Brief Definitive Report

B-LYMPHOCYTE ALLOANTIGENS IN CAUCASIANS*

BY ALAN TING, M. RAY MICKEY, AND PAUL I. TERASAKI

(From the Departments of Surgery and Biomathematics, School of Medicine, University of California, Los Angeles, California 90024)

Recently, it has been shown that human B lymphocytes possess a polymorphic antigen system distinct from the HLA system present on both T and B lymphocytes (1–4). These B-cell antigens are also present on cultured lymphoblastoid lines (1, 3, 5, 6), acute lymphoblastic leukemia cells (7), and chronic lymphoblastic leukemia cells (1 and footnote 1).

Earlier we described four distinct B-lymphocyte specificities which appeared to belong to one allelic system and which were distinct from HLA (8). The present study represents a refinement of those groups with multiple sera that are highly associated within each specificity. Evidence for allelism by fit in the Hardy-Weinberg equilibrium is also provided here.

Materials and Methods

B-cell-enriched and T-cell-enriched lymphocyte populations were isolated from 59 normal unrelated Caucasians. The method used has been described in detail elsewhere.[2] Briefly, lymphocytes were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation. The B cells were isolated by rosette formation of the T cells with neuraminidase-treated sheep blood cells and relayering on Ficoll-Hypaque. After centrifugation the rosetted T cells sedimented to the bottom of the tube and the B cells remained at the interface. The T cells were recovered by lysis of the sheep red cells with hypotonic Hanks' Balanced Salt Solution. The proportion of the B lymphocytes in the B-cell-enriched population of some samples was measured by fluorescent staining of surface immunoglobulin using polyvalent rabbit antihuman immunoglobulin. The B-cell-enriched and T-cell-enriched lymphocyte suspensions were adjusted to 1.5×10^6/ml in McCoy's medium.

The microcytotoxic test was performed by adding 1 μl of serum to 1 μl of cell suspension (1,500 cells) in a microtest plate. The mixture was incubated at room temperature for 30 min. 5 μl of pooled rabbit complement was added, and incubation was carried out at room temperature for an additional 3 h. The reaction was then stained, fixed, and read for cytotoxicity.

The serum samples used to identify the B-cell specificities were obtained from multiparous women. Initially over 400 serum samples were screened for B-cell antibodies. The 32 serum samples described in this report were selected from those in the initial screening program. The majority of the sera contained HLA antibodies. Removal of such antibodies was performed by two

* Supported by grant AM02375 from the National Institute of Arthritis and Metabolic Diseases. Computing assistance was obtained from Health Sciences Computing Facility, University of California at Los Angeles, supported by National Institutes of Health Special Research Resources grant RR-3.

[1] Billing, R., A. Ting, and P. Terasaki. 1976. Human B lymphocyte antigen expressed by a majority of lymphocytic and myelocytic leukemia cells. II. Detection by human pregnancy sera. Submitted for publication.

[2] Ettenger, R. B., R. N. Fine, A. Ting, and P. I. Terasaki. 1976. The presence of anti-B-cell activity in renal allograft recipients. Submitted for publication.

absorptions of each serum sample with an equal amount of pooled packed platelets. Each absorption was carried out at room temperature for 60 min. The specificities described in this report were derived from Mickey's Boolean analysis.

Results

The 32 serum samples used to define 11 specificities did not react with T lymphocytes after platelet absorption, indicating lack of HLA antibodies. Reactivity with the B-cell-enriched lymphocyte suspensions varied from 0% kill to approximately 95% kill. Killing of 35–95% of the cells was considered a positive reaction. Fluorescence staining of surface immunoglobulin showed that between 30 and 70% of the cells in the B-cell-enriched fraction were B cells. Rerosetting with neuraminidase-treated sheep red blood cells showed less than 10% to be T cells.

