We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Immunogenetics of Type 1 Diabetes

Rajni Rani

*National Institute of Immunology, New Delhi,
India*

1. Introduction

Type 1 diabetes (T1D), also known as Insulin dependent diabetes mellitus (IDDM) is an incurable multi-factorial autoimmune disorder. The disease is characterized by the loss of insulin producing beta cells of the pancreas resulting in abnormal metabolism of glucose which may lead to ketoacidosis and several other complications like retinopathy, nephropathy and even cardio-vascular diseases and pre-mature deaths (Pociot & Mcdermott, 2002). World-wide disease affects 1 in 300-400 children (Todd, 1995). Population based data from South India shows the incidence of T1D for four year period to be 10.5/100,000/ year (Ramachandran et al., 1996). Similar prevalence of type 1 diabetes has been observed in North India. A study from district of Karnal in North India reported the prevalence to be 10.20/100,000 population, with a higher prevalence in urban (26.6/100,000) as compared to rural areas (4.27/100,000) (Kalra et al. 2010). T1D develops as a result of complex interaction of many genetic and environmental factors leading to autoimmune destruction of the insulin producing pancreatic beta cells. While 20 genomic intervals have been implicated for the manifestation of the disease (Pociot & Mcdermott, 2002), role of an intricate network of the products of these genes cannot be ruled out. However, unravelling different factors involved and how they interact in integrated networks is like solving a jigsaw puzzle which is the aim of our studies. Basic problem with T1D patients is that by the time they first report to the physician, most of their pancreatic beta cells are already destroyed which leaves the clinician with no option but to give daily insulin injections. So, there is a need to identify the prediabetics before the onset of the disease and devise ways to inhibit autoimmunity in them. Following sections will show the work done in our laboratory to understand the intricate networks in which the genes involved in immune responses interact and their implications.

2. Role of Major Histocompatibility Complex (IDDM1)

The Major Histocompatibility Complex (MHC) region on chromosome 6p21.31 has been shown to have major role in predisposition to get type 1 diabetes. It is also called IDDM1.

2.1 Genes and proteins of the Major Histocompatibility Complex (MHC)

The human MHC, Human Leukocyte Antigen (HLA) system is the most polymorphic system of the human genome with more than 5000 alleles. The alleles of HLA loci are co-dominant i.e. both the alleles at a particular locus are equally expressed. The genes of HLA code for

glycoprotein molecules which are expressed on nucleated cells and are responsible for the recognition of non-self from self. The function of MHC molecules is to present exogenous and endogenous antigens in the form of peptides to the T cells for subsequent immune response to take place. The gene map of the MHC region of man on chromosome 6p21.3 shows that it spans about 4 megabases (3,838,986 bp to be precise). It is the most gene-dense region of the human genome with 224 genes of which 128 are known to be expressed. 40% of the expressed genes in this region have immune related functions (Horton et al., 2004).

There are two types of MHC molecules : MHC Class-I and Class-II which differ from each other in their constituents as well as their functions.

2.1.1 MHC class-I genes and proteins

MHC class-I genes are expressed on all nucleated cells in the form of cell surface glycoproteins. Function of MHC class-I molecules is to present antigenic peptides to CD8⁺ cytotoxic T cells (CTLs). The classical class-I genes in humans are HLA-A, HLA-B and HLA-C. All these genes are very polymorphic with 1519 alleles for HLA-A locus, 2069 alleles for B-locus and 1016 alleles for C-locus and these numbers are increasing with the discovery of new alleles everyday.

The MHC class-I molecule is a hetero-dimer of a heavy alpha chain (about 40-45 KDa) and the light chain, beta 2 microglobulin (β_2m) of 12 KDa (Bjorkman et al., 1987). While the genes for the heavy chains i.e. the alpha chains are encoded on chromosome 6, the gene for β_2m is encoded on human chromosome 15. The alpha chain of the MHC class-I molecule has three domains alpha 1 (α_1), alpha 2 (α_2) and alpha 3 (α_3). Alpha 1 (α_1) and alpha 2 (α_2) domains are the most polymorphic domains since they constitute the peptide binding groove of the MHC molecule. The genes encoding MHC class-I alpha chain have 8 exons with second and the third exons of the alpha chain gene being most polymorphic since they code for the α_1 and α_2 domains. The peptides that are presented by the MHC molecules have allele specific motifs, which means that certain peptides can be presented by certain MHC molecules. The affinity of the peptide to bind to the peptide binding groove is determined by the anchors present on the peptide binding groove where the peptides go and bind through hydrogen bonds. Specific motifs on the peptides determine which peptides would bind to which MHC molecule (Falk et al., 1991, Garrett et al., 1989).

2.1.2 MHC class-II genes and proteins

MHC class-II glycoproteins in humans are HLA-DR, -DP and -DQ. The MHC class-II molecule is a heterodimer of two polypeptide chains: an alpha (25-33 KDa) and a beta chain (24-29KDa) (Brown et al., 1993, De Vries & Van Rood, 1985). Unlike MHC class-I, both alpha and beta chains of the class-II molecule are encoded on chromosome 6. DRB1 gene encodes DR beta chain while DRA1 encodes DR alpha chain with 966 DRB1 alleles and 3 DRA1 alleles. Similarly DQB1 and DPB1 encode beta chains of DQ and DP molecules with 144 and 145 alleles respectively and DQA1 and DPA1 encode the alpha chains of DQ and DP molecules with 35 and 28 alleles respectively (Robinson et al., 2009).

While HLA class-I molecules are expressed on all nucleated cells, HLA class-II molecules are expressed on antigen presenting cells like macrophages, dendritic cells, B cells, thymic epithelium and activated T cells (Holling et al., 2004). The function of MHC class-II molecules is to present antigenic peptides to the CD4⁺ T helper cells (Th cells) which in turn initiate a cascade of immunological events resulting in activation of CD8⁺ cytotoxic T cells

(Horton et al., 2004). When a non-self antigen is presented to CD4⁺ T helper cells, they get activated and secrete certain cytokines like Interferon gamma and TNF-alpha in case of Th1 cells and IL-4, IL-5 and/or IL-6 in case of Th2 cells. While the cytokines secreted by Th1 cells activate the cytotoxic T cells which have already seen the antigen in the context of HLA class-I, Th2 cytokines activate the B cells to become plasma cells which make the antibodies against antigen they have seen. Thus an immune response takes place which varies in strength depending on the host factors and the peptides being presented. There are about 50,000-100,000 MHC molecules on each cell. Most MHC molecules are occupied by self peptides and the T cells are tolerized against them during thymic education so that auto-immune responses do not take place, however, some times something goes awry and there is a break in the tolerance resulting in recognition of self as non-self by the immune system resulting in an auto-immune response. This could be due to low expression of some antigens in the thymus which may result in self-reactive T cells to reach the peripheral circulation. Or it could be due to escape of self-reactive T cells from clonal deletion during T cell development.

2.2 MHC and Type 1 diabetes

Despite so much polymorphism, significant increase of one or more alleles of HLA in a disease population as compared to healthy controls, suggests functional implications due to their role in antigen presentation. We observed a significant increase of *DRB1*03:01* ($p<10^{-6}$, Odds Ratio (OR) =11.0), *DRB1*04:01* ($p<0.01$, OR=6.4) and *DRB1*04:05* ($p<0.03$, OR=5) in the patients (Figure 1a) using high resolution typing method of polymerase chain reaction followed by hybridization with sequence specific oligonucleotide probes(PCR-SSOP) (Rani et al., 2004, Rani et al., 1999).

Rani et al., Tissue Antigens : 64:145-155, 2004

Fig. 1. Distribution of *HLA-DRB1* alleles significantly increased in Type 1 diabetes. **a.** *DRB1*03:01*, *DRB1*04:01*, *DRB1*04:05* showing significant increase and *DRB1*04:03*, *DRB1*04:04* and *DRB1*07:01* showing significant reduction in T1D patients as compared to healthy controls. **b.** shows the homozygosity and heterozygosity of predisposing and protective alleles significantly increased or reduced in the T1D patients. Homozygous *DRB1*03:01/03:01*, heterozygous *DRB1*03:01/04:05*, *DRB1*03:01/04:01* and *DRB1*03:01/X* were significantly increased and *DRB1*04:03/X* and *DRB1*07:01 X* were significantly reduced in the T1D patients as compared to controls.

Our results were in concordance with earlier studies in North Indians (Gupta et al., 1991, Kanga et al., 2004, Mehra et al., 2002, Sanjeevi et al., 1999, Witt et al., 2002). However, we also observed *DRB1*07:01* ($p < 7 \times 10^{-6}$, OR = 0.16), *DRB1*04:03* ($p < 0.02$, OR = 0.25) and *DRB1*04:04* ($p < 0.05$, OR = 0.2) to be significantly decreased in the patients as compared to controls. We did not find any significant reduction of HLA-DR2 haplotype *DRB1*15:01-DQB1*06:02* which has been shown to confer strong protection from T1D in most ethnic groups (Baisch et al., 1990, Pugliese et al., 1995), probably because this haplotype has been found with a low frequency of only 1.06% in North Indians (Rani et al., 1998). On the other hand, we observed a marginally reduced frequency of *DRB1*15:06* in patients as compared to controls, which did not remain significant when p was corrected for the number of alleles tested for *DRB1* locus (Rani et al., 2004).

