

NEW INSIGHTS INTO THE HLA-B14 CROSS-REACTIVE GROUP (CREG) USING HLAMatchmaker



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Introduction

Serological cross-reactivity (CR) is a well-known feature of the HLA system. In the early 1980ies we demonstrated 'bidirectional' CR between the HLA-B14 CREG antigens (B8, B64, B65, B18, B39); showed CR between these 5 antigens and B42, B54, B55 and CR between B14 and B51 and between B39 and B37. We proposed that most B14 CREG antisera contain antibodies against many 'shared determinants' and that their specificity will depend on, e.g., the number of different antibodies present, their concentration and their degree of synergism.

HLAMatchmaker, a computer algorithm, was originally developed for use with highly sensitized patients (Duquesnoy, 2001; Duquesnoy, 2002) and has since been proven of use in non-sensitized patients (Duquesnoy *et al.*, 2003). The program assesses HLA compatibility by intra- and inter-locus comparisons of polymorphic amino acid triplets in the alloantibody accessible regions of HLA-A, -B and -C molecules. HLAMatchmaker can, in most instances, identify mismatched HLA antigens that share all their polymorphic triplets with the patient's HLA antigens, and thus considers them fully compatible. Since antibodies are not produced against shared triplets on mismatched HLA antigens we used this program to identify likely epitopes involved in the HLA-B14 CREG.

Method

Using HLAMatchmaker (Ser 1.2) we:

- 1) Determined the triplet mismatches between the stimulating antigen and the HLA-A,B types of groups of women who had made B14 CREG antibodies during pregnancy (stimulated by B8 n=9, B14 (B64 or B65) n=24, B18 n=3, B39 n=5).
- 2) Identified all the triplet mismatches within the 4 groups of women and determined their presence or absence on all HLA-A and -B specificities.
- 3) Searched for single or combinations of these triplets present on one or more antigens of the 'classical' B14 CREG but absent from antigens well established as *not* being part of the B14 CREG, e.g. HLA-A antigens, B7, B35.
- 4) Identified the single or combinations of triplet mismatches (from 3) that were generated by more than one stimulating antigen.

Results

The total number of triplet mismatches in each group was B8 n=12, B14 (B64 n=11 and B65 n=12), B18 n=6, and B39 n=6. Overall these covered 16 different positions and 22 different amino acid triplets. Only one triplet, 62Rn, was common to all groups of stimulating antigens and different triplets were common to groups of two or three.

Overall we found 54 combinations of triplets - some:

- 1) Were unique to B8, B14, B18, B39.
- 2) In *different combinations* covered all the B14 CREG antigens B8, B14, B18, B37, B38, B39, B54, B55, B56, B51, B52.
- 3) Included 'rare' antigens, e.g. B67, B73, B78, B81, B82.
- 4) Were largely (44/54) generated by one of the four antigens only: B8 n=18, B65 n=1, B14 (B64 and B65) n=12, B18 n=9, and B39 n=4. The remainder being stimulated by two in various combinations, e.g. B14, B18 and B8, B39.

Comment

Importantly, these findings identified using the HLAMatchmaker program suggest that B14 cross-reactivity depends on a multitude of different epitopes rather than a few epitopes shared by many antigens.

References

- Duquesnoy, R. J. (2001) HLA Matchmaker: a molecularly based donor selection algorithm for highly alloimmunized patients. *Transplantation Proceedings* **33**, 493.
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