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Human Immunology  


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Helicobacter pylori infection is linked to chronic gastritis, peptic ulcer and gastric carcinoma. During H. pylori infection, class II MHC expression by the gastric epithelium increases, as does the number of local CD4+ T cells, which appear to be important in the associated pathogenesis. These observations suggested that the epithelium might present antigens to T cells. Thus, we sought to determine whether gastric epithelial cells process antigens to establish their function as local antigen presenting cells (APC). We examined a panel of gastric epithelial cell lines for expression of the antigen processing cathepsins B (CB), L (CL), S (CS), and D (CD). The mRNA for these enzymes were detected by RT-PCR and the enzymes in the gastric epithelial cells were identified by various independent methods. We corroborated the expression of CB and CD on gastric epithelial cells from human biopsy samples. The functions of these proteases were confirmed by assessing their ability to digest ovalbumin, a conventional dietary antigen, and proteins from H. pylori. In summary, multiple lines of evidence suggest gastric epithelial cells process antigens for presentation to CD4+ T cells. To our knowledge, these are the first studies to document the antigen processing capacity of human gastric epithelial cells.


http://www.sciencedirect.com/science/article/B6T3B-47K22SY-3/2/5e38780a07861c76a744c5c6f1e86919

Natural killer (NK) cell-mediated cytolysis is stimulated and downregulated through the interaction of distinct human leukocyte antigen (HLA) class I molecules on target cells with specific killer cell immunoglobulinlike receptors (KIRs) on NK cells. Killer cell immunoglobulinlike receptors are highly polymorphic and are clonally distributed on NK cell populations within individuals. However, the regulation of KIR expression by individual HLA class I phenotypes is not well understood. To examine a potential influence of the HLA class I phenotype on KIR expression patterns we studied the KIR expression in individuals that were subgrouped according to the major HLA-C encoded KIR-epitopes (group C1 versus C2). In these individuals, NK cells were analyzed for KIR expression using flow cytometry and RNA-based expression analysis. Our results demonstrate that KIR genes are transmitted very heterogeneously with two main patterns of KIR genotypes as previously described; group A and group B (with 21 different genotypes).
There are distinct populations exhibiting different densities of CD158a and/or CD158b positive NK cells that coexist in all individuals. A clear correlation between KIR expression and the currently known HLA class I ligands was not observed. In conclusion, the surface expression of KIRs in individuals with different HLA class I genotypes indicates that other non-HLA class I encoded factors contribute to the shaping of the KIR repertoire.


http://www.sciencedirect.com/science/article/B6T3B-3S6D0TN-7/2/54e9c1c636002b53294ea89e95253d54

ABSTRACT: Conventional matching is based on numbers of alleles shared between donor and recipient. This approach, however, ignores the degree of relationship between alleles and haplotypes, and therefore the actual degree of difference. To address this problem, we have compared family members using a block matching technique which reflects differences in genomic sequences. All parents and siblings had been genotyped using conventional MHC typing so that haplotypes could be assigned and relatives could be classified as sharing 0, 1 or 2 haplotypes. We trained an Artificial Neural Network (ANN) with subjects from 6 families (85 comparisons) to distinguish between relatives. Using the outputs of the ANN, we developed a score, the Histocompatibility Index (HI), as a measure of the degree of difference. Subjects from a further 3 families (106 profile comparisons) were tested. The HI score for each comparison was plotted. We show that the HI score is trimodal allowing the definition of three populations corresponding to approximately 0, 1 or 2 haplotype sharing. The means and standard deviations of the three populations were found. As expected, comparisons between family members sharing 2 haplotypes resulted in high HI scores with one exception. More interestingly, this approach distinguishes between the 1 and 0 haplotype groups, with some informative exceptions. This distinction was considered too difficult to attempt visually. The approach provides promise in the quantification of degrees of histo-compatibility.


http://www.sciencedirect.com/science/article/B6T3B-3RWW785-6/2/e3d5059c3752afe4ec4d39d79161ffbb7d

ABSTRACT: Recent advances in the understanding and identification of chemokines and their receptors have provided evidence for their consideration as candidate loci with respect to genetic susceptibility/resistance to MS. Increased levels of the chemokine, macrophage inflammatory protein (MIP)-1[alpha], have been demonstrated in the cerebrospinal fluid of both patients with MS and mice with EAE, and anti-MIP-1[alpha] antibodies have been shown to prevent EAE. Recently, a common deletion mutation in the gene for the major receptor for MIP-1[alpha], chemokine receptor 5 (CCR5) has been described. Homozygotes for the mutation fail to express this receptor. Moreover, homozygotes are highly protected against HIV infection; this has potential implications for the cell entry of infectious agents in other multifactorial diseases where a viral component may be involved. In view of these aspects, a group of 120 unrelated Australian relapsing/remitting MS and 168 unrelated control subjects were screened for the CCR5[Delta]32 mutation. There was no significant difference in the allele frequency of CCR5[Delta]32 gene between the MS patients (0.1125) and the control population (0.0921). The presence of two CCR5[Delta]32 homozygotes in the MS patients indicates that the absence of CCR5 is not protective against MS. These data suggest that CCR5 is not an essential component in MS expression, though this may be due to redundancy in the chemokine system where different
chemokine receptors may substitute for CCR5 when it is absent.


http://www.sciencedirect.com/science/article/B6T3B-45CTM7Y-B/2/f27fb4fe01b4261983153d4c987b8094

Interleukin-4 (IL-4) is a cytokine of the Th2 subtype. It is suggested that Th2 cytokines are involved in induction of tolerance towards the graft after organ transplantation. Therefore, we studied the association between the frequencies of IL-4 producing helper T lymphocytes (IL-4 HTL) and acute rejection in a panel of 31 cardiac transplant patients. It was also investigated whether these frequencies were influenced by: (1) a single nucleotide polymorphism (SNP) at position -590 in the promoter region of the IL-4 gene, which influences the production level of IL-4; and (2) the expression of an IL-4 splice variant (IL-4[delta]2), which inhibits the IL-4 receptor. Frequencies of IL-4 HTL were determined by limiting dilution analysis. Genotyping for the SNP was carried out by sequencing. The ratio of wild type versus IL-4[delta]2 mRNA was determined by quantitative RT-PCR of mRNA isolated from stimulated MNC of cardiac transplant patients. Frequencies of IL-4 HTL were significantly higher in patients who did not suffer from acute cardiac transplant rejection, than in patients that suffered from at least one rejection episode requiring treatment in the first year after heart transplantation. The genotype of the promoter SNP and the ratio between wild type/splice variant IL-4 mRNA did not influence the measured frequencies of IL-4 HTL or the presence of transplant rejection itself.


http://www.sciencedirect.com/science/article/B6T3B-47PPRNH-3/2/5ddf9a5d0659bbe9e12c82a84c2eb27d2

The location of the human TNF genes within the MHC complex has prompted much speculation about the role of TNF alleles in the etiology of MHC-associated autoimmune diseases. On sequencing the 5' regulatory region of the human TNFA gene a G (TNFA-308G) to A (TNFA-308A) transition polymorphism at position -308 was discovered. We have developed a simple PCR assay to facilitate the screening of the -308 polymorphism at the DNA level. In view of the possible linkage between the TNFA-308A allele and a certain MHC type, TNFA-308 genotypes in HLA-typed healthy individuals (n = 88) were determined. A statistically significant association between the TNFA-308A allele and HLA-DR3, DQB1*0201, DQA1*0501, A1, B8, and the Ncol 5.5-kb RFLP of the TNFB gene was observed. In addition, we determined the frequency of the TNFA-308A allele in patients with FS (n = 13), an HLA-DR4-associated disease. In this study, no association was found of Felty's syndrome with the TNFA-308A allele, indicating that this allele does not appear to be a susceptibility factor for FS. Human Immunology 41, 259-266 (1994).


http://www.sciencedirect.com/science/article/B6T3B-47J6V1H-
Molecular genotyping of HLA class II genes using group-specific DNA amplification by the PCR followed by probing with (PCR-SSO) probes is too time consuming for the typing of cadaveric organ donors. Recently, amplification of DNA using PCR-SSP has proved a reliable and rapid method for typing HLA-DRB1 genes. PCR-SSP takes 2 hours to perform and is therefore suitable for the genotyping of cadaveric donors. We have designed a set of primers that in eight PCR reactions will positively identify the HLA-DQB1 alleles corresponding to the serologically defined series HLA-DQ2, DQ4, DQ5, DQ6, DQ7, DQ8, and DQ9. Presently, 30 homozygous cell lines and 138 individuals have been typed by the DQB1 PCR-SSP technique and compared with a combination of serology and RFLP with 100% concordance. No false-negative or false-positive amplifications were recorded. All combinations of DQB1 can be readily identified. DQB1 PCR-SSP can take as little as 130 minutes from start to finish, including DNA preparation.


Polymerase chain reaction (PCR)-based human leukocyte antigen (HLA) typing methods currently used in most histocompatibility laboratories, such as PCR-sequence-specific primers (PCR-SSP) and PCR-sequence-specific oligoprobes (PCR-SSO), are time-consuming and are at risk of contamination during the post-PCR process. The aim of this study was to develop a real-time PCR-based HLA-DRB1 and DRB3/4/5 low-medium resolution typing method to avoid these problems. This new method combined the use of specific primers and probes for HLA-DRB alleles. One pair of DRB gene primers and two DRB-specific probes (FAM and VIC) were used per reaction in each of a set of 16 PCR reaction tubes. To provide an internal positive control, each tube also contained a pair of primers and a TET probe for glyceraldehyde phosphate dehydrogenase. This allowed a very significant reduction in the number of reactions and the processing time, whereas typing resolution increased. After being successfully tested on 100 samples, the technique was validated in 200 clinical samples that had previously been typed for HLA-DRB using a standard PCR-based method. Identical results were obtained with all samples. This new method also reduced ambiguous results and was faster and less cumbersome than currently used PCR-SSP or PCR-SSO techniques.

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The tumor necrosis factor (TNF) and TNF receptor (TNF-TNFR) superfamily plays crucial roles in immune regulation and host immune responses. The superfamily has been also associated with many immune-mediated diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease, and diabetes. In order to investigate genetic variants of the TNF-TNFR superfamily, a total of 63 known single nucleotide polymorphisms (SNPs) in the coding region (cSNPs) of the TNF-TNFR superfamily genes were selected from the public SNP database. Among 63 cSNPs tested in this study, only 24 SNPs (38%) were validated to be polymorphic in the Korean population by primer extension-based SNP genotyping. By means of the new enhanced single strand conformational polymorphism (SSCP) method, we also identified a total of 78 SNPs, including 48 known SNPs and 30 novel SNPs, in the 44 human TNF-TNFR superfamily genes. The newly discovered SNPs in the TNF-TNFR superfamily genes revealed that the Korean population had very different patterns of allele frequency compared with African or white populations, whereas Korean allele frequencies were highly similar to those of Asian (correlation coefficient r = 0.88, p r = 0.90, p < 0.001). The validated SNPs in the TNF-TNFR superfamily would be valuable for association studies with several immune-mediated human diseases.


