HLA-A,-B,-C AND -DR ANTIGENS AND JUVENILE-ONSET DIABETES MELLITUS

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ABSTRACT

The genetic region which exerts a major influence on graft rejection is known as the major histocompatibility complex or MHC. The MHC in man, the HLA system, includes at least five distinct multiallelic loci, HLA-A,-B,-C,-D and -DR. The HLA system possesses three fundamental characteristics: it is highly polymorphic; its alleles are in linkage disequilibrium with one another as well as with other loci; and the HLA antigens demonstrate extensive cross-reactivity.

A number of previous reports have established associations between certain HLA-B antigens and juvenile-onset insulin-dependent diabetes mellitus (JOD). In this investigation we have studied the distribution of HLA antigens in patients with diabetes mellitus with a special emphasis on HLA antigens belonging to the DR locus. We observed a significant increase in the incidence of HLA-DR4 in JOD patients as compared to its frequency in the normal controls. The present data also confirm earlier reports of the increased incidence of HLA-B15, -B40 and -CW3 in the patient group. Further analyses of the data showed that the primary association of JOD was with HLA-DR4 and the associations with the HLA-B and -C loci antigens were secondary in nature.

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LIST OF ABBREVIATIONS AND SYMBOLS

ÁDP	adenosine diphosphate
^B f	properdin factor B
BSA	bovine serum albumin
B ₂ m	beta 2 microglobulin ,
Chido	chido red blood cell group
CLL	chronic lymphocytic leukemia
Con A	Concanavalin A
C-2	complement component 2
C-4	complement component 4
Сзн	third constant region of immunoglobulin heavy chain
DM	Diabetes Mellitus
g	gravity; the number of times the force of gravity at the equator at sea level
GL0	g]yoxylase .
HBSS	Hanks' balanced salt solution
HLA	Human leukocyte system A
H-2	histocompatibility 2, mouse MHC
ICA .	islet (of Langerhans) cell antibodies
IgA	immunoglobulin A
IgG 🕓	immunoglobulin G
IgG-Fc	immunoglobulin G-Fc receptor
IgM-Fc	immunoglobulin M-Fc receptor
JOD	juvenile-onset insulin dependent diabetes mellitus

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LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

ME _s	malic enzyme
мнс	major histocompatibility comples
min	minutes
ml	millilitre
MLC	mixed lymphocyte culture
MOD	maturity-onset diabetes mellitus
MODY	maturity-onset diabetes mellitus of youth
N	the number of subjects in a study population
N terminus	the amino terminus of a protein
NIH	• National Institutes of Health, USA
NS	not significant
Р	probability
PBL .	peripheral blood lymphocyte
PGs	urinary pepsinogen
PGM ₃	phosphoglucomutase-3
PHA	ohytohemagglutinin
ρI	isoelectric pH (negative logarithm of the hydrogen ion concentration)
PPD	punified protein derivative of Music Jaams tradericodia
r	coefficient of correlation
Roger	Roger red blood_cell group
RR	relative risk
RT	room temperature
sec	second

Shāhu light sheep anti-human light chain-flourescein conjugated antibody chain + FITC

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

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SLE	systemic lupus erythematosus
SODm _	superoxide dysmutase
sp. g.	specific gravity
SRBC	sheep red blood cell
Tγ	T lymphocytes with receptors for IgG-Fc domain
Tμ .	T lymphocytes with receptors for IgM-Fc domain
v/v	volume per volume
WHO	World Health Organization
w/w	weight per weight
X2	chi-square

SYMBOLS

°C degrees Centigrade

% percent

< less than

µl microliters

= equals

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I. INTRODUCTION

I.1. The Major Histocompatibility Complex (MHC)

I.l.l. <u>General</u>

The genetic region which exerts a major influence on graft rejection is known as the major histocompatibility complex or MHC. In all vertebrates studied so far, the MHC has been described as a genetic system of closely linked genes. The MHC in man is called the Human Leukocyte system A or the HLA system. It is located on the short arm of chromosome 6 (Breuning et al., 1977; Francke and Pellegrino, 1977; Lamm et al., 1974). At present, it includes at least 5 distinct multiallelic loci: HLA-A,-B,-C,-D and -DR. Of these loci, HLA-A,-B and -C, code for alloantigens which are detectable by serological methods. The fourth locus, HLA-D, controls lymphocyte responses in the *in vitro* mixed lymphocyte culture (MLC) reactions. Locus HLA-DR codes for alloantigens, expressed predominantly on B lymphocytes, which are also serologically detectable. In addition to these loci, there are other immune response loci. Also, several, different genetic traits have been linked to HLA. The arrangement of the loci of the HLA supergene on chromosome 6 is shown in Figure 1 (Allen, Jr., 1974; Francke and Pellegrino, 1977; Fu et al., 1974; Gill et al., 1928; Lamm and Petersen, 1979; Mann and Murray, 1979; Raum et al., 1976; Schreuder et al., 1979; Teisberg et al., 1976; Weitkamp et al., 1975).



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Figure 1: CHROMOSOME 6

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The identification, definition and genetic resolution of HLA antigens has resulted from the studies of many investigators over the last two decades. The rapid progress in the development of knowledge in this area could be attributed to international collaboration during eight International Histocompatibility Workshops. The results of these collaborative efforts have produced the identification of 90 alloantigens coded for by 5 HLA loci (Table 1). The existence of such a multiallelic system arose from the primordial need for a self-recognition and self-defense system (Dausset and Contu, 1980; Klein, 1975). Subsequent evolutionary advances incorporated spontaneous mutations in this genetic region. These mutations may have conferred selective advantage or disadvantage upon individuals carrying these alleles (Bodmer *et al.*, 1973; Gill *et al.*, 1978; Pious and Soderland, 1977).

I.1.2. The HLA-A, -B_ and -C antigens

The HLA-A,-B and -C antigens are the products of three multiallelic loci, respectively. There are at the present 20 antigens serologically detectable on the A locus, 40 antigens on the B locus, and 8 antigens on the C locus (Table 1). The products on these 3 loci are detectable on all nucleated cells in the body, on platelets, in saliva (Barnstable *et al.*, 1978; Gill *et al.*, 1978; van Rood *et al.*, 1975), and in serum (Allison *et al.*, 1977). They are also present on the developing embryo, however, not until the 6th week of fetal life (Gill *et al.*, 1978).

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WHO-Recognized HLA Specificities - 1980

HLA-A	HLA-B	HLA-C	
A1 A2 A3	85 B7 88	CW1 CW2 CW3	
A9 ·	B12	CW4 .	
A10	· B13	CW5	
A11	· B14	. CW6	
AW1 9	B15	CW7	
AW23(9)	BW16	CW8	
AW24(9)	B17		
A25(10)	B18		
A26(10)	BW21		
A28	BW22	HLA-D	
A29	B27		
AW30	BW35	. ` DW1	
AW31	B37	DW2	
AW32	BW38(W16)	DW3	
AW33	BW39(W16)	· DW4	,
AW34	B40 -	DW5	
AW36	BW41	J CDW6	
AW43	BW42	V DW7	
	BW44(12)	- DW8	
*	BW45(12)	DW9 ×	
	BW46	DWIÓ	
¥	BW47	- DW11	
•	BW48	DW12	
	BW49(W21)		
	BW50(W21)		
	BW51(5)		
	BW52(5)	HLA-DR	
	BW53		
	BW54(W22)	DR1	
•	BW55(W22)	DR2	
	BW56 (W22)	DR3	
	BW57(17)	DR4	
	• BW58(17)	DR5	
	BW59	DRW6	
•	BW60(40)	DR7	
	BW61(40)	DRW8	
•	BW62(15)	DRW9	
	BW63(15)		
		DKWIO	
	BW4	• 、•	
ه	· BW6 .		

The existence of these antigens was first described by Dausset, who found white-cell agglutinins in polytransfused patients (Dausset, 1954). Payng and van Rood independently identified leukocyte agglutinins produced by fetal-maternal stimulation (Payne and Rolfs, 1958; van Rood *et al.*, 1958). Currently, the lymphocyte cytotoxicity reaction is the most widely used method for serologic testing (Terasaki.*et al.*, 1978).

I.1.3. The HLA-D antigens

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There are 12 alleles which have been assigned to the HLA-D locus (Table 1). The strong proliferative responses seen in the MLC are due to disparity at the D locus. The MLC test is considered to be the *in vitro* homologue of the *in vivo* immune response (Smith and Royston, 1975). This technique, originally reported independently by Bach and co-workers (Bach and Hirschhorn, 1964) and by Lowenstein and co-workers (Bain *et al.*, 1964), involved the mixing of peripheral blood lymphocytes from two unrelated people and subsequent examination of the cells for morphological evidence of transformation from small to large blast cells. The value of the MLC test was proposed as a means of evaluating the histocompatibility and immunologic competence in patients receiving homografts (Rubin *et al.*, 1964).

The HLA-D antigens are present on B lymphocytes, macrophages, sperm and epidermal cells (Thorsby *et al.*, 1977).

I.1.4. The HLA-DR antigens

The HLA-DR locus is also multiallelic and is responsible for the coding of the 10 HLA-DR antigens which are, to date, detectable. These antigens are serologically detectable.

The existence of the DR antigens was first reported by Walford and co-workers (Walford *et al.*, 1973). They described these alloantigens as "extra" reactions by HLA typing sera, against chronic lymphocytic leukemia (CLL) cells. These authors referred to these antigens as belonging to the "Merrit" system. However, it was the identification of a group of alloantigens present on murine B cells but absent from T cells (Duckler and Sachs, 1974; Hauptfeld *et al.*, 1974) which greatly stimulated the search to find a comparable situation in humans. The reports confirming the existence of the HLA-DR antigens were presented independently by Bodmer *et al.* (1975), Terasaki *et al.* (1975) and van Rood *et al.* (1975) at the 6*th* International Histocompatibility Workshop and Conference in Arhus, Denmark, in 1975.

Figure 1 shows that the exact location of the HLA-DR locus is not yet known. To date, it is unclear whether the HLA-D and -DR antigens are the same (Albrechtsen *et al.*, 1977a,b; Bodmer, J., 1978; Fradelizi *et al.*, 1978; Fuller *et al.*, 1978; Mann and Murray, 1979; Reinsmoen *et al.*, 1978; Shackelford and Strominger, 1980; Suciu-Foca *et al.*, 1978b; Terasaki *et al.*, 1978). Also, it is unknown whether the HLA-DR antigens are coded by one or more loci (Albrechtsen *et al.*, 1977b; Park *et al.*, 1978; Tosi *et al.*, 1978).

There are many reports confirming the presence of DR antigens on B lymphocytes (Albrechtsen et al., 1977b; Bodmer, J., 1978; Hoffman et al., 1977; Mann et al., 1975; Reinsmoen et al., 1978; van Rood et al., 1975) and a report noting their absence from immunoglobulinproducing plasma cells (Honig, 1976). These antigens are also detectable on monocytes (Albrechtsen, 1977; Bodmer, J., 1978; Mann et al., 1975; van Rood $et \ al.$, 1975), on epidermal Langerhans cells (Forsum et al., 1978; Hirschberg and Thorsby, 1976; Klareskog et al., 1977b; Rowden ext al., 1977; Stingl et al., 1978), on the other cells belonging to the reticuloendothelial system, which reside in various organs of the body (e.g., liver -Kupffer cells; kidney - mesangial cells) (Koyama et al., 1979) and on sperm (Halin and Festenstein, 1975). B lymphoid cell lines (Halper et al., 1978; Honig et al., 1976) and. lymphocytic and myelocytic leukemia cells (Billing et al., 1976a; Mann and Sharrow, 1979) are DR positive. The DR antigens have been detected on myeloblasts, which are precursor cells to granulocytes. but not on mature granulocytes (Ross et al., 1978).

Earlier works reported the absence of murine B cell alloantigens from T cells (Duckler and Sachs, 1974; Hauptfeld *et al.*, 1974). Similar results were obtained in the earlier studies on human T cells (Bodmer *et al.*, 1975; Simpson, 1975; Terasaki *et al.*, 1975; Winchester *et al.*, 1975). At that time, T blasts were also considered to be negative for HLA-DR alloantigens (Billing *et al.*, 1976b). Recently, however, investigations have shown that although "resting" T cells may be DR negative, such is not the case for "activated" T cells and for certain

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subsets of T cells. T cells reactive with HLA-DR antisera have been found after sensitization in MLC (DeWolf et al., 1979; Evans et al., 1978; Greaves et al., 1979; Suciu-Foca et al., 1978a; Zimmerman et al., 1979) and following phytohemmagglutinin (PHA) or Concanavalin A (Con A) stimulation (DeWolf et al., 1979; Ferrone et al., 1980; Gerbase-Delima, 1980; Kaszubowski et al., 1980; Metzgar et al., 1979). These T cells belong to the T_Y subpopulation (suppressor cell function, IgG-Fc receptor positive). However, Ferrone et al. (1980) reported that following PHA stimulation, both Tu (helper cell function; IgM-Fc receptor positive) and Ty subpopulations were DR positive. In addition, Yu et al. (1980) have demonstrated an increase in the number of DR positive T lymphocytes during certain diseases, particularly in rheumatoid arthritis, systemic lupus erythematosus, Staphylococcus aureus infection, osteomyelitis, pneumococcal pneumonia and acute cholecystitis. They also found an increase in the DR positive T cell population following immunization with tetanus toxoid or PPD (purified protein derivative - Mycoplasma tuberculosis) at the injection site in people who reacted to the immunization.

The requirement for strong proliferative responses in MLC has long been taken as disparity at the D locus. In fact, the MLC test has been employed in the definition of the alleles of the D locus. However, as the DR antigens were identified, not only was the unitary control by the D locus questioned, but the identity of the D and DR loci as well. Studies suggest that although the D locus may effect control over MLC reactivity, the products of the DR locus are responsible

for the stimulus for cells to proliferate in the MLC (Albrechtsen et al., 1978; Bergholtz and Thorsby, 1979; Bonderik et al., 1975; Ceopellini et al., 1971; Fradelizi et al., 1978; Fuller et al., 1978; Hirschberg et al., 1977; Humphreys et al., 1976; Jonker et al., 1975; Opelz et al., 1975; van Leeuwen et al., 1973). In addition, a second MLC locus associated with HLA-A has been reported (Johnson et al., 1975; Sheehy, 1978; van Rood et al., 1977b).

I.2. Characteristics of HLA Antigens

Three fundamental characteristics best describe the HLA system. One, it is the most polymorphic system ever described in man. This is evidenced by the number of alloantigens coded by the 5 HLA loci (Table 1).

Secondly, the alleles of the different loci are in linkage disequilibrium, i.e., sets of alleles occurring together more frequently than would be expected by random assortment. This increased frequency of certain haplotypes (a group of alleles of linked genes contributed by either parent) is statistically significant and different patterns exist in different ethnic populations. For example, the most common haplotypes in North American Caucasians are:

> HLA-A1-B8-DR3 HLA-A3-B7-DR2 HLA-B15-DR4

The third characteristic of the HLA system is the existence of cross-reactivity among various HLA specificities belonging to a locus.

Cross-reactivity is the phenomenon whereby an antibody reacts with an antigen that is similar to that antigen which specifically stimulated the antibody's synthesis. The purpose of a system having such a characteristic is more the result of its polymorphic nature and natural selection than an attempt to have a cross-reactive pattern. Studies comparing the cross-reactivity of the HLA-A, -B and -C antigens by xenogeneic antisera give credence to the view that the HLA antigens arose through gene duplication of a common ancestral gene (Rask *et al.*, 1976). The amino acid sequences of HLA-A2 and -B7 show that there is on the order 'of an 80% average in the homology between the two antigens (Herring, 1978; Orr *et al.*, 1979a; Parham *et al.*; 1977).

Examples of cross-reactivities amongst the HLA-A and HLA-B antigens are given in Figure 2 and amongst the HLA-DR antigens in Figure . 3.

I.3. Biochemistry of the HLA Antigens

I.3.1. <u>HLA-A</u>, <u>-B</u>, <u>-C</u>

The physical structure of the HLA-A and -B antigens has shown that following papain solubilization there is a 54,000 dalton component, and a 12,000 dalton component (Kvist *et al.*, 1978; Orr *et al.*, 1979b; Strominger *et al.*, 1976; Tragardh *et al.*, 1979). The heavy component, a glycoprotein, carries the antigenic nature of the HLA antigens as well as all the carbohydrate moeities which have no antigenic function (Barnstable *et al.*, 1978; Feinstein, 1979; Herring, 1978; Parham *et al.*, 1977). Studies of the HLA-B7 molecule show that it posssesses 271

HLA-A, -B AND -C ANTIGENS, THEIR SEROLOGY AND CROSS-REACTION

<u>A ANTIGENS</u>

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Figure 3: Cross-reactive patterns of some of DR antigens.