Figure 1b shows the homozygosity and heterozygosity of *DRB1*03:01* and *DRB1*04* alleles significantly increased in T1D. Homozygous *DRB1*03:01* ($p < 10^{-7}$, OR = 14.54), heterozygous *DRB1*03:01/*04:05* ($p < 0.03$, OR = 10.9) and *DRB1*03:01/*04:01* ($p < 0.01$, OR = 13) were significantly increased in the patients as compared to controls who lacked this heterozygous combination. Heterozygous *03:01/X* (i.e. any other allele) ($p < 0.04$, OR = 1.89) was also significantly increased in the patients as compared to controls. Heterozygous *DRB1*04:03/X* ($p < 0.04$, OR = 0.22) and *DRB1*07:01/X* ($p < 10^{-7}$, OR = 0.066) were significantly reduced in the T1D patients as compared to controls suggesting their protective role. Significant protection has been shown to be associated with *DRB1*04:03* allele in a Belgian study of diabetes (Van Der Auwera et al., 1995). *DRB1*03:01*, *DRB1*04:01* and *DRB1*04:05* have also been shown to be associated with T1D patients in Sardinians, black population from Zimbabwe, Lithuanians, Czechs, Lebanese, Brazilians and African Americans (Alves et al., 2009, Cucca et al., 1995, Ei Wafai et al., , Fernandez-Vina et al., 1993, Garcia-Pacheco et al., 1992, Skrodeniene et al., , Tait et al., 1995, Weber et al.).

Cucca et al suggested that amino acid position $\beta 74$ and $\beta 86$ in DR beta chain are the key residues in the P4 and P1 pockets of the peptide binding groove of HLA-DR molecules (Cucca et al., 2001). A combined presence of Asp, Glu and Val in positions $\beta 57$ (P9), $\beta 74$ (P4) and $\beta 86$ (P1) in protective *DRB1*04:03* has been shown to be different from high risk *DRB1*04:05* which has Ser, Ala and Gly at these positions. However, in the North Indians we observed *DRB1*03:01* to be at highest risk and this allele has Asp, Arg, and Val in the three positions (Figure 2). A less predisposing allele in North Indians, *DRB1*04:01* has Asp, Ala, Gly and the protective *DRB1*04:04* and *DRB1*07:01* have Asp, Ala, Val and Val, Gln and Gly in the three positions respectively. Thus, Asp, Arg and Val in *DRB1*03:01* is entirely different from Val, Gln, and Gly in *DRB1*07:01* which seems to be important in our study since all the four DR4 alleles are present in less than 10% of the patients or control samples. In essence, these data suggest that it is probably not $\beta 74$ and $\beta 86$ alone, rather an integration of all the pockets of the peptide binding groove that determines which peptide of an auto-antigen would bind to the MHC molecule and result in auto-aggression based on the thymic education.

We also studied the alleles of *DQB1* locus. *DQB1*02:01* which is linked to *DRB1*03:01* was significantly increased ($p < 1 \times 10^{-8}$, OR = 5.08) in patients (Figure 3a). However *DQB1*03:02* and *DQB1*03:07*, alleles linked with *DRB1*04:01*, *DRB1*04:03*, *DRB1*04:04* and *DRB1*04:05* were not significantly increased in the patients because two of these alleles *DRB1*04:01* and *DRB1*04:05* were increased in the patients and the other two DR4 alleles *DRB1*04:03* and *DRB1*04:04* were significantly reduced in the patients. *DQB1*03:01* ($p < 6 \times 10^{-4}$, OR = 0.27) and

Fig. 2. Peptide binding groove of the predisposing and protective HLA-DRB1 alleles showing positions β57 (P9), β74 (P4) and β86 (P1) for predisposing *DRB1*03:01*, *DRB1*04:01* and *DRB1*04:05* and protective *DRB1*07:01*, *DRB1*04:04* and *DRB1*04:03* alleles.

*DQB1*05:03* (6×10^{-4} , OR=0.28) were significantly reduced in the patients. Homozygosity of *DQB1*02:01* was significantly ($p < 1 \times 10^{-5}$, OR=5.4) increased in the patients (Figure 3b). *DQB1*03:02* which was not significantly increased in the patients, showed a significant increase in heterozygous combination with *DQB1*02:01* ($p < 2 \times 10^{-5}$, OR=34.16). In fact none of the controls had *DQB1*02:01*/**03:02* heterozygous combination. In a Swedish study, *DQA1*03:01*/*DQB1*03:02* and heterozygous combinations of *DQA1*03:01*/*DQB1*03:02* and *DQA1*02:01*/*DQB1*05:01* have been shown to confer the highest susceptibility (Sanjeevi et al., 1995).

Some critical residues within the peptide binding sites of HLA-DQ beta chain have been proposed to play a crucial role in conferring predisposition to and protection from the diseases (Nepom & Kwok, 1998, Sheehy, 1992, Todd et al., 1987). Several studies have suggested that aspartic acid at DQβ residue 57 confers protection while DQB1 alleles with alanine at that position (*DQB1*02:01* and *DQB1*03:02*) and DQA1 with arginine at position 52 (R⁵²) confer susceptibility (Badenhoop et al., 1995, Chauffert et al., 1995, Todd et al., 1987). However, an individual can be either homozygous or heterozygous for alleles carrying

Fig. 3. Distribution of *HLA-DQB1* alleles in T1D patients and controls. **a** shows *DQB1*02:01* was significantly increased and *DQB1*03:01* and *DQB1*05:03* were significantly reduced in T1D patients as compared to controls. **b**. Homozygous and heterozygous *DQB1* alleles in T1D. Homozygous *DQB1*02:01/*02:01* and heterozygous *DQB1*02:01/*03:02* were significantly increased and *DQB1*03:02/X* were significantly reduced in T1D patients as compared to controls (Rani et al., 2004).

Asp⁵⁷ in *DQB1* or Arg⁵² in *DQA1*. Our in-depth investigation revealed that when *DR3* homozygosity was considered along with codon 57 of *DQB1* and codon 52 of *DQA1*, the only combination that was significantly increased in the patients group as compared to the controls was *DRB1*03:01,03:01-DQB1*XX-DQA1*RR*, suggesting that *DRB1*03:01* association is primary since the *DQB1* and *DQA1* alleles which are in linkage disequilibrium with *DRB1*03:01* have non-Asp⁵⁷ (*DQB1*X*) and Arg⁵² (*DQA1*R*), respectively(Rani et al., 1999).

3. Insulin linked polymorphic region in T1D (IDDM2)

Insulin linked polymorphic region (*IDDM2*) consists of a highly polymorphic stretch of 14-15 base pair repeats of DNA lying 365 bp upstream of the initiation of transcription of the *insulin* (*INS*) gene. *IDDM2* has been shown to have a role in transcription of insulin in thymus. Several forms of *IDDM2* have been reported based on the number of repeats (Bell et al., 1981, Kennedy et al., 1995). These *INS-variable number of tandem repeats* (*VNTR*) are divided into three different classes based on their sizes: *class-I* (26-63 repeats), *Class II* (about 85 repeats) and *class III* (141-209 repeats) (Bell et al., 1982, Bennett et al., 1995, Rotwein et al., 1986). T1D is associated with class I homozygosity (Bell et al., 1981, Bennett et al., 1995, Kennedy et al., 1995, Lucassen et al., 1993). We studied *INS-VNTR Class-I* and *Class-III* alleles based on typing for *Insulin gene 1127 Pst I* site (3'end) by PCR-RFLP as described by Pugliese et al (Pugliese et al., 1997)

Table 1 shows the frequencies of *Insulin VNTR* in T1D patients and healthy controls. While the frequency of *class-I VNTR* was increased significantly in the patients, *class-III VNTR* was decreased in them as compared to the controls. However, when the genotypes were studied, *class I* homozygosity was considerably increased in the patients as compared to controls,

giving an Odds ratio of 7.8. *Class I, III* heterozygosity was significantly reduced in the patients (Rani et al., 2004).

INS-VNTR	DIABETES		CONTROLS		p value	OR
	No.	%	No.	%		
<i>Class I</i>	108	98.2	85	89.47	0.008	6.35
<i>Class III</i>	64	58.2	87	91.57	2 X 10 ⁻⁸	0.13
Genotypes						
<i>Class I, I</i>	46	41.8	8	8.42	2X10 ⁻⁸	7.8
<i>Class I, III</i>	62	56.4	77	81.05	10 ⁻⁵	0.301
<i>Class III, III</i>	2	1.8	10	10.52	0.008	0.157

Table 1. INS-VNTR allele frequencies and Genotype frequencies in T1D patients and controls.

3.1 Simultaneous presence of predisposing *HLA-DRB1* and *INS-VNTR* alleles

MHC and VNTR are encoded on two different chromosomes. However, they may have integrated roles in manifestation of T1D due to the functional implications of these genes. So, we studied if simultaneous presence of the predisposing alleles of the two genes had any role to play in manifestation of T1D.

Our investigation revealed that homozygous *Class-I INS-VNTR* along with homozygous or heterozygous *DRB1*03:01* were significantly increased in the T1D patients ($p<1\times10^{-8}$) with a Relative Risk of 70.81 (Rani et al., 2004). In fact, none of the controls had homozygous *Class-I INS-VNTR* along with *DRB1*03:01* in homozygous or heterozygous state. This combination gives a positive predictive value (PPV) of 100% with a specificity of 100% and sensitivity of 32.63% since only 32.63% of the patients showed this combination. Since *DRB1*03:01* homozygosity is significantly increased in the patients, homozygous *DRB1*03:01* and heterozygous *DRB1*03:01* only with *DRB1*04:01* and *DRB1*04:05* along with heterozygous *I, III-INS-VNTR* may also be considered as predisposing since it gives a relative risk of 10.55 (Rani et al., 2004).

If we add all these predisposing combinations i.e. simultaneous presence of homozygous or heterozygous *HLA-DRB1*03:01* along with homozygous (Class-I, I) or heterozygous (I, III) VNTR class-I and III, 50.53% of the patients as compared to only 1.4% of the controls had these combinations giving a relative risk of 48.67. This combination gives a PPV of 97.96% with a specificity of 98.6% and sensitivity of 50.5% since only 50.5% of the patients showed this combination. Thus, our results showed that: (1) homozygous or heterozygous *DRB1*03:01* along with homozygous Class-I INS-VNTR and (2) homozygous *DRB*03:01* and heterozygous *DRB1*03:01* only with *DRB1*04:01* or *DRB1*04:05* with heterozygous *Class-I/III INS-VNTR* may be used to predict a pre-diabetic before the onset of the disease in North Indian high risk group (Rani et al., 2004). However, typing a larger cohort may be required to confirm such a major increase in risk.