We present our results in the identification of polymorphic sites within the second exon of the human leukocyte antigen A (HLA-A) region using the DNA microarray technology. Allele specific detection was performed by polymerase chain reaction followed by ligase detection reaction (LDR) in combination with a universal array, a powerful method for high throughput DNA sequence analysis. By this approach we confirmed 32 human samples previously characterized by direct DNA sequencing, thus demonstrating the interest of this approach.

hypervariable region of the T cell receptor [beta] (TCRB) chain, and it is the region that has been predicted to confer fine specificity of the TCR for peptide-MHC complexes. For this reason analysis of TCRB CDR3 heterogeneity may provide insight into immune mechanisms operative in infectious and autoimmune diseases. PBMC stimulated with either mitogen (PHA), superantigen (TSST-1), or nominal antigen (tetanus toxoid) have been compared with unstimulated PBMC using a two-dimensional approach. Analysis of the expressed TCRBV gene repertoire CDR3 length profile coupled with SSCP methodology enabled the discrimination of sequences with the same CDR3 length. For both freshly isolated and PHA-stimulated PBMC, a normally distributed spectrum of CDR3 lengths (five or more products) was observed. These products differed by 3 bp (1 amino acid) due to the strict requirement for in-frame rearrangements in the CDR3 region of TCR. By contrast, tetanus toxoid-stimulated PBMC had restricted profiles for most TCRBV families after as few as 7 days of incubation. The oligoclonal nature of samples showing CDR3 length restriction was revealed by SSCP analysis and confirmed by sequence determination. Superantigen stimulation resulted in unique patterns of diversity, which included polyclonal expansion of specific TCRBV families as well as oligoclonal expansion of most other TCRBV families. These data reveal complex yet distinct patterns of TCR diversity in response to different T cell activation stimuli.


http://www.sciencedirect.com/science/article/B6T3B-3W78C15-4/2/87ea0c65e5a6377b74bf1580f5ac9f34

Diversity in the peripheral T cell receptor repertoire of rhesus (Macaca mulatta) and pig-tailed macaques (Macaca nemestrina) has been studied by examining the profile of CDR3 lengths in TCR [beta] chains. Expressed CDR3 length distribution profiles for individual TCRBV families were obtained from total peripheral blood mononuclear cells (PBMC) and T cell subsets isolated from PBMC. These studies reveal that the T cell receptor repertoire of PBMC from healthy macaques often exhibits skewing in TCRBV family CDR3 profiles. The skewing of TCRBV family CDR3 profiles was evident as discrete expanded length(s) and was detected in up to 50% of the PBMC profiles. Analyses of separated T cell populations demonstrated that the CD8+ T cell subset was responsible for the majority of observed skewing in CDR3 length profiles. However, CD4+ T cells were also shown to contribute to the skewed peripheral PBMC repertoire in these animals. While certain TCRBV families frequently displayed skewed profiles, there was no concordance in the particular CDR3 lengths expanded among the different animals. Furthermore, an additional feature of the peripheral blood of the animals studied was the presence of an unusual population of extrathymic CD4+ and CD8+ (double-positive) T cells (up to 9.6% in the PBMC of rhesus macaques). The double-positive T cells could be differentiated from CD4 single-positive and CD8 single-positive T cells by their increased surface expression of LFA-1 and decreased CD62L expression. The percentage of the double-positive T cells was higher in rhesus than pig-tailed macaques and contributed substantially to the peripheral T cell repertoire.


http://www.sciencedirect.com/science/article/B6T3B-3S2BWT5-5/2/24c30a74cc3bf611c91aca9fbc0fee75

ABSTRACT: Pemphigus vulgaris (PV) is an autoimmune disease of the skin and mucous membranes characterized by an autoantibody response against an epidermal cadherin. We
performed high resolution HLA class II typing in 19 patients with PV from Rawalpindi, Pakistan and 19 non-Jewish European PV patients from Boston by sequence-specific oligonucleotide probe hybridization. The results were compared with two separate ethnically matched control populations. We found that PV patients from Pakistan had significantly increased frequencies of DRB1*1404 (p = 0.01), DQA1*0101 (p = 0.02), and DQB1*0503 (p = 0.01). Among the patients of non-Jewish European ancestry, DRB1*1401 (p = 0.06), DQA1*0101 (p = 0.05) and DQB1*0503 (p = 0.06), were increased in PV patients. Formal linkage analysis between the major histocompatibility complex and the PV antibody was performed in 67 relatives of the 19 Pakistani patients. The results showed strong evidence for linkage of HLA-DRB1*1404, DQA1*0101, DQB1*0503, with the presence of PV antibody in relatives' families with a significant logarithm of the odds score of 6.06. Based on the three dimensional structure of class II molecules, we propose that HLA-DQA1*0101 and DQB1*0503, encode a negatively charged P9 peptide binding pocket of the DQ molecule and are significantly associated with susceptibility to PV in non-Jewish populations.


Natural killer (NK) cell alloreactivity observed during stem cell transplantation (SCT) can be either beneficial (graft-versus-leukemia effect) or detrimental to the host (graft-versus-host disease). Killer immunoglobulin-like receptors (KIR), expressed on NK and CD8 memory T cells, are regulated at a posttranscriptional level and, because there are currently no KIR-specific antibodies available, the analysis of these receptors remains elusive. To better define the role of cells expressing KIR after SCT, we studied KIR transcript repertoires in 29 grafted patients who received myeloablative or nonmyeloablative regimens. We restricted our analysis to 3DL1, 3DL2, 2DL4, 2DS3, and 2DS4 KIR transcripts 6 months after SCT. Absolute counts of NK and CD8 T cells were determined by flow cytometry, and KIR transcripts were quantified by real-time reverse transcription polymerase chain reaction at days 14, 28, 60, 100, and 180 after transplantation. Three groups of patients were identified. Groups I and III were characterized by the absence or a delayed appearance of KIR transcripts, which correlated with the highest risk of acute graft-versus-host disease (aGvHD). In contrast, in group II, a significant transcript peak was observed early, and only one patient suffered from aGvHD (p = 0.025). Thus determining the kinetics of KIR transcription should make it possible to identify transplanted patients at a high risk of developing aGvHD.


http://www.sciencedirect.com/science/article/B6T3B-3WY9RK0-7/2/9afaee3672d14cf82fe68af9d2b4626f

In cryostat sections of 84 head and neck squamous cell carcinomas (HNSCC) HLA class I and [beta]2m expression was analysed using monomorphic and locus specific monoclonal antibodies. Loss of expression was heterogeneous and none of the tumours tested showed a total loss of HLA class I and/or [beta]2m when analysed with W6/32, which recognises HLA class I determinants and anti-[beta]2m MoAbs. Weak HLA class I and [beta]2m expression was found in 9 tumours (11%) and heterogeneous expression was found in 2 tumours (2%). When analysed with locus-specific antibodies (HCA2 and HC10, anti-HLA-A and anti-HLA-B/C, respectively) 37 tumours (44%) showed a loss, weak or heterogeneous expression of one or both loci. Tumours showing a down-regulated HLA class I expression were analysed for mutations in either allele of the [beta]2m gene by sequencing based mutation analysis (SBMA). Exon 1 and exons 2 and 3
were amplified separately by PCR using M13-tailed intron-specific primers. PCR products were sequenced in two directions. In none of the tumours mutations in the \( \beta 2m \) gene were detected. In 59% of the tumours with down-regulated HLA class I expression, lost or down-regulated TAP 1 expression was found when analysed with anti-TAP 1 antibodies. This indicates an important role for TAP in down-regulation of HLA class I expression in HNSCC.


http://www.sciencedirect.com/science/article/B6T3B-48BM4HF-1/2/d408d38850bc2603158fd1eaadddad19

It is well known that type 1 diabetes mellitus (T1DM) is a complex genetic disease resulting from the autoimmune destruction of pancreatic beta cells. Several genes have been associated with susceptibility and/or protection for T1DM, but the disease risk is mostly influenced by genes located in the class II region of the major histocompatibility complex. The attraction of leukocytes to tissues is essential for inflammation and the beginning of autoimmune reaction. The process is controlled by chemokines, which are chemotactic cytokines. Some studies have shown that CCR2-64I and CCR5-\&Delta;32 might be important for protection of susceptibility to some immunologically-mediated disorders. In the present study, we demonstrate the lack of association between CCR2-64I and CCR5-\&Delta;32 gene polymorphism and T1DM and we describe a new method for a simple and more precise genotyping of the CCR2 gene.


http://www.sciencedirect.com/science/article/B6T3B-445B3YR-H/2/95d92e34e6b234f75bc0ef745077ff38

Human papillomaviruses type 16 and 18 are the major cause of cervical cancer. However, genetic factors contribute to the propensity of persistent HPV infection and cervical carcinoma. Allelic variants of the human leukocyte genes have shown to be associated with cervical neoplasia. The strongest associations have been found with the genes in the HLA class II region. The aim of this study was to analyze the association of two non-HLA class II markers with invasive cervical cancer. Microsatellite polymorphism of the TNFA gene located in the class III region and a short tandem repeat polymorphism of the MICA gene located in the centromeric end of the HLA class I region were analyzed. Eighty-five patients and 120 matched control individuals from a population-based cohort from Northern Sweden participated in this nested case-control study. MICA was not associated with cervical carcinoma. TNFa-11 frequency was increased in the HPV18 DNA positive patients (OR = 2.84, \( p = 0.0481, CI = 1.04-7.78, pc = NS \)). TNFa-11 was not associated with susceptibility to HPV16 infection, but it increased the risk for cervical cancer with the HLA DQ6 (DQA 1*0102-DQB 1*0602) haplotype. Our findings indicate that the association of TNFA with cervical cancer is different with CIN. The extended HLA DQ6-TNFa-11 haplotype is increasing the risk for development of cervical cancer significantly (OR = 3.08, \( p = 0.0104, CI = 1.30-7.31 \)).


