cell donor search protocol.

In addition, haplotypic associations may differ for EVs and this may also influence matching strategies in unrelated stem cell donor selection.

Rationale

Although EVs have been identified for 8 HLA-A (16 alleles) and 10 HLA-B (13 alleles) gene families (July 2002), little is known of their frequency in random populations.

Frequency of expression variants in Wales

Population typed by both serology and DNA-based methods. We identified 6,528 subjects who were comprehensively HLA-A and -B typed by serology and tested by PCR-SSP to the 'split specificity' level as a minimum.

These were 5,026 random blood donors from the Welsh Bone Marrow Donor Registry and 1,502 unrelated potential bone marrow or solid organ transplant and other patients.

Subjects were typical of our local population. There were no significant differences ($p_{\text{corr.}} < 0.05$) in the frequency of HLA 'specificities' between the blood donors and patients and both groups showed a good fit to Hardy-Weinberg equilibrium and expected homozygosity (all p-values > 0.25). Thus, the total group appeared typical of the local northern European Caucasoid population resident in Wales.

Expression variants found. Six likely EVs were identified by failure to detect an HLA-A or -B specificity by standard serology but the detection of an HLA gene by PCR-SSP.

All six cases were retested by both serology and PCR-SSP using new samples. In all instances the original findings were confirmed.

'High resolution' PCR-SSP typing and nucleotide sequencing were used to further delineate the likely EVs.

Two were known null alleles:

- B*1501102N
- B*4423N

However, the remaining four did not correspond to any of the currently identified null alleles detailed in the IMGT/HLA Sequence Database (July 2002). These were:

- A*01 but not A*0104N
- B*08 but not B*0808N
- Two A*03 but not A*0303N

Note: The two HLA-A*03 null alleles did not possess the same mutation.

Comments

This study indicates that the phenotype frequency of individual HLA-A and -B EVs in the local population resident in Wales, is approximately 0.015% or less and that their corresponding gene frequencies are < 0.0001 .

The identification of four new EVs, at a frequency of about 1 in 1,700 of our northern European Caucasoid subjects, suggests that with careful testing many more new EVs will be found in the Caucasoid population.

Further work is required to establish the full sequences and extent of expression of these new EVs. This will help to determine their relevance in the selection of, for example, unrelated stem cell donors.