Pathogenesis of T1D is extremely complex. Significant association with *HLA-DRB1*03:01* and *INS-VNTR Class-I* may have functional implications. Increase in frequency of particular MHC allele suggests that these molecules may be preferentially presenting certain auto-peptides to the T cells resulting in subsequent autoimmune responses. Studies on *INS-VNTR*, however, have shown that *class-III* alleles are associated with 2 to 3 fold higher

mRNA levels of insulin than *Class-I* in thymii of fetuses, suggesting poor expression of thymic INS expression resulting in poor thymic education for insulin in people with homozygous *Class-I,I* and *class-I,III* VNTRs resulting in break of tolerance in predisposed individuals. However, higher expression of insulin in the thymii of individuals with homozygous *class-III, III* may be able to facilitate immune tolerance induction, as a mechanism for dominant protective effect of *Class-III* alleles (Pugliese et al., 1997, Vafiadis et al., 1997).

Our results are contrary to that of Veijola et al. (Veijola et al., 1995) on Finnish children who showed that both 5' and 3' INS loci showed an association with T1D in *non-DR3/non-DR4* patients (Veijola et al., 1995). However, in our studies only 9.47% of the *non-DR3/DR4* patients were homozygous for *Class-I* INS-VNTR as compared to 4.2% controls and this difference was not significant statistically. Julier et al. (Julier et al., 1991), on the other hand, had reported that the risk contributed by the INS region was increased in *DR4*-positive patients. Again, in our study only 6.32% of the patients as compared to 1.39% of controls had INS-VNTR *class-I* homozygosity with *DRB1*04:01* and *DRB1*04:05* alleles and this difference was not significant statistically.

4. Cytokine genes

Cytokines are the coordinators of the immune system that interact in integrated networks and functions of one cytokine may be modulated or substituted by another (Bidwell et al., 1999). A cascade of cytokines are involved in pro-inflammatory auto-immune responses in T1D. Single nucleotide polymorphisms (SNPs) in different pro-inflammatory and anti-inflammatory cytokine genes at certain defined regions have been shown to be associated with differential amount of their production (Asderakis et al., 2001, Awad et al., 1998, Bittar et al., 2006, Burzotta et al., 2001, Fishman et al., 1998, Louis et al., 1998, Pociot et al., 1993). Pro-inflammatory cytokines and their integrated influences are known to regulate complex immune responses during autoimmune destruction of tissues (Rabinovitch, 1994). Hence it is necessary that they are studied and analysed in context of each other and not in isolation from each other. We had reported for the first time the integration and interaction of *TNF- α* gene with other cytokine genes and *HLA-DRB1* and *B* loci alleles (Kumar et al., 2007).

We studied the cytokine gene polymorphism using XIIIth International Histocompatibility Workshop's (IHWK, Heidelberg kit) and One lambda's cytokine typing kits (Canoga Park, CA, USA) based on Polymerase Chain reaction (PCR) with sequence specific primers (PCR-SSP). PCR-SSPs were done for *IFN- γ* (*A⁺⁸⁷⁴T*) (14), *TNF- α* (*G⁻³⁰⁸A*) (15), *IL-6* (*G⁻¹⁷⁴C*) (9), *IL-10* (*A⁻¹⁰⁸²G*, *T⁻⁸¹⁹C*, *C⁻⁵⁹²A*) (16), and *TGF β 1* (*T^{cdn10}C*, *G^{cdn25}C*) (11). *T*→*C* substitution in nucleotide 29, codon 10 of the first exon of *TGF β 1*, changes the amino acid Leu → Pro. Similarly *G*→*C* substitution in nucleotide 74, codon 25 of first exon of *TGF β 1*, changes the amino acid Arg → Pro. However, since we are studying the SNPs in the two codons, we will refer to the SNPs in codons 10 and 25 hereafter. and not the resultant amino acids to avoid any confusion and to maintain consistency with the other SNPs.

Our results showed that the high producing genotype of *TNF- α -308GA* and *AA* were significantly increased and low producing genotype *GG* was significantly reduced in T1D patients as compared to controls ($p < 7 \times 10^{-6}$). None of the other cytokine genes showed any significant difference between the patients and controls.

4.1 Simultaneous presence of TNF-α genotypes with IFN-γ, IL-6, IL-10 and TGF-β1 genotypes and haplotypes

TNF-α, *IFN-γ*, *IL-10*, *IL-6* and *TGF-β1* genes are localized on different chromosomes. *TNF-α* is encoded on chromosome 6p21.3, *IFN-γ* is encoded on 12q14, *IL-10* is encoded on 1q31-q32, *IL-6* is encoded on 7p21 and *TGF-β1* is encoded on 19q13.2. However, the products of these genes interact in integrated networks. Since only *TNF-α* showed a significant association with T1D, we studied whether simultaneous presence of *TNF-α* genotypes with different genotypes of the other cytokines in an individual could suggest an interaction between these cytokine genes.

Other cytokines Genotype / haplotype	TNF-α GA/ AA		p	OR (95% CI)	TNF-α GG		p	OR (95% CI)
	T1D No. (%)	Controls No. (%)			T1D No. (%)	Controls No. (%)		
<i>IFN-γ Int +874</i>	N=235	N=128			N=235	N=128		
AA (L)	41 (17.4)	9 (7.0)	0.003@	2.79 (1. 25- 6.42)	46 (19.6)	44 (34.4)	0.001@	0.465 (0.28- 0.77)
TA+TT (H)	56 (23.8)	15 (11.07)	0.003@	2.39 (1.24- 4.66)	92 (39.1)	60 (46. 9)	0.188	0.729 (0.461- 1.15)
<i>IL-6 -174</i>	N=235	N=127\$			N=235	N=127\$		
CC (L)	11 (4.7)	1 (0.78)	0.03#	4.3 (1. 21- 14.56)	14 (5.95)	8 (6.29)	0.531	0.919 (0.357- 2.53)
GG+GC (H)	86 (36.6)	23 (18.1)	0.0001@	2.61 (1.5-4.56)	124 (52.75)	95 (74.8)	0.000004@	0.76 (0.227- 0.621)
<i>IL-10 Haplotypes*</i>	N=235	N=128			N=235	N=128		
Low secretor	45 (19.2)	16 (12.7)	0.068	1.65 (0.86- 3.22)	77 (32.76)	57 (44.5)	0.03#	0. 607 (0. 38- 0.96)
High Secretor	52 (22.1)	8 (6. 25)	0.0001@	4.26 (1.9- 10.1)	61 (25.95)	47 (36.7)	0.04#	0.6 (0.37- 0.98)
<i>TGF-β1 Haplotypes*</i>	N=235	N=128			N=235	N=128		
Low secretor	8 (3.4)	1 (0.8)	0.11	3.17 (0.87- 12.11)	7 (2.98)	3 (2.3)	0.506	1.17 (0.443- 3.23)
High Secretor	89 (37.8)	23 (18.0)	0.00004@	2.8 (1.6- 4.86)	131 (55.7)	101 (78.9)	0.000006@	0. 336 (0.198- 0.568)

N=Total number of samples studied, \$Number of control samples studied for IL-6 were 127, one sample could not be typed due to PCR failure. TNF-α GA/ AA have been combined as high secretor genotypes.
Corrected p value (pc) not significant, @ Corrected p value (pc) significant,
*IL-10 : halpotype combinations -1082/-819/-590 : GCC,GCC; GCC,ACC; GCC,ATA= high secretors; ACC,ACC; ACC,ATA = Low secretors.
TGF- β1 halpotype combinations Cdn10/Cdn25 : TG,TG; TG,CG; TG,CC; CG,CG =High secretors, CG,CC, CC,CC = Low secretors.

Table 2. Simultaneous presence of *TNF-α* genotypes with *IFN-γ*, *IL-6*, *IL-10* and *TGF-β1* genotypes and haplotypes (Kumar et al., 2007).

Table 2 shows the simultaneous presence of high and low secreting genotypes of *TNF-α*, along with *IFN-γ*, *IL-6*, *IL-10* and *TGF-β1* genotypes and haplotypes. When *IFN-γ* was studied by itself, it did not show any significant difference between patients and controls. However, when studied in the context of *TNF-α* -308G/A, both low and high secretor genotypes, *IFN-γ* (+874AA and TA+TT respectively) along with high secretor genotypes of *TNF-α* -308 GA+AA were significantly increased in patients as compared to controls, suggesting its effect is rather neutral. However, low producer genotypes of *TNF-α* -308GG along with low producer genotype of *IFN-γ* +874 AA seems to be protective. Interestingly, 66. 7% of the patients who had low producer genotype of *TNF-α* -308GG had high producer genotype of *IFN-γ* +874 TA+TT. Hence, in the absence of high secretor genotype of *TNF-α*, *IFN-γ* may have a role in autoimmune destruction of pancreatic beta cells. *IFN-γ* acts singularly as well as synergistically with other inflammatory stimuli to induce NO production which can be cytotoxic and thus has been implicated in pathogenesis of certain autoimmune and inflammatory diseases (Mccartney-Francis et al., 1993). Similarly, promoter SNPs of *IL-6* -174G/C did not show any significant difference between patients and controls, but when studied in the context of *TNF-α* -308G/A, high producer genotypes, *IL-6* -174 GG+GC (Fishman et al., 1998) were increased in patients with *TNF-α* -308 GA+AA. Kristiansen and Mandrup-Poulsen (Kristiansen & Mandrup-Poulsen, 2005) have shown that IL-6 promotes islet inflammation but is unable to promote β-cell destruction for which other pro-inflammatory cytokines are needed. The other pro-inflammatory cytokine playing a role in destruction β-cells in the present scenario could be

HLA-B-DRB1-haplotypes	Patients N=210		Controls N=91.		p	OR (95% C.I.)
	No*	%	No*	%		
B*8-DRB1*03	69	32.85	3	3.3	10 ⁻⁸	14.35 (4.19 -37.93)
B*8- Non DRB1*03	2	0.95	1	1.1	0.662	0.865 (0.498-1.5)
B*50 – DRB1*03	41	19.5	3	3.3	6x10 ⁻⁵	7.11 (2.04-21.47)
B*50 – Non-DRB1*03	6	2.86	4	4.4	0.355	0.639 (0.155-2.77)
B*58 – DRB1*03	36	17.1	5	5.5	0.003	3.55 (1.27-10.72)
B*58 – Non DRB1*03	3	1.4	8	8.8	0.003	0.165 (0.06-0.433)
NonB8/B50/58-DRB1*03	35	16.7	6	6.6	0.01	2.83 (1.09-7.82)
NonB8/B50/B58/non DRB1*03	38	18.1	59	64.8	10 ⁻⁸	0.12 (0.06-0.216)

* No. of patients /Controls with the haplotypes shown in the first column.