An association of HLA-DQ3 with SCC of the cervix has been reported by researchers in Germany and Norway. This article documents a similar-sized study with patients and controls from northwest England. We report in detail on serologically determined HLA polymorphism in SCC patients with respect to HPV 16 infection, MHC class II expression within the tumor, serologic response to HPV, and other relevant clinical variables. We have also extended our studies to include DNA-based analysis using PCR and SSO probes for HLA-DQ. No significant association of any HLA-A, -B, -C, -DR, or -DQ antigen with SCC patients was found. While a possible explanation of the differences among studies could be a reflection of disease heterogeneity, the several tumor and clinical factors examined do not account for the observed differences from previous reports. Further studies are needed for a greater understanding of the interaction of HPV and HLA type in the development of cervical neoplasia.


To evaluate the long-term reconstitution of the T cell immune repertoire in recipients of an allogeneic Bone Marrow Transplantation (allo-BMT), we have analyzed the T cell receptor (TCR) repertoire in the periphery and the T cell response against tetanus toxoid in two T-B+ Severe Combined Immunodeficiency Disease (SCID) patients more than 11 years after HLA haplo-identical allo-BMT. Our studies demonstrate that in the periphery of allo-BMT recipients, on the basis of TCR V-gene segment usage, the T cell immune repertoire long after allo-BMT is diverse, as is that of the donor. However, when donor and allo-BMT recipient were compared, differences were noted in the TCR Complementarity Determining Region 3 (CDR3) size distributions and in the T cell response against tetanus toxoid. In particular, the tetanus toxoid specific T cell clones differed in their use of HLA restriction elements, and expressed different T cell receptors. Moreover, we have uncovered donor-type tetanus toxoid specific T cell clones which were established from allo-BMT recipient derived peripheral blood lymphocytes and were found to be restricted by the non-shared recipient allele. This observation suggests a role for recipient-mediated T cell selection processes, in the thymus or at extra-thymic sites.


Susceptibility to autoimmune hepatitis type I (AIH-1) has been associated with HLA-DR3, DR52, and DR4 antigens in Caucasian and Oriental patients. However, in Brazil, disease susceptibility is primarily linked to DR13 and DR52. In this highly admixed population, we find different DR13-associated haplotypes, presenting a unique opportunity to discriminate relevant genes within a tightly linked genomic region. To identify the primary susceptibility locus, we sequenced DR13 alleles of 39 patients with AIH-1 and 22 controls. Patients were almost exclusively DRB1*1301, but half of controls typed DRB1*1302. HLA-DQ haplotypes were varied. Oligotyping of DRB3
locus of all patients and also within the HLA-DR13 positive group showed an allele distribution comparable to controls, confirming that the stronger association lies in the DRB1 locus. On the other hand, if DRB1*1301 is the major susceptibility factor in our sample, the only amino acid different from DRB1*1302 in position 86, corresponding to pocket 1 in the peptide-presenting groove, may be important. We propose that peptide presentation leading to pathogenesis of AIH-1 may be quite stringent, but will also be affected by other strong genetic or environmental susceptibility factors, which would explain the various HLA molecules associated to the disease in the different populations.

http://www.sciencedirect.com/science/article/B6T3B-3Y0HP3C-G/2/126cd280f492608b5912fe7334485f04

Cumulative evidence indicates that the human interleukin-4 receptor [alpha] chain gene (IL-4R[alpha], CD124) is highly polymorphic in contrast to other cytokine receptor genes. Our group recently identified the IL-4R[alpha] variant R551 as being strongly associated with decreased kidney allograft survival. Due to the key immunoregulatory role of IL-4 and controversial reports on the association of IL-4R[alpha] variants with atopy, we present here the development of polymerase chain reaction-primer sets for sequence-specific amplification of all seven hitherto described amino acid polymorphisms, and we investigated 158 blood donors prospectively. By using an Expectation-Maximization algorithm, we calculated the presence of 11 putative human IL-4R[alpha] haplotypes and identified 4 putative IL-4R[alpha] haplotypes with a cumulative frequency of >90%. None of the polymorphisms showed a significant association with the phenotype atopy. All mutant alleles showed a trend toward decreased total IgE levels. This association was only significant (p U-test) for the A375, R406, and P478 variants in non-atopic blood-donors (n = 90), presumably due to the high variance of IgE levels among the smaller group of atopic individuals. We postulate that IL-4R[alpha] mutations are associated to different extents with a decrease in function of the receptor but do not present a major atopy locus.

http://www.sciencedirect.com/science/article/B6T3B-3S966VT-3/2/1d3f887c96c8bdb5777775abb0d985c4

Genes may be silenced at the transcriptional level by 'genomic imprinting' in such a way that only one of the parental alleles is expressed. Imprinting may be tissue-specific and in some cases it seems also to be time-dependent during development. The phenomenon has been studied in pre- and post-implantation developmental processes. Animal studies of genomic imprinting of major histocompatibility complex (MHC) antigens in the placenta have shown discordant results. To address this issue in the human placenta, we examined the expression of the non-classical human leukocyte antigen (HLA) class I gene, HLA-G. Genomic imprinting of the HLA-G locus could have implications for the interaction in the feto-maternal relationship. Restriction Fragment Length Polymorphism (RFLP), allele-specific amplification and Single Strand Conformation Polymorphism (SSCP) analysis followed by DNA sequencing were performed on Reverse Transcription (RT) Polymerase Chain Reaction (PCR) products of HLA-G mRNA to examine the expression of maternal and paternal alleles. Our results demonstrate that HLA-G is co-dominantly expressed in first trimester trophoblast cells. A "new" non-synonymous base substitution in exon 4 was detected. We also investigated the different alternatively spliced forms of HLA-G mRNA in
first trimester trophoblast and found the full-length transcript to be the far most abundant.


http://www.sciencedirect.com/science/article/B6T3B-49D1MHV-3/2/afe731ae0bfc91d87d9e86a6e3b2e84

Stromal-cell derived factor-1 (SDF-1) is a powerful chemokine that upregulates T-cell migration and activation. The gene for SDF-1 is located near type 1 diabetes susceptibility locus IDDM10, suggesting a contribution by SDF-1 to the induction of diabetes. Recently the role of SDF-1 gene polymorphism in the clinical presentation of type 1 diabetes in French population has been reported. To test the putative involvement of SDF-1 gene polymorphism in predisposition to or clinical heterogeneity of type 1 diabetes in Japanese population, we conducted the case-control study. The SDF1-3'A variant (801 G to A in the 3'-untranslated region) was determined by the polymerase chain reaction-restriction fragment length polymorphism technique in 184 patients with abrupt-onset type 1 diabetes and 106 healthy control subjects. No significant difference in allele and genotype frequencies of SDF1-3'A variant was found between type 1 diabetic patients and healthy controls. However, the SDF1-3'A variant was strongly associated with early-onset diabetes in a recessive model (AA versus AG + GG, p = 0.017). The mean age-at-onset in patients carrying SDF1-3'AA genotype was significantly younger than that in patients with SDF1-3' AG or GG genotype (p = 0.028). The frequencies of SDF1-3'A variant were significantly increased in HLA-DR4/9 patients compared with non-DR4/9 patients (p = 0.008). These results suggest that the SDF-1 gene polymorphism is associated with the age-at-onset of type 1 diabetes in Japanese population.


http://www.sciencedirect.com/science/article/B6T3B-4625RR1-2/2/0a4bd6b568578595cf87585cd380dbde

This study investigated whether interleukin-10 (IL-10) gene promoter region polymorphisms are associated with susceptibility to or clinical presentation of type 1 diabetes. The frequency of -1082G/A, -819C/T, and -592C/A polymorphisms was analyzed in 128 Japanese patients with type 1 diabetes and in 107 healthy control subjects in a case-controlled study. The allelic and haplotypic frequencies of the IL-10 gene promoter region polymorphisms were similar in patients with type 1 diabetes and in control subjects. However, the -819T and -592A allele were associated with adult-onset (>18 years) of the disease (p = 0.037). Furthermore, the frequency of ATA haplotype was increased in adult-onset patients than that in early-onset patients (p = 0.037). Among the genotypes comprising ATA haplotype, the frequency of ATA/ATA was significantly higher in adult-onset patients than in early-onset patients (p = 0.004). These results suggest that the IL-10 gene promoter polymorphisms are associated with the age-at-onset in Japanese patients with type 1 diabetes.

The central class III region of the human major histocompatibility complex contains highly polymorphic genes that are associated with immune disorders and may serve as susceptibility factors for viral infections. Many HLA haplotype specific rearrangements, duplications, conversions and deletions, occur frequently in the C4 gene region. Genetic deficiencies of complement components are associated with recurrent occurrence of bacterial infections. We have studied the complement profile and the class III genes 5'-RP1-C4A-CYP21A-TNXA-RP2-C4B-CYP21B-TNXB-3' in a 4-year-old Caucasian patient. He has suffered from several pneumonias caused by respiratory viruses, eight acute otitis media, prolonged respiratory infections and urinary tract infection. Complement C4 was constantly low, but the other complement components, from C1 to C9, C1INH, factor B and properdin, were within normal limits. Immunological evaluation gave normal lymphocyte numbers and functions with the exception of subnormal T cell response to pokeweed mitogen. Molecular studies of the C4 gene region in the patient revealed homozygous deletion of CYP21A-TNXA-RP2-C4B generating total deficiency of C4B and the flanking 5' region up to C4A, and in the father a missing CYP21A gene. Further investigations are needed to elucidate the relationship between C4B deficiency and susceptibility to infections.


Association frequencies of TCR J[beta] gene segments with six V[beta] families (V[beta] 3, 6.1-3, 8, 9, 12, and 18) were analyzed in T-cell populations obtained from healthy blood donors. The six selected V[beta] families are located at various chromosomal positions relative to other recombinatorial elements (D[beta], J[beta], C[beta]). We report here that in CD4+ as well as CD8+ T-cell subsets, all 13 J[beta] gene segments were used in combination with all the V[beta]s tested and that no correlation between the genomic position of the individual V[beta]s and J[beta] gene segment usage was observed. J[beta] gene segment usage was found to be nonrandom in general, with J[beta] 2.7 and J[beta] 2.4 exhibiting highest and lowest frequency of utilization, respectively. J[beta] family 2 was used more frequently than J[beta] family 1 by the two T-cell subsets. Some individual J[beta] gene segments were skewed toward either CD4+ or CD8+ T cells. Thus, J[beta] 1.3 and J[beta] 1.6 were consistently biased toward expression in CD4+ T cells. In contrast, when combined with V[beta]8 or V[beta]9, J[beta] 2.1 results were skewed dramatically toward expression in CD8+ T cells. We also found 70 cases of expanded individual V[beta]/J[beta] associations in a total of 1092 investigated combinations, 62 of which were confined to the CD8+ T-cell populations. CD8+ T-cell populations are thus much more likely to contain TCR V[beta]/J[beta]-restricted expansions than CD4+ T cells.


