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Proposal to determine cDNA nucleotide sequences in HLA class I alleles in certain participants in the Groote Eylandt study.

Scientific Background

Hypothesis: That HLA class I alleles in Aboriginal Australians may have subtle variation at the nucleotide level that is not necessarily detectable by serological analysis but nevertheless may significantly change the structure of the peptide-binding cleft of the HLA class I molecule.

Longer term aims, not part of this proposal:

To improve the quality of donor-recipient matching for HLA antigens in bone-marrow and organ transplantation. To develop an HLA class I typing protocol based on sequence-specific oligonucleotide (SSO) hybridizations of polymerase chain reaction (PCR) products, capable of detecting HLA class I alleles in all Australians.

Short term aims, specific to this proposal:

To determine cDNA sequences for HLA class I alleles in Aboriginal Australians, specifically, for HLA-A2, A11, A24, Aw34 and HLA-B13, B15, Bw56, B40.

Background:

The outcome for bone-marrow transplantation in Aboriginal Australians is so poor that this is now rarely attempted (J. Chapman, pers. comm.). There have been regrettably few studies of HLA antigens in Aborigines, even though exquisite matching of HLA antigens from donors and recipients can radically improve the outcome of bone-marrow transplantation,

(Bradley, 1991). Recent analysis of HLA class II genes in Aborigines from the Kimberley and Cape York regions by the Human Genetics Group, JCSMR (Gao *et al.* submitted) has made it clear why the prognosis for bone-marrow transplantation in Aborigines is so poor. Nucleotide sequence polymorphisms of exon 2 of HLA-DRB1, DRB3, DRB5, DQA1 and DQB1 alleles were determined in 217 Aboriginal study participants. Four novel HLA-DRB1 alleles were found, accounting for nearly 50% of the HLA-DRB1 allele frequency in Aborigines from north western Australia. DRB1*A01 was similar to DRB1*1401 but with Asp (GAT) instead of Ala (GCT) at position 57, and DRB1*AB4 was similar to DRB1*1402 but with Asp (GAC) instead of Glu (GAG) at position 28. Another allele, DRB1*AB3, had DRB1*04 sequences at codons 7-16 but the rest of the exon was the same as DRB1*1401, apparently arising by intra-exonic recombination in the segment encoding the β-pleated sheet. DRB1*AB2 was a combination of DRB1*04 and DRB1*0803 nucleotide sequences, with apparent segmental transfer of some DRB1*0803 nucleotides (codons 67-74) to a DRB1*04 recipient allele. In addition to the four novel HLA-DR alleles described above, other HLA-DR alleles found commonly in Aborigines include DRB1*1402, 0410 and 0411, described previously only in Hispanics or Amerindians (Gorski 1989, Petersdorf 1991) and 0803, described originally in Orientals (Watanabe *et al.* 1990).

The DNA sequencing of these alleles has permitted development of an HLA class II PCR-SSO typing protocol (Gao *et al.* submitted) appropriate for typing Aboriginal donors and recipients.

No novel HLA-DQ alleles have been detected in Aborigines, either by RFLP or PCR-SSO analysis, but SSOs give only a partial view of nucleotide sequences and DNA sequencing of exon 2 of HLA-DQB alleles is in progress in the Human Genetics Group. In contrast to HLA-DQ, PCR-SSO patterns at the HLA-DPB1 locus revealed a new allele, now recognized as DPB1*2201 (Gao *et al.* 1992), present in one third of the 172 Aboriginal study

participants tested. The new allele has a DNA sequence identical to DPB1*0501 throughout exon 2 except for one nucleotide substitution at codon 69, resulting in the acidic amino acid glutamine (GAG) instead of the basic amino acid lysine (AAG). The DPB1*2201 allele is apparently confined to Australian Aborigines and has not been detected in other populations of Asia-Oceania (Easteal *et al.* unpublished).

At the polymorphic HLA class I loci, serological studies (Hay *et al.* 1986) have shown HLA-Aw34 and HLA-Bw56 as the most common alleles in Australian Aborigines. The most recent compilation of HLA class I nucleotide sequences (Zemmour and Parham, 1991), includes 22 HLA-A and 31 HLA-B alleles, but sequences for HLA-Aw34 and -Bw56 are not available. Until these sequences are determined, a PCR-SSO class I typing protocol suitable for use in Aborigines cannot be developed. Further, for several of the serological specificities found in Aborigines, multiple allelic subtypes have been described in other populations. For instance, HLA-A2 has at least six variants (Zemmour and Parham, 1991), and subtypes have been described for A11 (two), B13 (two) and B40 (three).

The apparently high rate of mutation at coding sites of functional significance in HLA class I genes suggests that the serologically-defined alleles in Australian Aborigines may be associated with multiple allelic variants. This possibility is further suggested, somewhat laterally, by our reported observation (Serjeantson *et al.* 1983) of heterogeneity in serological reactions of HLA-A10 (Aw34) Bw56 and B15 alleles in Melanesians. Since there have been very few published studies of HLA serology in Australian Aborigines (Cross et al. 1973; Bashir et al. 1971; Hay et al. 1976), the hypothesis of heterogeneous serological subtypes awaits analysis at the molecular level.

Determination of exon 2 nucleotide sequences in HLA-DRB1 and HLA-DPB1 in Aborigines has permitted development of rapid PCR-SSO class II typing

protocols suitable for use in any molecular genetic laboratory associated with a transplantation program. Determination of cDNA HLA class I allele nucleotides will similarly permit future design of exon 2 and exon 3 SSOs for development of a PCR-SSO class I typing protocol suitable for use in all Australians.

Research Plan

HLA-A and HLA-B locus-specific 3′ untranslated sequences have recently been published by Dr P. Parham's laboratory. Nucleotide primers based on these sequences will be used in generating cDNA suitable for PCR amplification, from cell-lines established by Dr B. Currie and S. Ho, using blood provided by Dr T. Burt. The 1.2 kb PCR products will be cloned into M13 for nucleotide sequencing, using a strategy similar to that described by Ennis *et al.* (1990) DNA sequencing will be performed using the ABI automated nucleic acid sequencer at the ANU's Biomolecular Resource Facility.

Alleles HLA-A11, A24, Aw34, B13, Bw56 B40, Cw4 are well-represented in the Groote Eylandt kindred; HLA-A2,-B15 and -Cw3 have been introduced to the kindred by #30408

DNA sequencing of any particular allele will only be undertaken with the written informed consent of the particular donor. Donors invited to participate in the DNA sequencing project are listed by ID number in the attached sheet, together with the HLA serotypes of interest to the study.

A longer ~~item~~ term project, developing PCR/SSO HLA class I typing protocols, does not form part of this proposal although it will be based on the results generated by this study.

References

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ID numbers of participants in the HLA serology study of Groote Eylandt who will be invited to participate in the HLA class I DNA sequencing project.

ID	Serotypes for study	Reserve ID
40005	Aw34,- Bw56,-	40013
30011	Aw24,-	30010
50022	A11,- B13,B40	50025
50021	A11,?A10/19; Bw56,B40	---
40025	A24,Aw34 B13,- Cw4	30015
30408	A2 B15 Cw3	40015

Reserve IDs are possible substitute IDs if those selected are unavailable. The substitutes are generally heterozygotes and are not the preferred donors.