Table 3. Comparison of HLA-B-DRB1 haplotypes showing significant association, between patients and controls (Kumar et al., 2007)

TNF- α in patients with GA and AA genotypes and IFN- γ in patients with TNF- α GG genotype. High producer genotype IL-6 -174 GG+GC along with low producer genotype of TNF- α -308GG seems to be protective.

Different haplotypes of IL-10 based on SNPs in the promoter region have been shown to be associated with quantity of IL-10 production in-vitro (Asderakis et al., 2001, Stanilova et al., 2006). The frequency of low producer haplotype of IL-10 ATA (haplotype with positions -1082/-819/-590) has been shown to be increased in the adult onset patients in Japan, with no significant differences between T1D patients and controls (Ide et al., 2002). Reynier et al (Reynier et al., 2006) did not see any significant association of IL-10-1082G/A with T1D in French population. However, they did observe a significant association of IL-10 -1082 polymorphism to be associated with GAD and IA-2 antibody at clinical onset. In our study also, we did not observe any significant difference between T1D patients and controls when IL-10 was studied by itself. However, simultaneous presence of high producer genotypes of TNF- α -308 GA+AA and high producer haplotypes of IL-10 in the patients may have a role in recruitment of islet specific CD8⁺ T cells and thus may have a role in insulinitis through ICAM-1 dependent pathway. In non-obese diabetic (NOD) mice (animal model for human Type 1 diabetes) pancreatic IL-10 has been shown to hyper-induce ICAM-1 expression on vascular endothelium. However, in the absence of ICAM-1, insulinitis and diabetes could be prevented, thus providing evidence that IL-10 is sufficient to drive pathogenic autoimmune responses and accelerated diabetes via an ICAM-1 dependent pathway (Balasa et al., 2000). Presence of IL-10 during early stages of IDDM has also been shown to favor the generation of effector CD8⁺ T cells leading to accelerated diabetes in NOD mice (Balasa et al., 2000). Treatment of young mice with anti-TNF- α and anti-IL-10 mAb has also been shown to prevent diabetes and insulinitis (Lee et al., 1996, Yang et al., 1994).

Similarly, when TGF- β 1 was studied by itself, no significant difference was observed between patients and controls. A significant increase of high producer haplotypes of TGF- β 1 with TNF- α -308 GA+AA and a significant decrease of high producer haplotypes of TGF- β 1 with TNF- α -308GG in T1D patients as compared to controls was observed. These results show that different cytokines work in concert with each other and may alter or modulate their functions depending on the milieu. TGF- β 1 has been shown to be an extremely potent chemotactic factor in-vitro which influences monocyte recruitment and accumulation via increased expression of α and β integrins (Wahl et al., 1993). It has been shown to rapidly and transiently up-regulate α -4 integrin dependent adhesion of leukocyte cell lines and peripheral blood lymphocytes (Bartolome et al., 2003). α -4 integrin, in turn, has been shown to play a prominent role in the spontaneous development of insulinitis and diabetes in NOD mice (Yang et al., 1997). Increased levels of TGF- β 1 have been associated with destruction of pancreatic beta cells and pathogenesis of diabetic complications (Korpinen et al., 2001). Hence, in the presence of high secretors of TNF- α , the high secretor genotypes of TGF- β 1 may have a role both in destruction of pancreatic beta cells as well as in migration of CD4⁺ and CD8⁺ T cells into the pancreas (Insulinitis) through α -4 integrin, which act against pancreatic beta cells along with Nitric oxide mediated cytotoxicity. Under these circumstances, TGF- β 1 may not be able to arrest the pro-inflammatory functions of TNF- α and IFN- γ .

So, our data provides circumstantial evidence justifying the presence of high secretor genotypes of TNF- α -308GA and AA along with high secretor genotypes of IL-6, IL-10 and TGF- β 1 and provide an immunogenetic basis for the autoimmune responses in T1D. The data suggest that the beta cell destruction in T1D may be mediated by both CD4⁺ T helper

cells and CD8⁺ cytotoxic T cells recruited through Integrins and ICAM-1 dependent pathways in the pancreas for which cytokine genes seem to play a pivotal role.

4.2 *TNF-α* and *HLA* genes

TNF-α gene is very closely linked to the MHC. Deng et al (Deng et al., 1996) observed that the *TNF-α* associations in Caucasians and Chinese of Taiwan, may be due to its being in linkage disequilibrium with *DR3-DQB1*0201* haplotype. We too had observed *DRB1*03:01*, *DRB1*04:01* and *DRB1*04:05* to be associated with T1D in North Indians (Rani et al., 2004). Hence, we wanted to study if the *TNF-α* association was independent of these alleles or due to linkage disequilibrium (LD) between *TNF-α* -308A and the predisposing *DRB1* alleles. Interestingly, the LD analysis showed that both *TNF-α* -308A as well as *TNF-α* -308G alleles are in LD with *DRB1*03:01*, the most predisposing *HLA-DRB1* allele, suggesting that the effect of *TNF-α* -308A is not because of its being in LD with *HLA-DRB1*03:01*, the predisposing MHC allele.

Since *TNF-α* locus is very closely linked to *HLA-B* locus, we also studied the alleles of B-locus for a possibility of *TNF-α* -308A allele being in LD with one of the B-locus alleles . Surprisingly we observed LD between *TNF-α* -308G with *B*08* and *TNF-α* -308A allele with *HLA B*50:01* and *B*58:01*. All the three B-locus alleles *B*08:01*, *B*50:01* and *B*58:01* were in linkage disequilibrium with *DRB1*03:01* (Table 3). Because of *HLA-B*08* being in LD with *TNF-α* -308G, *B*08:01-DRB1*03:01* haplotype was also in LD with *TNF-α* -308G (Table 4).

HLA-B-TNF-α-DRB1-haplotypes	Number of haplotypes observed 2N=418	Haplotype frequencies	D _{abc} [#]
<i>B*8- TNF-α -308A-DRB1*03</i>	19	0.045	-0.013
<i>B*8- TNF-α -308G-DRB1*03</i>	75	0.179	0.015
<i>B*50- TNF-α -308A-DRB1*03</i>	42	0.1	0.0329
<i>B*50- TNF-α -308G-DRB1*03</i>	32	0.076	-0.0023
<i>B*58- TNF-α -308A-DRB1*03</i>	33	0.079	0.0141
<i>B*58- TNF-α -308G-DRB1*03</i>	28	0.067	-0.0032

Table 4. Linkage disequilibrium analysis of *HLA-B- TNF-a -DRB1* haplotypes prevalent in T1D patients from North India (Kumar et al., 2007).

However, *B*50:01-DRB1*03:01* and *B*58:01-DRB1*03:01* haplotypes were in LD with *TNF-α* -308A allele. We observed 48.8 % of the patients had *B*08/non-B*08/non-B*50:01/non-B*58:01-TNF-α-308G-DRB1*03:01* haplotypes as compared to 34 % with *B*50:01/ B*58:01-TNF-α -308A-DRB1*03:01* haplotypes. With this in-depth analysis, it becomes clear that the effect of *TNF-α* -308A allele is not because of its being in LD with *DRB1*03:01*, *B*08:01*, *B*50:01* or *B*58:01*, but due its functional implications and its integrated effect with other cytokines. In conclusion, while the MHC may be involved in auto-antigen presentation, *TNF- alpha* and other cytokines play an integrated role in destruction of the pancreatic beta cells though enrichment and recruitment of autoantigen specific CD4⁺ and CD8⁺ T cells which have immunogenetic bases (Figure 6).