Bodmer, J., (1978)

amino acid residues with 2 intrachain disulphide bridges with 2 loops, 65 and 55 amino acids long, respectively, separated by a 38 residue segment. There is internal homology between the N terminus (residues 1-90) and the region including the first disulphide loop, residues 91-180 (Orr *et al.*, 1979b).

The heavy chain is non-covalently bound to the small chain (Barnstable *et al.*, 1978; Strominger *et al.*, 1976) which has been identified as β_2 microglobulin (β_2 m) (Barnstable *et al.*, 1978; Herring, 1978). The β_2 m, although physically associated with the MHC-coded heavy chain, is known to be nonglycosylated and coded for by a gene located on chromosome 15 (Bodmer, 1978; Goodfellow *et al.*, 1975; Herring, 1978; Krangel *et al.*, 1979). The β_2 m is invariant in structure and rabbit antihuman β_2 m antiserum reacts with chicken β_2 m (Feinstein, 1979; Kvist *et al.*, 1978). Human β_2 m contains a single disulphide bridge (Strominger *et al.*, 1976). Although not directly involved in the antigenicity of the HLA-A, -B, -C antigens, β_2 m is essential to the structure as its absence leaves the heavy chain antigenically inactive (Barnstable *et al.*, 1978; Welsh *et al.*, 1977).

The HLA antigen complex is an integral membrane glycoprotein whose carboxyl terminal passes through the lipid bilayer to the cytoplasmic side of the plasma membrane (Barnstable *et al.*, 1978; Herring, 1978; Krangel *et al.*, 1979; Walsh and Crumpton, 1977). Quantitative studies have shown that B lymphocytes express three times as many HLA antigens as expressed on T cells (Pelligrino *et al.*, 1978).

Noteworthy are the similarities between the two components of the HLA-A, -B, -C antigens and the immunoglobulins. Consideration has

been given to the point that they arose from common ancestral genes (Barnstable *et al.*, 1978; Feinstein, 1979; Orr *et.al.*, 1979c; Strominger *et al.*, 1976). The B_2m , first isolated from human urine, shows strong homology to the C_3H domain of IgG (Strominger *et al.*, 1976). The heavy chain of HLA-B7 contains an 88 residue fragment which also bears strong homology to the immunoglobulin constant domains (Orr *et al.*, 1979c).

I.3.2. <u>HLA-DR</u>

. The biochemical nature of the HLA-DR antigens is fundamentally different from that of the HLA-A, -B, -C antigens. This difference is in the complete independence from $\beta_2 m$ (Barnstable *et al.*, 1978; Billing et al., 1976b; Snary et al., 1977; Sullivan et al., 1979). The DR antigens are composed of two glycoprotein chains non-covalently linked in a one to one subunit structure ratio (Springer et al., 1977a). One chain has an apparent molecular weight of 33,000-35,000 daltons and has been referred to as "heavy chain", "alpha chain" or "p34". This acidic chain (pI = 5.4) demonstrátes very little phenotypic variability. The second chain has an apparent molecular weight of 27,000 - 29,000 and it has been referred to as "light chain", "beta chain" or "p29" It is a neutral chain (pI = 6.1) with more variability in structure, size and charge (Barnstable et al., 1978; Billing et al., 1976b; Humphreys et al., 1976; Klareskog et al., 1977a; Shackelford and Strominger, 1980; Snary et al., 1977; Springer et al., 1977a, b; Sullivan *et al.*, 1979).

Studies where the HLA-DR antigens were separated into their component "alpha" and "beta" chains, anti-DR antisera bound preferentially to the "beta" subunit (Silver and Ferrone, 1979). Also, there appears to be both structural and antigenic similarities between HLA-DR antigens and their counterparts in the mouse (Billing *et al.*, 1976b; Kvist *et al.*, 1978; Nilsson *et al.*, 1977; Shackelford and Strominger, 1980; Silver and Ferrone, 1979; Springer *et al.*, 1977a; Sullivan *et al.*, 1979), the guinea pig (Barnstable *et al.*, 1978; Kvist *et al.*, 1978; Springer *et al.*, 1977a) and also the monkey, rat, cow, pig and chicken (Barnstable *et al.*, 1978).

Comparisons with the well studied murine MHC, the H-2 region, show that the products of the I-E/C region closely resemble the DR antigens, with "alpha" chains demonstrating as much as 90% homology and "beta" chains as much as 50% (Shackelford and Strominger, 1980; Silver and Ferrone, 1979).

The orientation of the DR antigens is such that the proteolysis sensitive carboxyl end of both chains passes through the lipid bilayer to reach the cytoplasm. A hydrophobic region, penultimate to the carboxyl terminal, is responsible for membrane binding. The amino terminal is completely extracellular (Barnstable *et al.*, 1978; Kaufman and Strominger, 1979). The relationship of orientation to function may allow these membrane components to act as a route for the direct transfer of information from extracellular to intracellular (Barnstable *et al.*, 1978).

I.4. Biological and Clinical Functions of HLA Antigens

The functional aspects of the HLA system have been studied with two physiologically integrated considerations. One approach concerned itself with the manner in which the HLA system could serve the practice of medicine, e.g., organ transplantation, disease associations. The second approach examined the biological significance of the HLA system, e.g., control of cell-cell interaction, and cell differentiation (Bach $et \alpha l$, 1979).

I.4.1. <u>Clinical</u>

The concept of matching cell surface antigens for organ transplantation dates back to Carl Landsteiner. More recently, studies have shown that the matching of donors and recipients for HLA-D and -DR has provided a significant improvement in graft survival rates. Van Rood *et al.* (1979) have demonstrated that matching for either one or two DR alleles improved one year kidney survival rates to about 80%, while also matching for HLA-A, and -B alleles increased one year survival rates to 90%. While antibodies to the HLA-A, -B, -C antigens probably result in hyperacute rejection, reports are contradictory with respect to the effects of pre-transplant anti-HLA-DR antibodies on graft survival (Thorsby, 1979). Studies have shown that with respect to graft rejection the HLA-A, -B, -C antigens function primarily as target antigens, although DR antigens may also be involved for cytotoxic (killer) T cells. The products of the HLA-D and -DR loci are responsible for initiation of allograft immunity (Thorsby, 1979).

The association of HLA with diseases is discussed separately (Section 1.5).

I.4.2. <u>Biological</u>

Studies of the biological significance of the HLA system have shown that the products of the HLA-A, -B, -C loci are involved in restricting cytotoxic T cell responses to virus-infected targets or to target cells expressing minor histocompatibility antigens (McMichael and McDevitt, 1977; Thorsby, 1979).

The interactions between B cells and T cells, as well as between T cells and macrophages are strongly influenced by self-HLA-D/DR antigens (Albrechtsen, 1977; Bergholtz and Thorsby, 1978, 1979; Bergholtz *et al.*, 1980; Friedman *et al.*, 1979; Simpson and Matsunaga, 1979; Strelkauskas *et al.*, 1977). The pathophysiology behind contact dermatitis has been shown to possibly involve an altered-DR antigen on epidermal Langerhans cells (a reticuloendothelial cell) as the means of stimulating autologous killer cells (Forsum *et al.*, 1978; Klareskog *et al.*, 1977b). The expression of DR antigens also seems to involve regulatory mechanisms, carried out by T cells, particularly T_Y cells and suppression (Engleman *et al.*, 1978; Evans *et al.*, 1977; Yu *et al.*, 1980). The involvement of the DR antigens is present in immunological suppressor factor molecules (Engleman *et al.*, 1978).

Mann and Muchmore (1980) discuss the association of DR antigens with mono and oligosaccharides with respect to antigen-antibody reactions.

They suggest that the DR antigens act as receptors for these sugar molecules as a means of cellular interaction, whereby monocyte "recognition" of syngeneic T cells occurs through specific cell surface carbohydrate structures on the T cell surface.

B lymphocytes and neutrophils, during the course of their respective differentiation pathways, pass through a DR-positive period to a DR-negative period. In the case of B lymphocytes, the terminal plasma cell is DR-negative (Halper *et al.*, 1978). In the case of neutrophils, the DR-positive myeloblast loses its DR antigens in the promyelocyte stage (Ross *et al.*, 1978). The role of DR antigens in B cell differentiation is most dramatically portrayed in the congenital disorder "infantile agammaglobulinemia". Patients with this disorder completely lack detectable DR antigens and consequently fail to develop any B lymphocytes (Hoffman *et al.*, 1977).

I.5. HLA and Disease

The search for associations between the HLA system and disease can be said to take its origins from the report by Lilly *et al*. where they described the influence of the murine H-2 complex upon susceptibility to Gross leukemia virus and subsequent disease (Lilly *et al*., 1964).

I.5.1. HLA studies with respect to disease

The approach to studying the association between HLA and disease has been divided into four main areas with respect to disease. There

are those diseases that may involve hereditary components. The best known association in this group of diseases is between HLA-B27 and ankylosing spondylitis which occurs predominantly in males (Dick, 1978). The second group includes malignant diseases. Lilly et al. (1964) were the first to demonstrate an association between the murine histocompatibility complex and virus-induced leukemia. Unfortunately, such a strong association between malignant diseases and HLA has not been found in Thirdly, there are those diseases which involve immunological humans. phenomena as the basis of the pathology. Here, it seems that HLA-B8/DR3 have shown the most consistent pattern, particularly in those disorders in which autoantibodies are present. Examples include juvenile-onset diabetes, Graves' disease (hyperthyroidism) and Addison's disease. Finally, are the miscellaneous group of disorders which do not fall into any one of the previous categories. For example, Behcet's disease in .Japanese which is associated with HLA-B5 (Ohno et al., 1973).

Table 2 gives some of the currently known HLA and disease associations. The proposed mechanisms of HLA association with diseases can be considered in the following areas: immune responsiveness, cross reactivity, linkage disequilibrium, and receptor functions (Dick, 1978; Rosenberg and Kidd, 1977).

I.5.2. Immune responsiveness

The concept of immune response genes is a combination of both Burnet's clonal selection theory and Jerne's surveillance theory which proposes that a gene or group of genes control the response of the immune

Tab]	е	2
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ILA-Associa	ted Dis	sorders*
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HLA

D/DR3

D/DR3 D/DR4 D/DR2

D/DR3 B8

D/DR3

D/DR3

D/DR'2

D/DR2 D/DR2 B/18

D/DR2

D/DR4

D/DR4

D/DR4

D/DR4

D/DR5

D/DR5

D/DR5

A1 A3 B14 B5 B47 B27 B27 B27 B35 Cw6 D/DR3 D/DR3 D/DR3 D/DR3

· .
Hodgkin's disease Idiopathic hemochromatosis
Behcet's disease Congenital adrenal hyperplasia Ankylosing spondylitis Reiter's disease Acute anterior uveitis Subacute thyroiditis
Psoriasis vulgaris
Dermatitis herpetiformis Celiac disease
Sicca syndrome Idiopathic Addison's disease

Idiopathic Addison's disease Graves' disease Insulin-dependent diabetes

Myasthenia gravis

Condition

SLE

Idiopathic membraneous nephropathy

Multiple sclerosis Optic neuritis C2 deficiency

Goodpasture's syndrome Rheumatoid arthritis Pemphigus (Jews) IgA nephropathy Hydralazine-induced SLE Hashimoto's thyroiditis Pernicious anemia Juvenile rheumatoid arthritis: pauciart

* Taken from Svejqaard et al. (1981)

system to an antigenic challenge (Greenburg and Yunis, 1978; Levis and Berry, Jr., 1975; Sasazuki *et al.*, 1977). The phenomenon of aging assists in the study of immune responsiveness in that certain age-related changes appear to alter the control of the immune genes resulting in increased or decreased susceptibility to a disease (Greenburg and Yunis, 1978; Oliver, 1977). The group of autoimmune related diseases might best exemplify this point. This group of diseases, which have in common the presence of an antibody directed against a tissue of the body, are thought to be the result of a change in the control of the body's immune responsiveness. The pathophysiology of Addison's disease, a disorder consisting of adrenal hypofunction, involves the presence of antibodies directed against adrenal tissue. Similar to other diseases of this group, Addison's disease is associated with HLA-B8 and Dw3 (McMichael and McDevitt, 1977).

I.5.3. Cross-reactivity

Evidence seems to support a concept which would include immune responsiveness with the mechanism of cross-reactivity in the HLA and disease association (Dick, 1978). Antigen mimicry may explain as to why an agent is not "recognized" as foreign and thereby allowed to follow its course of pathology. Included in this concept of crossreactivity is the possibility that there is an interaction between a solubilized HLA gene product and a pathological entity (e.g., a virus or toxin) which subsequently confers host susceptibility or resistance to this entity, "altered-self" (Bias and Chase, 1977; Dick, 1978; Greenberg and Yunis, 1978; Mann and Murray, 1979; McMichael and McDevitt, 1977;

Sasazuki *et al.*, 1977; Svejgaard and Ryder, 1976). Ankylosing spondylitis, an arthropathy involving the sacro-iliac joint, demonstrates a significant association with HLA-B27 (Caffrey and Jarnes, 1973). A concept of antigen mimicry has been postulated between HLA-B27 and bacilli of the *Klebsiella* species, thought to be involved in the disease process (Seager *et al.*, 1979). The available evidence is, however, weak (Svejgaard *et al.*, 1981).

I.5.4. Linkage disequilibrium

The concept of linkage disequilibrium as a mechanism for the HLA and disease association suggests that the HLA gene is in linkage disequilibrium with a disease susceptibility gene(s). This phenomenon may be involved with genetic selection and survival of the fittest (Oliver, 1977; Sasazuki *et al.*, 1977; Thomsen and Bodmer, 1979). Although multiple sclerosis, a debilitating neurological disease, may not have materially altered evolutionary selection its association with the HLA-A3-B7-DR2 linkage group is a noteworthy example here, in that both the HLA linkage group and mutliple sclerosis are present in high frequencies in northern Europeans,

I.5.5. Receptor function

Finally, HLA antigens could function as membrane receptors for microorganisms, e.g., infectious agents (Rosenberg and Kidd, 1977) and body products, e.g., hormones (Svejgaard and Ryder, 1976). However, evidence for such a mechanism is not yet available.

I.6. Diabetes Mellitus

Diabetes mellitus (DM) was first recognized as occuring in families by the ancient Hindus (Tatersall *et al.*, 1980). One can attribute the clinical manifestations to metabolic disorders involving carbohydrates, fats and proteins (Robbins and Cotran, 1979). The complexity of DM is obvious upon examination of a tentative National Institutes of Health classification (Cahill, 1978) which herein includes the clinical types (Fajans *et al.*, 1978) (Table 3).

The common occurrence and potentially lethal nature of this disorder in which there is a great deal of evidence for familial predisposition has proded continuing search for answers in this "geneticist's nightmare" (Neel, 1977). Twin studies have best demonstrated that in fact this is a genetically heterogeneous disorder. The strong concordance, as high as 90%, among MOD diabetic twin pairs suggests this type of DM to be predominantly inherited. The low concordance in JOD diabetic twins lends itself better to a recessive or multifactorial mode of inheritance with varying degrees of penetrance (Cahill, 1978; Craighead, 1978; Ganda and Soeldmer, 1977; Ginsberg-Fellner, 1980; Neel, 1977; Rubenstein *et al.*, 1977; Tattersall *et al.*, 1980).

The division of DM for the purpose of HLA studies, for practical reasons, includes only JOD and MOD.

