ficoll-hypaque gradient. The HLA phenotype is determined by incubating the lymphocytes in a microtiter plate with complement antibodies to specific HLA antigens. If the lymphocytes carry molecules recognized by the antibody, the antibody will bind to the cell and the cell will be lysed. To detect lysis, a fluorescent compound, such as ethidium bromide, is added and fluorescence is measured. Reactions are graded based on the percentage of lysis. The greater the fluorescence the greater the degree of incompatibility between donor and recipient.

Mixed lymphocyte culture

The mixed lymphocyte reaction (MLR) is an in vitro assay in which leukocytes, from two genetically distinct individuals of the same species, are cultures resulting in cell blast transformation, DNA synthesis, and proliferation. Generation of the MLR occurs as a consequence of the incompatibility of the allogeneic determinants which are expressed on the surface of cell populations and which are encoded by the MHC. MLRs can identify discrepancies in the HLA class II loci which microcytotoxicity may not detect. The recipient's lymphocytes are mixed with the potential donor's lymphocytes and incubated. The donor's lymphocytes are treated so that they will not proliferate in the presence of the recipient's lymphocytes. A radioactive DNA compound is added to the mixture. If the recipient lymphocytes react to the donor lymphocytes they will uptake the DNA and their radioactivity can be measured and is a measure of the responsiveness of the recipient's lymphocytes to the donor cells. MLR assays are labor intensive and costly.

DNA techniques for compatibility testing

Restriction fragment length polymorphism (RFLP) is a difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples with specific restriction endonucleases. The initial step in RFLP is the extraction and purification of DNA, next the purified DNA is digested using restriction endonucleases. Then the reaction fragments produced during DNA fragmentation are analyzed using gel electrophoresis, the fragments are negatively charged and can be easily separated by electrophoresis, which separates molecules based on their size and charge. The gel is treated with luminescent dyes in order to make DNA bands visible. An RFLP probe is a labelled DNA sequence, frequently a cDNA clone. An RFLP probe is added and hybrides with one or more fragments of the digested DNA sample after they were separated by gel electrophoresis, thus revealing a unique blotting pattern characteristic to a specific genotype at a specific locus. RFLP can be used for HLA typing.

Sequence specific oligonucleotide typing can be used for HLA typing. The initial step is the HLA-locus-specific amplification of DNA by polymerase chain reaction. Amplified DNA is then immobilized on a solid support, usually a nylon membrane, and then hybridized with a battery of sequence specific oligonucleotide probes (SSOP) (direct hybridisation). Fluorochromes are linked with the probes to allow their detection by chemiluminescence. Alternatively, SSO probes can be immobilized on a solid support, for example colour-coded microspheres, and hybridized with labelled polymerase chain reaction products (reverse hybridisation). The higher the number of probes the better the resolution level. Only the probe completely matched with the target sequence amplified will hybridize and give a positive signal. Most of the vast polymorphism of the HLA system results from conversion