5. Vitamin D receptor

Vitamin D Receptor (VDR) is a ligand dependent transcription factor that belongs to the super family of the Nuclear Hormone Receptors (Evans, 1988). The ligand for VDR is

Vitamin D₃ i.e., 1,25-(OH)₂D₃ which mediates its biological actions through VDR. When 1,25-(OH)₂D₃ binds to VDR, it induces conformational changes in VDR promoting its heterodimerization with Retinoid X Receptor (RXR), followed by translocation of this complex into the nucleus. The RXR-VDR heterodimer in turn binds to the vitamin D₃ responsive elements (VDRE) in promoter regions of vitamin D responsive genes (Boonstra et al., 2001). This results in the regulatory function of Vitamin D₃. In the absence of classical responsive elements, 1,25-(OH)₂D₃ may controls the expression of some genes like cytokine genes by targeting inducible transcription factors like NFAT in IL-2 in a sequence specific manner (Takeuchi et al., 1998). 1,25-(OH)₂D₃ has been shown to have an important immunomodulatory role since it represses transcription of Th1 cytokines like IL-2 (Alroy et al., 1995, Bhalla et al., 1984), IFN- γ (Cippitelli & Santoni, 1998) and IL-12 (D'ambrosio et al., 1998) and up regulates the production of Th2 cytokines IL-4 and TGF- β 1 (Cantorna et al., 1998). It has been shown to enhance the development of TH2 cells via a direct effect on naive CD4⁺ cells (Boonstra et al., 2001). Besides, 1,25-(OH)₂D₃ has also been shown to modulate the expression of HLA class-II alleles on monocytes and human bone cells (Rigby et al., 1990, Skjodt et al., 1990)

Studies have shown that administration of Vitamin D₃ in NOD mice, before the onset of Insulinitis, can effectively prevent the disease progression. However, when administered after the establishment of insulinitis, vitamin D₃ was not as effective. Similarly, in humans too, vitamin D supplementation in early childhood has been shown to reduce the incidence of T1D (Hypponen et al., 2001, Jones et al., 1998). Since 1,25-(OH)₂D₃ mediates its effect through VDR, we studied the VDR gene polymorphisms and their interaction with the most predisposing MHC alleles to investigate their role, if any, in the pathophysiology of T1D.

The VDR SNPs studied include the T>C SNP in exon2 initiation codon detected with *FokI* restriction enzyme (Gross et al., 1996), the A>G SNP detected with *BsmI* (Morrison et al., 1992) and G>T SNP detected with *ApaI* (Faraco et al., 1989) located in Intron 8, and a silent C>T SNP (Durrin et al., 1999) detected with *TaqI*, located in Exon 9. These SNPs were studied using PCR amplification and restriction digestion by the aforesaid enzymes as described earlier (Faraco et al., 1989, Hustmyer et al., 1993). We also studied the interaction between VDR alleles and predisposing HLA alleles using LD based statistics (Zhao et al., 2006) and subsequently sequenced the promoter region of the predisposing MHC allele to detect the VDRE sequence which has been shown to modulate the expression of the HLA alleles (Ramagopalan et al., 2009), suggesting the functional implications of the statistically significant interaction (Israni et al., 2009). We further provided documentary evidence that expression of HLA class-II molecules was being modulated by vitamin D₃.

5.1 VDR *FokI*, *BsmI*, *ApaI* and *TaqI* genotypes and haplotypes in T1D patients

While there were no significant differences in the genotypes of *ApaI* and *TaqI* in patients and controls. *FokI* 'ff' was significantly increased in the patient group as compared to controls and *BsmI* 'bb' was significantly decreased in the patient group. However, these differences did not remain significant after Bonferroni's correction (Israni et al., 2009).

Haplotype analysis was carried out for the four restriction sites studied in the VDR gene in patients and controls using SHEsis program (<http://202.120.7.14/analysis/myAnalysis.php>) (Shi & He, 2005). Additionally, Famhap (<http://famhap.meb.uni-bonn.de>) was used to confirm the frequencies of the haplotypes. Haplotype *FBA*t and *fBA*T were significantly increased in T1D patients and *fBA*T was significantly reduced in them as compared to controls.

5.2 Gene to gene interaction of VDR haplotypes with predisposing HLA alleles

Simultaneous presence of different VDR haplotypes along with the predisposing *HLA* alleles was studied in patients. Interestingly, simultaneous presence of haplotypes *FBAT* and *FbaT* along with the predisposing *DRB1* alleles was significantly increased while the same haplotypes were protective when associated with non-predisposing alleles of *DRB1*. Similar results were obtained with other haplotypes like *FBA*t, *fBA*T and *fba*T in association with the predisposing *HLA-DRB1* alleles (Israni et al., 2009).

To study the interaction between two unlinked loci i.e., *VDR* and the predisposing *HLA-DRB1* alleles, we used LD based statistics as described by Zhao et al (Zhao et al., 2006). The analysis revealed that *F* and *T* alleles in the exons 2 and 9 for *FokI* and *TaqI* restriction sites respectively showed significant interactions with predisposing *HLA-DRB1* allele *DRB1*03:01* (Israni et al., 2009).

Fig. 4. *HLA- DRB1*03:01* promoter sequence from 3 subjects suffering from T1D and 3 normal healthy individuals homozygous for *DRB1*03:01*. Important regulatory elements like S-box, X-box, Y-box, CCAAY-box, TATA-box and VDRE are highlighted. Star (*) in the last row shows homology and dots (.) shows nucleotide substitution in one or more samples at that particular site and dashes(-) represent gaps inserted to maximize the homology. (Israni et al., 2009).

5.3 Sequence analysis of HLA DRB1*03:01 promoter region

Amongst the predisposing *HLA-DRB1* alleles, majority of the patients (85.9%) had *DRB1*03:01*. Thus, we sought to look for the VDRE in the promoter region of the allele. The promoter regions of 3 T1D subjects and 3 healthy controls homozygous for *HLA-DRB1*03:01* were amplified and sequenced to determine the VDRE variants in the North Indian population. Sequences were aligned using ClustalW2, and the presence of a VDRE was confirmed in-silico using JASPAR_CORE version 3.0 database using default conditions (Sandelin et al., 2004). Figure 4 shows the *HLA-DRB1*03:01* promoter sequences showing the localization of vitamin D response element (VDRE) in the promoter region of *HLA-DRB1*03:01* from the 6 subjects.

Interestingly, the alignment showed exactly the same sequence of VDRE in the promoter region of *HLA-DRB1*03:01* which has been shown to influence the expression of *HLA* allele *DRB1*15:01* by Ramagopalan et al (Ramagopalan et al., 2009) suggesting the bases for interaction of VDR with *HLA-DRB1*03:01*

5.4 Altered expression of *HLA-DRB1*03:01* by 1,25-(OH)₂D₃ (Calcitriol)

5.4.1 Flow cytometry

To study if vitamin D3 administration would alter the expression of MHC class-II, we stimulated *HLA-DRB1*03:01* homozygous B-lymphoblastoid cell (B-LCL) line VAVY (International Histocompatibility Workshop cell line Number IHW09023) with 100nM of calcitriol (Sigma) for 24 hours and stained with anti-HLA DR-PE antibody (BD Biosciences) and acquired on BD-LSR to study the expression of HLA-DR on stimulated and unstimulated B-LCL. The data was analysed using WinMDI 2.9 software. The results showed significantly higher expression of HLA-DR in the B-LCL stimulated with calcitriol as compared to the unstimulated one (Figure 5A and B).

5.4.2 Real time PCR

We also studied the levels of transcripts for *HLA-DRB1* in B-LCL VAVY after 24 hour stimulation with calcitriol and compared it to unstimulated B-LCL using real time PCR. The data shows 1.89 fold increase in the *HLA-DRB1* transcripts from B-LCL stimulated with calcitriol as compared to the unstimulated one. These results were confirmed on peripheral blood mononuclear cells (PBMCs) derived from a normal healthy control homozygous for *HLA-DRB1*03:01*.

Our results showed enhanced expression of HLA-DR on the B-LCLs stimulated with calcitriol as compared to the unstimulated one confirming that indeed the interaction of VDR with *HLA-DRB1*03:01* is occurring through the VDRE present in the promoter region of the gene. Based on the earlier studies and the present data one can speculate that in the absence of required amount of Vitamin D in early life in the predisposed individuals with *HLA-DRB1*03:01*, the expression of the allele may be impaired in the thymus (Ramagopalan et al., 2009) resulting in escape from thymic deletion of autoreactive T cells leading to T1D manifestations.

6. Conclusions

Our studies show that simultaneous presence of *DRB1*03:01* along with homozygous *INS-VNTR* class-I was significantly increased ($p < 10^{-8}$) in T1D patients, giving a relative risk of 70.81 (Rani et al., 2004). *INS-VNTR* class-I has been shown to be associated with lower

Fig. 5. Flow cytometric analysis of HLA-DR expression. A: Histogram of HLA-DR-PE staining of B-LCL-VAVY cells treated with and without 100nM calcitriol. The figure shows enhanced expression of HLA-DR in stimulated B-LCL as compared to unstimulated one. B: VAVY cells show a significant increase in surface HLA-DR expression as determined by the geometric mean fluorescence intensity of antibody staining (Israni et al., 2009).

expression of Insulin in thymii of fetuses as compared to Class-III alleles (Pugliese et al., 1997, Vafiadis et al., 1997) which may be responsible for poor thymic education for insulin resulting in autoimmunity against pancreatic beta cells. Our studies provide additional evidence based on the statistically significant interaction between the predisposing *HLA* allele and high producer alleles of *VDR* which may be detrimental for the manifestation of T1D in the absence of 1,25-(OH)₂D₃ in early childhood and/or *in-utero* and this interaction is mediated by VDRE present in the promoter region of *DRB1*03:01* (Israni et al., 2009). With poor thymic education for insulin and *HLA-DRB1*03:01* protein, environmental factors like viral infections, vitamin D deficiency and some milk proteins may be involved in initiation of the autoimmune responses against the pancreatic beta cells. While HLA class-II molecules may be involved in auto-antigen presentation to T helper cells, higher producing genotypes of pro-inflammatory cytokines like *IFN-gamma* and *TNF-alpha* may be involved in enhancing the cell mediated immune responses through proliferation of CD4⁺ and CD8⁺ T cells, while higher producing genotypes of *IL-10* and *TGF-beta* may have a role in recruitment of these autoreactive T cells in the pancreas through ICAM-1 and Integerin dependent pathways. Final destruction of pancreatic beta cells may occur through CD4⁺ and CD8⁺ T cells and nitric oxide production since IFN- γ may act singularly as well as synergistically with other inflammatory stimuli to induce NO production which can be cytotoxic and thus may have a role in pathogenesis of T1D (Figure 6). Future studies should focus on developing approaches to inhibit autoimmunity before the onset of the disease.