The heterogeneity of DM is further supported by the significant increase in certain HLA antigens in JOD but not in MOD (Barbosa vt al., 1978). Various studies report the significant increase in HLA-B8, -B15, -B18, -Cw3, -Dw3, -Dw4, -DR3 and -DR4 (Bertrams vt al., 1979; Cudworth
Diabetes Mellitus - Tentative NIH Classification

' and Clinical Forms*

NIH Cla	assification	Clinical Types			
			stage		age
Type 1	insulin dependent	i)	ketosis prone	i)	classic in juveniles (JOD)
		ii)	preketosis prone	ii)	at any age
Type 2	non-insulin dependent		le la	?	
	 a) non obese i) insulin requiring ii) non-insulin requiring b) obese i) insulin requiring ii) non-insulin requiring iii) gestational iv) impaired glucose tolerance 	i) ii)	requiring insulin for correction of fasting hyper- glycemia non requiring insulin	i) ii)	maturity onset diabetes of middle age or older age groups (MOD) maturity onset diabetes of the young (MODY)
	of glucose tolerance vi) potential abnormality				
Diabete	es or Glucose Intolerance Assoc	iated	with Certain Condi	tion	s or Syndromes
i) ii) iiï) iv) v)	hormonal drug-induced pancreatic disease insulin receptor abnormalities other genetic and chromòsomal s	syndr	omès		·
* Taken	from Cahill, 1978; Fajans et gi	2.(19	78)		

et al., 1979; Duquesnoy et al., 1976; Farid et al., 1978; Hammond and Asmal, 1980; Ilonem et al., 1980; Landgraf et al., 1976; Ludwig et al., 1976a; Nerup et al., 1974; Richens et al., 1979; Sachs et al., 1980; Singal and Blajchman, 1973; Solow et al., 1979), as well as a significant decrease in HLA-B7, -Dw2 and -DR2 in JOD. In Japanese patients, Bw22 is significantly increased (Wakisaka et al., 1976). In South African Blacks (Xhosa tribs) the increase was observed in Bw35 and A2 with a decrease in Bw42 in JOD. This report also found the first markers for MOD as being an increase in both B12 and B41 (Briggs et al., 1980). It is also known that HLA-B8 and -Dw3, as well as HLA-B15, -Cw3 and -Dw4 respectively, exist in linkage disequilibrium. Therefore, it has been proposed that JOD is associated with two different alleles and their concurrent presence increases the likelihood of JOD greater than either locus separately (Svejgaard et al., 1975).

The immunological responses are quite different between JOD and MOD. The presence of antibodies to the Islets of Langerhans (ICA) in JOD is associated with the onset of disease and may even be involved in its etiology. These antibodies, of the IgG class, are reactive to an antigen common to all types of islet cells (Doniach and Bottazzo, 1977) and are directed towards the cytoplasmic components of these cells (Rayfield and Seto, 1978). The tendency is for high levels of ICA's to be present initially upon diagnosis but this level declines over the years (Doniach and Pouplard, 1976; Irvine *et al.*, 1977; Kaldany, 1979; Lendrum *et al.*, 1976; Singal and Blajchman, 1973). The presence of ICA antibodies also varies proportionally to different forms of DM - one

in which the antibodies are transient in nature and a second, in which the disorder is of primarily autoimmune nature where the ICA's are persistent (Cudworth, 1978).

The presence of ICA's lends itself to a discussion of the relationship between autoimmunity and DM. Huang and MacLaren (1976) first postulated DM as being an autoaggressive disorder when they demonstrated cytoadherance and cytotoxicity against human insulinoma cells by lymphocytes from JOD. This theory was expanded to demonstrate that in fact DM was a polyendocrine autoimmune disease. This follows with its HLA-B8 association and the latter's association with other endocrine autoimmune disorders (Bottazzo *et al.*, 1978; Doniach and Bottazzo, 1977; Drash, 1980; Fajans *et al.*, 1978; Ganda and Soeldmer, 1973; Irvine *et al.*, 1977; Kaldany, 1979, Ludwig *et al.*, 1979).

The other aspects of ICA antibodies and cell-mediated immunity also suggest a viral involvement in the etiology of the disorder. It is not clear as to whether there is a susceptibility to the virus which perpetrates the islet cell destruction or whether islet cells possess an inherent predilection for viral infection with a subsequent immune response bringing about their destruction. The control of this phenemenon could possibly involve the immune response genes and the HLA antigens (Doniach, 1975; Doniach and Pouplard, 1976; Drash, 1980; Ganda and Soeldmer, 1973; Nerup *et al.*, 1976). The first recorded observation of a viral infection resulting in a case of DM was made by Stang in 1864 following a case of mumps (myxovirus). More than a quarter of a century later, in 1899, Harris noted a similar phenomenon (Koyama *et al.*, 1979;

Sinaniotis et al., 1975). More recently, Gamble and co-workers reported the seasonal increase in DM cases, peaking in the autumn and winter, and associated this with Coxsackie virus infection, particularly type B4 (Gamble and Taylor, 1969, 1976) as detected by antiviral antibodies (Gamble et al., 1969). Subsequently, reports of DM involving infectious agents, or even their attenuated forms in vaccines, include the other B types of Coxsackie virus (B1.-3 and B5), cytomegalovirus, rubella, varicella, Epstein-Barr virus, as well as Mycoplasma pneumonia (Champsaur et al., 1980; Nelson et al., 1975; Ray et al., 1980; Rayfield and Seto, 1978; Sinaniotis et al., 1975). An important point is that all of these reports involve the indirect finding of antiviral antibodies or the isolation of virus agents from specimens other than the pancreas. However, Yoon and co-workers have recovered a variant of Coxsackie type B4 virus from the pancreatic islets of a 10-year old boy whose islets showed lymphocytic infiltration and beta cell necrosis. The patient's serum had a neutralizing antibody titre of 1:32 on the day of his death (Yoon et al., 1979). This indicates an immune response to viral infection but does not clarify the exact role of the virus in the resultant DM (Jansen et al., 1977).

It would seem that irrespective of the level of control diabetic patients achieve over their diabetes, they are prone to atherosclerosis, microangiopathies, and infection more so than a non-diabetic. Lesions involving the kidneys, merve and eyes are much more prominent in diabetics (Malone, 1977). The alteration of the active site of many enzymes by reactive ketones binding to an arginyl residue present in that site may be a further possibility causing altered body metabolism (Bottazzo *et al.*,

1978; Cahill, 1978; Deckert, 1979; Moller *et al.*, 1978). The increased susceptibility to infection seems understandable in light of an impaired cell-mediated immunity. This includes decreased responses to PHA stimulation and allogeneic stimulation as well as impaired killer cell activity and granulocyte adherence, chemotaxis and phagocytosis (Bagdade *et al.*, 1978; Delespesse *et al.*, 1974; Han and Dadey, 1976; Ludwig *et al.*, 1976b; Schernthaner *et al.*, 1978a).

Anti-insulin antibodies may have a role in the etiology of JOD, however, they also appear to be a component of the autoimmune phenomenon observed in established cases. An association between HLA-DR antigens and anti-insulin antibodies has been observed by some investigators (Egeberg *et al.*, 1976; Schernthaner *et al.*, 1978b). The most interesting point is the increased incidence of allergic reactions to insulin in JOD patients who carry HLA-B7 (B7 is decreased in JOD). In addition, it has been postulated that high and low responders to insulin may show an association with HLA-B15 (Bertrams *et al.*, 1976; Schernthaner *et al.*, 1976). The presence of autoantibodies directed against insulin receptors on monocytes correlates with a rare form of insulin-resistant DM (Doniach and Bottazzo, 1977).

This disorder of carbohydrate, fat and protein metabolism, which clinically manifests itself as a carbohydrate intolerance, has a primary focus of insulin metabolism insufficiency. The destruction of the pancreas islets of Langerhans either through viral infection and/or an immune response appear to be one etiological route. However, as DM is as complicated a disorder as the number of its possible etiologies (Fajans

et αl ., 1978), one must consider "other" possibilities such as a congenital deficiency somewhere in the long process from precursor to functional product (Doniach, 1975).

I.7. Objectives of Proposed Study

The objectives of the proposed study included detailed analyses of the relationship between alleles of the HLA system and juvenile-onset diabetes mellitus. Previous work in this and other laboratories demonstrated a significant increase in the incidence of HLA-B8, -B15, -Cw3 and Dw3 antigens in diabetic patient populations (Cudworth and Woodrow, 1975; Cudworth, 1978; Duquesnoy et al., 1976; Farid et al., 1978; Landgraf et al., 1976; Ludwig et al., 1976a; Nerup et al., 1974; Rubenstein et al., 1977; Singal and Blajchman, 1973; Wakisaka $et \ all$, 1976). The products of the HLA-DR locus have not been examined for an association with juvenileonset diabetes. Since HLA-DR antigens are thought to be associated or linked with immune response genes, we studied the distribution of HLA-DR antigens in juvenile-onset diabetes patients and in a control population. In addition, the objectives of the proposed work included studies of the nature of the associations of HLA-DR versus HLA-A, -B, -C antigens with juvenile-onset diabetes.

II. MATERIALS AND METHODS

II.1. Cell Preparation

II.1.1. <u>Peripheral blood lymphocyte preparation (PBL) for HLA-A,-B,-C</u> <u>typing</u>

The isolation of PBL was based upon a technique described by Boyum (1968). A 10 ml sample of blood was collected by venipuncture into a heparinized Vacutainer tube (Becton Dickinson Co., Mississauga). The blood was diluted 1 in 3 with a Hanks' balanced salt solution (HBSS) (Grand Island Biological, N.Y.) and layered over a Ficoll-Hypaque solution (sp. q. = 1.077) (Pharmacia Fine Chemicals, Uppsala, Sweden). The cell suspension was spun at 400 g for 20 min in an MSE GT-2 centrifuge (Measuring & Scientific Equipment Ltd., Sussex, England). The interface, consisting primarily of PBL, platelets and monocytes, was retrieved. One drop of a 0.5% adenosine diphosphate (ADP) solution (Sigma Chemical Co., St. Louis) was added per ml of the cell suspension and this was mixed gently until large aggregates of platelets were observed. The suspension was spun at 1000 g for 5 sec in a Fisher Model 59 centrifuge (Fisher Scientific Co., N.J.) to remove the platelet aggregates. The supernatant was recovered and the cells washed twice with HBSS. The final cell suspension was adjusted to a concentration of 1×10^{6} cells/ml in HBSS.

II.1.2. <u>B lymphocyte preparation for HLA-DR typing</u>

Human PBL were prepared as described above. The lymphocytes were washed with HBSS and resuspended in approximately 5 ml of a 1% bovine serum albumin (BSA) (Pentex BSA-fraction V, Miles Lab., Inc., Elkhart, Ind.) solution. To this, one drop of a 5% v/v suspension of neuraminidase-treated sheep red blood cells (SRBC) (Woodlyn Labs. Ltd., Guelph) was added per 10⁶ lymphocytes so as to give a cell ratio of 10 lymphocytes to one SRBC. The lymphocyte-SRBC cell mixture was incubated at room temperature (RT) for 30 min, then spun at 200 g for 5 min and further incubated for 60 min at 4°C. The cells were gently resuspended and layered over Ficoll-Hypaque solution, as described above. The cell suspension was spun for 20 min at 400 g. B lymphocyte enriched cell population was retrieved from the interface, washed twice in HBSS and resuspended to a final concentration of 1 X 10⁶ cells/ml. The T lymphocyte enriched population was recovered from the pellet by shock lysis of the SRBC with distilled water, washed twice and resuspended at a final cell concentration of 1 X 10⁶ cells/ml in HBSS. The B cell suspension was evaluated for enrichment using a sheep anti-human light chain fluoresceinlabelled antiserum (Shāhu light chain-FITC) (McMaster University Host Resistance Programme) and a Leitz Orthoplan microscope fitted with a Ploempak II vertical fluorescence illuminator and an HBO 50 mercury lamp.

II.1.3. Preparation of sheep red blood cells (SRBC).

A 5% v/v suspension of SRBC was prepared using HBSS and washed twice. The cells were treated with 25 units of neuraminidase (GIBCO, N.Y.)

for 30 min at 37°C and were washed three times. The final concentration was 5% v/v.

II.2. Cytotoxicity Assays

II.2.1. <u>HLA-A, B and C antigen typing</u>

The HLA-A, -B and -C antigen typing was performed using the microdroplet cytotoxicity assay of Terasaki (Terasaki *et al.*, 1978). One μ l of PBL cell suspension was added to HLA typing trays containing 1 μ l of monospecific human anti-HLA antisera. The trays were incubated at RT for 30 min and then 5 μ l of a pooled rabbit complement were added to each well. The trays were incubated for an additional 60 min at RT. The cells were stained with 1 μ l of eosin Y (Fisher Scientific, N.J.) and fixed with 5 μ l of formaldehyde (37% w/w, class II) (Fisher Scientific, N.J.). The cells were allowed to gravity settle for a minimum of 30 min at which time a cover slip (Gold Seal Coverglass, Clay Adams, Parsippany, N.Y.) was applied. The reactions were scored using a Zeiss inverted phase-contrast microscope (Carl Zeiss, W. Germany). The scoring was performed with the following standard:

Percentage o	fˈce	<u>11s</u>	stained	(killed)	<u>Score</u>
, Q	-	· 5		• •		1
6	-	10	-		X	2
11	-	20		` •	х р т	4
21	- .	80			,	6
81	- 1	00				8
	•				۵	•

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The reactions scored as "8" and "6" were considered positive, a reading of "4" was weakly positive and both "2" and "1" were negative. The patient and control subjects were typed for the following HLA-A, -B and -C antigens: A1, A2, A3, A9, Aw23(9), Aw24(9), A10, A25(10), A26(10), A11, A19, A28, A29, Aw30, Aw31, Aw32, B5, B7, B8, B12, B13, B14, B15, B17, B18, B27, B37, B40, Bw16, Bw38(16), Bw39(16), Bw21, Bw22, Bw35, Cw1, Cw2, Cw3, Cw4 and Cw5.

II.2.2. <u>HLA-DR antigen typing</u>

The microdroplet lymphocyte cytotoxicity test was performed using a B cell enriched population with the following modifications. Cells were incubated with typing sera for 60 min and with the complement source for 120 min, respectively. The HLA-DR alloantigens were defined on the basis of reactivity with antisera defined by the Seventh International Histocompatibility Workshop. Test reactions were scored as described above.

B lymphocytes were typed for HLA-DR antigens -DR1, DR2, DR3, DR4, DR5, DR6 and DR7.

II.3. Subjects

II.3.1. Patient population

Ninety-four unrelated Caucasians with juvenile-onset. insulindependent (JOD) diabetes were typed for HLA-A, -B and -C antigens. Sixty-two of the patients were also typed for HLA-DR antigens. The patients were referred from the Diabetic Clinic of the McMaster University. Medical Centre and the Henderson General Hospital.

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II.3.2. Control population

Two hundred unrelated, nondiabetic Caucasians were typed for HLA-A, -B and -C antigens. Fifty-four of these were also typed for HLA-DR specificities.

II.4. Statistics

The frequency distributions of the HLA-A, -B, -C and -DR antigens in the diabetic population were compared with those in the control population.

The following approximate test of significance was used to test the differences (Colton, 1974):

•	· · ·	Patients	Controls
	HLA antigen present	a	b
,	HLA antigen absent	с	ď
•		, `	

The total, N, is equal to a+b+c+d.

The probability values were determined using standard tables relating probability with chi-square as below:

> chi-square X^2 (1 degree of = $\frac{[|ad-bc|-N/2]^2N}{(a+c)(b+d)(a+b)(c+d)}$

The multiallelic nature of each HLA locus allows for a chance occurrence of an increased incidence of one of the antigens. To correct for this, the probability value for each comparison was multiplied by the number of alleles at that locus. A corrected probability value of less than 0.05 was considered significant. Correlation of coefficient values (r) were calculated as follows:

The relative risk (RR) of developing JOD with the presence of an HLA antigen was determined as follows (Woolf, 1955):

$$RR = \frac{(a) X d}{(c) X b}$$

To test the possibility of primary and secondary associations of the HLA-A, -B, -C and -DR alleles with JOD, the two populations were subdivided into groups of antigen-positive and antigen-negative subgroups. The frequency distributions of the remaining antigens were subsequently analyzed in these subgroups using the method described by Woolf (1955).

t.

 $r = \sqrt{\frac{\chi^2}{N}}$

III. RESULTS

The frequencies of the HLA-A, -B and -C antigens in JOD were compared with those in the control population. The frequencies for some of these antigens in these two populations are given in Table 4. The incidence of B15 was significantly increased $(X^2 = 9.34; corrected)$ P = 0.035; RR = 2.61) in the JOD group (30%) as compared with the controls (14%). Similarly, the incidence of -Cw3 was significantly higher $(X^2 = 7.07; \text{ corrected P} = 0.047; \text{ RR} = 2.34)$ in the patients (28%)than in the control subjects (14%). The incidences of B8 and Cw4 were higher in the patients than in the controls; the increases, however, were not statistically significant. Similarly, the decrease in the incidence of B7 in the JOD group was not significant. In addition, the data show a significant increase ($X^{2^{-}} = 7.12$; corrected P = 0.046; RR = 2.58) in the frequency of B40 in the patients (22%) as compared with the controls (10%). The remaining HLA-A, -B and -C alleles did not demonstrate any significant differences in their frequencies between the patient and the control populations.

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The incidences of HLA-DR specificities in JOD and control populations are given in Table 5. The incidence of DR4 was significantly increased ($X^2 = 7.53$; corrected P = 0.043; RR = 3.47) in the diabetics (44%) as compared with control subjects (18%). Also, the data show an increase in the frequency of DR3 and a decrease in the incidence of DR2 in the patients. These differences are not significant, however.