The antigen and gene frequencies of the 11 specificities and the number of sera used to define each specificity are given in Table I. The terminology used here for specificity assignment is the same as that used for HLA specificities. "Main" specificities are designated by a number, and closely associated or included specificities are given the same number followed by a decimal progression. Group 1 has the highest frequency (50.8%) and group 2.2 the lowest (3.4%). Five specificities (1–5) were defined by multiple serum samples. The coefficient of correlation values among the sera within each group are given in Table II. The correlation coefficients are generally quite high, in the range of 0.55–0.90. Certain exceptions were seen, and these in some cases could be attributed to duospecific antibodies. For example, serum 3 in group 3 appeared to also contain anti-2, and serum 3 in group 5 also reacted with group 2. The five specificities were further analyzed to determine the number of genetic loci needed for their expression. Fig. 1 gives the phenotypic distribution of these five specificities in 59 unrelated Caucasians. Two sets of triplets were found, one cell having 1, 2, and 3 and the other 2, 3, and 4. 10 cells did not have any of the five specificities.

TABLE I

Antigen and Gene Frequencies of B-Cell Specificities in 59 Unrelated Caucasians

Group	No. Sera	Frequency	Gene frequency
		%	
1	3	50.8	0.299
2	4	22.0	0.117
3	4	16.9	0.088
4	5	16.9	0.088
5	5	11.9	0.061
1.1	4	32.2	0.177
1.2	1	22.0	0.117
2.1	1	10.2	0.052
2.2	2	3.4	0.017
3.1	1	13.6	0.070
6	1	10.2	0.052

The main B-cell groups are designated by a number and closely associated or included groups are given the same number followed by a decimal progression.

TABLE II

Coefficient Values (× 100) Among the Sera Defining Each of the Five Groups. Values of 30 or Greater Indicate a Significant Correlation (P value < 0.05)

Sera	1	2	3	4	5	Sera	1	2	3	4	5
Group 1						Group 4					
1		76	66			1		72	90	48	72
2			48			2			72	72	63
3						3				41	80
						4					35
Group 2						5					
1		67	54	53	63						
2			60	75	77	Group 5					
3				68	73	1		46	26	41	40
4					82	2			19	54	54
5						3				26	35
						4					54
Group 3						5					
1		85	37	54							
2			49	46							
3				24							
4											

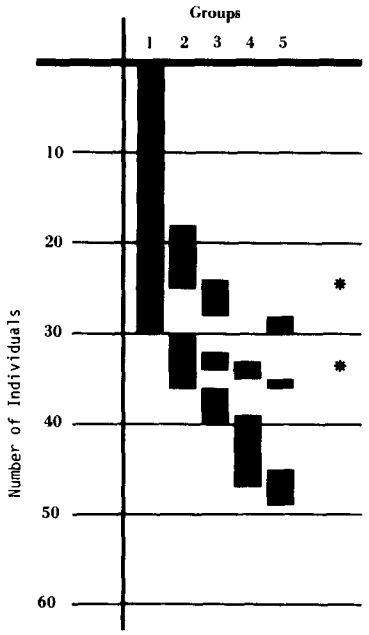

FIG. 1. Distribution of B-lymphocyte groups in 59 unrelated Caucasians. Two sets of "triplets" were found, each marked by an asterisk. Each bar represents individuals possessing the particular B-lymphocyte group given at the top of the figure. 49 of the 59 individuals studied could be typed for at least one of the five groups.

Goodness of fit of the five specificities into one allelic system was estimated by the Hardy-Weinberg equilibrium analysis (Table III). The X^2 value for the comparison of the number expected and observed for each phenotype was in each case less than 3.8. Furthermore, the total X^2 value, utilizing all phenotypes, was

TABLE III
Distribution of Phenotypes in 59 Unrelated Caucasians

Phenotype	No. expected	No. observed	X^2 value
1, –	17.5	18	0
1, 2	4.1	7*	2.1
1, 3	3.1	3	0
1, 4	3.1	0	3.1
1, 5	2.1	2	0
2, –	5.6	2	2.3
2, 3	1.2	2‡	0.5
2, 4	1.2	1	0
2, 5	0.8	1	0.1
3, –	4.1	3	0.3
3, 4	0.9	1	0
3, 5	0.6	0	0.6
4, –	4.1	5	0.2
4, 5	0.6	2	3.3
5, –	2.7	2	0.2
–, –	7.1	10	1.2
	58.8	59	13.9

$P > 0.18$; degrees of freedom (df) = 10.
* One cell 1, 2, 3 (treated as 1.2).
‡ One cell 2, 3, 4 (treated as 2.3).