Fig. 6. Conclusions of our studies. Predisposing genetic factors like MHC, INS-VNTR and VDR may be involved in poor thymic education for insulin and *HLA-DRB1*03:01* protein resulting in recognition of self proteins as non-self by T cells. These genetic factors along with environmental factors like viral infections, vitamin D deficiency and some milk proteins may be involved in initiation of the autoimmune responses against the pancreatic beta cells. While HLA class-II molecules may be involved in auto-antigen presentation to T helper cells, higher producing genotypes of pro-inflammatory cytokines like *IFN-γ* and *TNF-α* may be involved in the cell mediated immune responses. Higher producing genotypes of *IL-10* and *TGF-β*, may have a role in recruitment of the autoreactive CD4⁺ and CD8⁺ T cells in the pancreas through ICAM-1 and Integerin dependent pathways respectively. Final destruction of pancreatic beta cells may occur through CD4⁺ and CD8⁺ T cells and nitric oxide production since *IFN-γ* may act singularly as well as synergistically with other inflammatory stimuli to induce NO production which can be cytotoxic and thus may have a role in pathogenesis of T1D.

7. Acknowledgment

The studies were funded in part by grants from Department of Science and Technology (DST), Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India and partly by Core funds of National Institute of Immunology, New Delhi, India. The patient sample for this work came from All India Institute of Medical Sciences (AIIMS), New Delhi and I would like to acknowledge Dr. Ravinder Goswami, the endocrinologist from AIIMS for the same. I would like to acknowledge the students and project fellows who have been involved in doing this work: Avinash Kumar, Rashmi Kumar, Shruti Agarwal, Neetu Israni, Shailendra Kumar Singh. I am thankful to Dr. Alberto Pugliese for valuable suggestions and INS-VNTR protocol. I would like to thank Dr. Joannis Mytilineos and the technical staff of Heidelberg University for the cytokine typing kit supplied for cytokine gene polymorphism component of XIIIth International Histocompatibility Workshop. We are thankful to Yong Yong Shi for providing the SHEsis program (<http://202.120.7.14/analysis/myAnalysis.php>) for haplotype analysis. We are thankful to the Fred Hutchinson Cancer Research Center IHWG Cell and Gene Bank for providing *HLA-DRB1*03:01* homozygous lymphoblastoid Cell lines for studies showing

effect of vitamin D on HLA-DR expression. Mr. Kapoor Chand's technical support is acknowledged. Help of Dr. Narendra Kumar, Georgia Institute of Technology, USA, in making the ribbon diagrams for HLA-DRB1 peptide binding pockets is acknowledged.

8. References

- Alroy, I., Towers, T.L. & Freedman, L.P. (1995). Transcriptional repression of the interleukin-2 gene by vitamin D3: direct inhibition of NFATp/AP-1 complex formation by a nuclear hormone receptor. *Mol Cell Biol*, 15, 10,(Oct, 1995) 5789-99.
- Alves, C., Toralles, M.B. & Carvalho, G.C. (2009). HLA class II polymorphism in patients with type 1 diabetes mellitus from a Brazilian racially admixed population. *Ethn Dis*, 19, 4,(Autumn, 2009) 420-4.
- Asderakis, A., Sankaran, D., Dyer, P., Johnson, R.W., Pravica, V., Sinnott, P.J., Roberts, I. & Hutchinson, I.V. (2001). Association of polymorphisms in the human interferon-gamma and interleukin-10 gene with acute and chronic kidney transplant outcome: the cytokine effect on transplantation. *Transplantation*, 71, 5,(Mar 15, 2001) 674-7.
- Awad, M.R., El-Gamel, A., Hasleton, P., Turner, D.M., Sinnott, P.J. & Hutchinson, I.V. (1998). Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation*, 66, 8,(Oct 27, 1998) 1014-20.
- Badenhoop, K., Walfish, P.G., Rau, H., Fischer, S., Nicolay, A., Bogner, U., Schleusener, H. & Usadel, K.H. (1995). Susceptibility and resistance alleles of human leukocyte antigen (HLA) DQA1 and HLA DQB1 are shared in endocrine autoimmune disease. *J Clin Endocrinol Metab*, 80, 7,(Jul, 1995) 2112-7.
- Baisch, J.M., Weeks, T., Giles, R., Hoover, M., Stastny, P. & Capra, J.D. (1990). Analysis of HLA-DQ genotypes and susceptibility in insulin-dependent diabetes mellitus. *N Engl J Med*, 322, 26,(Jun 28, 1990) 1836-41.
- Balasa, B., La Cava, A., Van Gunst, K., Mocnik, L., Balakrishna, D., Nguyen, N., Tucker, L. & Sarvetnick, N. (2000). A mechanism for IL-10-mediated diabetes in the nonobese diabetic (NOD) mouse: ICAM-1 deficiency blocks accelerated diabetes. *J Immunol*, 165, 12,(Dec 15, 2000) 7330-7.
- Bartolome, R.A., Sanz-Rodriguez, F., Robledo, M.M., Hidalgo, A. & Teixido, J. (2003). Rapid up-regulation of alpha4 integrin-mediated leukocyte adhesion by transforming growth factor-beta1. *Mol Biol Cell*, 14, 1,(Jan, 2003) 54-66.
- Bell, G.I., Karam, J.H. & Rutter, W.J. (1981). Polymorphic DNA region adjacent to the 5' end of the human insulin gene. *Proc Natl Acad Sci U S A*, 78, 9,(Sep, 1981) 5759-63.
- Bell, G.I., Selby, M.J. & Rutter, W.J. (1982). The highly polymorphic region near the human insulin gene is composed of simple tandemly repeating sequences. *Nature*, 295, 5844,(Jan 7, 1982) 31-5.
- Bennett, S.T., Lucassen, A.M., Gough, S.C., Powell, E.E., Undlien, D.E., Pritchard, L.E., Merriman, M.E., Kawaguchi, Y., Dronsfield, M.J., Pociot, F. & et al. (1995). Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat Genet*, 9, 3,(Mar, 1995) 284-92.
- Bhalla, A.K., Amento, E.P., Serog, B. & Glimcher, L.H. (1984). 1,25-Dihydroxyvitamin D3 inhibits antigen-induced T cell activation. *J Immunol*, 133, 4,(Oct, 1984) 1748-54.

- Bidwell, J., Keen, L., Gallagher, G., Kimberly, R., Huizinga, T., McDermott, M.F., Oksenberg, J., McNicholl, J., Pociot, F., Hardt, C. & D'Alfonso, S. (1999). Cytokine gene polymorphism in human disease: on-line databases. *Genes Immun*, 1, 1,(Sep, 1999) 3-19.
- Bittar, M.N., Carey, J.A., Barnard, J.B., Pravica, V., Deiraniya, A.K., Yonan, N. & Hutchinson, I.V. (2006). Tumor necrosis factor alpha influences the inflammatory response after coronary surgery. *Ann Thorac Surg*, 81, 1,(Jan, 2006) 132-7.
- Bjorkman, P.J., Saper, M.A., Samraoui, B., Bennett, W.S., Strominger, J.L. & Wiley, D.C. (1987). Structure of the human class I histocompatibility antigen, HLA-A2. *Nature*, 329, 6139,(Oct 8-14, 1987) 506-12.
- Boonstra, A., Barrat, F.J., Crain, C., Heath, V.L., Savelkoul, H.F. & O'Garra, A. (2001). 1 α ,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. *J Immunol*, 167, 9,(Nov 1, 2001) 4974-80.
- Brown, J.H., Jardetzky, T.S., Gorga, J.C., Stern, L.J., Urban, R.G., Strominger, J.L. & Wiley, D.C. (1993). Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature*, 364, 6432,(Jul 1, 1993) 33-9.
- Burzotta, F., Iacoviello, L., Di Castelnuovo, A., Gliaca, F., Luciani, N., Zamparelli, R., Schiavello, R., Donati, M.B., Maseri, A., Possati, G. & Andreotti, F. (2001). Relation of the -174 G/C polymorphism of interleukin-6 to interleukin-6 plasma levels and to length of hospitalization after surgical coronary revascularization. *Am J Cardiol*, 88, 10,(Nov 15, 2001) 1125-8.
- Cantorna, M.T., Woodward, W.D., Hayes, C.E. & DeLuca, H.F. (1998). 1,25-dihydroxyvitamin D3 is a positive regulator for the two anti-encephalitogenic cytokines TGF-beta 1 and IL-4. *J Immunol*, 160, 11,(Jun 1, 1998) 5314-9.
- Chauffert, M., Cisse, A., Chevenne, D., Parfait, B., Michel, S. & Trivin, F. (1995). HLA-DQ beta 1 typing and non-Asp57 alleles in the aborigine population of Senegal. *Diabetes Care*, 18, 5,(May, 1995) 677-80.
- Cippitelli, M. & Santoni, A. (1998). Vitamin D3: a transcriptional modulator of the interferon-gamma gene. *Eur J Immunol*, 28, 10,(Oct, 1998) 3017-30.
- Cucca, F., Lampis, R., Congia, M., Angius, E., Nutland, S., Bain, S.C., Barnett, A.H. & Todd, J.A. (2001). A correlation between the relative predisposition of MHC class II alleles to type 1 diabetes and the structure of their proteins. *Hum Mol Genet*, 10, 19,(Sep 15, 2001) 2025-37.
- Cucca, F., Lampis, R., Frau, F., Macis, D., Angius, E., Masile, P., Chessa, M., Frongia, P., Silveti, M., Cao, A., De Virgiliis, S. & Congia, M. (1995). The distribution of DR4 haplotypes in Sardinia suggests a primary association of type I diabetes with DRB1 and DQB1 loci. *Hum Immunol*, 43, 4,(Aug, 1995) 301-8.
- D'Ambrosio, D., Cippitelli, M., Cocciolo, M.G., Mazzeo, D., Di Lucia, P., Lang, R., Sinigaglia, F. & Panina-Bordignon, P. (1998). Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-kappaB downregulation in transcriptional repression of the p40 gene. *J Clin Invest*, 101, 1,(Jan 1, 1998) 252-62.
- de Vries, R.R. & van Rood, J.J. (1985). Immunobiology of HLA class-I and class-II molecules. Introduction. *Prog Allergy*, 36, 1985) 1-9.
- Deng, G.Y., Maclaren, N.K., Huang, H.S., Zhang, L.P. & She, J.X. (1996). No primary association between the 308 polymorphism in the tumor necrosis factor alpha