CD28-CD4+ T-cell subpopulation is expanded in kidney allograft patients with long graft survival. To seek for the roles of CD28-CD4+ T cells in the long-term acceptance of kidney allografts, we
characterized this population by analyzing cell surface molecules, TCR V[beta] repertoire, mixed lymphocyte reaction (MLR), and cytokine production. The number of CD28-CD4+ T cells increased correlatively with time after transplantation in this group of patients. The CD28-CD4+ T cells did not express detectable levels of CD25, CD69, V[alpha]24, or CTLA-4 but expressed heterogeneous amounts of CD45 RA on the surface. Freshly sorted CD28-CD4+ T cells revealed a restricted V[beta] repertoire, whereas the V[beta] usage of CD28+CD4+ T cells from the same patients was much diversified. Expression levels of TGF-[beta] and IFN[gamma] gene were significantly higher in the CD28- CD4+ T cells than in the CD28+CD4+ T cells from the kidney allograft patients. These findings suggest that an oligoclonal CD28- CD4+ T-cell population is continuously activated in patients with long allograft survival, which may be linked with the long-term acceptance.


http://www.sciencedirect.com/science/article/B6T3B-47J6T30-6B/2/42202191fe442eeeca88b4b240ab33d33

Mhc-DRB and -DQA1 second-exon and -DRB 3'-untranslated-region nucleotide sequences of three lowland gorillas with no known family relationship with each other and of two HLA homozygous typing cell lines were determined and compared with published primate Mhc-DRB and -DQA1 sequences. Eleven distinct MhcGogo-DRB second-exon sequences were found, which represent the gorilla counterparts of the HLA-DRB1*03, -DRB1*10, -DRB3, -DRB5, -DRB6 allelic lineages. One Gogo-DRB second-exon sequence does not have an obvious human counterpart and is tentatively designated Gogo-DRBY*01. The gorilla equivalents of the HLA-DRB2 and -DRB8 loci were identified as judged on Mhc-DRB 3' - untranslated-region sequences. In addition, four different Gogo-DQA1 alleles belonging to three different allelic lineages were detected. The Mhc-DRB-DQA1 haplotypes of these gorillas were deduced based on the obtained Mhc-DRB and -DQA1 sequences and the two published Mhc-DRB haplotypes of the lowland gorilla Sylvia. All deduced Gogo-DRB-DQA1 haplotypes show gene constellations different from known HLA-DRB-DQA1 haplotypes, while some of the Gogo-DRB haplotypes presented here contain more DRB genes than the HLA-DRB haplotypes. Based on phylogenetic trees, bootstrap analyses, and the gorilla, chimpanzee, and human Mhc-DRB haplotypes described, we propose that at least two Mhc-DRB loci, here tentatively designated Mhc-DRBI and -DRBII, existed on an ancient primate Mhc-DRB haplotype. The Mhc-DRB1*01, DRB1*02 (-DRB1*15 and DRB1*16), -DRB1*03 (-DRB1*03, -DRB1*08, -DRB1*11, -DRB1*12, -DRB1*13, and DRB1*14), and -DRB1*10 allelic lineages and -DRB3 and -DRBY loci probably evolved from the hypothetical primate Mhc-DRBI locus, whereas the present primate Mhc-DRB2, -DRB4, and -DRB6 loci originate from the ancient Mhc-DRBII locus of this core primate Mhc-DRB haplotype. Human Immunology 36, 205-218 (1993)


http://www.sciencedirect.com/science/article/B6T3B-3VPC8M5-9/2/39a263fa5e083ac5ecebe216b0c2296d0

Block matching is a valuable tool for selecting donors for bone marrow transplantation. Identical, electrophoretic profiles of unrelated bone marrow donor-recipient pairs have been shown to be associated with long-term survival and a reduction of graft versus host disease (GVHD). This study was undertaken to determine the sequences of the PCR products which are generated. PCR products obtained with beta-block primers following the amplification of DNA extracted from
cell lines homozygous for 7.1 and 8.1 ancestral haplotypes were cloned and sequenced. The PCR products were characterised and the beta block profiles reconstructed. The data indicate that the profiles consist of homoduplexes and heteroduplexes which are formed by the products of probably 3 different sequence locations.


http://www.sciencedirect.com/science/article/B6T3B-4DS9X0M-2/2/9a85f1dee284292a58efd7a9b4168b7f

The heterodimeric TAP (transporter associated with antigen processing) complex plays a key role in immune surveillance. By forming the portal between the cytoplasm and the endoplasmic reticulum, it enables cells to present antigenic peptides by human leukocyte antigen class I antigens to cytotoxic T cells, so infected and malignant cells can be eradicated. Because the nature of the peptide determines whether an immune response is evoked, peptide selective transport by TAP may influence immune surveillance. Currently, seven TAP1 and four TAP2 alleles are known. Each may have its own effect on peptide transport. In this study, we investigated whether TAP1 and TAP2 alleles are associated with the development of head and neck squamous-cell carcinoma (HNSCC). We developed a high-throughput SNaPshot\[trademark] assay to determine the frequencies of the TAP1 and TAP2 alleles in 79 Dutch control subjects and 94 patients with HNSCC. Strikingly, all control and HNSCC samples contained a TAP1*0101 allele, with the exception of one tumor patient. The most frequent alleles were TAP1*0101 (88.2%) and TAP2*0101 (81.2%). No significant difference was found between control subjects and patients with HNSCC. Here, we report the TAP1 and TAP2 allele frequencies in the Dutch population, the development of a high-throughput TAP typing technique, and a new TAP1*0501 allele.


http://www.sciencedirect.com/science/article/B6T3B-47J6T1Y-5T/2/92616852a86a3bb0570b653a0097141

Antibodies to the platelet HPA-1a antigen can elicit in the newborn a condition known as neonatal alloimmune thrombocytopenic purpura (NAITP). Previous studies based on RFLP analysis showed that 100% of HPA-1a-negative women who produced anti-HPA-1a antibodies (responders) were HLA-DRw52a (DRB3*0101). However, this specificity could also be found in some HPA-1a-negative women not producing anti-HPA-1a antibodies (nonresponders). We have analyzed in detail by PCR-SSOP the HLA-DR, -DQ, and -DP loci of 36 responders and 10 nonresponders. We found that while the allele DRB3*0101 was present in the vast majority of responders (91%), there were exceptions. Furthermore, the DQB1*0201 allele was found to be present in almost all responders (94%), but again was also found in nonresponders. The risk of alloimmunization to HPA-1a in an HPA-1b homozygous mother significantly increases with the presence of either allele, the odds ratio being 39.7 for DQB1*0201 and 24.9 for DRB3*0101. Sequencing of exon 2 of these two alleles from responders indicated no sequence difference when compared with the consensus sequences. This indicates that they do not represent variants when compared with the same alleles found in some nonresponders.

http://www.sciencedirect.com/science/article/B6T3B-47J6V0T-JR/2/d78ce7a96e857abc26b65c7cd33f5e94

In this study we analyzed the impact of a MHC class-II-deficient environment on the differentiation of CD4+CD8- T lymphocytes into functional defined subsets of lymphokine-producing T-helper cells. To this end a CD4+CD8- T-cell line and CD4+CD8- T-cell clones, isolated from PBMCs of a type III BLS patient, were stimulated in vitro with anti-CD3 and PMA and assessed for lymphokine transcription patterns. The results of these analyses show that CD4+CD8- T cells that have matured in a MHC class-II-deficient environment display lymphokine transcription patterns that resemble those of MHC class-II-expressing family control-derived CD4+CD8- T cells.


http://www.sciencedirect.com/science/article/B6T3B-3WY9RK0-C/2/69b7d6d3b63450f458b42c0eb3183fb1

Diversity in the HLA-B22 group was investigated in the Korean population using PCR-SSOP and DNA sequencing analyses. Allelic typing of the B22 gene was performed by gene amplification of the polymorphic exons 2 and 3 of the HLA-B genes from 91 B22 positive individuals followed by a hybridization assay using 63 digoxigenin-labelled probes. Five different SSOP patterns including an unexpected pattern were identified and correlated well with the observed serologic types and with data obtained from DNA sequencing analyses. Novel allele, B*5507, was identified from two unrelated individuals who exhibited standard B54 serologic reactivity but an unexpected SSOP pattern. The DNA sequence of B*5507 is identical to B*5502 in exons 2 and 3 except for a single nucleotide substitution at codon 45 (GAG -> GGG) altering glutamic acid to glycine. Among the already known B molecules, this substitution has been observed only in the B54 molecule encoded by B*5401 allele. This is the evidence that Gly-45 is a crucial site forming the B54 serologic epitope. Interestingly, both alleles (B*5401 and B*5507) exhibit strong association with Cw*0102. Along with previous data, B22 appears to be a very diverse group in the Korean population consisting of at least seven different alleles. B*5401, B*5502, and B*5601 are the most frequent alleles. B*5507, B*5501, B*5504, and B*5604 appear at lower frequencies. Data obtained from this study will be useful in hematopoietic stem cell donor searches as well as in determination of a typing strategy for the HLA-B22 types in this population.


http://www.sciencedirect.com/science/article/B6T3B-47J6SN6-B/2/775dd3c0b2ad967e3a341886ac51a909

The HLA-DPB1 alleles in 93 Australian aborigines, from two geographically separate areas within Australia, were studied by AFLP analysis. There was a restricted range of DPB1 alleles seen in the aboriginal population, and the distribution of alleles varied between the two aboriginal groups. DPB1*0501 was the most common allele in the aborigines from the Central Desert, whereas DPB1*0401 was the most frequent allele in the Northern Coast aborigines. A new AFLP pattern was observed, and was found to correspond to the allele DPB1*2201, recently identified by SSO analysis. The DPB1 allele frequency distribution for both of the aboriginal groups was different.
from that seen for the Australian Caucasoid population.


http://www.sciencedirect.com/science/article/B6T3B-402K8YX-D/2/87a253d2cafd15a7861cd01a922069b7

This study was designed to investigate how antiendothelial antibodies (EAbs) are involved in acute irreversible renal graft rejection. Eluates from 25 renal allografts, lost by irreversible rejection (n = 22) and by renal vein thrombosis (controls n = 3), were tested against a panel of cultured human umbilical vein endothelial cells (HUVEC). All patients were under immunosuppression at the time of nephrectomy. EAbs binding and membrane expression of adhesion molecules ELAM-1 and VCAM-1 were analyzed by flow cytometry (FACS) and by semiquantitative RT-PCR for mRNAs coding for those molecules. The absence of anti-HLA antibodies against the donor was ascertained at transplant, and before and after nephrectomy by the negativity of specific crossmatches performed using the most sensitive techniques. EAbs eluted from eight rejected kidneys bound to HUVEC. They did not induce any cytotoxicity, but their incubation with HUVEC (4 h at 37[deg]C; 2.5 mg/ml) led to upregulation of mRNAs coding for VCAM-1 (35- to 60-fold increases) and ICAM-1 (8- to 12-fold increases) as compared with control EAbs. Membrane expression of adhesion molecules was also strikingly increased, with 80% of the cells expressing VCAM-1 and 65% expressing ELAM-1 upon incubation. EAbs were detected in eight out of nine (88.8%) eluates from kidneys lost from acute vascular rejection, but in none of the 13 (0.0%) kidneys lost from other types of rejection (p < 0.0001). We conclude that EAbs, capable of activating human endothelial cells, can be recovered from acutely rejected kidneys and may play a direct role in the pathogenesis of acute rejection.


