refractory to amplification. Thus, this assay allows highly specific direct analysis of every homo- or heterozygous combination of HLA-DR by the detection of amplified DNA after agarose gel electrophoresis without subsequent procedures. Our new typing technology will improve the speed and reliability of the selection of optimally matched unrelated donors for renal transplantation.

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M.19 Inefficient peptide binding accounts for low surface expression of the HLA-E antigen

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The classical HLA alleles, HLA-A, -B, -C, are highly polymorphic and code for heavy chains which, complexed with β_2m and peptide represent T cell restriction elements and are present on almost all nucleated cells. In contrast, the cell surface expression, tissue distribution and function of non-classical HLA antigens is not known. We used a locus-specific oligonucleotide to investigate the transcription of the non-polymorphic HLA-E gene in tissues and cell lines. HLA-E was found to be ubiquitously described in two mRNA of 1.8 kb and 2.7 kb. Their ratio differs depending on the tissue and cell line tested and on the stimulation conditions. Sequence analysis of the appropriate cDNA clones and the corresponding genomic region demonstrated that the two HLA-E transcripts differ in the extent of the 3'untranslated region. S1 nuclease protection assays confirmed identity of the polypeptides encoded by the two mRNA. Transfection of the HLA-E cosmid into human β_2m expressing mouse L929 cells (J27) or together with human β_2m into mouse myeloma X63 cells resulted in the transcription of mainly the 1.8 kb message. Only the transfected X63 cells showed a reproducible low level cell surface expression of a human β_2m associated HLA-E α-chain irrespective of the amount of HLA-E mRNA present in the transfectant clones. Pulse chase experiments demonstrated that the human β_2m associated HLA-E α-chains are not processed into an endoglycosidase H resistant compartment. Lowering the culture temperature to 26 °C resulted in an increase of surface expression of HLA-E but not of HLA-B27 in the corresponding X63 transfectants indicating that empty HLA-E α-chains are expressed on the cell surface. The implications of these findings with regard to the expression of HLA-E *in vivo* and a possible role of the 2.7 kb transcript will be discussed.

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M.20 New developments in biodegradable microspheres for magnetic separation techniques

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Magnetic microspheres (MMS) for *in vitro* and *in vivo* applicability should possess the following properties: biodegradable, homogenous size, slow sedimentation rate in biocompatible systems, smooth and inert surface to avoid unspecific interactions with assay components,

nontoxic, covalent coupling to various ligands by uncomplicated chemistry. In addition, we ask for five classical criteria to determine the efficacy of the magnetic separation technique: specificity, sensitivity, viability, recovery, and function. These properties and criteria are fulfilled in our recently improved 0.5, 0.8 and 1.5 μm diameter albumin MMS. They have been particularly tested for pancreatic islet preparations, since islet transplantation requires large amounts of viable islets separated from the highly immunogenic exocrine tissue. Exocrine tissue-specific lectins are covalently coupled to MMS and used in an electromagnetic reactor (EMR): (1) UEA-I with binding specificity for rat exocrine tissue, used in extensive model studies, (2) WFA for human exocrine tissue, (3) GSA-I, RCA-I, and WFA for pig exocrine tissue, and (4) BPA and RCA-I for bovine and canine exocrine tissues. Difficulties to obtain sufficient human material prompted us to change to the other large animal species. Magnetically separated islets were functionally tested *in vitro* by insulin secretion and will be further tested *in vivo* by autologous islet transplantation in pigs. The data on large scale separation should provide information about (1) a new, most efficient approach to lectin/ligand-dependent EMR in general, and (2) an effective way to yield purified allo-/xenogenic islet for (clinical) transplantation, in particular.