13.9 with a P value of greater than 0.18. These results indicate that the five specificities could fit as an allelic system controlled by one genetic locus. The gene frequencies of the five specificities total 0.653, indicating that there are still a large number of alleles to be defined.

Certain specificities appear to be included in other specificities. Groups 1.1 and 1.2 are almost completely included in group 1, although there were four exceptions. Group 2.2 is included in group 2.1, and both are completely included in group 2. Group 3.1 is included in group 3 with one exception. These serologic inclusions indicate that the "broad specificities," 1, 2, and 3 may be split into smaller components, and this inclusion phenomenon is similar to that encountered in HLA serology. Partial inclusion of groups 1 and 1.1 may also indicate splits, with the reactions that do not fit representing technical errors or additional antibodies. On the other hand, the partial inclusions may indicate the existence of more than one genetic locus and a high degree of linkage disequilibrium between certain specificities as in HLA, e.g., HLA-A1 and HLA-B8, HLA-BW35 and HLA-CW4. It can not be discounted that the "short" groups are caused by weakly reacting sera.

The association among the B-cell specificities and HLA specificities was analyzed. No significant associations were seen. The highest was between group 1.1 and HLA-BW38 (r value of 0.14). However, it must be pointed out that the number of cells possessing certain B-cell specificities was small, and perhaps associations may become more apparent with larger numbers of cases.

Discussion

With the recent demonstration of excess antigenic reactivity with B lympho-

cytes (1–4) it is important to establish the specificities found on B lymphocytes, much as the HLA antigens have been defined. We have demonstrated here that typing for B specificities can be carried out in a relatively simple way by methodology already developed for the HLA system. The basic microlymphocyte cytotoxicity test was modified only by increasing the incubation time from 1.5 h to 3.5 h. Actually, the time can be shortened to the standard 1.5 hours, although relatively fewer sera would be suitable as reagents because of loss of sensitivity.

The principal technical difficulty encountered has been in the preparation of the B lymphocytes. Although the preparations are often not ideal and the surface immunoglobulin in staining cells are between 30 and 70%, by rerosetting, 90% of the cells can be shown to be non-T cells. Despite the problem that the B-cell preparations are probably contaminated with null cells and monocytes, it has been our experience that many of the positive reactions obtained show 90% of the test cells killed. Weak reactions in which a low preparation of cells are killed by all of the antisera are found in poor preparations with high T-cell contamination. By strict monitoring of the B-cell suspension to eliminate T cells, strong "B-cell" reactions in which 80–90% of cells are killed can routinely be obtained.

We attribute the increase in correlation coefficients among sera of the same specificity found in this study over the previous one (8) to the higher quality of non-T cells isolated and the ensuing stronger reactions obtained. With the procurement of multiple serum samples for each specificity from different donors, greater confidence can be attached to the validity and stability of the specificities defined.

With respect to the relationship of the B-lymphocyte specificities to HLA, no significant association has been found in this series or in the previous one (8). Thus, although the original serum samples contained HLA antibodies, after absorption with platelets, the remaining antibodies active against B cells no longer have HLA specificities. This does not mean, however, that the B-cell antigens are not determined by the HLA chromosome. Family studies are now being performed to examine this important point.

Summary

Human B lymphocytes have been shown to have at least five polymorphic specificities defined by 32 antisera. The antisera were produced by absorption with pooled platelets to remove HLA activity and were selected out of over 400 tested sera. The sera that defined the five specificities had high correlation coefficients within a group (generally in the range of 0.6–0.9). As shown by the fit in the Hardy-Weinberg equilibrium, the five specificities appear to be determined by alleles at one genetic locus. No association between these specificities and HLA was noted.

We wish to thank Mr. John McClelland, Mr. Min Sik Park, Ms. Judy Lesch, and Ms. Marilyn Makimoto for their excellent technical assistance.

Received for publication 14 January 1976.

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