http://www.sciencedirect.com/science/article/B6T3B-3YS8MPG-H/2/07a3e477a40e0a1aa595e15ea0d1118d

A highly polymorphic (CA)n microsatellite marker (DQCAR), located between the DQA1 and the DQB1 genes, was characterized in four ethnic groups. Based on length polymorphism, 12 alleles could be defined. The marker is located 1- to 2-kb telomeric to the DQB1 gene and 10 kb centromeric to the DQA1 gene and was shown to be in tight linkage disequilibrium with HLA-DQ. Analysis of the linkage disequilibrium pattern revealed little additional diversity in DQ1-associated haplotypes. Almost all DQ1 subjects examined were DQCAR 103 or DQCAR 107 (13 and 15 CA repeats, respectively). In contrast, significant haplotypic diversity was observed for most DQ2-, DQ3-, and DQ4-associated haplotypes. These haplotypes often had longer allele sizes (DQCAR > 111, more than 17 CA repeats) and more DQCAR alleles per haplotype. These haplotypes also carried DQCAR alleles of different sizes, even though they bore the same DQA1 and DQB1 alleles, and sometimes the same DRB1 allele as well. These results indicate that DQCAR could be a useful marker to better define disease associations with HLA. Our results are also consistent with the hypothesis that CAR alleles with higher numbers of repeats have higher mutation rates and that recombination within the HLA-DR/DQ region is haplotype dependent.

The aim of the study was to assess the influence of constraints of V-D-J rearrangement on the nonrandom junctional diversity of productive T-cell receptor [beta]-chain genes in peripheral T-cells. Mature peripheral T lymphocytes are expected to display a biased repertoire of T cell receptors (TCRs), enriched for those that can recognize peptides presented by the major histocompatibility complex (MHC) molecules. Therefore, functional TCR rearrangements of peripheral T-cells are unsuitable to reveal the bias of the TCR repertoire, introduced by V-D-J rearrangement. To overcome this problem, we have studied nonfunctional TCR genes representing a repertoire of rearranged TCR gene sequences without any known post-rearrangement selection. Detailed molecular analysis of a database generated from more than 500 functional (TCRBV20S1) and nonfunctional (TCRBV10S1P and TCRBV19S1P) T-cell receptor genes from peripheral blood T-cells permitted a comparative analysis of recombination frequencies of each germline-encoded V, D, and J-segments, as well as exonucleolytic nibbling and addition of nucleotides in functional and nonfunctional transcripts. Our data demonstrate that V-D-J recombination generates a more diverse CDR3 length distribution than found among productive TCRBV genes, suggesting that selection constrains the CDR3 to an optimal junctional region length. Furthermore, the well established biased patterns of D- and J-usage in the rearranged TCRBV genes in human peripheral blood lymphocytes were also present in nonfunctional transcripts. Therefore, V-D-J diversity is biased mainly by constraints of the rearrangement process rather than intrathymic T-cell selection and peripheral expansion of particular T-cell clones.


Clonally expanded T cells might be involved in the pathogenesis of Crohn's disease (CD). To test the impact of CD on the regional distribution of expanded T cells, this study analyzed the T cell receptor [beta] (TCRB) repertoire within colonic biopsy specimens from 12 CD patients and 6 noninflammatory controls by TCR spectratyping. Migration characteristics of dominant CDR3 bands from different sites of the normal mucosa suggested focal, segmental, or ubiquitous spreading of individual expanded clones. Similar patterns were observed when inflamed and noninflamed areas of the colon of CD patients were compared, suggesting that regional expansion of T cells was more closely related to anatomic proximity than to local inflammatory activity. CDR3-sequence analysis of TCRBV12+ T cells, which were selectively expanded in the inflamed colon of 3 CD patients, failed to reveal a public CDR3 motif. Our data indicate the existence of distinct patterns of regional T cell expansions in the normal gut mucosa, which are not significantly disrupted by chronic intestinal inflammation. This does not exclude a pathogenic role of expanded T cells in CD through more subtle changes, but emphasizes the need to distinguish them from a discontinuous distribution of clonally expanded T cells in normal colon.

Naumov, Y. N., E. N. Naumova, et al. (1996). "CD4+ and CD8+ circulating [alpha][beta] T-cell repertoires are equally complex and are characterized by different levels of steady-state TCR expression." Human Immunology 48(1-2): 52.
The repertoire complexity of CD4+ and CD8+ T cells was measured in three healthy blood donors for a number of TCR BV gene families by TCR spectra-typing. This method subdivides V family-specific PCR products based on CDR3 length. Genomic DNA was analyzed to determine the distribution of the cells bearing particular V-J rearrangements. cDNA was analyzed to measure the levels of transcripts arising from those same cells. The complexity and distribution of T cells in each lineage were equal for most BV families. Certain families showed frequent skewing in CD8 cells. Analysis of the intensity profiles of RNA versus DNA spectratypes indicated that in general, there is a constant ratio of transcript per cell for all rearranged sizes within a particular family. This ratio appeared higher in CD4 cells. Thus, steady-state levels of TCR mRNA were measured and found to be higher in CD4+ than in CD8+ cells.


We studied tumor necrosis factor (TNF), lymphotoxin-[alpha] (LT-[alpha]), and TNF receptors type 1 (TNFR-1) and type 2 (TNFR-2) gene polymorphisms as well as HLA class II DRB1 alleles in Japanese patients with human T-cell lymphotropic virus type I (HTLV-I) associated myelopathy (HAM) (n = 51), patients with adult T-cell leukemia/lymphoma (ATL) (n = 48), asymptomatic HTLV-I carriers (n = 50), and HTLV-I seronegative, normal controls (n = 112). There were significant differences between HAM patients and normal controls in the distributions of TNF promoter region polymorphism at position -857, the LT-[alpha] gene NcoI polymorphism, and the T-G substitution in exon 6 of the TNFR-2 gene. The distribution of the NcoI polymorphism of the LT-[alpha] gene was also significantly different between HAM patients and asymptomatic HTLV-I carriers. In contrast, we failed to detect any difference in the frequency of DRB1, TNF promoter at position -1031, -863, or the TNFR-1 promoter -383 polymorphism. The results suggest that the TNF/LT-[alpha] gene region within the HLA class III of chromosome 6 and the TNFR-2 gene region located on chromosome 1p36 might contribute to susceptibility to HAM, and that aberrant expression or function of these cytokines and the receptor could be involved in the development of HAM.


The human leukocyte antigen (HLA) class III region, located on chromosome 6p21, has been regarded as one of the susceptible loci for type 1 diabetes. Because it contains many genes related to inflammatory and immune responses, including tumor necrosis factor (TNF), lymphotoxin-[alpha] (LT-[alpha]), and allograft inflammatory factor 1 (AIF-1) genes, it is unclear which gene within the class III region is responsible for the susceptibility to the disease. We sequenced the AIF-1 gene region and detected three novel polymorphisms, all of which were diallelic and localized at introns. Then, we investigated AIF-1, TNF, and LT-[alpha] gene polymorphisms in 165 patients with type 1 diabetes, consisting of 90 patients with young-onset
type 1 diabetes, 75 patients with adult-onset type 1 diabetes, and 200 control patients. We also analyzed TNF receptors type 1 (TNFR1) and type 2 (TNFR2) gene polymorphisms, located on chromosome 12p13 and 1p36, respectively. Although there were significant differences between type 1 diabetes patients and controls in the distributions of TNF promoter polymorphisms at position -1031 and -857, and LT-[alpha] gene NcoI polymorphism, none of them was independently associated with the disease after two-locus analysis with HLA class II alleles. We detected the significantly increased frequency of the -383C allele, located in the TNFR-1 promoter region, in both young-onset and adult-onset diabetes patients compared with controls. In addition, the -383C allele was found to be associated with higher expression of the TNFR1 gene than that of -383A allele in in vitro expression. These results suggest that the TNFR1 gene region might be a susceptible locus to type 1 diabetes in Japanese.


http://www.sciencedirect.com/science/article/B6T3B-47J6SN6-G/2/f5d24409809431835816dd045d88a917

We designed a primer for the PCR directed against a highly conserved sequence of the TCR V[beta] gene. The V[beta]-universal primer, in combination with a constant region-specific primer, enabled us to amplify TCR[beta] cDNA of allo-HLA class-II-reactive T-cell clones by PCR without prior knowledge of their V[beta] sequences. The amplified TCR cDNA was purified by agarose gel electrophoresis and subjected to direct sequencing. In nine of ten T-cell clones analyzed, direct TCR sequencing gave readable sequence ladders, including two-thirds of V[beta], junctional, and J[beta] regions. One T-cell clone gave an unreadable mixed-profile sequence ladder, indicating that this clone expressed more than one major TCR[beta] transcript. Even in this case, however, it was possible to determine two different TCR[beta] sequences separately using sequence primers specific to one of the 13 J[beta] segments deduced from the mixed ladder. Thus, direct sequencing utilizing the single V[beta]-universal primer enabled a simple, rapid, and reliable sequence determination of TCR[beta] cDNA of all T-cell clones analyzed.


http://www.sciencedirect.com/science/article/B6T3B-3XRY8CD-8/2/ed15b5c69f3d7737368f8a10b6a66abd

The TNF-[alpha] gene is located in the HLA region and has been implicated in the pathogenesis of Type I (insulin-dependent) diabetes mellitus (IDDM). We investigated the frequency of TNFa microsatellite alleles in 76 young-onset IDDM patients, 65 adult-onset IDDM patients, and 90 control subjects. We also examined the association of these TNFa alleles with HLA-DRB1 alleles, HLA-class I alleles, and TNF-[alpha] production. The frequency of the TNFa2 and TNFa9 alleles was increased in the young-onset IDDM patients compared to control subjects, but the increased frequency of TNFa2 was not significant after the correction for the number of comparisons was made. We did not find any association of TNFa2 or TNFa9 with any of the HLA-DRB1 alleles. In contrast, the frequency of the TNFa13 allele was decreased in both the young-onset and the adult-onset IDDM patients compared to the control subjects, but the difference lost significance after the correction was made in the adult-onset IDDM. The TNFa13 allele was strongly associated with DRB1*1502. Patients with TNFa2 or TNFa9 had greater TNF-[alpha] production, while those positive for TNFa13 had lower TNF-[alpha] production than patients with non-TNFa2, a9, and a13 alleles. These results suggest that TNF polymorphisms are associated with age-at-
onset of IDDM and influence the inflammatory process of pancreatic \( \beta \) cell destruction in the development of IDDM.