- promoter region and insulin-dependent diabetes mellitus. *Hum Immunol*, 45, 2,(Feb, 1996) 137-42.
- Durrin, L.K., Haile, R.W., Ingles, S.A. & Coetzee, G.A. (1999). Vitamin D receptor 3'-untranslated region polymorphisms: lack of effect on mRNA stability. *Biochim Biophys Acta*, 1453, 3,(Mar 30, 1999) 311-20.
- Ei Wafai, R.J., Chmairie, H.N., Makki, R.F. & Fakhoury, H. Association of HLA class II alleles and CTLA-4 polymorphism with type 1 diabetes. *Saudi J Kidney Dis Transpl*, 22, 2,(Mar, 273-81.
- Evans, R.M. (1988). The steroid and thyroid hormone receptor superfamily. *Science*, 240, 4854,(May 13, 1988) 889-95.
- Falk, K., Rotzschke, O., Stevanovic, S., Jung, G. & Rammensee, H.G. (1991). Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature*, 351, 6324,(May 23, 1991) 290-6.
- Faraco, J.H., Morrison, N.A., Baker, A., Shine, J. & Frossard, P.M. (1989). ApaI dimorphism at the human vitamin D receptor gene locus. *Nucleic Acids Res*, 17, 5,(Mar 11, 1989) 2150.
- Fernandez-Vina, M., Ramirez, L.C., Raskin, P. & Stastny, P. (1993). Genes for insulin-dependent diabetes mellitus (IDDM) in the major histocompatibility complex (MHC) of African-Americans. *Tissue Antigens*, 41, 2,(Feb, 1993) 57-64.
- Fishman, D., Faulds, G., Jeffery, R., Mohamed-Ali, V., Yudkin, J.S., Humphries, S. & Woo, P. (1998). The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest*, 102, 7,(Oct 1, 1998) 1369-76.
- Garcia-Pacheco, J.M., Herbut, B., Cutbush, S., Hitman, G.A., Zhonglin, W., Magzoub, M., Bottazzo, G.F., Kiere, C., West, G., Mvere, D. & et al. (1992). Distribution of HLA-DQA1, -DQB1 and DRB1 alleles in black IDDM patients and controls from Zimbabwe. *Tissue Antigens*, 40, 3,(Sep, 1992) 145-9.
- Garrett, T.P., Saper, M.A., Bjorkman, P.J., Strominger, J.L. & Wiley, D.C. (1989). Specificity pockets for the side chains of peptide antigens in HLA-Aw68. *Nature*, 342, 6250,(Dec 7, 1989) 692-6.
- Gross, C., Eccleshall, T.R., Malloy, P.J., Villa, M.L., Marcus, R. & Feldman, D. (1996). The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. *J Bone Miner Res*, 11, 12,(Dec, 1996) 1850-5.
- Gupta, M.M., Raghunath, D., Kher, S.K. & Radhakrishnan, A.P. (1991). Human leucocyte antigen and insulin dependent diabetes mellitus. *J Assoc Physicians India*, 39, 7,(Jul, 1991) 540-3.
- Holling, T.M., Schooten, E. & van Den Elsen, P.J. (2004). Function and regulation of MHC class II molecules in T-lymphocytes: of mice and men. *Hum Immunol*, 65, 4,(Apr, 2004) 282-90.
- Horton, R., Wilming, L., Rand, V., Lovering, R.C., Bruford, E.A., Khodiyar, V.K., Lush, M.J., Povey, S., Talbot, C.C., Jr., Wright, M.W., Wain, H.M., Trowsdale, J., Ziegler, A. & Beck, S. (2004). Gene map of the extended human MHC. *Nat Rev Genet*, 5, 12,(Dec, 2004) 889-99.

- Hustmyer, F.G., DeLuca, H.F. & Peacock, M. (1993). ApaI, BsmI, EcoRV and TaqI polymorphisms at the human vitamin D receptor gene locus in Caucasians, blacks and Asians. *Hum Mol Genet*, 2, 4,(Apr, 1993) 487.
- Hypponen, E., Laara, E., Reunanen, A., Jarvelin, M.R. & Virtanen, S.M. (2001). Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet*, 358, 9292,(Nov 3, 2001) 1500-3.
- Ide, A., Kawasaki, E., Abiru, N., Sun, F., Takahashi, R., Kuwahara, H., Fujita, N., Kita, A., Oshima, K., Sakamaki, H., Uotani, S., Yamasaki, H., Yamaguchi, Y. & Eguchi, K. (2002). Genetic association between interleukin-10 gene promoter region polymorphisms and type 1 diabetes age-at-onset. *Hum Immunol*, 63, 8,(Aug, 2002) 690-5.
- Israni, N., Goswami, R., Kumar, A. & Rani, R. (2009). Interaction of vitamin D receptor with HLA DRB1 0301 in type 1 diabetes patients from North India. *PLoS One*, 4, 12,(2009) e8023.
- Jones, G., Strugnell, S.A. & DeLuca, H.F. (1998). Current understanding of the molecular actions of vitamin D. *Physiol Rev*, 78, 4,(Oct, 1998) 1193-231.
- Julier, C., Hyer, R.N., Davies, J., Merlin, F., Soularue, P., Briant, L., Cathelineau, G., Deschamps, I., Rotter, J.I., Froguel, P. & et al. (1991). Insulin-IGF2 region on chromosome 11p encodes a gene implicated in HLA-DR4-dependent diabetes susceptibility. *Nature*, 354, 6349,(Nov 14, 1991) 155-9.
- Kalra, S., Kalra, B. & Sharma, A. (2010). Prevalence of type 1 diabetes mellitus in Karnal district, Haryana state, India. *Diabetol Metab Syndr*, 2, 14.
- Kanga, U., Vaidyanathan, B., Jaini, R., Menon, P.S. & Mehra, N.K. (2004). HLA haplotypes associated with type 1 diabetes mellitus in North Indian children. *Hum Immunol*, 65, 1,(Jan, 2004) 47-53.
- Kennedy, G.C., German, M.S. & Rutter, W.J. (1995). The minisatellite in the diabetes susceptibility locus IDDM2 regulates insulin transcription. *Nat Genet*, 9, 3,(Mar, 1995) 293-8.
- Korpinen, E., Groop, P.H., Fagerudd, J.A., Teppo, A.M., Akerblom, H.K. & Vaarala, O. (2001). Increased secretion of TGF-beta1 by peripheral blood mononuclear cells from patients with Type 1 diabetes mellitus with diabetic nephropathy. *Diabet Med*, 18, 2,(Feb, 2001) 121-5.
- Kristiansen, O.P. & Mandrup-Poulsen, T. (2005). Interleukin-6 and diabetes: the good, the bad, or the indifferent? *Diabetes*, 54 Suppl 2, Dec, 2005) S114-24.
- Kumar, R., Goswami, R., Agarwal, S., Israni, N., Singh, S.K. & Rani, R. (2007). Association and interaction of the TNF-alpha gene with other pro- and anti-inflammatory cytokine genes and HLA genes in patients with type 1 diabetes from North India. *Tissue Antigens*, 69, 6,(Jun, 2007) 557-67.
- Lee, M.S., Mueller, R., Wicker, L.S., Peterson, L.B. & Sarvetnick, N. (1996). IL-10 is necessary and sufficient for autoimmune diabetes in conjunction with NOD MHC homozygosity. *J Exp Med*, 183, 6,(Jun 1, 1996) 2663-8.
- Louis, E., Franchimont, D., Piron, A., Gevaert, Y., Schaaf-Lafontaine, N., Roland, S., Mahieu, P., Malaise, M., De Groote, D., Louis, R. & Belaiche, J. (1998). Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol*, 113, 3,(Sep, 1998) 401-6.