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Frequency of HLA-B15, -B40, -B8, -B7, -Cw3 and -Cw4 in Normal and Juvenile-Onset Diabetic Patient Populations

		•	•	······································	
HLA	Patients	Controls	•	Correcto	ed `.
	(%)	(%),	χ2	, Р	RR*
B15	30	-14	9,34	0,035	2.61
B40	22	10	7.12	0.046	2.58
Cw3	28	- 14 -	7.07	0.047	2.34
Cw4	21	11	6.39	• NS	2.18
B8	29	22 .	1.50	· NS	1.47
B7	18	29	3.15	NS	0.55

*Value for relative risk

NS = not significant

Frequency of HLA-DR Antigens in Normal and Juvenile-Onset Diabetic Patient Populations

Antigen	Patients	Controls		Corrected	
	n = 62 (%)	n = 54 (%)	X2	Р	RR*
DR1	18	13	0.20	NS	1.47
DR2	18	38	5.12	NS	0.35
DR3	39	18	5.00	NS	2.84
DR4	44	18	7.53	0.043	3.47
DR5	5	9	0.92	NS	0.05
DR6	21	32	1.48	NS	0.55
DR7	18	27	1.05	NS	0.58
				•	

*Value for relative risk

NS = not significant

The existence of linkage disequilibrium between HLA-B and -C alleles and -DR specificities has been reported. In the present data we found a strong linkage disequilibrium between B8 and DR3 and between B7 and DR2 in the control population (Table 6). However, similar associations were not observed in the diabetics. On the other hand, an association between Cw3 and DR4 was observed in both groups. Also, B15 and Cw3 showed linkage disequilibrium in both populations (JOD, r = 0.25; controls, r = 0.51).

Alleles of the gene loci in the HLA region are well recognized to be associated with JOD. The data reported here raise important questions. One of these questions is which alleles of the HLA loci are most strongly associated with juvenile diabetes? Therefore, we attempted to characterize our results by Woolf's analysis. Table 7 compares the strength of the association of B40, -Cw3 and DR4 with JOD. The two groups of subjects were divided into B15-positive and B15-negative subgroups, and the incidences of B4O, Cw3 and DR4 were subsequently tabulated. It was observed that DR4 was significantly associated with the diabetogenic gene(s), whereas B40 and Cw3 were not. Both B15 and DR4 were significantly associated with the diabetogenic gene(s) when the two groups were divided into B40-positive and B40-negative subgroups and the incidences of B15, Cw3 and DR4 were evaluated (Table 8). DR4 was again significantly associated with the diabetogenic gene(s) when the two populations were first subdivided into Cw3-positive and Cw3negative subpopulations and the B15, B40 and DR4 frequencies subsequently tabulated (Table 9). Neither B15 nor B40 was significantly associated

Association Between HLA-DR and HLA-B and HLA-C Specificities in Normal and in Juvenile-Onset Diabetic Patient Populations*

		Patients	Controls
HĽA-DR2	HLA-B7	0.17	0.37
HLA-DR3	HLA-B8	0.19	0.63
HLA-DR4	HLA-Cw3	0.32	0.30

*The data are expressed as correlation of coefficient (r) values.

Chi-Square Test for an Association Between B40, Cw3 or DR4 and Juvenile-Onset Diabetes After Having Divided the Two Populations into B15-Positive and B15-Negative Subgroups

Antigens	Chi-Square	Homogeneity Chi-Square
B40	NS	0.1365 (p < 0.7114)
Cw3	NS ·	0.7118 (p < 0.4009)
DR4	6.99 (p < 0.0083)	0.0631 (p < 0.8026)

NS = Not significant.

Chi-Square Test for an Association Between B15, Cw3 or DR4 and Juvenile-Onset Diabetes After Having Divided Patients and Controls

into B40-Positive and B40-Negative Subgroups

Antigen	Chi-Square	Homogeneity Chi-Square
B15	5.479 (p < 0.0193)	0:1365 (p < 0.7114)
Cw3	NS	0.3450 (p < 0.5552)
DR4	7.1889 (p < 0.0042)	0.0783 (p < 0.7795)

NS = Not significant.

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Chi-Square Test for an Association Between B15, B40 or DR4 and Juvenile-Onset Diabetes After Having Divided Patients and Controls into Cw3-Positive and Cw3-Negative Subgroups

Antigen	Chi-Square	Homogeneity Chi-Square
	****	UNI-Square
B15	NS	0.7118 (p < 0.3789)
B40	NS	0.3450 . (p < 0.5552)
DR4	5.94 (p < 0.0147)	0.075 (p < 0.7872)

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NS = Not significant

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with the diabetogenic gene(s) in this analysis. When similar calculations were performed by first dividing the populations into DR4-positive and DR4-negative groups and then tabulating the incidences of B15, B40 and Cw3, only B15 was significant (Table 10).

Chi-Square Test for an Association Between B15, B40 or Cw3 and Juvenile-Onset Diabetes After Having Divided Patients and Controls

into DR4-Positive and DR4-Negative Subgroups

Antigen	Chi-Square	Homogeneity Chi-Square		
B15	3.85 (p < 0.05)	0.0631 (p < 0.8026)		
B40	NS _	0.0783 (p < 0.7795)		
Cw3	NS	0.0796 (p < 0.7795)		

NS = Not significant

IV: DISCUSSION

We have studied the distribution of HLA-A, -B, -C and -DR antigens in juvenile-onset diabetic patients. The present data show that the frequencies for HLA-B15, -B40, -Cw3 and -DR4 were significantly increased in the patient group as compared with the control population. Analysis of the data indicates that HLA-DR4 possesses the strongest association with JOD. The data suggest that HLA-DR4 has the primary association with the diabetogenic gene(s) and the associations of B15, B40 and Cw3 with the diabetogenic gene(s) are secondary.

The present data support earlier observations of an association of HLA-B15 and Cw3 with JOD (Cudworth and Woodrow, 1975; Ludwig *et al.*, 1976; Nerup *et al.*, 1974; Singal and Blajchman, 1973; Solow *et al.*, 1977a; Thomsen *et al.*, 1975). In relation to HLA-B8, although the data . suggest a slight increase in its frequency in the patient population, the difference found was not statistically significant. Similarly, the "protective association" of HLA-B7 with the absence of JOD as reported by Ludwig *et al.* (1976a) is not present in our data. In addition, the present data show a significant increase in the frequency of B40 in the patients as compared with controls.

The focus of our present work was the human B lymphocyte alloantigen locus, HLA-DR. The data presented here show a significantly higher incidence of HLA-DR4 in the patients. Also, the data show an

increase in the incidence of DR3 and a decrease in the incidence of DR2 in the patients. These data are in general agreement with our earlier observations (Solow *et al.*, 1977a, b) and those of other workers (Bodmer, J. *et al.*, 1977; Cattaneo *et al.*, 1977; Garovay *et al.*, 1977; Jeannet *et al.*, 1977; Mayer *et al.*, 1977; Suciu-Foca *et al.*, 1977). We reported a decrease in the frequency of locally defined B cell group 7, UKII, and Thorsby 2 (all comparable to DR2) and an increase in locally defined B cell group 4 (comparable to DR4 and DR7) and UKIII (comparable to DR3) in the JOD patients.

The presence of linkage disequilibrium between certain alleles of the HLA supergene have been reported and may account for either a primary or a secondary association of HLA alleles with a disease. It is known that HLA-B15. Cw3 and DR4 are in linkage disequilibrium and so are B40 and Cw3. Analysis of the present data by Woolf's method (1955) indicates that DR4 possesses the strongest association with JOD. It could be interpreted to mean that DR4 has the primary association with the diabetogenic gene(s) and the associations of B15, B40 and Cw3 with the diabetogenic gene(s) are secondary.

Nerup *et al.* (1974) presented evidence which indicated that the level of relative risk (RR) for developing JOD varied with respect to homogeneity and heterogeneity for HLA-B8 and -B15. They suggested that the level of RR associated with the presence of the diabetogenic HLA antigens increased as follows: B8/x or B15/x < B8/B8 or B15/B15 << B8/B15. Svejgaard *et al.*(1975) proposed a model which stated that two HLA-linked diabetogenic genes existed, one associated with the HLA-B8-Dw3 linkage

group and a second associated with the linkage group HLA-B15-Dw4. Svejgaard subsequently modified the model to comply with more current evidence to say that the diabetogenic genes were associated with HLA-DR3 and -DR4, respectively (Svejgaard *et al.*, 1980).

The heterogeneity of diabetes mellitus is widely accepted and as well that JOD itself is a heterogeneous disorder (Rotter and Rimoin, 1978). Rotter and Rimoin (1979) theorized the existence of three forms of JOD. Form 1 is the HLA-B8 (-DR3) associated autoimmune form which involves the diminishing presence of pancreatic islet-cell antibodies from the time of diagnosis. This form also demonstrates a greater incidence of microangiopathies. In general, the association of autoimmune diseases with the HLA-B8-DW3-DR3 linkage group has been examined and several disease associations have been established (McMichael and McDevitt, 1977). The second form is associated with B15 (DR4) and the patients with this form of disease tend to produce antibodies to insulin. Form 3 is a compound form in which both B8 (DR3) and B15 (DR4) are present.

Several theories have been proposed in an attempt to assign a genetic model to JOD susceptibility. Briefly, the first of these theories is based on a model involving two alleles at one locus - a diabetes susceptibility allele and a normal allele. This model varies with either recessive, dominant or intermediate modes of inheritance (Svejgaard *et al.*, 1980).

Rubenstein *et al.* (1977) and Thomsen *et al.* (1977) proposed a recessive model with incomplete penetrance. A dominant model with incomplete penetrance was proposed by Spielman *et al.* (1979). Both of these models

have recently been rejected by Svejgaard *et al.* (1980). They observed that the minimal 'diabetes' gene frequency was incompatible with these two models. These workers went on to propose an intermediate model of inheritance, however, it too had some shortcomings. The intermediate model was unable to account for an age-related distribution of DR3 and DR4. HLA-DR3 was found in equal frequency in all ages-at-onset groups. The increased relative risk for the DR3, DR4 heterozygote was also found to be higher than for DR3 / DR3 and DR4 / DR4 homozygotes. This intermediate model is also incompatible with differences in relative risks between HLA-DR3, -DR4 heterozygotes and DR3/DR3 or DR4/DR4 homozygotes (Svejgaard *et al.*, 1980).

The next level of complexity in genetic models involves a three allele model at one locus - two susceptibility alleles and one normal allele. Hodge *et al.* (1980) have presented such a model which appears suitable to the available data. Their model is able to account for various penetrance modes associated with the different HLA allele combinations previously mentioned.

The work presented in this thesis supports the concept of two modes of disease susceptibility - the HLA-DR3 allele associated with one mode and the DR4 allele associated with another mode. The results indicated that although both DR3 and DR4 were present in increased frequency in the JOD population, only DR4 was significantly increased. This could be explained by the fact that our JOD patient population belonged to a diabetic clinic which consisted of younger patients. On the other hand, other investigators who found an increase in both B8-DR3

and B15-DR4 had studied a mixed population. Similarly, it would appear that as a result of the support presented here to the two gene theory of diabetes susceptibility, that the genetic model of three alleles at one locus (Hodge *et al.*, 1980) would also be a more logical model to accept.

Future work in this area should emphasize better genetic definition of JOD. Although the heterogeneity of this disorder is well known and several genetic models have been proposed, the attempt to fit a unitarian, simplistic model of HLA association may not be appropriate. Therefore, it remains to refine the definition of the association of HLA alleles with JOD and this could be accomplished by better genetic and pathogenetic definitions of JOD.

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APPENDIX

The results presented in this thesis have been published under separate cover. Copies of the papers are included here.

It is with the approval of the senior author, Dr. D.P. Singal, that the papers are included in this thesis. As well, it is recognized by Dr. Singal that the author of this thesis was the major contributor to the published manuscripts.

APPENDIX

HLA-A,B,C and B-Lymphocyte Alloantigens in **Insulin-Dependent Diabetes**

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H. Solow, R. Hidalgo, M. Blajchman, and D. P. Singal

RESULTS

TUDIES of the HLA supergene have S identified significant associations between HLA-B alleles and insulin-dependent diabetes. Our findings of an association between Bw15 and insulin-dependent diabetes have been confirmed by other investigators¹⁴ In addition, antigens B8. Cw3, and Dw3 have been found in significantly higher frequency in this disorder.24 In conjunction with these increases, Ludwig et al demonstrated a statistically significant decrease in the frequency of HLA-B7⁴

Evidence has been presented that demonstrates the presence of genetically determined B-cell alloantigens linked to, but distinct from, those of the HLA system. The objectives of the present investigation were to study whether these associations of HLA-B alleles with diabetes were due to a close linkage of HLA-B alleles with B-lymphocyte alloantigens.

MATERIALS AND METHODS

Sixty one unrelated white patients with insulindependent diabetes were typed for HLA and B-cell alloantigens. The results were compared to a control population of 55 healthy unrelated white adults from the same geographic region-

The HLA typing was performed on peripheral blood lymphocytes by the microdroplet lymphocyte cytotoxicity test of Terasaki

The B-cell alloantigen typing was performed on a B-cell-enriched lymphocyte population by the techinque described elsewhere? Fight B-cell alloantigen groups are presently recognized in our laboratory

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The phenotype frequencies of HLA-B7, B8, and Bw15 in insulin-dependent diabetic patients and in control populations are given in Table 1A. In order to diminish the probability of committing a type II error, we combined the present data with that from our previously published report (Table 1B) ' The frequency of Bw15 in the patients (28°_{σ}) was higher than that in the control population (11°,) in the present study Although the frequency of Bw15 in the respective pooled patient and control populations remained essentially the same as in the present data, the increased frequency of Bw15 in patients was statistically significant (corrected p < 0.001) The combined relative risk of developing insulin-dependent diabetes associated with the presence of this antigen was calculated to be 3.7 times greater than in its absence. B8 was more trequent in patients (29%) as compared to normal controls (15°_{a}) in the present series However, no differences were observed in the incidence of B8 in patient and control groups in the pooled data. The incidence of B7 was lower in the patients (15°) than in the normal controls (31°,) However, no differences were observed in the phenotype frequency of B7 in patients and control populations in the combined data -

The incidence of HLA-Bw40 was increased in patients in the present series. However, this increase was not statistically significant. No differences were observed in incidence of Bw40 in patient and control groups in the combined data

The frequencies of antigens belonging to the HLAsC locus were increased in patients for Cw1 (23^a, patients versus 9^a, controls) and for Cw3 (28°, patients versus 19°, controfs). Antigen Cw4 was decreased in the patient group (6°, patients versus 19°, con-

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		Insulin Dependent			
	Antigen	Diabetes ≮ ∈ (n.⇔ól)	Controls (n = 55)	x ²	Relative Risk
A Present data	Ŷ	,			
	Bw 15	28	11	4 18	3,16
	88	29	15	289	2.46
	87	15	31	3 36	0 39
8 Combined data*		(n = 111)	(n = 200)		
	Bw 15	32	11	16 42	374
	88	26	24	0 23	111
	87	17	17	00	, 1.00

Table 1. Incidence (%) of Certain MLA-B Antigens in Insulin-Dependent Diabetes and Control Population

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*Present data plus data from Singal and Blajchman¹

trob) The corrected p value in all instances was greater than 0.50

No differences were observed in the frequencies of the remaining HLA antigens in insulin-dependent diabetic patients as compared to control population,

Phenotype frequencies for B-lymphocyte alloantigens in patient and control populations are given in Fig. 1. It is evident that the frequency of antigen 4 was increased in insulin-dependent diabetic patients (35°_{o}) as compared to its frequency in controls



Fig. 1. Phenotype frequencies of B-lymphocyte alloantigens in insulin-dependent diabetic patients (IDD) and in normal controls.

 (20°_{o}) . On the other hand, B-cell specificity 7 was decreased in patients (23°_{o}) patients versus 33°_{o} controls). These differences in the incidence of these antigens were not statistically significant

The B-lymphocyte alloantigens did not show any significant association with antigens of the HLA system in the present data.

DISCUSSION

Our data demonstrate a strong association of HLA-Bw15 with insulin-dependent diabetes. This association is stronger than the association of B8 and Cw3 with this disease. The analyses of results presented here and those of other investigators suggest that there may be differences in various patient populations studied. These data emphasize the possibility of two genes being involved in the etiology of insulin-dependent diabetes, one linked to B8 and the other to Bw15. This is supported by a considerable additive (overdominance) effect of B8 and Bw15 on the relative risk for insulin-dependent diabetes. Also, the increased frequency of Bw15 in insulin-dependent diabetes but not in other investigated organ-specific autoimmune diseases suggests that a gene associated with Bw15 might have a different function²

No differences in the frequency of B-lymphocyte alloantigens in insulin-dependent diabetes and in control population were found in the present investigation. The analysis of results did not show any signifiHLA-A,B,C AND B-LYMPHOCYTE ALLOANTIGENS IN DIABETES

cant association of B-lymphocyte antigens with those of the HLA system.

The present data indicate that the HLA-B alleles have a closer association with insulin-dependent diabetes than the B-cell alloantigens. We are grateful to Dr. W. Spaulding, Director of the Diabetic Day Care Unit, McMaster University Medical Centre, for allowing us to study his patients, and to Sarah Joseph for excellent technical assistance and Mary Fuhringer for secretarial assistance.

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REFERENCES

J Singal DP, Blajchman MA Diabetes 22 429, 1973

2 Thomsen M, Platz P, Ortved Andersen O, et al Transplant Rev 22 125, 1975

3 Nerup J, Platz P, Ortved Andersen O, et al Lancet 2 864, 1974

4 Ludwig H, Schernthaner G, Mayr W N Engl J Med 294 1066, 1976

5 Singal DP, Naipaul N, Hidalgo R. In Ray JG, Hare DB, Pederson PD, et al (eds). NIAID Manual of Tissue Typing Techniques. DHEW Publication No-(NIH) 76-545, 1976, p. 126

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APPENDIX

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Juvenile-onset Diabetes: HLA-A, -B, -C, and -DR Alloantigens

H SOLOW R HIDALGO, AND D P SINGAL

SUMMARY

We studied the distribution of HLA-A, -B, and -C antigens in 94 juvenile-onset diabetic patients and of HLA-DR antigens in 62 of these patients. The frequencies for HLA-B15, -B40, and -Cw3 were significantly increased in the patient group as compared with the control group. With respect to the B-cell specificities, DRw4 was significantly increased in the patients. Analysis of the data to detect the possible presence of primary and secondary associations between HLA alleles and diabetogenic gene(s) indicated that DRw4 possessed a primary association with the diabetogenic gene(s). As a result, B15, B40, and Cw3 possessed secondary associations, DIABETES 28:1-4, January 1979.

uvenile-onset diabetes is a disease of dramatic onset and, often, fatal outcome. It results from disturbances in the normal insulin and carbohydrate metabolism ¹ Several hypotheses have been proposed to explain the mechanism(s) of abnormal insulin metabolism, for example, viral infection and autoimmune processes could impair insulin production by destroying the islet cells of the pancreas.² The latter have been suggested by the demonstration of autoantibodies reactive with islet cells ³

The involvement of the major histocompatibility complex (MHC) in controlling immunologic responses has been considered. Evidence obtained in the mouse MHC, the H-2 gene complex, suggested that an area referred to as the immune (I) region is involved in the immunologic responses of the mouse to single synthetic antigens.⁴ Also, the H-2^k phenotype, in the mouse, is positively associated with susceptibility to Gross leukemia virus and negatively to Tennant leukemia virus.⁵ In recent years evidence has accumulated that the immune response to ordinary complex antigens and the susceptibility of laboratory animals to experiment-

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ally induced diseases, such as allergic thyroiditis⁶ or allergic encephalomyelitis,⁷ is also under the control of genes closely linked with the histocompatibility locus. The genes thought to control these responses have been labeled immune response (Ir) genes and their products, immune-associated (Ia) antigens.

The human counterpart to the mouse la antigens has been studied independently by many workers.⁸ Recently, the locus controlling the human la antigens was defined as the HLA-DR locus, and seven alleles were described DRw1, DRw2, DRw3, DRw4, DRw5, DRw6, and DRw7 * The DR antigens have been serologically detected an B lymphocytes, macrophages, sperm, and epithelial tissue ¹⁰⁻¹² Functionally the DR antigens have been hypothesized to be antigen receptors, cell-cell interaction components, or selfrecognition determinants ¹³

The HLA-DR locus is a part of the HLA supergene, which is located on the short arm of chromosome 6 ¹⁴ At the present time, four other highly polymorphic loci (A, B, C, and D) are recognized ¹⁵ These loci, although closely linked, are distinct from each other. The gene products of the A, B, and C loci are glycoprotein structures that are serologically detectable in a cytotoxicity assay. The D-locus gene products control lymphocyte responses, which are assayed in vitro by measuring the proliferative response of one person's lymphocytes to those of a second person incompatible at that locus. This procedure is called a mixed lymphocyte culture test (MLC). Recent advances have suggested that the D-locus gene products are also serologically detectable.¹⁶

During the past few years the studies of the HLA supergene have demonstrated an association between antigens HLA-B8, -B15, -Cw3, and -Dw3 and juvenile-onset diabetes (JOD) ¹⁷⁻²² The association of B-cell alloantigens defined in our laboratory and JOD has been previously reported ²² B-cell group 4 was increased and group 7 was decreased in the patients as compared with control subjects

Presently we report the results of typing for the HLA-DR antigens in a larger series of Caucasian, juvenile-onset insulin-dependent diabetic patients than previously Also the HILAA B C AND DRAULOANTIGENSING/ABRES.

TABLE 1

Frequency of HLA-B15, -B40, -B8, -B7, -Cw3, and -Cw4 in normal and juvenile-onset diabetic patient populations

HLA antigen	Patients n = 94 (%),	Controls n = 200 → (%)	X²	Corrected P	RR*
B15	30	14	9 34	0 035	2 61
B40	22	10	11,77	0.012	2 58
Cw3	28	14	7.07	0 047 ·	2 34
Cw4	21	* 11	6 39	. NS	2 18
B8	29	22	1 50	NS	1 47
87	18	29	3 15	NS	0 55

* Value for relative risk

DR antigen specificities were defined on the basis of cytotoxicity using the antisera of the Seventh International Histocompatibility Workshop. We found the incidence of HLA-DRw4 to be increased significantly in the diabetic group. DRw3 showed an increase in the diabetics and DRw2 was decreased in the diabetics, however, neither difference was statistically significant. In also studying the HLA-A, -B, and -C alloantigen frequencies, we found B15, B40, and Cw3 to be significantly increased in the patients. Further analyses of our data showed that the primary association of the MHC alleles with JOD was with HLA-DRw4 and that the associations with B15, B40, and Cw3 were secondary.

MATERIALS AND METHODS

Ninety-four unrelated Caucasians with juvenile-onset, insulin-dependent diabetes were typed for HLA-A, -B, and -C antigens. Sixty-two of these patients were also typed for HLA-DR antigens. The results were compared with 200 unrelated adult Caucasians-typed for HLA-A, -B, and -C antigens and with 54 subjects of the control population typed for HLA-DR specificities.

The HLA-A, -B, and -C typing was performed on peripheral blood lymphocytes by the microdroplet lymphocyte cytotoxicity test.²³ The patients and the controls were typed for the following HLA-A, -B, and -C antigens. A1, A2, A3, A9, Aw23(9), Aw24(9), A10, A25(10), A26(10), A11, A19, A28, A29, Aw30, Aw31, Aw32, B5, B7, B8, B12, B13, B14, B15, B17, B18, B27, B37, B40, Bw16, Bw38(16), Bw39(16), Bw21, Bw22, Bw35, Cw1, Cw2, Cw3, Cw4, and Cw5

The HLA-DR typing was performed on a B-cell-enriched lymphocyte population we have previously described ²⁴ DR specificities were defined on the basis of reactivity with

TABLE 2 Frequency of HLA-DR antigens in normal and juvenile-onset diabetic patient populations

Antigen		Patients n ≠ 62 (%)	Controls n = 54 (%)	۱ ۲ ^۲	Corrected P	RR
DRw1		18	13	3 80	NS	1 47
DRw2		18	38	5 12	NS	0 35
DRw3		39	18	5 00	NS	2 84
DRw4	-	44 . •	· 18	7 53	0 043	3 47
DRw5	-	5	9	0 92	NS	0 05
DRw6		21	32 [,]	1 48	NS	0 55
DRw7	·	18	27	1 05	· NS	0 58
*						

RR, relative risk

sera in the Seventh International Histocompatibility Workshop.

The frequency distributions of the HLA-A, -B, -C, and -DR antigens in diabetics were compared with those in control subjects. An approximate test of significance was used to test the differences.²⁵ The data were analyzed for linkage disequilibrium between alleles of the DR locus with those of the B and C loci. The correlation coefficient values (*r*) were calculated using the method referred to by Colton ²⁵ To test the possibility of primary and secondary associations of the HLA-B, -C, and -DR alleles with JOD the two populations were subdivided into groups of antigen-positive and antigen-negative subgroups. The frequency distributions of the remaining antigens were subsequently analyzed in these subgroups using the method described by Woolf.²⁶

RESULTS

The frequencies of the HLA-A, -B, and -C antigens in JOD were compared with those in the control population. The frequencies for some of these antigens in these two populations are given in Table 1. The incidence of B15 was significantly increased ($X^2 = 9.34$, corrected P = 0.035, relative risk (RR) = 2.61) in the JOD group (30%) as compared with the controls (14%) Similarly, the incidence of Cw3 was significantly higher ($X^2 = 7.07$, corrected P = 0 047, RR = 2 34) in the patients (28%) than in the control subjects (14%). The incidences of B8 and Cw4 were higher in the patients than in the controls, the increases, however, were not statistically significant. Similarly, the decrease in the incidence of B7 in the JOD group was not significant In addition, the present data show a significant increase $(X^2 = 11.77, \text{ corrected } P = 0.012, RR = 2.58)$ in the frequency of 840 in the patients (22%) as compared with the controls (10%) No difference in the incidence of B18 was observed in JOD (10%) and controls (9%). The remaining HLA-A, -B, and -C alleles did not demonstrate any significant differences in their frequencies between the patient and the control populations

The incidences of HLA-DR specificities in JOD and control populations are given in Table 2. The incidence of DRw4 was significantly increased ($X^2 = 7.53$, corrected P = 0.043, RR = 3.47) in the diabetics (44%) as compared with control subjects (18%). However, an increase in the frequency of DRw3 and a decrease in the incidence of DRw2 in the patients were not statistically significant.

The existence of linkage disequilibrium between HLA-B and -C alleles and HLA-DR specificities has been reported " In the present data we found a strong association between B8 and DRw3 and between B7 and DRw2 in the control group (Table 3) These associations were not observed in the diabetics. On the other hand, an association between

TABLE 3
Association between HLA-DR and HLA-B and -C specificities
in normal and in juvenile-onset diabetic patient populations*

		Patients	Controls
HLA-DRw2	HLA-B7	0 17	0 37
HLA-DRw3	HLA-B8	0 19	0 63
HLA-DRw4	HLA-Cw3	0 32	0 30

* The data are expressed as correlation coefficient (r) values

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Cw3 and DRw4 was observed in both groups Also, B15 and Cw3 showed linkage disequilibrium in both populations (JOD, r = 0.25, controls, r = 0.51)

Alleles of the gene loci in the HLA region are now well recognized to be associated with JOD. The data in the present report raise important questions. One of these questions is which alleles of the HLA loci are most strongly associated with juvenile diabetes. Therefore, we attempted to characterize our results by Woolf's analysis, as has been done for diabetes and celiac disease²⁷ Table 4 compares the strength of the association of B40, Cw3, and DRw4 with JOD The two groups of subjects were divided into B15-positive and B15-negative subgroups, and the incidences of B40, Cw3, and DRw4 were subsequently tabulated. It was observed that DRw4 was significantly associated with the diabetogenic gene(s), whereas B40 and Cw3 were not. Both B15 and DRw4 were significantly associated with the diabetogenic gene(s) when the two groups were divided into B40-positive and B40-negative subgroups and the incidences of B15, Cw3, and DRw4 were evaluated (Table 5) DRw4 was again significantly associated with the diabetogenic gene(s) when the two populations were first subdivided into Cw3-positive and Cw3-negative subpopulations and the B15, B40, and DRw4 frequencies subsequently tabulated (Table 6) Neither B15 nor B40 was significantly associated with the diabetogenic gene(s) in this analysis: When similar calculations were performed by first dividing the two populations into DRw4-positive and DRw4-negative groups and then tabulating the incidences of B15, B40, and Cw3, only B15 was significant (Table 7)

TABLE 4

Chi-square test for an association between B40, Cw3, or DRw4 and juvenile-onset diabetes after having divided the two populations into B15-positive and B15-negative subgroups

e	
Chi-square	Homogeneity Chi-square
NS	0 1365 (P < 0 7114)
, NS	0 7118 (P < 0 4009)
6 99 (P < 0 0083)	0 0631 (P < 0 8026)
	Chi-square NS NS 6 99 (P < 0 0083)

DISCUSSION

The present data support earlier observations of an association of HLA-B15 and -Cw3 with juvenile-onset diabetes.^{17-22,28,29} In relation to B8 although the data suggest a slight increase in its frequency in the patient population, the difference found was not statistically significant. Similarly the "protective association" of B7 with the absence of JOD as reported by Ludwig et al.²¹ is not present in our data. In addition, the present data show a significant increase in the frequency of B40 in the patients as compared with controls

The focus of our current work was the human B lymphocyte alloantigen locus, HLA-DR. The data presented here show a significantly higher incidence of HLA-DRw4 in the patients. Also the data show an increase in the incidence of DRw3 and a decrease in the incidence of DRw2 in the patients.

TABLE 5

Chi-square test for an association between B15, Cw3, or DRw4 and juvenile-onset diabetes after having divided the patients and the controls into B40-positive and B40-negative subgroups

Antigen	Chi-square	Homogeneity chi-square
B15	5 479 (P < 0 0193)	0 1365 (P < 0 71 14)
Cw3	NS	0 3450 (P < 0 5552)
DRw4	7 1889 (P < 0 0042)	0 0783 (P < 0 7795)

These data are in general agreement with our earlier observations^{22,23} and those of other workers.^{32–37} We reported a decrease in the frequency of locally defined B cell group 7, UK II, and Thorsby 2 (all comparable to DRw2) and an increase in the frequency of locally defined B cell group 4 (comparable to DRw4 and DRw7) and UK III (comparable to DRw3) in the JOD patients.

The presence of linkage disequilibrium between certain alleles of the HLA supergene have been reported⁹ and may account for either a primary or a secondary association of HLA alleles with a disease. It is known that HLA-B15, -Cw3, and -DRw4 are in linkage disequilibrium and so are B40 and Cw3^{9,30} Analysis of the present data by Woolf's method²⁵ indicates that DRw4 possesses the strongest association with JOD. It could be interpreted to mean that DRw4 has the primary association with the diabetogenic gene(s) and the associations of B15, B40, and Cw3 with the diabetogenic gene(s) are secondary.

The numerous reports of HLA associations with JOD can be divided into two groups On one side are those who feel that JOD is associated with a single recessive gene and that any HLA association(s) found is with this recessive gene.³¹ On the other side are those that feel there are two diabetogenic genes and two different HLA associations, one with B8, Dw3, and DRw3 and the other with B15, CW3, and DRw4 The present data extend the two-genehypothesis concept in the etiology of juvenile-onset diabetes¹⁹ in that the one diabetogenic gene is associated with DRw4 and the second with Dw3, and the latter possibly more strongly with DRw3

It seems likely from the evidence presented here that a major factor determining susceptibility to juvenile-onset diabetes is the presence of a diabetogenic gene or genes at

TABLE 6

Chi-square test for an association between B15, B40, or DRw4 and juvenile-onset diabetes after having divided the patients and controls into Cw3-positive and Cw3-negative subgroups

Antigen	· Chi-square	'Hômogeneity Chi-square
B 15	NS	0 7118 (P <.0 3789)
B 40	NS	0 3450 (P < 0 5552)
DRw4	, 5 94 (P < 0.0147)	0 075 (P < 0.7872)

TABLE 7

Chi-square test for an association between B15, B40, or Cw3 and juvenile-onset diabetes after having divided the patients

and controls into DRw4-positive and DRw4-negative subgroups

	~	Homogeneity
Antigen	Chi-square	Chi-square
-B15	3 85 · (P < 0 05)	0 063 i (P < 0.8026)
· B40	NS	· 0 0783 (P < 0 7795)
Cw3	NS	0 0796 (P < 0 7795)

a locus closely linked to the HLA chromosomal region. The mechanism of the association between HLA and insulindependent diabetes remains speculative and has been discussed elsewhere 19

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REFERENCES

Bondy P.K. Diabetes mellitus Disorders of carbohydrate metabolism In Cecil-Loeb Textbook of Medicine, Beeson, P1 and McDermott, W1

Eds Philadelphia, W B Saunders, 1968, p. 1175 ² Cudworth A. White G. Woodrow, J. et al. Aetiology of juvenile-onset diabetes. Lancet 1 385–88, 1977

Irvine, W., McCallum, C. Gray, R., et al. Pancreatic işlet cell antibodies in diabetes mellitus correlated with duration and type of diabetes, coexistent autoimmune disease and HLA type Diabetes 26 138-47, 1977

Benacerral B, and McDevitt, H. Histocompatibility-linked immune response ganes Science (Wash DC) 175 273-79, 1972

'Tennant J R Susceptibility and resistance to viral leukogenesis in the mouse II Response to the virus relative to histocompatibility factors carried by the prospective host J Natl Cancer Inst 34 633-41, 1965 "Vladuliu A O, and Rose, N R Autoimmune murine thyroiditis

relationship to histocompatibility (H-2) type_Science (Wash D.C.) 174 1137-39 1971

7 Gasser D. Newbin C., Palm J. et al. Genetic control of susceptibility to experimental allergic encephalomyelitis in rats. Science (Wash, D,C.). 181 872-73 1973

*Arbeit, R. Sachs D. Amos B., et al. Human lymphocyte alloantigen(s) similar to murine Ir region-associated (Ia) antigens, J. Immunol. 115

1173-75 1975 * "Bodmer W F Joint report of the Seventh International Histocompatibility Workshop In Histocompatibility Testing 1977 Bodmer W F. Batchelor, J. R. Bodmer, J. G., et al., Eds. Copenhagen, Munksgaard, 1978, pp. 351-57

Winchester, R. Fu, S., Wernet P. et al. Recognition by pregnancy

serums of non-HLA alloantigens selectively expressed on B lymphocytes J Exp Med 141 924-29, 1975 "Rowden, G , Lewis, M , and Sullivan, A la antigen expression on

human epidermal Langerhans cells Nature (London) 268 247-48, 1977. ¹⁸ Kamoun, M., Wiels, J., Lepage, V., et al. Presence of la equivalent

on human spermatozoa detected by specific reagents prepared by the absorption-elution technique on B cell lymphoid lines or PHA stimulated cells Tissue Antigens 10 245, 1977 (Abstr.)

"Rosenberg L, and Kidd, K HLA and disease susceptibility a primer N Engl J Med 297 864-66, 1977

¹¹Francke, U., and Pellegrino, M. Assignment of the major histocompatibility complex to a region of the short arm of human chromosome 6 Proc Natl Acad Sci USA 74 1147-51, 1977 ¹⁵Nomenclature for Factors of the HLA System Tissue Antigens 11

81-86, 1978

⁶¹ van Rood, J. van Leeuwen, A. Termijtelen, A. et al. Recognition of HLA-D by serology Tissue Antigens 10 125, 1977 (Abstr.)
 ¹⁷ Singal, D.P., and Blajchman, M. Histocompatibility (HL-A) antigens

lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus Diabetes 22 429-32, 1973

**Nerup, J., Platz P., Onved-Anderson, O., et al, HLA antigens and diabetes mellitus Lancet 2 864-66 1974

"Thomsen M Platz, P Ortved-Anderson O, et al MLC lyping in juvenile diabetes and idiopathic Addison's disease. Transplant Rev 22 125-47, 1975. ²⁹ Cudworth, A., and Woodrow, J. HLA system and diabetes mellitus.

Diabetes 24 345-49 1975

²¹ Ludwig, H., Schernthaner, G., and Mayr, W. Is HLA-B7 a marker associated with a protective gene in juvenile-onset diabetes mellitus? N Engl J Med 294 1066, 1976

** Solow, H., Hidalgo, R., Blajchman, M., et al., HLA-A, B. C and B. mphocyte alloantigens in insulin dependent diabetes. Transplant Proc 9 1859-61, 1977

23 Mittal, K., Mickey, M., Singal, D., et al., Serotyping for homotransplantation XVIII Refinement of microdroplet lymphocyte cytotoxicity test Transplantation 6 913-27 1968

24 Singal, D., Naipaut, N., and Hidalgo R. In NIAID Manual of Tissue Typing Techniques Ray J., Hare, D., Pederson, P., et al. DHEW publication no (NIH) 76-545, 1976 pp 126-29

23 Colton, T. Statistics in Medicine, Boston, Little, Brown & Co., 1974 24 Woolf, B. On estimating the relation between blood group and dis-

ease Ann Human Genet 19 251-53, 1955 27 Keuning, J., Pena, A., van Hooff, J., et al. HLA-DW3 associated

with coeliac disease Lancet 7 506-08, 1976 2* Solow, H., Hidalgo, R., and Singal, D. Insulin dependent diabetes

HLA-A B. C and B cell alloantigens Tissue Antigens 10 195 1977 (Abstr.) ** Schernthaner G. Ludwig H. and Mayr W. Juvenile diabetes meltitus HLA-antigen-frequencies dependent on the age of onset of the disease J Immunogenet 3 117-21, 1976

¹⁰ Bodmer, J., Curtoni, E., van Leeuwen, A., et al., The A.B.C. of HLA In Histocompatibility Testing 1975, Kissmeyer-Nielsen, F. Ed 1975, pp

21-29 ³¹ Rubinstein, P., Suciu-Foca, N., and Nicholson, J. Genetics of juvenife diabetes mellitus. A recessive gene closely linked to HLA-D and with

50 per cent penetrance N Engl J Med 297 1036-40, 1977 ³⁸ Mayr W R, Schernthaner G, Ludwig H, et al. la type alloantigens in insulin-dependent diabetes mellitus. Tissue Antigens 10, 194, 1977. (Abstr.) 33 Jeannet, M. Ralfoux, C., de Meerlosse, P. et al. HLA and Ia-liké

antigens in juvenile-onset diabetes. Tissue Antigens 10:196, 1977. (Abstr.) 34 Cattaneo, R. Busetto, F., Mazzoli, M. et al. Associations between

A B, C and la antigens with juvenile onset diabetes (JOD) Tissue Antigens 10 196, 1977 (Abstr.) ³⁵ Bodmer, J. Mann, J. Hill, A., et al. The association of la antigens ³⁶ Bodmer, J. Mann, J. Hill, A., et al. The association of la antigens 10

with juvenile onset diabetes and rheumatoid arthritis. Tissue Antigens 10

197, 1977 (Abstr) ¹⁹ Sucu-Foca N, Rubenstein P, Susinno E, et al. la typing of J D M. families Tissue Antigens 10, 199, 1977 (Abstr.) ¹⁷ Garovay, M, R, Carpenter C, Reddish M, et al. La specificities ¹⁹ Garovay, M, R, Carpenter C, Reddish M, et al. La specificities ¹⁰ Successful to the balance mellitus. Tissue Antigens, 10, 200, 1977

associated with juvenite diabetes mellitus. Tissue Antigens 10 200, 1977 (Abstr)

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BIBLIOGRAPHY

ž

∽.	· ·
	Allen, Jr. F.: Linkage of HLA and GBC. Vox. Sang. <u>27</u> : 382-384, 1974.
~	Allison, J., Pelligrino, M., Ferrone, S., Callahan, G. and Reisfeld, R.: Biologic and chemical characterization of HLA antigens in human serum. J. Immunol. <u>118</u> : 1004-1009, 1977.
,	Albrechtsen, D.: HLA-D-associated "Ia-like" antigens on human macrophages. Scand. J. Immunol. 6: 907-912, 1977.
	Albrechtsen, D., Solheim, B. and Thorsby, E.: Human Ia-like antigens associated with HLA-D. Scand. J. Immunol. <u>6</u> : 419-425, 1977a.
	Albrechtsen, D., Solheim, B. and Thorsby, E.: Serological identification of 5 HLA-D associated (Ia-like) determinants. Tissue Antigens <u>9</u> : 153-162, 1977b.
	Albrechtsen, D., Solheim, B. and Thorsby, E.: Relationship of HLA-D associated B lymphocyte (Ia-like) antigens in the cell membrane. Tissue Antigens <u>11</u> : 372-383, 1978.
	Bach, F.H., Gose, J.E., Alter, B.J., Sondel, P.M. and Bach, M.L.: Past, present and future aspects of histocompatibility. Transplant. Proc. XI(1): 1207-1211, 1979
•	Bach, F., and Hirschhorn, K.: Lymphocyte interaction: A potential histocompatibility test <i>in vitro</i> . Science 143: 813-814, 1964.
	Bagdade, J., Stewart, M. and Walters, E.: Impaired granulocyte adherence. A reversible defect in host defense in patients with poorly controlled diabetes. Diabetes <u>27</u> : 677-681, 1978.
	Bain, B., Vas, M. and Lowenstein, L.: The development of large immature mononuclear cells in MLC. Blood <u>23</u> : 108-116, 1964.
	Barbosa, J., King, R., Goetz, F., Noreen, H. and Yunis, E.: HLA in maturity-onset type of hyperglycemia in the young. Arch. Intern. Med. <u>138</u> : 90-93, 1978.

Barnstable, C., Jones, E. and Crumpton, M.: Isolation, structure and genetics of HLA-A, -B, -C and -DR2 (Ia) antigens. Br. Med. Bull. 34: 241-246, 1978.

60

Benacerraf, B. and McDevitt, H.: Histocompatibility linked immune response genes. Science 175: 273-279, 1972.

Bergholtz, B., Thoresen, A. and Thorsby, E.: HLA-D/DR restriction of macrophage-dependent antigen activation of immune T lymphocytes: crossreacting allogeneic HLA-D/DR may partly substitute for self HLA-D/DR. Scand. J. Immunol. 11: 541-548, 1980.

Bergholtz, B. and Thorsby, E.: HLA-D restriction of the macrophage-dependent response of immune human T lymphocytes to PPD *in vitro*. Inhibition of anti-HLA-DR antisera. Scand. J. Immunol. 8: 63-73, 1978.

- Bergholtz, B. and Thorsby, E.: HLA-D restricted antigen activation of sensitized T lymphocytes: studies on the ability of HLA-D/DR expressing B lymphocytes to substitute for macrophages in antigen activation. Scand. J. Immunol. 10: 267-274, 1979.
- Bertrams, J., Baur, M., Gruneklee, D. and Gries, F.: Association of BfF₁, HLA-B18 and insulin-dependent diabetes mellitus. Lancet 2: 98, 1979.
- Bertrams, J., Jansen, F., Gruneklee, D., Reis, H., Drost, H., Beyer, J., Gries, F. and Kuuert, E.: HLA antigens and immunoresponsiveness to insulin-dependent diabetes mellitus. Tissue Antigens 8: 13-19, 1976.
- Bias, W. and Chase, G.: Genetic implications of HLA and disease associations. Transplant. Proc. 9: 531-533, 1977.
- Billing, R., Rafizadeh, B., Drew, I., Hartman, G., Gale, R. and Terasaki, P.: Human B-lymphocyte antigens expressed by lymphocytic and myelocytic leukemia cells. I. Detection by rabbit antisera. J. Exp. Med. <u>144</u>: 167-178, 1976a.
- Billing, R., Safani, M. and Peterson, P.: Isolation and characterization of human B cell alloantigens. J. Immunol. 117: 1589-1593, 1976b.

Bodmer, J.: Ia antigens. Definition of the HLA-DRw specificities. Br. Med. Bull. 34: 233-240, 1978.

Bodmer, J., Mann, J. and Hill, A.: The association of Ia antigens with juvenile-onset diabetes and rheumatoid arthritis. Tissue Antigens 10: 197, 1977 (Abstract).

Bodmer, W.: The HLA system: Introduction. Br. Med. Bull. 34: 213-216, 1978.

Bodmer, W., Cann, H. and Piazza, A.: Differential genetic variability among polymorphisms as an indicator of natural selection. IN: Histocompatibility Testing, (ed. J. Dausset and J. Colombami) Munksgaard, Copenhagen, pp. 753-757, 1973. Bodmer, W., Jones, E., Yound, D., Goodfellow, P., Bodmer, J., Dick, H. and Steel, C.: Serology of human Ia type antigens detected on lymohoid cell lines: An analysis of the VI workshop sera. IN: Histocompatibility Testing, (ed., F. Kissmeyer-Nielsen) Munksgaard, Copenhagen, pp. 677-684, 1975.

Bonderik, H., Albrechsten, D., Solheim, B. and Thorsby, E.: Dissociated antibody inhibition of responding and stimulating cells in one-way mixed lymphocyte culture (MLC). IN: Histocompatibility Testing, (ed. F. Kissmeyer-Nielsen), Munksgaard, Copenhagen, pp. 604-607, 1975.

- Bottazzo, G., Mann, J., Thorogood, M., Baum, J. and Doniach, D.: Autoimmunity in juvenile diabetics and their families. Br. Med. J. <u>2</u>: 165-168, 1978.
- Boyum, A.: Isolation of mononuclear cells and granulocytes from blood. Scand. J. Immunol. 21: 77-89, 1968.
- Breuning, M., van den Berg-Loosen, E., Bernini, L., Bijlsma, J., van Loghem, E., Khan, P. and Nijenhuis, L.: Localization of HLA on the short arm of chromosome 6. Human Genet. 37: 131-139, 1977.
- Briggs, B., Jackson, W., DuToit, E. and Botha, M.: The histocompatibility (HLA) antigen distribution in diabetes in Southern African Blacks (Xhosa). Diabetes 2: 68-71, 1980.
- Caffrey, M. and Jarnes, D.: Human lymphocyte antigen association in ankylosing spondylitis. Nature 242: 121, 1973.
- Cahill, Jr., G.: Diabetes mellitus: A brief overview. The Johns Hopkins Med. J., 143: 155-159, 1978.
- Cattaneo, R., Busetto, F., Mazzoli, M. and Bosco, P.: Associations between A,B,C and Ia antigens with juvenile onset diabetes (JOD). Tissue Antigens 10: 196, 1977 (Abstract).
- Ceppellini, R., Bonnard, G., Coppo, F., Miggiano, V., Pospisil, M., Curtoni, E. and Pelligrino, M.: MLC'C and HL-A antigens. II. Inhibition by anti-HLA sera. Transplant. Proc. 3: 63-70, 1971.

Champsaur, H., Dussaix; E., Samolyk, D., Fabre, M., Bach, C. and Assan, R.: Diabetes and Coxsackie virus B5 infection. Lancet i: 251, 1980.

Colton, T.: Statistics in Medicine, Little, Brown & Co., Boston, 1974.

Craighead, J.: Current views on the etiology of insulin-dependent diabetes mellitus. New Engl. J. Med. 299: 1439-1445, 1978.

Cudworth, A.: Editorial: The HLA system, autoimmune endocrinopathy and diabetes mellitus. Eur. J. Clin. Invest. 8: 355-357, 1978.

Cudworth, A., Wolf, E., Gorsuch, A. and Festenstein, H.: A new look at HLA genetics with particular reference to type 1 diabetes. Lancet ii: 389-391, 1979.

Cudworth, A. and Woodrow, J.: HLA system and diabetes mellitus. Diabetes 24: 345-349, 1975.

Dausset, J.: Leuco-agglutinins. IV. Leuco-agglutinins and blood transfusion. Vox Sang. 4: 190, 1954.

Dausset, J. and Contu, L.: Is the MHC a general self-recognition system playing a major unifying role in an organism? Human Immunol. 1: 5-17, 1980.

Deckert, T., Egeberg, J., Frimodt-Møller, C., Sander, E. and Svejgaard, A.: Basement membrane thickness, insulin antibodies and HLA antigens in long standing insulin dependent diabetes with and without severe retinopathy. Diabetologia 17: 91-96, 1979.

Delespesse, J., Duchateau, J., Bastenie, P., Lauvaux, J., Collet, H. and Govaerts, A.: Cell-mediated immunity in diabetes mellitus. Clin. Exp. Immunol. 18: 461-467, 1974.

DeWolf, W., Schlossman, S. and Yunis, E.: DRw antisera react with activated T cells. J. Immunol. 122: 1780-1784, 1979.

Dick, H.: HLA and disease: Introductory review. Br. Med. Bull. <u>34</u>: 271-274, 1978.

Doniach, D.: Pathology of pancreas in diabetes mellitus. Proc. Roy. Soc. Med. 68: 255-256, 1975.

Doniach, D. and Bottazzo, G.: Autoimmunity and the endocrine pancreas. Pathobiol. Ann. 7: 327-346, 1977.

Doniach, D. and Pouplard, A.: Humoral autoimmunity in diabetes mellitus. Acta endocrinol. (Scand.) 83 (Suppl.) 205: 55-61, 1976.

Drasch, A.: The etiology of diabetes mellitus. New Eng. J. Med. 300: 1211-1213, 1979.

Duckler, H. and Sachs, D.: Evidence for identity or close association of the F_c receptor of B lymphocytes and alloantigens determined by the Ir region of the H-2 complex. J. Exp. Med. 140: 779-796, 1974. Duquesnoy, R., MacDonald, M., Mullins, P., Hackbarth, S., Trausman, H. and Levitsky, L. Increased frequency of HLA-Dw3 in North American Black patients. Tissue Antigens 13: 369-372, 1976.

Egeberg, J., Junker, K., Kromann, H. and Nerup, J.: Autoimmune insulinitis. Pathological findings in experimental animal models and juvenile diabetes mellitus. Acta endocrinol. (Scand.) 83 (Suppl.) 205: 129-137, 1976.

- Engleman, E., McMichael, A. and McDevitt, H.: Suppression of the mixed lymphocyte reaction in man by a soluble T-cell factor. J. Exp. Med. 147: 1037-1043, 1978.
- Evans, R., Faldetta, T., Humphreys, R., Pratt, D., Yunis, E. and Schlossman, S.: Activated T cells synthesize and express Ia like antigens that may play a critical role in cell-cell interaction. DR⁺ T cells in MLC. J. Exp. Med. 148: 1440-1445, 1978.
- Fajans, S., Cloutier, M., Crowther, R. and Arbor, A.: Clinical and etiologic heterogeneity of idiopathic diabetes mellitus. Diabetes 27: 1112-1125, 1978.
- Farid, N., Barnard, J., Peeper, B., Noel, E., Kelly, F., Davis, A., Hobeika, C. and Marshall, W.: The association of HLA with juvenile diabetes mellitus in Newfoundland. Tissue Antigens 12: 215-222, 1978.
- Feinstein, A.: Immunoglobulins and histocompatibility antigens. Nature 282: 230, 1979.
- Ferrone, S., Indiveri, F., Russo, C., Molinaro, G., Quaranta, V., Wilson, B. and Pelligrino, M.: HLA-DR antigens on PHA-stimulated T lymphocytes: structural and functional analysis with HLA-DR monoclonal antibodies. VIII International Congress of the Transplantation Society. Abstract F 5.4., June 29-July 5, 1980, Boston, Mass., U.S.A.
- Forsum, U., Klareskog, L., Tjernlund, U. and Peterson, P.: Significance of the expression of HLA-DR antigen on epidermal Langerhans cells. Acta Dermatol. (Stockholm) Suppl. 79: 37-40, 1978.
- Fradelizi, D., Nunez-Roldan, A. and Sasportes, M.: Human Ia-like DRw lymphocyte antigens stimulating activity in primary mixed lymphocyte reaction. Eur. J. Immunol. 8: 88-93, 1978.
- Francke, U. and Pellegrino, M.: Assignment of the major histocompatibility complex to a region of the short arm of human chromosome C. Proc. Natl. Acad. Sci. USA 74: 1147-1151, 1977.

Friedman, S., Kuhns, J., Irigoyen, O. and Chess, L.: The induction of TNP-altered, self-reactive human cytotoxic T cells by soluble factors: The role of Ia antigens. J. Immunol. 122: 1302-1309, 1979.

Fu, S.M., Kundel, H., Brusman, H., Allen, Jr., F. and Fotino, M.: Evidence for linkage between HLA histocompatibility genes and those involved in the synthesis of second component of complement. J. Exp. Med. <u>140</u>: 1108-1111, 1974.

 $\dot{\omega}$

Fuller, T., Einarson, M., Pinto, C., Ahern, A. and Yunis, E.: Genetic evidence that HLA-DR(Ia) specificities include multiple HLA-D determinants on a single haplotype. Transplant. Proc. <u>10</u>: 781-784, 1978.

- Gamble, D., Kinsley, M., Fitzgerald, M., Bolton, R. and Taylor, R.: Viral antibodies in diabetes mellitus. Br. Med. J. 3: 627-630, 1969.
- Gamble, D. and Taylor, K.: Seasonal incidence of diabetes mellitus. Br. Med. J. 3: 631-633, 1969.
- Ganda, O. and Soeldmer, S.: Genetic, acquired and related factors in the etiology of diabetes mellitus. Arch. Intern. Med. <u>137</u>: 461-469, 1977.
- Garovay, M.R., Carpenter, C., Reddish, M., Fagan, G., Olivier, D. and Gleason, R.: "Ia" specificities associated with juvenile diabetes mellitus. Tissue Antigens 10: 200, 1977 (Abstract).
- Gill, T., Cramer, D. and Kunz, H.: The major histocompatibility complex comparison in the mouse, man and the rat. Amer. J. Pathol. <u>90</u>: 735-777, 1978.
- Ginsberg-Fellner, F.: Insulin dependent diabetes mellitus. Ped. Ann. 9: 142-153, 1980.
- Greaves, M., Verbi, W., Festenstein, D., Papasteridas, C., Jaraquemada, D. and Hayward, A.: "Ia-like" antigens on human T cells. Eur. J. Immunol. 9: 356-362, 1979.
- Greenberg, L. and Yunis, E.: Histocompabitility determinants, immune responsiveness and aging in man. Fed. Proc. 37: 1258-1262, 1978.
- Gerbase-Delima, M., Kraemer, M., Novo, N., Paiva, E. and Mendes, N.: Expression of HLA-DR on T lymphocytes. Tissue Antigens <u>15</u>: 257-266, 1980.
- Goodfellow, P., Jones, E., van Heyringer, V., Solomon, E., Bobrow, M., Miggiano, V. and Bodmer, N.: The B₂ microglobulin gene is on chromosome 15 and not in the HLA region. Nature 254: 267, 1975.
- Halim, K. and Festenstein, H.: HLA-D on sperm is haploid, enabling use of sperm for HLA-D typing. Lancet ii: 1255-1256, 1975.

Halper, J., Fu, S., Wang, C., Winchester, R. and Kunkel, H.: Patterns of expression of human "Ia-like" antigens during the terminal stage of B cell development. J. Immunol. 120: 1480-1484, 1978.

Hammond, M. and Asmal, A.: HLA and IDD in South African Indians. Tissue Antigens 15: 244-248, 1980.

- Han,T. and Dadey, B.: T and B lymphocytes. Exclusive role as responders and stimulators in human 'one way' lymphocyte reaction. Immunology 13: 643-648, 1976.
- Hauptfeld, V., Hauptfeld, J. and Klein, J.: Tissue distribution of I region-associated antigens in the mouse. J. Immunol. <u>113</u>: 181-188, 1974.
- Herring, R.: The chemistry of transplantation antigens. Blut <u>36</u>: 199-209, 1978.
- Hirschberg, H., Albrechtsen, D., Solheim, B. and Thorsby, E.: Inhibition of human mixed lymphocyte culture stimulation by anti-HLA-Dassociated antisera: studies with primed responding cells. Eur. J. Immunol. 7: 26-29, 1977.
- Hirschberg, H. and Thorsby, E.: The presence of HLA-D determinants on human skin cells. Transplantation 21: 343-347, 1976.
- Hodge, S., Rotter, J. and Lange, K.: A three allele model for the heterogeneity of juvenile-onset insulin-dependent diabetes. Ann. Human Genet. 43: 399-412, 1980.
- Hoffman, T., Wang, C., Winchester, R., Ferrarini, M. and Kunkel, H.: Human lymphocytes bearing "Ia-like" antigens; absence in patience with infantile agammaglobulinemia. J. Immunol. <u>119</u>: 1520-1524, 1977.
- Honig, R., Billing, R., Matsuyama, M. and Terasaki, P.: B lymphocyte alloantigen specificities present on cultured lymphoblastoid cell lines. J. Immunol. 117: 1300-1302, 1976.
- Humphreys, R., McCune, J., Chess, L., Herrman, H., Malenka, D., Mann, D., Parham, S., Schlossman, S. and Strominger, J.: Isolation and immunologic characterization of a human, B-lymphocyte-specific, cell surface antigen. J. Exp. Med. 144: 98-112, 1976.
- Huang, S.W. and MacLaren, N.: Insulin-dependent diabetes: a disease _ of autoaggression. Science 192: 64-66, 1976.

Ilonem, J., Herva, E., Tiilikainem, A., Akerblom, H., Mustonem, A., Koivukangas, T. and Kouvalainem, K.: HLA genetics of insulin dependent juvenile onset diabetes mellitus in North Finland. Tissue Antigens 15: 381-388, 1980.

- Irvine, W., McCallum, C., Gray, R., Campbell, C., Duncan, L., Farquhar, J., Vaughan, H. and Morris, P.: Pancreatic islet-cell antibodies in diabetes mellitus correlated with the duration and type 1 diabetes, coexistent autoimmune disease, and HLA type. Diabetes <u>26</u>: 138-147, 1977.
- Jansen, F., Muntertering, H. and Schmidt, W.: Virus-induced diabetes and immune system. Suggestion that appearance of diabetes depends on immune reactions. Diabetologia 13: 545-549, 1977.
- Jeannet, M., Raffoux, C., de Meeiosse, P., Debry, G., Bally, C., Streiff, F. and Sisonenko: HLA and Ia-like antigens in juvenile-onset diabetes. Tissue Antigens 10: 196, 1977 (Abstract).
- Johnson, A., Amos, D., Noreen, H. and Yunis, E.: Strong MLR associated with the LA or first locus HL-A. Transplantation 20: 291-295, 1975.
- Jonker, J., Persyn, G. and van Rood, J.: MLC inhibition studies with sera from multiparous women. IN: Histocompatibility Testing (ed. F. Kissmeyer-Nielson), Munksgaard, Copenhagen, pp. 594-603, 1975.
- Kaldany, A.: Autoantibodies to islet cells in diabetes mellitus. Diabetes 28: 102-105, 1979.
- Kaufman, J. and Strominger, J.: 'Both chains of HLA-DR bind to membrane with a penultimate hydrophobic region and the heavy chain is phosphorylated at its hydrophilic carboxyl terminus. Proc. Natl. Acad. Sci. USA 76: 6304-6308, 1979.
- Kaszubowski, P., Goodwin, J. and Williams, Jr. R.: Ia antigen on the surface of a subfraction of T cells that bear Fc receptors for IgG. J. Immunol. 124: 1075-1078, 1980.
- Klareskog, L., Sandberg-Tragardh, L., Rask, L., Lindbrom, J., Curman, B. and Peterson, P.: Chemical properties of human Ia antigens. Nature 265: 249-251, 1977a.
- Klareskog, L., Tjernland, U., Forsum, U. and Peterson, P.: Epidermal Langerhans cells express Ia antigens. Nature 268: 248-250, 1977b.
- Klein, J.: Many questions (and almost no answers) about the phylogenetic origin of the major histocompatibility complex. Adv. Exp. Med. Biol. 64: 467-478, 1975.
- Krangel, M., Orr, H. and Strominger, J.: Assembly and maturation of HLA-A and HLA-B antigens *in vivo*. Cell 18: 979-991, 1979.

- Koyama, K., Fukunishi, T., Barcos, M., Tanigaki, N. and Pressman, D.: Human Ia like antigens in nonlymphoid organs. Immunology 38: 333-341, 1979.
- Kvist, S., Klareskog, L. and Peterson, P.: Identification of H-2 and Ia antigen analogues in several species by immunological cross-reactions of xenoantisera. Scand. J. Immunol. 7: 447-452, 1978.
- Lamm, L., Friedrich, V., Peterson, G., Jorgensen, J., Njelson, J., Therkelsen, A. and Kissmeyer-Nielsen, F.: Assignment of the major histocompatibility complex to chromosome no. 6 in a family with a pericentric inversion. Human Hered. 24: 273-284, 1974.
- Lamm, L. and Petersen, G.: The HLA genetic linkage group. Transplant. Proc. 11: 1692-1696, 1979.
- Landgraf, R., Landgraf-Leurs, M., Lander, T., Scholz, S., Kuntz,/B. and Albert, E.: HLA haplotypes and glucose tolerance in families of patients with juvenile-onset diabetes mellitus. Lancet <u>ij</u>: 1084-1085, 1976.
- Lendrum, R., Walker, G., Cudworth, A., Woodrow, J. and Gamble, D.: HLAlinked genes and islet cell antibodies in diabetes mellitus. Br. Med. J. 1: 1565-1567, 1976.
- Levis, W., Berry, Jr., F.: Mechanism of HLA "predispositions" to specific diseases. New Eng. J. <u>Mad.</u> 293: 1323-1324, 1975.
- Lilly, F., Boyse, E. and Old, L.: Genetic basis of susceptibility of viral leukemogenesis. Lancet ii: 1207, 1964.
- Ludwig, H., Schernthaner, G. and Mayr, W.: Is HLA-B7 a marker associated with a protective gene in juvenile-onset diabetes mellitus? New Engl. J. Med. 294: 1066, 1976a.
- Ludwig, H., Schernthaner, G., Erd, A. and Mayr, W.: Humoral immunodeficiency to bacterial antigens in patients with juvenile-onset diabetes mellitus. Diabetologia 12: 259-262, 1976b.
- Ludwig, H., Schernthaner, G. and Mayr, W.: Ia-type alloantigens and humoral autoimmune responsiveness in insulin-dependent diabetes mellitus. Metabolism 28: 797-800, 1979.

Malone, J.: Newer aspects of diabetes. Adv. Pediat. 24: 1-41, 1977.

Mann, D., Abelson, L., Henkart, P., Harris, S. and Amos, D.: Specific human B lymphocyte alloantigen-linked to HLA. Proc. Natl. Acad. Sci. USA 72: 5103-5106, 1975. Mann, D. and Muchmor[®]e, A.: Inhibition of DRw antisera reactivity by mono and oligosaccharides. J. Immunol, <u>124</u>: 2218-2221, 1980.

Mann, D. and Murray, C.: HLA alloantigens: disease association and biologic significance. Sem. Hematol. <u>16</u>: 293-308, 1979.

Mann, D. and Sharrow, S.: B cell alloantigens in T lymphocytes. Transplant Proc. 11: 1774-1775, 1979.

Mayr, W.R., Schernthaner, G., Ludwig, H., Pausch, V. and Dub, E.: Ia type alloantigens in insulin-dependent diabetes mellitus. Tissue Antigens 10: 194, 1977 (Abstract).

McMichael, A. and McDevitt, H.: The association between the HLA system and disease. Prog. Med. Genetics 2: 39-100, 1977.

Metzgar, R., Bertoglio, J., Anderson, J., Bonnard, G. and Ruscetti, F.: Detection of HLA-DRw (Ia-like) antigens on human T lymphocytes grown in tissue culture. J. Immunol. 122: 949-953, 1979.

Moller, E., Persson, B. and Sterny, G.: HLA phenotypes and diabetic retinopathy. Diabetologia 14: 155-158, 1978.

Neel, J.: The genetics of juvenile-onset diabetes mellitus. New Eng. J. Med. 297: 1062-1063, 1977.

Nelson, P., Pyke, D. and Gamble, D.: Viruses and the etiology of diabetes: a study in identical twins. Br. Med. J. 4: 249-251, 1975,

Nerup, J., Anderson, O., Christy, M., Platz, P., Ryder, L., Thomsen, M., Svejgaard, A.: HLA, autoimmunity, virus and the pathogenesis of juvenile diabetes mellitus. Acta endocrinol. (Scand.) 83 (Suppl.) 205: 167-175, 1976.

Nerup, J., Platz, P., Andersen, O., Christy, M., Lyngsoe, J., Poulsen, J., Ryder, L., Thomsen, M., Nielsen, L. and Svejgaard, A.: HLA antigens and diabetes mellitus. Lancet ii: 864-866, 1974.

Nilsson, S., Schwartz, B., Waxdal, M., Green, I., Cullen, S. and Mann, D.: A human alloantiserum precipitates Ia-like molecules from chronic lymphocytic leukemia cells. J. Immunol. 118: 1271-1274, 1977.

Ono, S.: Study on HLA antigens in Behcet's disease. Acta Soc. Ophthalmol. Jpn: 78: 458-463, 1974.

Oliver, R.: Histocompatibility antigens and human disease. Br. J. Hospital Med. 18: 449-459, 1977.

69

Opelz, G., Kiuchi, M., Takasugi, M. and Terasaki, P.: Autologous stimulation of human lymphocyte subpopulations. J. Exp. Med. <u>142</u>: 1327-1333, 1975.

Orr, H., Lopez de Castro, J., Parham, P., Ploegh, H. and Strominger, J.: Comparison of amino acid sequences of two human histocompatibility antigens, HLA-A2 and HLA-B7: 10cation of putative alloantigenic sites. Proc. Natl. Acad. Sci. USA 76: 4395-4399, 1979a.

- Orr, H., Lopez de Castro, J., Lancet, D. and Strominger, J.: Complete amino acid sequence of a papain solubilized human histocompabitility antigen, HLA-B7. 2. Sequence determination and search for homologies. Biochemistry 18: 5711 - 5720, 1979b.
- Orr, H., Lancet, D., Robb, R., Lopez de Castro, J. and Strominger, J.: The heavy chain of human histocompatibility antigen HLA-B7 contains an immunoglobulin-like region. Nature 282: 266-270, 1979c.
- Payne, R. and Rolfs, M.R.: Fetomaternal leukocyte incompatibility. J. Clin. Invest. 37: 1756-1763, 1958.
- Parham, R., Albert, B., Orr, H. and Strominger, J.: Carbohydrate moiety of HLA antigens. Antigenic properties and amino acid sequences around the site of glycosylation. J. Biol. Chem. 252: 7555, 1977.
- Park, M., Terasaki, P., Bernoco, D. and Iwaki, Y.: Evidence for a second B cell locus separate from the DR locus. Transplant. Proc. <u>10</u>: 823-828, 1978.
- Pelligrino, M., Belvedere, M., Pelligrino, A. and Ferrone, S.: B peripheral lymphocytes express more HLA antigens than T peripheral lymphocytes. Transplantation 25: 93-95, 1978.
- Pious, D. and Soderland, C.: HLA variants of cultured human lymphoid cells: evidence for mutational origin and estimation of mutation rate. Science 197: 769-771, 1977.

Rask, L., Lindblom, J. and Peterson, P.: Structural and immunological similarities between antigens from three loci. Eur. J. Immunol. 6: 93-100, 1976.

Ray, C., Palmer, J., Crossley, J. and Williams, R.: Coxsackie B virus antibody responses in juvenile onset diabetes mellitus. Clin. Endocrinol. 12: 375-378, 1980.

Rayfield, E. and Seto, Y.: Viruses and the pathogenesis of diabetes mellitus. Diabetes 27: 1126-1140, 1978. Raum, D., Glass, D., Carpenter, C., Alper, C. and Schur, P.: The chromosomal order of genes controlling the major histocompatibility complex, properdin factor B, and deficiency of the second component of complement. J. Clin. Invest. 58: 1240-1248, 1976.

Remsmoen, N., Noreen, A., Greenberg, L. and Kersey, J.: Stimulatory determinants expressed on B cells which produce specific PLT reactivity. Transplant. Proc. 10: 767-769, 1978.

Richens, E., Quilley, J. and Hartog, M.: Immunological features of juvenile-onset diabetic patients correlated to HLA type. Clin. exp. Immunol. 36: 198-204, 1979.

Robbins, S. and Cotran, R.: Pathologic Basis of Disease, 2nd ed. Saunders Co., p. 327, 1979.

Rosenberg, L. and Kidd, K.: HLA and disease susceptibility: A primer. New Eng. J. Med. 297: 1060-1062, 1977.

Ross, G., Jarowski, C., Rabellino, E. and Winchester, R.: The sequential appearance of Ia-like antigens and two different complement receptors during the maturation of human neutrophils. J. Exp. Med. <u>147</u>: 730-744, 1978.

Rotter, J. and Rimoin, D.: Heterogeneity in diabetes mellitus - update 1978. Evidence for further genetic heterogeneity within juvenileonset insulin-dependent diabetes mellitus. Diabetes 27: 599-608, 1978.

Rotter, J. and Rimoin, D.: Diabetes mellitus - the search for genetic markers. Diabetes Care 2: 215-226, 1979.

Rowden, G., Lewis, M. and Sullivan, A.: Ia antigen expression on human epidermal Langerhans cells. Nature 268: 247-248, 1977.

Rubenstein, P., Suciu-Foca, N. and Nicholson, J.: Genetics of juvenile diabetes mellitus. A recessive gene closely linked to HLA D and with 50 percent penetrance. New Eng. J. Med. 297: 1036-1040, 1977.

Rubin, A., Stenzel, K., Hirschhorn, K. and Bach, F.: Histocompatibility and immunologic competence in renal homotransplantation. Science 143: 815-816, 1964.

Sachs, J., Cudworth, A., Jaraquemada, D., Gorsuch, A. and Festensteim, H.: Type 1 diabetes and the HLA-D locus. Diabetologica 18: 41-43,*1980.

Sasazuki, T., McDevitt, H. and Grumet, C.: The association between genes in the MHC and disease susceptibility. Ann. Rev. Med. <u>28</u>: 425-452, 1977. Schernthaner, G., Ludwig, H., Mayr, W. and Willverseder, R.: Genetic factors on insulin antibodies in juvenile-onset diabetes. New Eng. J. Med. 295: 622, 1976.

Schernthaner, G., Mehnert, H., Ludwig, H., Scheiner, O. and Kraft, D.: Impairment of killer cell activity in insulin-dependent diabetes mellitus. Diabetes 27: 459, 1978a (Abstract).

Schernthaner, G., Ludwig, H. and Mayr, W.: Immunoglobulin G - insulin antibodies and immune region-associated alloantigens in insulindependent diabetes mellitus. J. Clin. Endocrin. Metabol. <u>48</u>: 403-407, 1978b.

Schreuder, I., Meo, T., Termijtelen, A. and Khan, P.: The Bf locus is between HLA-B and D/DR. Eighth International Histocompatibility Workshop Newsletter 19: December, 1979.

Schuurman, R., Gelfand, E., Matheson, D., Zimmerman, B. and Dosch, H.: Identification of Ia on a subpopulation of human T lymphocytes that stimulate in a mixed lymphocyte reaction. J. Immunol. 124: 1924-1928, 1980.

Seager, K., Bashir, H., Géczy, A., Edmonds, J. and de Vere-Tyndall, A.: Evidence for a specific B27-associated cell surface marker on lymphocytes of patients with ankylosing spondylitis. Nature 277: 68-70, 1979.

Shackelford, D. and Strominger, J.: Demonstration of structural polymorphism among HLA-DR light chains by two-dimensional gel electrophoresis. J. Exp. Med. 151: 144-165, 1980.

Sheehy, M.: Reconsideration of genetic mapping of the weak LD locus. Tissue Antigens 11: 188-189, 1978.

Silver, J. and Ferrone, S.: Structural organization of human DR antigens and their murine analog, I-E antigens: Serologic, genetic and functional implications. Transplant. Proc. 11: 1743-1744, 1979.

Simpson, E.: Stimulation of MLC and cytotoxic responses: evidence that T cells express SD but not LD antigens, whereas B cells express both. Eur. J. Immunol. 456-461, 1975.

Simpson, E. and Matsunaga, T.: Physiological function of major histocompatibility complex macromolecules. Transplantation <u>27</u>: 295-297, 1979.

Sinaniotis, C., Daskalpoulou, E., Lapatsanis, P. and Doxiadis, S.: Diabetes mellitus after mumps. Arch. Dis. Childhood <u>50</u>: 749-750, 1975. Singal, D. and Blajchman, M.: Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. Diabetes 22: 429-432, 1973.

Smith, R. and Royston, I.: Human lymphocytes: participation of T and B cells in mixed lymphocyte reactions. Transplant. Proc. 7 (Suppl.)]: 63-64, 1975.

- Snary, D., Barnstable, C., Bodmer, W., Goodfellow, P. and Crumpton, M.: Cellular distribution, purification and molecular nature of human Ia antigens. Scand. J. Immunol. 6: 439-452, 1977.
- Solow, H., Hidalgo, R., Blajchman, M. and Singal, D.P.: HLA-A,-B,-C and B lymphocyte alloantigens in insulin dependent diabetes. Transplant. Proc. 9: 1859-1861, 1977a.
- Solow, H., Hidalgo, R. and Singal, D.P.: HLA-A,B-C and B cell alloantigens. Tissue Antigens 10: 195, 1977b (Abstract).
- Solow, H., Hidalgo, R. and Singal, D.P. Juvenile-onset diabetes: HLA-A, -B,-C and -DR alloantigens. Diabetes 28: 1-4, 1979.
- Spielman, R., Baker, L. and Zmijewski, C.: Inheritance of susceptibility to juvenile onset diabetes. IN: Genetic Analysis of Common Diseases. Applications to Predictive Factors in Coronary Disease, (eds. C. Sing and M. Skolnick), Alan R. Liss, New York, pp. 567, 1979.

Springer; T., Kaufman, J., Terhorst, C. and Strominger, J.: Purification and structural characterization of human HLA-linked B-cell antigens. Nature 268: 213-218, 1977a.

Springer, T., Kaufman, J., Siddoway, L., Mann, D. and Strominger, J.: Purification of HLA-linked B lymphocyte alloantigens in immunologically active form by preparative sodium dodecyl sulfate-gel electrophoresis and their studies on subunit associations. J. Biol. Chem. 252: 6201-6207, 1977b.

Stingl, G., Katz, S., Abelson, L. and Mann, D.: Immunofluorescent detection of human B cell alloantigens on S-Ig-positive lymphocytes and epidermal Langerhans cells. J. Immunol. 120: 661-664, 1978.

Strelkauskas, A., Wilson, B., Callery, R., Chess, L. and Schlossman, F.: T-cell regulation of human peripheral blood B-cell responsiveness. J. Exp. Med. 146. 1765-1772, 1977.

Strominger, J., Humphreys, R., McCune, J., Parham, P., Robb, R., Springer, T. and Terhorst, C.: The immunoglobulin-like structure of human histocompatibility antigens. Fed. Proc. 35: 1177-1182, 1976.

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- Suciu-Foca, N., Rubenstein, P., Sussino, E., Weiner, J., Martin, M., Fotino, M., Day, B. and Nicholson, J.: Ia typing of J.D.M. families. Tissue Antigens <u>10</u>: 199, 1977 (Abstract).
- Suciu-Foca, N., Susinno, E., McKierman, P., Dohwsky, C., Weiner, J. and Rubenstein, P.: DRw determinants on human T cells primed against allogeneic lymphocytes. Transplant. Proc. 10: 845-848, 1978a.
- Suciu-Foca, N., Werner, Rohowsky, C., McKierman, P., Susermo, E. and Rubenstein, P.: Indications that Dw and DRw determinants are controlled by distinct (but closely linked) genes. Transplant. Proc. 10: 799-804, 1978b.
- Sullivan, A., Jerry, L., Ikeman, R., Maccari, R., Thi, H. and Sylvester, C.: Isolation of a human B lymphocyte protein with Ia-like properties. Can. J. Biochem. 57: 21-31, 1979.
- Svejgaard, A., Platz, P., Ryder, L., Nielsen, L. and Thomsen, M.: HLA and disease association - a survey. Transplant. Rev. 22: 3-43, 1975.
- Svejgaard, A., Platz, P. and Ryder, L.: Insulin-dependent diabetes mellitus. IN: Histocompatibility Testing (ed. P. Terasaki), UCLA Tissue Typing Laboratory, Los Angeles, Ca., pp. 638-656, 1980.
- Svejgaard, A., Morling, N., Platz, P., Ryder, L. and Thomsen, M.: HLA and disease associations with special reference to mechanisms. Transplant. Proc. 13: 913-917, 1981.
- Svejgaard, A. and Ryder, L.: Interaction of HLA molecules with nonimmunological ligands as explanation of HLA and disease association. Lancet i: 547-549, 1976.
- Tattersall, R., Pyke, D. and Nerup, J.: Genetic patterns in diabetes mellitus. Human Pathol. 11: 273-283, 1980.
- Teisberg, P., Akesson, I., Olaisen, B., Gedde-Dahl,Jr., T. and Thorsby, E.: Genetic polymorphism of C4 in man and localization of a structural C4 locus to the HLA gene complex of chromosome 6. Nature 264: 253-254, 1976.
- Terasaki, P., Bernoco, D., Park, M., Ozturk, G. and Iwaki, Y.: Microdroplet testing for HLA-A, B and D antigens. The Philip Levine Award Lecture. Amer. J. Clin. Pathol. 69: 103-120, 1978.
- Terasaki, P., Opelz, G., Park, M. and Mickey, M.: Four new B lymphocyte specificities. IN: Histocompatibility Testing, (ed. F. Kissmeyer-Nielsen), Munksgaard, Copenhagen, pp. 657-664, 1975.

Thomsen, G. and Bodmer, W.: HLA haplotype associations with disease. Tissue Antigens 13: 91-102, 1979.

Thorsby, E.: The major histocompatibility complex HLA: some recent developments. Transplant. Proc. 11: 616-623, 1979.

Thorsby, E., Albrechtsen, D., Hirshberg, H., Kaakinen, A. and Solheim, B.: MLC-activating HLA-D determinants: identification, tissue distribution and significance. Transplant.Proc. 9: 393-400, 1977.

Tosi, R., Tanigaki, N., Centis, D., Ferrara, G. and Pressman, D.: Immunological dissection of human Ia molecules. J. Exp. Med. <u>148</u>: 1592-1611, 1978.

- Tragardh, L., Curman, B., Wiman, K., Rask, L. and Peterson, P.: Chemical, physical-chemical and immunological properties of papain-solubilized human transplantation antigens. Biochemistry 18: 2218-2226, 1979.
- van Leeuwen, A., Schmit, H. and van Rood, J.: Typing for MLC(LD): the selection of nonstimulator cells by MLC inhibition tests using SD identical stimulator cells (MISIS) and fluorescent antibody studies. Transplant. Proc. 5: 1539-1542, 1973.
- van Rood, J.J., Eernisse, J.G. and van Leeuwen, A.: Leukocyte antibodies in sera of pregnant women. Nature 181: 1735, 1958.

van Rood, J., van Leeuwen, A., Keuning, J. and Termijtelen, A.: Evidence for two series of B cell antigens in man and their comparison with HLA-D. Scand. J. Immunol. 6: 373-384, 1977b.

- van Rood, J.; van Leeuwen, A., Parleviet, J., Termijtelen, A. and Keuning, J.: LD typing by serology. IV Description of a new locus with three alleles. IN: Histocompatibility Testing (ed. F. Kissmeyer-Nielsen), Munksgaard, Copenhagen, pp. 629-631, 1975.
- van Rood, J., van Leeuwen, A., Termijtelen, A. and Keuning, J.: The genetics of the major histocompatibility complex in man, HLA. IN: The Role of Products of the Histocompatibility Gene Complex in Immune Response, (eds. D. Katz and B. Benacerraf), Academic Press, N.Y., pp. 31, 1976.

van Rood, J.J., Persijn, G., van Leeuwen, A., Goulney, E, and Gabb, B.: A new strategy to improve kidney graft survival. The induction of CML nonresponsiveness. Transplant. Proc. 11: 737-742, 1979.

Wakisaka, A., Aizawa, M., Matsuura, M., Nakagawa, S., Nakayama, E., Ktskura, K., Okuro, A. and Wagatsuma, Y.: HLA and juvenile diabetes mellitus in the Japanese. Lancet ii: 970, 1976. Walford, R., Waters, H., Smith, G. and Sturgeon, P.: Anomalous reactivity of certain HLA typing sera with leukemic lymphocytes. Tissue Antigens 3: 222-234, 1973.

Walsh, F. and Crumpton, M.: Orientation of cell surface antigens in the lipid bilayer of lymphocyte plasma membrane. Nature 269: 307-311, 1977.

Weitkamp, L., May, A. and Johnston, E.: The linkage relationships of HLA with other genetic marker systems. Human Hered. 25: 337-345, 1975.

Welsh, K., Dorval, G., Nilsson, K., Clements, G. and Wigzell, H.: Quantitation of B₂-microglobulin and HLA on the surface of human cells. II. In vitro cell lines and their hybrids. Scand. J. Immunol. 6: 265-271, 1977.

Woolf, B.: "On estimating the relation between blood group and disease. Ann. Human Genet. 19: 251-253, 1955.

Yoon, J., Austin, M., Onodera, T. and Notkins, A.: Virus-induced diabetes mellitus. Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. New Eng. J. Med. 300: 1173-1179, 1979.

Yu, D., Winchester, R., Fu, S., Gibofsky, A., Ko, H. and Kunkel, H.: Peripheral blood Ia-positive T cells. Increases in certain diseases and after immunization. J. Exp. Med. <u>151</u>: 91-100, 1980.

Zimmerman, R., Ozer, H., deWolf, W., Evans, R., Yunis, E. and Schlossman, S.: The detection of Ia-like and DRw antigens on activated T cells. Transplant. Proc. 11: 1770-1773, 1979.