

# **Human Leukocyte Antigen-DP in Acute Nonlymphocytic and Acute Lymphoblastic Leukemia**

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## **ABSTRACT**

Acute leukemias are characterized by the clonal proliferation of haemopoietic progenitor cells arrested in maturation. Clinical manifestations are related to the infiltration of the bone marrow and other tissues by the leukemic blasts.

Peripheral blood mononuclear cell (PBMC) from leukemic patients (29)ANLL,(36)ALL and from normal donors(55)were typed for HLA-DP specificities using microlymphocytotoxicity assay.

Results showed frequencies of DPw alleles in ANLL patients were not significantly different from controls ,except that in DPw1 was absent .In contrast ,in ALL,frequencies of DPw2 and DPw5 were significantly increased ( $p < 0.05$ , relative risk (RR) = 1.95 and  $p < 0.01$ , RR=4.27, respectively.

These results ,therefore ,demonstrate both positive negative associations between major histocompatibility complex (MHC) gene products which are in only very weak linkage with the rest of HLA ,and ALL,ANLL patients.The HLA-DP region could thus contain long sought –after genes influencing susceptibility and resistance to leukaemogenesis.

## **INTRODUCTION**

Although the first report of an association between human major histocompatibility complex (MHC) products and disease concerned the prevalence of human leukocyte antigen A1(HLA-A1) in Hodgkins disease(1) ,significant associations between HLA alleles and any types of tumor are rare .Several studies have reported (usually weak) associations between both Class I and Class II MHC products and various tumors and,in addition ,family analyses have on occasion suggested genetic susceptibility factors in tumorigenesis.(2,3)

HLA-DP products(4) are encoded centromeric to HLA-DQ but show only very weak linkage disequilibria with DR,DQ,probably due to a recombination hotspot.(5) DP molecules are typical Class II structures sharing many of the features common to these immunologically active moieties(4),for example in their function as alloantigens stimulating

lymphocyte proliferative and cytotoxic responses ,and as restriction elements for antigen presentation .However ,they behave as surprisingly weak primary stimuli, and even in the context of allogenic transplantation are probably of minor importance .(6)

The frequencies of DP alleles have been Investigated in several diseases with known HLA association ,usually with the conclusion that are not altered compared to control populations.(7,8) however ,this might be as expected given the lack of linkage between DP and the rest of HLA .Despite this ,positive association between certain disease states and DP specificities have been alluded to or recently reported.(9,10) It would perhaps be more appropriate to examine diseases with a suspected genetic susceptibility component but no clear HLA –associations .

## **MATERIALS AND METHODS**

### **Patients Study Group:**

A total of 65 Iraqi patients with ANLL and ALL. (29) ANLL, 12 females and 17 males, age ranged (13-47) years,with an average age 30 years. (36) ALL ,17 females and 19 males, age ranged (12-33) years,with an average age 22.° years.

### **Control Group:**

Healthy control, was consisted of 55 healthy individuals age ranged (20-50) years,with an average age 35.5 years who have no history or clinic evidence of malignant disease.

### **Collection of blood sample:**

Twenty ml of venous blood were drawn from patients preoperatively and controls. The blood sample collected in plastic heparinized tube (10 Iu/ml).

### **HLA-typing:**

The microlymphocytotoxicity test was established by (11) and modified by(12,13).

HLA- DP antigens are present on B-lymphocytes but not on T-lymphocytes therefore, B-cells and T-cells have to separated before testing for HLA -typing.

### **A- Setting up typing plates:**

The Terasaki plates were filled with mineral oil or liquid paraffin up to the brim, and by using Hamilton syringe, one microliter (1µl) of each typing sera was dispensed into each well of labeled plates, then the plates were stored at -70°C till use.

### **B- Isolation of lymphocytes:**

The lymphocytes were isolated from the whole blood by density gradient centrifugation, which was developed by ( 14).

- 1- Heparinized blood sample was diluted with an equal volume of PBS.

- 2- In a universal tube one volume of diluted blood was carefully layered over equal volume of lymphoprep, without mixing the layers.
- 3- After that the tubes were centrifuged for 30 min at 3000 rpm.
- 4- By Pasteur pipette, lymphocytes which had been appeared as a white ring were carefully removed and put into a new plastic centrifuge tubes containing HBSS, then the tubes filled with HBSS, the contents were gently mixed.
- 5- The cells were washed twice with HBSS by centrifugation for 10 min at 1000 rpm. Supernatant was discarded and pellet was resuspended with HBSS and mixed well, followed by third centrifugation at 1500 rpm for 10 min, the supernatant was completely discarded and the pellet was resuspended with 1 ml of warm media (RPMI-1640) supplemented with 10% inactivated FCS.
- 6- Viability of the cells was checked with 0.2% trypan blue dye. One drop was mixed with the cell suspension, and 200 cells were counted and those cells, which exclude the dye, were considered viable .

N.: Before use of FCS, it should be inactivated by incubation for 30 min at 56°C in water bath.

#### **C- Separation of T and B lymphocytes:**

1. Two ml disposable syringe was loosely filled with 0.15 gm nylon wool, and washed with 10 ml HBSS, then 2 ml of RPMI-1640 medium at 37°C has been added to warm the nylon wool.
2. The suspension of the mixed lymphocyte was poured into the separation column and allow to soak in well, both ends of syringe were sealed with parafilm and the column was incubated at 37°C for 30 min.
3. Non-adherent T-cells were washed out with 10 ml of warm RPMI-1640 and collected in a tube.
4. The adhering B-cells were eluted with the same volume of warm medium and collected in test tube containing 1 ml of warm medium by compressing the nylon wool with syringe plunger, this step was repeated for several times, adding 2 ml of medium to the column each time. B cells suspension were centrifuged for 10 min at 1000 rpm, the supernatant was discarded. The cells were washed again with warm medium RPMI-1640.
5. The cells were resuspended by using warm medium and the numbers of cells adjusted to 2000-3000 cell/ $\mu$ l. the B-cells used for DP typing (15).

**Microlymphocytotoxicity Assay:**

1. One  $\mu$ l of lymphocytes suspension was dispensed per well by Hamilton syringe. The plates were incubated at room temperature for 60 min.
2. Five  $\mu$ l rabbit complement were added to each well followed by incubation of plates at room temperature for 120 min.
3. Three  $\mu$ l of eosin solution ( 5%) were added to each well and allowed 2 min for staining.
4. Six  $\mu$ l of formaldehyde were added per well to fix the cells.

**Scoring of the reactions:**

Reading of reactions of HLA typing was done under phase contrast microscope, the living cells are bright and luminous (negative reaction), while the dead cells were dark and larger than the live cells (positive reaction ).

The number of dead lymphocytes were compared with the total number of cells and then quoted as score value ( 16).

The HLA-DP positive control should produce 75-100% dead cells while all cells in the negative control should be live, if it is weak positive (the negative control), the background which should be taken into account when scoring (Table 1 ).

**Table 1: Scoring of the HLA reaction**

<i>Lysed cells %</i>	<i>Evaluation</i>
0-10%	Score 1 negative
11-20%	Score 2 doubtful negative
21-50%	Score 4 weak positive
51-80%	Score 6 positive
81-100%	Score 8strong positive

**Statistical data analysis:** Data were statistically analyzed using SPSS statistical software (version 11.5).

**Results and discussion:**

HLA-DP typing data were satisfactory for 29 of the ANLL patients and 36 of the ALL patients .The frequencies of assigned DP specificities for ANLL and ALL patients compared to the normal group are shown in tables 2 and 3 respectively .

In ANLL patients there was a significant decrease in frequency of DPw1 ;in fact ,in 27 patients DPw1 was absent. There was a significant

increase in the frequency of DPb1 (13.7%) when compare with control group (1.8%).

**Table 2: distribution of HLA-DP specificities in ANLL patients with control group.**

Antigen	Control N=55		Patients N=29		P	RR
	NO	%	NO	%		
DPw1	12	21.8	2	6.8	<0.05	0.26
DPw2	18	32.7	10	34.4	NS	1.08
DPw3	15	27.2	7	24.1	NS	0.84
DPw4	39	70.9	21	72.4	NS	0.07
DPw5	2	3.6	1	3.4	NS	0.94
DPb1	1	1.8	4	13.7	<0.01	<b>8.64</b>

NS: not significant ,RR:relative risk ,RR= a\*d/b\*c.

a= patients number which carry DP-antigen (positive).

b= patients number which don't carry DP-antigen (negative).

c= control number which carry DP- antigen (positive).

d= control number which don't carry DP-antigen (negative).

Interestingly ,a two –specificity analysis for DR-DP frequencies in ANLL patients compared to normals group ,as the only significant difference, a remarkably high RR for the combination of DR7 and DPb1(RR=26.11,p<0.01) .However ,this may be by the finding that DR7 itself was increased in the ANLL patients (RR=2.829, p<0.05). (17). Further experiments must resolve the question of the meaning of the increased of DPb1 in ANLL patients.

In ALL patients frequencies of DP alleles were very different from control group .Again ,a decreased of DPw1 was observed (Table3).Because of the linkage between DR3 and DPw1,the decrease in DPw1 in ALL patients is therefore even more remarkable than the RR of only 0.36 already suggests.

**Table 3: distribution of HLA-DP specificities in ALL patients with control group**

Antigen	Control N=55		Patients N=36		P	RR
	NO	%	NO	%		
DPw1	11	20.0	3	8.3	<0.05	0.36
DPw2	16	29.0	16	44.4	<0.05	1.95
DPw3	13	23.6	4	11.1	NS	0.40

DPw4	38	69.0	25	60.4	NS	1.01
DPw5	2	3.6	5	13.8	<0.01	4.24
DPb1	1	1.8	1	2.7	NS	<b>1.54</b>

NS:not significant ,RR:relative risk .If RR less than (1) ,mean neagative association between disease and antigen,while if RR more than (1),mean positive association between disease and antigen .

The presence of HLA-DPw1 may therefore be associated with resistance genes for ALL leukemogenesis .The frequency of DPw3 was also decreased in ALL patients ,and ,although this did not reach significance ,may also suggest the presence of resistance linked to DPw3.In ALL patients ,positive as well as negative associations with DP alleles were observed .There was a slight but significant increase in DPw2 giving a RR of 1.95 ,and a much more marked increase in DPw5 giving a RR of 4.27.In white persons,the DPw5 specificity is rare ,but despite this,the significance of the increase in ALL patients reached<0.01p value.The increase in DPw2 and DPw5 in ALL are not due to an increase in the frequency of DRw6, with which both these DP-types are linked in our normal group (18).

The results presented here ,therefore, suggest that in patients ,that HLA-DPw alleles can serve as markers both for genes of "susceptibility" and resistance to leukemogenesis.

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#### الخلاصة

مستضد خلايا الدم البيض (DP) لمرضى ابيضاض الدم اللمفاوي الحاد يعرف ابيضاض الدم بمجموعة من الاختلالات الخبيثة في الانسجة المكونة للدم ويرتبط من الناحية التشخيصية بزيادة عدد خلايا الدم البدائية .

في هذه الدراسة تم التحري عن مستضد خلايا الدم البيض (DP) لمرضى ANLL وكان عددهم ٢٩ مريضا ومرضى ALL وعددهم ٣٦ مريضا. ول ٥٥ شخص كمجموعة سيطره. حيث سحبت عينات الدم الوريدي وفصلت خلايا الدم المحيطي احادية النواة واجري عليها فحص سمية الخلايا اللمفاوية للتحري عن مستضد خلايا الدم البيض (DP). أظهرت النتائج وجود اختلافات غير معنوية لمرضى ANLL عند مقارنة النتائج بمجموعة السيطرة ماعدى المستضدين (DPw1,DPb1).فيما اظهرت نتائج مرضى ALL وجود فروق معنوية لمستضدي (DPw2,DPw5) عند مقارنة النتائج بمجموعة السيطرة.

هذه النتائج تشير الى اهمية HLA-DP في عملية الاستعداد للاصابة ومقاومة مرض ابيضاض الدم اللمفاوي الحاد.