http://www.sciencedirect.com/science/article/B6T3B-456FC2M-3/2/8f464cabee33a3b3449adb6442f6e40d

Human leukocyte antigen (HLA) incompatibilities are the most important immunological barriers to bone marrow transplant success when using unrelated donors. Until recently, standards for donor selection included serological methods for HLA class I antigens and DNA-based typing for HLA class II alleles. In our center cytotoxic T-lymphocyte precursor (CTLp) assays have been an integrated part of the search selection procedure as well. More recently, DNA-based typing for HLA class I became available. This allowed us to determine the correlation of CTLp frequencies directed against incompatibilities at the HLA-A, -B, and -C locus in 211 donor-recipient pairs. HLA class I incompatibilities are significantly \( p < 0.001 \). However, this is mainly due to Cw*0303-0304 mismatches. In conclusion, although there is a highly significant correlation between the outcome of the CTLp frequency test and HLA allele class I typing, exceptions occur. It is unclear whether they are all clinically relevant but they certainly provide additional insight in allograft recognition.


http://www.sciencedirect.com/science/article/B6T3B-47NVYHF-C/2/14bfbfc4ec71ca297a763f020a5cc98

Evolutionary relatedness among the highly polymorphic DR[\( \beta \)] genes has been established based on shared nucleotide sequences and structural organization of DR[\( \beta \)] loci. The evolution of promoter regions of the B1*0701, B1*0101, B1*1501, B5*0101 genes was analyzed by cloning and sequencing. This shows that the polymorphism and isomorphism of HLA DR[\( \beta \)] genes extend into the 5' flanking promoter region of the genes and that evolutionary relatedness also exists among the DR[\( \beta \)] gene promoters. This suggests that DR[\( \beta \)] gene promoters and coding regions coevolved. The effect of the naturally occurring nucleotide substitutions in the polymorphic and isomorphic DR[\( \beta \)] promoters on transcriptional activity has been determined in a transient expression system. The transcriptional activity of two polymorphic DR[\( \beta \)] promoters, B1*1501 and B1*0701, and two isomorphic DR2 promoters, B1*1501 and B5*0101, is the same for these promoters. Together these data suggest that naturally occurring substitutions do not significantly affect the transcriptional activity of these promoters. Human Immunology 41, 112-120 (1994)


http://www.sciencedirect.com/science/article/B6T3B-47NVYJD-S/2/22ca6632e5c66d578d988b5df083055d

The HLA-DRB1, -DRB3, -DRB4, and -DRB5 alleles of the Guarani and Kaingang Amerindians
were characterized. Our previous serologic analyses detected three class II haplotypes among the Kaingang: DR2-DQ3, DR4-DQ3, and DR8-DQ4. In addition to these, the Guarani presented haplotype DR6-DQ3. Individuals typed serologically (67 Kaingang and 34 Guarani) were selected for molecular analyses. Using a set of 23 SSOs for hybridization of PCR products from generic DRB amplification six different haplotypes were identified, of which only three are shared by the two tribes. The oligonucleotide hybridization patterns are compatible, with haplotypes DRB1*1602-DRB5*02, DRB1*0404-DRB4*0101, DRB1*0802, and DRB1*0901-DRB4*0101 in the Kaingang tribe, and haplotypes DRB1*1602-DRB5*02, DRB1*0411-DRB4*0101, DRB1*1413-DRB3*0101, DRB1*0802, and DRB1*0901-DRB4*0101 among the Guarani. DRB1*1413 is a new allele, most closely related to DRB1*1402, which is common among South and North American Indians. At the segments analyzed, they differ solely at position 57, which is GAT (aspartic acid) in DRB1*1402 and AGC (serine) in DRB1*1413. This allele probably originated in South American Indians, resulting from a single segmental exchange event between alleles DRB1*1402 (the acceptor) and DRB1*0411.


Sequencing-based typing (SBT) is the most comprehensive method for characterizing human leukocyte antigen gene polymorphisms. Development of a SBT method for DQA1 is hampered because of a deletion of codon 56 in nearly half of the known DQA1 alleles. Sequence electropherograms of heterozygous samples comprising a deletion allele and a non-deletion allele display misalignment after codon 56 because of a three base-pair shift in the deletion allele. To overcome this problem, we have designed three group-specific primer sets to selectively amplify the deletion alleles from the nondeletion alleles. DNA samples are initially polymerase chain reaction (PCR)-typed using these primer sets along with an internal positive control primer set specific to growth hormone gene 1 (hGH1). The positive group-specific PCR reactions were selectively repeated without hGH1 control primers, and the amplicons were used as template in sequencing reactions. The sequence data were analyzed to obtain DQA1 types using ABI MatchTools software as well as the newly available Conexio Genomics Assign SBT Genotyping Software. The method was validated using a panel of reference DNA from the University of California, Los Angeles, International DNA Exchange Program. We conclude that the present SBT method is a technically simple and robust procedure to characterize the sequence polymorphisms in exon 2 of DQA1 gene.


The role of the DPB1 gene in genetic susceptibility to type I diabetes has yet to be elucidated. Studies of DPB1 alleles are conflicting. Analysis at the amino acid level, rather than consideration of allelic polymorphism, has been informative in determining disease susceptibility encoded by the DRB1 and DQ genes. In this study, therefore, amino acid variation at polymorphic sites of the DP[beta] peptide chain encoded by the second exon of the DPB1 gene was analyzed in diabetic and control subjects from white Caucasian, North Indian Asian, and Jamaican populations. Human leukocyte antigen genotypes and haplotypes were analyzed using a logistic-regression approach and the data were conditioned for the effects on disease risk of the DRB1, DQA1, and
DQB1 genes. Eight DP[beta] amino acid residues were significantly associated with type I diabetes independent of DR and DQ (DP[beta] 9, 33, 35, 36, 55, 56, 57, and 69). None of these residues, however, correlated consistently with disease risk in all three racial groups. This contrasts with findings for the DR[beta], DQ[alpha] and DQ[beta] peptide chains, where the identity of the amino acid at particular sites has been found to correlate with predisposition to type I diabetes.


http://www.sciencedirect.com/science/article/B6T3B-47J6SMM-7/2/99145dd667a94c3c6628695879c465de

Certain T-cell receptor (TCT) [beta]-chain variable (V), joining (J), and constant (C) gene segments, as well as TCR[alpha]-chain V gene segments, are disproportionally represented in TCR [alpha] and [beta] cDNA libraries derived from PHA-stimulated peripheral blood lymphocytes. Sequences of 138 TCR[alpha] clones and 96 TCR[beta] clones were determined and of these 128 TCR[alpha] clones and 88 TCR[beta] clones were found to contain unique combinations of V, J, and C gene segments or to display diversity in N region nucleotides. The frequency of the V, J, and C genes used in the assembly of unique transcripts was ascertained. Of the 24 reported V[beta] genes, families, 21 were observed among the 88 TCR[beta] clones including four V[beta] families (V[beta]1, V[beta]2, V[beta]3, and V[beta]4) that were represented in the sample times more frequently than would be expected on the basis of copy number within the gene complex. Seventy-eight percent of the clones were positive for C[beta]2 and more than half of the clones (53%) used one of two J[beta]2 genes: J[beta]2.1 was present in 27 clones and J[beta]2.7 in 20 clones. TCR V[alpha] families were also disproportionately represented in this sample. Twenty-five of 30 V[alpha] families were observed in the sample of 128 clones including six recently reported V[alpha] families. Three V[alpha] families, V[alpha]2, V[alpha]8, and V[alpha]23, accounted for 40% of the TCR[alpha] clones and were represented at 18%, 9.4%, and 13.3%, respectively. Both V[alpha]2 and V[alpha]8 gene families contain more than one gene; thus the number of clones observed in these families may, in part, be related to gene number. However, V[alpha]23, which appears to be a single-copy gene family, is significantly overrepresented in this sample. Although disproportional usage of V[beta] genes may be accounted for by superantigen exposure, reasons for disproportional usage of J[beta], C[beta], and V[alpha] genes are presently unknown.


http://www.sciencedirect.com/science/article/B6T3B-44SHDT2-B/2/ca2e0d5b565a5ff00c71514a97ce7743

Cytotoxic T-lymphocyte antigen 4 (CTLA4) gene polymorphism located in the 3’ untranslated region (UTR) was investigated in 141 Spanish patients (38 men and 103 women) with rheumatoid arthritis (RA) and in 194 ethnically-matched healthy controls. Twenty alleles having different numbers of (AT) repeats (from 7 to 32) were found in this population. (AT)7 and (AT)16 were the most frequent alleles, and accounted for almost two-thirds of the allelic frequency in the control population. Consequently, alleles were assigned as L (large: 16 or more AT repeats) or S (short: less than 16 AT repeats). When the L/S distribution in patients and controls were compared, an increase of L alleles was observed among patients (49.9% vs. 39.7%; p = 0.02; pc = 0.04, odds ratio [OR] = 1.46; 95% confidence interval [CI], 1.06-2.01). Hence, the frequency of S alleles was decreased among patients (51.1% vs. 60.3%; p = 0.02; pc = 0.04; OR = 0.69; 95%CI, 0.50-0.95).
Moreover, a statistically significant decrease in the frequency of S/S individuals was observed among RA patients (27.7% versus 40.7%; p = 0.01; pc = 0.03; OR = 0.56; 95%CI, 0.34-0.91). These differences were irrespective of the HLA "shared epitope" (SE) status, and were observed similarly among SE+ as well as among SE- patients. After combining these data with other reported previously by us, from studies of CTLA4 49 (A/G) and -318 (C/T) polymorphisms, we conclude that the strongest association between CTLA4 gene polymorphisms and RA susceptibility occurs with the 3' UTR polymorphism.


In an attempt to define the role of HLA class II genes in predisposition to primary Sjorgen's syndrome, patients of two different ethnic groups (Israeli Jews and Greeks of non-Jewish origin) suffering from this disorder were studied. Oligonucleotide genotyping revealed the majority in both groups to carry either DRB1*1101 or DRB1*1104, alleles that are in linkage disequilibrium with DQB1*0301 and DQA1*0501. The high frequency of the two alleles in these SS patients is in contrast with the accepted association of primary SS with HLA-DR3 in Italian and American individuals. Molecular analysis of DQB1 and DQA1 alleles found in American Caucasian and American black SS (or SLE) patients demonstrated high frequencies of DQB1*0201 and DQA1*0501. The fact that the majority of SS patients, across racial and ethnic boundaries, carry a common allele, DQA1*0501, implies its involvement in the predisposition to primary SS. Based on sequence analysis and the computer imaging of the HLA class II molecule structure, a hypothetical model for the role of the DQ molecule in promoting primary SS is proposed.


ABSTRACT: Sequencing Based Typing (SBT) is a generic approach for the identification of HLA-A polymorphism. This approach includes the high resolution typing of the HLA-A broad reacting groups, HLA-A subtypes and will identify new alleles directly. The SBT approach described here uses a locus specific amplification of DNA from exon 1 to exon 5. The resulting 2,022 bp PCR product serves as a template for the subsequent sequencing reactions. Amplification is followed by direct sequencing of exons 2, 3 and 4 in both orientations with fluorescently labeled primers to define all polymorphic positions leading to a high resolution typing result. In this study the sequence of exons 2 and 3 of a panel of 49 cell lines was determined. In addition, the exon 4 region of 35 cell lines was also sequenced to evaluate the exon 4 polymorphism. The HLA-A type of most of the cells could be identified by sequencing only exons 2 and 3. However, the sequence of exon 4 was required to discriminate A*0201 from A*0209 and A*0207 from A*0215N. In this panel, an identical new "HLA-A*0103" was identified in two Caucasian samples.

The function of the TAP gene products appears to be the transport of antigenic peptides into the lumen of the endoplasmic reticulum where peptides are loaded onto HLA molecules. The polymorphisms within the TAP genes and potential disease associations are the subject of intense current study. While several methods have been described for TAP1 genotyping, most of these methods are unable to definitively assign TAP1 genotypes to individuals heterozygous at more than one polymorphic position. A combination named TAP1U was observed in approximately 25% of study subjects. We developed a restriction enzyme based method that allows definitive TAP1 genotypes assignment to 100% of subjects. We also further developed and optimized TAP genotyping by PCR amplification of specific alleles (PASA) that resulted in significant time and cost savings. Hence, we report a novel method for assigning TAP genotypes for TAP1U subjects and the modified PASA reactions. These improvements facilitate the rapid and efficient assignment of TAP genotypes useful for large human disease-gene association studies.


Cytokine genetic polymorphisms are the subject of disease-association studies that require large-scale human genotyping. Polymerase chain reaction based custom microarrays and microfluidics systems were used to develop genotyping assays for following cytokine polymorphisms: tumor necrosis factor-α G-308A, interleukin-4 (IL-4) C-589T, interferon-γ (CA)n repeats, IL-1RN 86-bp variable number of tandem repeats (VNTR), and CCR5 32-bp indel. For G-308A, 70.9% of DNA samples assayed were homozygous for wild type, 25.5% were heterozygous, and none were homozygous for variant allele. For C-589T, 35.5% of DNA samples were homozygous for wild type, 38% were heterozygous, and 22% were homozygous for variant. For IL-1RN VNTR, 71% of DNA samples were homozygous and the remainder were heterozygous. For CCR5, 96.4% of amplicons were homozygous for wild type, and 3.6% were heterozygous containing deletion. For IFN-γ (CA)n repeats, 35.6% had 2, 2 alleles, 42.2% had 2, 3 alleles, and 11% had 3, 3 alleles with alleles 1 through 5 corresponding to 11 through 15 repeats, respectively. There was good concordance between the results we obtained and current "gold-standard" methodologies for analyzing single nucleotide polymorphisms and size polymorphisms. Electronic DNA concentration with high stringency predisposes microarray technology to hybridization fidelity and accuracy, and microfluidics systems outperform conventional methodologies for size polymorphisms. Comprehensive genotyping can be achieved for clinical epidemiologic studies on cytokine gene polymorphisms using this approach.


More than 590 human leukocyte antigen (HLA)-B alleles have been identified by sequence analysis. Although the polymorphic exon 2 and 3 sequences of all HLA-B alleles are described, the sequences of the other exons of a number of infrequent B-alleles are unknown. In this study, the exon 1, 4, or 5 sequences of 39 different HLA-B alleles were elucidated by allele-specific sequencing. Overall, these exon sequences showed identity with the majority of the known
sequences from the corresponding allele groups, except for four alleles B*4010, B*4415, B*4416, and B*5606. The exon 1 sequence of B*4010 had nucleotide differences with all B*40 alleles, but was identical to the B*54, *55, *56, and *59 allele groups. B*4416 differed from B*440201 at position 988, which was previously considered a conserved position. B*4415 showed exon 1, 4, and 5 sequences deviating from the other B*44 alleles, but identical to B*4501. The exon 1 and 4 sequences of B*5606 differed from other B*56 alleles, but were in complete agreement with B*7801. The deviating exon sequences of B*4415 and B*5606 confirmed the evolutionary origin of these alleles suggested by the sequences of exons 2 and 3. The polymorphism observed in exons 1, 4, and 5 merely reflects the lineage-specificity of HLA-B.


http://www.sciencedirect.com/science/article/B6T3B-4DSPW36-4/2/d6179c0d3306b6c31254dd94847233ac

An unusual haplotype was detected in a family of a caucasian transplant patient. Human leukocyte antigen (HLA) analysis of the family demonstrated the absence of HLA-A on one of the haplotypes present in two family members. One was serologically typed A24, the other A2. Because they had one haplotype in common, the HLA-A allele of the shared haplotype was supposed to be a null allele. Different molecular typing methods identified only one allele in both individuals. The results suggest a deletion of the complete HLA-A gene or a major part of it. For confirmation, microsatellite analysis of the HLA-A region was performed with six microsatellite markers. Both family members were heterozygous for all markers, and a deletion of HLA-A could not be proven. Fluorescent in situ hybridization (FISH) was performed with cosmid and PAC probes encompassing the HLA-A gene. Both probes demonstrated an identical normal distribution pattern for diploid results. The absence of any serologic and molecular reaction with the results of the microsatellite and FISH analysis make a deletion of a narrow region, encompassing the HLA-A gene, the most plausible explanation.

Swelsen, W. T. N., C. E. M. Voorter, et al. "Sequence-based typing of the HLA-A10/A19 group and confirmation of a pseudogene coamplified with a*3401." Human Immunology In Press,

Uncorrected Proof http://www.sciencedirect.com/science/article/B6T3B-4FG4842-1/2/21364969d1d36980249c26ee55446f29

The strategy for sequencing human leukocyte antigen (HLA)-A was based on separate amplification of exons 2 and 3, followed by forward and reverse heterozygous sequencing of the alleles. Validation of the method was obtained by sequencing 11 individuals carrying alleles from all different HLA-A allele groups, except *43. All alleles could be correctly identified except A*3401. Unexpected polymorphic positions were identified in exon 3, even in individuals homozygous for A*3401. The pseudogene HLA-COQ or HLA-DEL linked to A*3401 was coamplified and sequenced in addition. The problem was solved by using different amplification primers for exon 3 with mismatches for the two pseudogenes. A total of 252 unrelated individuals with at least one allele belonging to the A10 or A19 group were typed for HLA-A by this strategy. Ten different alleles were identified in the A10 group and 14 in the A19 group. As second allele a further 30 different subtypes from all different groups were sequenced. In 21 individuals, sequencing exon 1 was necessary to distinguish A*7401 from A*7402. The sequencing strategy, with separate amplification of the exons, has proven to be a robust method, resulting in reliable and efficient high-resolution HLA-A typing.
Genetic variations in the locus encoding the transporter associated with antigen processing, subunit 1 (TAP1), were systematically studied using samples from Caucasians, Africans, Brazilians, and compared with data from chimpanzees. PCR-amplified genomic sequences corresponding to the 11 exons were analyzed by single-strand conformation polymorphism (SSCP) and sequencing. Six nonsynonymous and 2 synonymous single nucleotide polymorphisms (SNPs) were found to be common in one ethnic group or another, and they involved codons 254 (Gly-GGC/Gly-GGT) in exon 3, 333 (Ile-ATC/Val-GTC) in exon 4, 370 (Ala-GCT/Val-GTT) in exon 5, 458 (Val-GTG/Leu-TTG) in exon 6, 518 (Val-GTC/Ile-ATC) in exon 7, 637 (Asp-GAC/Gly-GGC), 648 (Arg-CGA/Gln-CAA) and 661 (Pro-CCG/Pro-CCA) in exon 10. At each SNP site the sequence listed first was predominant in all ethnic groups. Several SNPs segregated on the same chromosome regardless of populations and species. Together, the SNPs produced 5 major human TAP1 alleles, 4 of which matched the officially recognized alleles *0101, *02011, *0301, and *0401; the 5th allele differed from each of those by at least 4 SNPs. Overall, TAP1*0101 was the predominant allele in all ethnic groups, with frequencies ranging from 0.667 in Zambians to 0.808 in US Caucasians. The TAP1*0401 frequency showed the greatest difference between Africans (0.221-0.254) and Caucasians (0.033), with Brazilians (0.058) fitting in the middle. Consistent with earlier work based on Caucasians and gorillas, *0101 appeared to be the newest human TAP1 allele, suggesting a dramatic spread of *0101 into all human populations examined. Characterization of TAP1 polymorphisms allowed the design of a PCR-based genotyping scheme that targeted 7 SNP sites and required 2 separate genotyping techniques.

Genetic susceptibility to multiple sclerosis (MS) has so far been strongly localized to the MHC class II region encoding the alleles of the haplotype HLA-DRB1*1501, -DQA1*0102, -DQB1*0602. However, this haplotype is not carried by approximately 40% of MS patients; a potential explanation could be that they carry other MHC class II alleles with similar function due to the sharing of nucleotide sequences encoding critical amino acid residues. The DRB1 gene is polymorphic at residue 86, encoding valine or glycine. In view of the increasing evidence for a functional role for DRB1 aa86 in the binding and presentation of autoantigenic peptides such as myelin basic protein, this study investigated associations with the residue 86 polymorphism in an Australian MS population. A significant increase in the Val86/Val86 genotype was observed in the MS patients, which was still present in the absence of the DRB1*1501 allele (p = 0.032). This suggests that DRB1 aa86 may have an independent role in contributing to MS susceptibility. The Val86/Val86 genotype was correlated with genotyping for other putative MS susceptibility genes, including T cell receptor beta chain germline polymorphisms, HLA-DMB alleles, and -DQA1 and -DQB1 alleles encoding critical amino acid residues, with a significant interaction only observed with DQB1 Leu26 (p = 0.014). Additional studies of the HLA-DRB1 aa86 polymorphism in MS, and its function, are needed to more fully understand this association.
CD8 deficiency is a rare primary immunodeficiency caused by a defect of ZAP-70, which plays a pivotal role in T cell activation. We previously reported the existence of memory phenotype-CD4+ T cells in a case of CD8 deficiency, which demonstrates that activation signals through ZAP-70 are not essential to the phenotypic conversion of T cells from "naive" to "memory." In this study, we further characterized CD45RO+ T cells in a CD8 deficient patient. We showed that the patient's CD45RO+ T cell population had a wide variety of T cell receptor V[beta]-chain gene usage, and contained few clonally expanded T cells, while many clonally expanded T cells were present in the memory T cell population of age-matched healthy children. These results suggest that various kinds of antigens were involved in the differentiation of the patient's T cells, and that the differentiation into memory T cells was not accompanied by profound T cell proliferation. Moreover, our findings confirmed that the patient's CD45RO+CD4+ T cells had acquired effector-cytokine producing ability, indicating that there exists an alternative activation pathway which is independent of ZAP-70 for the acquisition of effector-cytokine producing ability.

van den Elsen, P. J., N. van der Stoep, et al. (2000). "Lack of CIITA expression is central to the absence of antigen presentation functions of trophoblast cells and is caused by methylation of the IFN-[gamma] inducible promoter (PIV) of CIITA." Human Immunology 61(9): 850.

Lack of MHC-mediated antigen presenting functions of fetal trophoblast cells is an important mechanism to evade maternal immune recognition. In this study we demonstrated that the deficiency in MHC expression and antigen presentation in the trophoblast cell lines JEG-3 and JAR is caused by lack of class II transactivator (CIITA) expression due to hypermethylation of its interferon-[gamma]-responsive promoter (PIV). Circumvention of this lack of CIITA expression by introduction of exogenous CIITA induced cell surface expression of HLA-DR, -DP, and -DQ, leading to an acquired capacity to present antigen to antigen-specific T cells. Transfection of CIITA in JEG-3 cells also upregulated functional HLA-B and HLA-C expression. Note- worthy, this lack of IFN-[gamma]-mediated induction of CIITA was also found to exist in normal trophoblast cells expanded from chorionic villus biopsies. Together, these observations demonstrate that lack of CIITA expression is central to the absence of antigen presentation functions of trophoblast cells.

to specific amplification with two types of the 3'-sided primers. The amplified DNAs were hybridized with 23 nonradioactively labeled SSOPs. Based on the hybridization patterns with the SSOPs, 34 HLA-B specificities were divided into 26 epitope combination (EC) groups. Fifteen HLA-B specificities were classified into four EC groups and these HLA-B specificities could not be distinguished from one another in the same EC group. Another 16 HLA-B specificities corresponded one by one to 16 distinct EC groups, and two subtypes of HLA-Bw 75, B27, and Bw48 were also identified enabling the accurate typing of 22 HLA-B alleles at the DNA level. Single-strand conformation polymorphism (SSCP) of the PCR products from group I HLA-B alleles was also investigated. The HLA-B allelics showed distinct electrophoretic patterns in nondenaturing polyacrylamide gels, depending on the nucleotide sequences of the exon 2, indicating that the SSCP analysis may be an alternative, useful and practical HLA-matching system of HLA-B specificity in tissue transplantation.


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MHC class II alleles and haplotypes were determined from unrelated individuals and families of the Arhuaco (n = 107), Kogi (n = 42), Arsario (n = 18), and Wayu (n = 88) tribes located in the northern part of Colombia. Class II DRB, DQA1, and DQB1 alleles were determined by PCR-SSO and PCR-RFLP based methods. Four haplotypes, [DRB1*0407, DRB4*0101, DQA1*03, DQB1*0302]; [DRB1*0403, DRB4*0101, DQA1*03, DQB1*0302]; [DRB1*1402/1406, DRB3*0101, DQA1*0501, DQB1*0301]; and [DRB1*0802, DQA1*0401, DQB1*0402], were observed among these four tribes. In addition to these haplotypes, the Wayu Indians showed a frequency of 21.3% for the [DRB1*1602, DRB5*02, DQA1*0501, DQB1*0301] haplotype, 13.1% for the [DRB1*0411, DRB4*0101, DQA1*03, DQB1*0302] haplotype, and 8.1% for the [DRB1*0411, DRB4*0101, DQA1*03, DQB1*0402] haplotype. Red cell antigen typing was used to calculate genetic admixture. The Kogi and Arsario showed no genetic admixture while the Arhuaco tribe showed admixture with genes of African origin and the Wayu showed admixture with Caucasians as well as genes of African origin. These findings were confirmed by the MHC class II allele and haplotype data obtained, as alleles and haplotypes of Caucasian and African origin were detected in the Wayu and Arhuaco and not in the Kogi or Arsario. These studies will be important in disease association and transplantation studies for Amerindian and Colombian populations and for correlating genetic traits with the anthropologic and linguistic data available in order to better understand the Amerindian populations.


http://www.sciencedirect.com/science/article/B6T3B-4D1359N-6/2/cbfeb63654cfe18c61943422260c92

Type 1 diabetes (T1D) is a complex autoimmune disease. Several genetic loci have been implicated in the susceptibility to this illness. Evaluated was the role of the CTLA4 exon 1 A49G polymorphism and its role as a risk factor for T1D in our population. DNA from 190 patients with T1D and their families and 96 control individuals were genotyped for CTLA4 exon 1 polymorphism and human leukocyte antigen (HLA)-DQB1*0201 and *0302 haplotypes by polymerase chain reaction (PCR) amplification-restriction enzyme analysis and PCR amplification that used sequence-specific primers, respectively. Patients were nonobese and 2 analysis and family-based association studies were performed and suggested the association of CTLA4 exon
1 G polymorphism with T1D ($p = 0.0229$). Furthermore, in HLA-DQB1*0201-positive patients with T1D, the GG and AA genotypes were higher and lower, respectively, than those found in control individuals. This study suggests that CTLA4 is a candidate susceptibility gene for T1D.

Zetterquist, H. and O. Olerup (1992). "Identification of the HLA-DRB1*04, -DRB1*07, and -DRB1*09 Alleles by PCR Amplification with sequence-specific primers (PCR-SSP) in 2 hours." Human Immunology 34(1): 64.

http://www.sciencedirect.com/science/article/B6T3B-47DTV94-2R/2/265159a9dd6e67f8ef4892c5b8b1bc20

The clinical applicability of genomic HLA class II typing techniques has increased after the introduction of PCR-based typing strategies. In typing by PCR amplification using sequence using sequence-specific primers (PCR-SSP), amplification of specific alleles or groups of alleles is achieved, provided that the mismatch(es) of the SSP is located in the 3' end of the primer. Thus, the specificity of the typing system becomes part of the amplification step, which reduces the total typing time to a minimum by simplifying the postamplification processing of samples. The set of primers presented here identifies all of the alleles of the DR4 group, DRB1*0401- DRB1*0411, as well as the DRB1*07 and DRB1*0901 alleles. In the present study of DR4 alleles, PCR-SSP was compared with hybridization with sequence-specific oligonucleotide probes following group-specific PCR amplification (PCR-SSO). The two typing strategies gave completely concordant results in the 90 DR4-positive and the 32 DR4-negative individuals and cell lines studied. DR7, DQ9/DR9,DQ9 discrimination using PCR-SSP, was compared with MspI DQA RFLP typing, also with concordant results in the 33 DR7- and/or DR9-positive and 36 DR7- and DR9-negative individuals and cell lines tested. No false-negative or false-positive typing results were obtained. Genomic typing by PCR-SSP was performed in the overall time of 2 hours, including rapid DNA preparation, PCR amplification, postamplification processing, documentation, and interpretation of results. This makes the PCR-SSP strategy for HLA class II typing attractive not only in population- and disease-association studies, but also in routine clinical practice, including donor-recipient matching prior to cadaveric transplantation.

Zipp, F., C. Windemuth, et al. (2000). "Multiple sclerosis associated amino acids of polymorphic regions relevant for the HLA antigen binding are confined to HLA-DR2." Human Immunology 61(10): 1021.

http://www.sciencedirect.com/science/article/B6T3B-41NCFTH-8/2/7c931e6c016ad71c7d071061ed1fb17b

Among the candidate genes for multiple sclerosis (MS), the strongest influence is conferred by human leucocyte antigen (HLA) class II genes, in particular the DR2, DQ6, Dw2 haplotype (DRB1*1501, DQA1*0102, DQB1*0602). Similar to other autoimmune diseases, it is not clear yet how the presence of a specific HLA-DR or -DQ molecule translates into an increased disease susceptibility. Previous observations by us and others imply a HLA-DR2 dependent propensity of antigen-specific T-cell lines to produce increased amounts of TNF-[alpha]/[beta] as one mechanism how DR2 could contribute to susceptibility. In this article, we investigated the distribution of polymorphic stretches of the DRB1, DQA1, and DQB1 chains known to be relevant for antigen binding, in 66 unrelated patients with relapsing remitting MS and 210 unrelated controls. We found a significant association with disease for the appearance of proline at position 11, arginine at position 13, and alanine at position 71 of HLA-DR[beta]1. Surprisingly, we identified only residues preferentially expressed in the MS group that were related to HLA-DR2. Thus, the contribution of HLA class II to the pathogenesis of MS is not mediated by allele-overlapping antigen binding sites, but is confined to the disease associated HLA allele.
IL-2 receptor is expressed at low levels on adult blood lymphocytes, and at lower levels on cord blood cells. IL-2 receptor [alpha] and [beta] chain expression increases gradually from 0-18 months of age. The level of soluble CD25 (IL-2 receptor [alpha] chain) has been reported to be elevated in cord blood. Quantitative RT-PCR showed that adult cells express 10 times as much CD25 mRNA as cord cells. Cord plasma showed only a marginal ability to strip CD25 from the membrane. To assess the functional consequences of low IL-2 receptor expression, cord and adult cells were activated in vitro. The response was stimulus-dependent, but cord cells upregulated CD25 readily. Cord and adult cells proliferated in an IL-2-dependent assay to a similar extent. Infants suffering acute infection showed marginally higher levels of membrane CD25 expression than infants without overt infection. Thus neonatal and infant lymphocytes express lower levels of IL-2 receptors than adult cells, reflecting lower mRNA concentrations at least for CD25; they are able to up-regulate receptors in response to in vitro stimulation and are able to respond in vitro to IL-2-dependent stimulation; however in vivo there may be a dampening down of the IL-2 system in infancy.