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

Majid Sakhi Jabir

ABSTRACT:

BACKGRAOUND:

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

METHODS:

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

RESULTS:

Results showed frequencies of (DPw) alleles in (ANLL) patients were not significantly different from controls ,exept 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 .

CONCLUSION:

These results ,demonstrate both positive and 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 HLD-DP region could thus contain long sought –after genes influencing susceptibility and resistance to leukemogenesis.

KEY WORDS: ANLL, ALL, HLA, DP-Antigen.

INTRODUCTION:

Although the first report of an association between human major histocompatibility complex (MHC) products and disease concernd 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 moietics[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]

Biochemical technology division –Applied sciences , Department-University of Technology .

The frequencies of DP alleles have been Investigated in sevsral 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 certin diseas 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. (36) ALL ,17 females and 19 males, age ranged (12-33) years.

Control Groups:

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

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 volum 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 cetrifuged 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 l0 min at 1000 rpm. Supernatant was discarded and pellet was resuspended with HBSS and mixed well, followed by third centrifugation at 1500 rpm for l0 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.B.: 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 nyloon 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.

5. 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. **6.** The cells were resuspended by using warm medium and the numbers of cells adjusted to 2000-3000 cell/µl. the B-cells used for DP typing (15).

- Microlymphocytotoxicity Assay:

1.One µl of lymphocytes suspension was dispensed per well by Hamilton syringe. The plates were incubated at room temperature for 60 min.

2.Five µl rabbit complement were added to each well followed by incubation of plates at room temperature for 120 min.

3.Three µl of eosin solution (5%) were added to each well and allowed 2 min for staining. 4.Six µl 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 luminus (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 .

Lysed cells %	Evaluation
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 S.P.S.S. statistical software (version 11.5).

RESULTS:

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 respectivily . 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%).

DISCUSSION:

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. 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 $p<0.01$ 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.

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	٢	6.8	<0.05	0.26
DPw2	١٨	32.7	١٠	34.4	N.S	1.08
DPw3	١٥	27.2	٧	24.1	N.S	0.84
DPw4	٣٩	70.9	٢١	72.4	N.S	0.07
DPw5	٢	3.6	١	3.4	N.S	0.94
DPb1	١	1.8	٤	13.7	<0.01	8.64

N.S:non significant ,RR:relative risk ,RR= a*d/b*c.(12)
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).

Table 3: distrebution 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	N.S	0.40
DPw4	38	69.0	25	60.4	N.S	1.01
DPw5	٢	3.6	5	13.8	<0.01	4.24
DPb1	١	1.8	1	2.7	NS	1.54

N.S:non 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 .

REFERENCES:

1. Moller-G ,editor Transplantation reviews .1975;22:82-97.
2. Jeannet-M Class II HLA antigen in autoimmune and immune mediated diseases / Solheim B,Moller E,Ferrone S,editors,Berlin 1986.pp489-514.
3. Albert-E;Baur-M;Mayr-W Histocompatibility testing.J-immunol.1989;2:68-77.
4. Sanchez-M; Shaw-S HLA-DP:Current status/ Sohleim B,Moller E,Ferrone S ,editors,Berlin 1986.pp83-108.
5. Bodmer-W HLA today.J-hum immunol. 1996; 17:490-503.
6. Pawelec-G;Ehninger-G;Schnmidt-H;Wernet-P HLA-DP matching and graft –versus-host disease in allogenic bone marrow transplantation.J-Transplantation.1990;42:558-560.
7. Moen-T;Stien-R;Bratile-A;Bondevik-E Distribution of HLA antigens in multiple sclerosis.J-Tissue Antigens.1987;15:126-129.
8. De Jongh-B; Termijtelen-A; Bruining-G Relation of Insulin-dependent diabetes and the HLA-linked SBsystem. J-Tissue Antigens. 1994;23:87-93.
9. Hoffman-R;Shaw-S;Francis-L HLA-DP antigens in patients with pauciarticular juvenile rheumatoid arthritis .J-Arthritis rheum. 1996;28:245-250.
10. Odum-N;Morling-N;Friss-J Increased HLA-DPw2 in pauciarticular onset juvenile arthritis.J-Tissue Antigens. 1995; 28: 254-250.
11. Terasaki-P;Meclelland-J Microdroplet assay of human serum cytotoxines. J-Nature. 1964; 204:998-1000.
12. Dick-H;Kissmeger-F;Nielson-F Histocompati-bility techniques .North holand,Biochemical press, Amsterdam ,New york, Oxford 1979.pp21-37.
13. Bender-K The HLA system .Biotest bulletin. 1984.Vol.2.pp64-116.
14. Boyum-A Isolation of mononuclear cells and granulocytes from human blood,isolation of mononuclear cells by centrifugation and of granulocytes by combind centrifugation at Ig.J-Clin.Lab.Inves.1968;21:77-78.
15. Danuilous-J;Terasaki-P;Park-M;Ayoub-G B-lymphocyte isolation by thrombin nylon wool in histocompatibility testing/Terasaki P,editor ,UCLA testing laboratory ,Los Angeles 1990:pp287-289.
16. Johanson-A;Hurley-C;Hartman-R HLA:MHC of human transplantation immunology/clinical diagnosis and management by labrotory methods.Sounder company ,Philidelphia 1996.Vol.2.pp958-978.
17. Graham-P;Gerhard-E;Claudia-M;Peter-W HLA(DR-DP)antigens in leukemia.J-Cancer. 1998;52:475-477.
18. Graham-P;Gerhard-E;Claudia-M;Peter-W Class II MHC antigens in leukemia .J-Cancer. 1999;61:312-325.