- Lucassen, A.M., Julier, C., Beressi, J.P., Boitard, C., Froguel, P., Lathrop, M. & Bell, J.I. (1993). Susceptibility to insulin dependent diabetes mellitus maps to a 4.1 kb segment of DNA spanning the insulin gene and associated VNTR. *Nat Genet*, 4, 3,(Jul, 1993) 305-10.
- McCartney-Francis, N., Allen, J.B., Mizel, D.E., Albina, J.E., Xie, Q.W., Nathan, C.F. & Wahl, S.M. (1993). Suppression of arthritis by an inhibitor of nitric oxide synthase. *J Exp Med*, 178, 2,(Aug 1, 1993) 749-54.
- Mehra, N.K., Kaur, G., Kanga, U. & Tandon, N. (2002). Immunogenetics of autoimmune diseases in Asian Indians. *Ann N Y Acad Sci*, 958, Apr, 2002) 333-6.
- Morrison, N.A., Yeoman, R., Kelly, P.J. & Eisman, J.A. (1992). Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphism and circulating osteocalcin. *Proc Natl Acad Sci U S A*, 89, 15,(Aug 1, 1992) 6665-9.
- Nepom, G.T. & Kwok, W.W. (1998). Molecular basis for HLA-DQ associations with IDDM. *Diabetes*, 47, 8,(Aug, 1998) 1177-84.
- Pociot, F., Briant, L., Jongeneel, C.V., Molvig, J., Worsaae, H., Abbal, M., Thomsen, M., Nerup, J. & Cambon-Thomsen, A. (1993). Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-alpha and TNF-beta by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. *Eur J Immunol*, 23, 1,(Jan, 1993) 224-31.
- Pociot, F. & McDermott, M.F. (2002). Genetics of type 1 diabetes mellitus. *Genes Immun*, 3, 5,(Aug, 2002) 235-49.
- Pugliese, A., Gianani, R., Moromisato, R., Awdeh, Z.L., Alper, C.A., Erlich, H.A., Jackson, R.A. & Eisenbarth, G.S. (1995). HLA-DQB1*0602 is associated with dominant protection from diabetes even among islet cell antibody-positive first-degree relatives of patients with IDDM. *Diabetes*, 44, 6,(Jun, 1995) 608-13.
- Pugliese, A., Zeller, M., Fernandez, A., Jr., Zalcberg, L.J., Bartlett, R.J., Ricordi, C., Pietropaolo, M., Eisenbarth, G.S., Bennett, S.T. & Patel, D.D. (1997). The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nat Genet*, 15, 3,(Mar, 1997) 293-7.
- Rabinovitch, A. (1994). Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM. Therapeutic intervention by immunostimulation? *Diabetes*, 43, 5,(May, 1994) 613-21.
- Ramachandran, A., Snehalatha, C. & Krishnaswamy, C.V. (1996). Incidence of IDDM in children in urban population in southern India. Madras IDDM Registry Group Madras, South India. *Diabetes Res Clin Pract*, 34, 2,(Oct, 1996) 79-82.
- Ramagopalan, S.V., Maugeri, N.J., Handunnetthi, L., Lincoln, M.R., Orton, S.M., Dyment, D.A., Deluca, G.C., Herrera, B.M., Chao, M.J., Sadovnick, A.D., Ebers, G.C. & Knight, J.C. (2009). Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1*1501 is regulated by vitamin D. *PLoS Genet*, 5, 2,(Feb, 2009) e1000369.
- Rani, R., Fernandez-Vina, M.A. & Stastny, P. (1998). Associations between HLA class II alleles in a North Indian population. *Tissue Antigens*, 52, 1,(Jul, 1998) 37-43.
- Rani, R., Sood, A. & Goswami, R. (2004). Molecular basis of predisposition to develop type 1 diabetes mellitus in North Indians. *Tissue Antigens*, 64, 2,(Aug, 2004) 145-55.

- Rani, R., Sood, A., Lazaro, A.M. & Stastny, P. (1999). Associations of MHC class II alleles with insulin-dependent diabetes mellitus (IDDM) in patients from North India. *Hum Immunol*, 60, 6,(Jun, 1999) 524-31.
- Reynier, F., Cazalis, M.A., Lecoq, A., Paye, M., Rosa, A., Durand, A., Jhumka, U., Mougin, B., Miossec, P., Bendelac, N., Nicolino, M. & Thivolet, C. (2006). Lack of association of IL-10 promoter gene variants with type 1 diabetes in a French population. *Hum Immunol*, 67, 4-5,(Apr-May, 2006) 311-7.
- Rigby, W.F., Waugh, M. & Graziano, R.F. (1990). Regulation of human monocyte HLA-DR and CD4 antigen expression, and antigen presentation by 1,25-dihydroxyvitamin D3. *Blood*, 76, 1,(Jul 1, 1990) 189-97.
- Robinson, J., Waller, M.J., Fail, S.C., McWilliam, H., Lopez, R., Parham, P. & Marsh, S.G. (2009). The IMGT/HLA database. *Nucleic Acids Res*, 37, Database issue,(Jan, 2009) D1013-7.
- Rotwein, P., Yokoyama, S., Didier, D.K. & Chirgwin, J.M. (1986). Genetic analysis of the hypervariable region flanking the human insulin gene. *Am J Hum Genet*, 39, 3,(Sep, 1986) 291-9.
- Sandelin, A., Alkema, W., Engstrom, P., Wasserman, W.W. & Lenhard, B. (2004). JASPAR: an open-access database for eukaryotic transcription factor binding profiles 10.1093/nar/gkh012. *Nucl. Acids Res.*, 32, suppl_1,(January 1, 2004, 2004) D91-94.
- Sanjeevi, C.B., Kanungo, A., Shtauvere, A., Samal, K.C. & Tripathi, B.B. (1999). Association of HLA class II alleles with different subgroups of diabetes mellitus in Eastern India identify different associations with IDDM and malnutrition-related diabetes. *Tissue Antigens*, 54, 1,(Jul, 1999) 83-7.
- Sanjeevi, C.B., Landin-Olsson, M., Kockum, I., Dahlquist, G. & Lernmark, A. (1995). Effects of the second HLA-DQ haplotype on the association with childhood insulin-dependent diabetes mellitus. *Tissue Antigens*, 45, 2,(Feb, 1995) 148-52.
- Sheehy, M.J. (1992). HLA and insulin-dependent diabetes. A protective perspective. *Diabetes*, 41, 2,(Feb, 1992) 123-9.
- Shi, Y.Y. & He, L. (2005). SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*, 15, 2,(Feb, 2005) 97-8.
- Skjodt, H., Hughes, D.E., Dobson, P.R. & Russell, R.G. (1990). Constitutive and inducible expression of HLA class II determinants by human osteoblast-like cells in vitro. *J Clin Invest*, 85, 5,(May, 1990) 1421-6.
- Skrodeniene, E., Marciulionyte, D., Padaiga, Z., Jasinskiene, E., Sadauskaite-Kuehne, V., Sanjeevi, C.B. & Ludvigsson, J. HLA class II alleles and haplotypes in Lithuanian children with type 1 diabetes and healthy children (HLA and type 1 diabetes). *Medicina (Kaunas)*, 46, 8,(505-10.
- Stanilova, S.A., Miteva, L.D., Karakolev, Z.T. & Stefanov, C.S. (2006). Interleukin-10-1082 promoter polymorphism in association with cytokine production and sepsis susceptibility. *Intensive Care Med*, 32, 2,(Feb, 2006) 260-6.
- Tait, B.D., Drummond, B.P., Varney, M.D. & Harrison, L.C. (1995). HLA-DRB1*0401 is associated with susceptibility to insulin-dependent diabetes mellitus independently of the DQB1 locus. *Eur J Immunogenet*, 22, 4,(Aug, 1995) 289-97.

- Takeuchi, A., Reddy, G.S., Kobayashi, T., Okano, T., Park, J. & Sharma, S. (1998). Nuclear factor of activated T cells (NFAT) as a molecular target for 1 α ,25-dihydroxyvitamin D₃-mediated effects. *J Immunol*, 160, 1,(Jan 1, 1998) 209-18.
- Todd, J.A. (1995). Genetic analysis of type 1 diabetes using whole genome approaches. *Proc Natl Acad Sci U S A*, 92, 19,(Sep 12, 1995) 8560-5.
- Todd, J.A., Bell, J.I. & McDevitt, H.O. (1987). HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature*, 329, 6140,(Oct 15-21, 1987) 599-604.
- Vafiadis, P., Bennett, S.T., Todd, J.A., Nadeau, J., Grabs, R., Goodyer, C.G., Wickramasinghe, S., Colle, E. & Polychronakos, C. (1997). Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat Genet*, 15, 3,(Mar, 1997) 289-92.
- Van der Auwera, B., Van Waeyenberge, C., Schuit, F., Heimberg, H., Vandewalle, C., Gorus, F. & Flament, J. (1995). DRB1*0403 protects against IDDM in Caucasians with the high-risk heterozygous DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 genotype. Belgian Diabetes Registry. *Diabetes*, 44, 5,(May, 1995) 527-30.
- Veijola, R., Vahasalo, P., Tuomilehto-Wolf, E., Reijonen, H., Kulmala, P., Ilonen, J., Akerblom, H.K. & Knip, M. (1995). Human leukocyte antigen identity and DQ risk alleles in autoantibody-positive siblings of children with IDDM are associated with reduced early insulin response. Childhood Diabetes in Finland (DiMe) Study Group. *Diabetes*, 44, 9,(Sep, 1995) 1021-8.
- Wahl, S.M., Allen, J.B., Weeks, B.S., Wong, H.L. & Klotman, P.E. (1993). Transforming growth factor beta enhances integrin expression and type IV collagenase secretion in human monocytes. *Proc Natl Acad Sci U S A*, 90, 10,(May 15, 1993) 4577-81.
- Weber, P., Meluzinova, H., Kubsova, H., Ambrosova, P., Polcarova, V., Cejkova, P. & Cerna, M. Type 1 diabetes and LADA--occurrence of HLA-DRB1 *03 and DRB1 *04 alleles in two age different groups of diabetics. *Adv Gerontol*, 23, 2,(243-8.
- Witt, C.S., Price, P., Kaur, G., Cheong, K., Kanga, U., Sayer, D., Christiansen, F. & Mehra, N.K. (2002). Common HLA-B8-DR3 haplotype in Northern India is different from that found in Europe. *Tissue Antigens*, 60, 6,(Dec, 2002) 474-80.
- Yang, X.D., Sytwu, H.K., McDevitt, H.O. & Michie, S.A. (1997). Involvement of beta 7 integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in the development of diabetes in obese diabetic mice. *Diabetes*, 46, 10,(Oct, 1997) 1542-7.
- Yang, X.D., Tisch, R., Singer, S.M., Cao, Z.A., Liblau, R.S., Schreiber, R.D. & McDevitt, H.O. (1994). Effect of tumor necrosis factor alpha on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. *J Exp Med*, 180, 3,(Sep 1, 1994) 995-1004.
- Zhao, J., Jin, L. & Xiong, M. (2006). Test for interaction between two unlinked loci. *Am J Hum Genet*, 79, 5,(Nov, 2006) 831-45.

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen