Brief Definitive Report

ASSOCIATION OF CERTAIN Ia ALLOTYPES WITH THE OCCURRENCE OF CHRONIC LYMPHOCYTIC LEUKEMIA

Recognition by a Monoclonal Anti-Ia Reagent of a Susceptibility Determinant Not in the DR Series*

By ANTONIO NUNEZ-ROLDAN, ILONA SZER,‡ TAKASHI TOGUCHI, JANET CUTTNER, and ROBERT WINCHESTER

From the Erwin S. and Rose F. Wolfson Laboratory of Cellular Mechanisms of Disease, The Hospital for Joint Diseases Orthopaedic Institute, Mount Sinai School of Medicine, New York 10003

The existence of human Ia antigen specificities distinct from those of the system of DR alleles has been documented (1). More recently, polyclonal human allosera have been used to define the MT and MB systems of alleles (2, 3) and monoclonal antibodies recognizing specificities related to certain of the MT and MB determinants have been described (4).[1] Although important questions concerning the precise molecular and genetic basis of the Ia specificities remain unanswered, evidence is accumulating that many of the alloantigens presumably occur on distinct molecules under the control of separate genes (5–9).[1]

Despite the association of murine viral leukemogenesis with certain major histocompatibility complex haplotypes (10) in man, a considerable effort has not revealed evidence of a strong association between HLA A, B, or DR alleles and susceptibility to chronic lymphocytic leukemia or other common forms of malignancy (11–15). However, because in certain families, multiple instances of chronic lymphocytic leukemia or Hodgkin's disease were correlated with the inheritance of particular HLA haplotypes (16, 17), the possibility remained that previously unstudied alleles of histocompatibility loci, such as the non-DR Ia specificities, might be associated with this susceptibility. The present study was undertaken among individuals with chronic lymphocytic leukemia to explore this hypothesis.

Materials and Methods

Population Characterization. The diagnosis of chronic lymphocytic leukemia was made in 29 American Caucasians by conventional blood and bone marrow examination and the presence of a monoclonal proliferation of B lymphocytes. 48% of the individuals were considered to be of Jewish extraction. 30% were Rai stage 0, 15% stage I, 20% stage II, 15% stage III, and 20% stage IV. Healthy laboratory and administration personnel served as controls. They were selected before study to include a similar percentage of ethnically matched persons. The average age of the control population was 33; that of the study was 60.3.

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‡ Fellow of the New York Rheumatism Association.

[1] T. Toguchi, G. R. Burmester, A. Nunez-Roldan. I. S. Szer, J. D. Capra, and R. J. Winchester. Definition of MT3-like and MB3-like determinants with monoclonal antibodies: molecular independence of the antigens. Manuscript submitted for publication.

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Cell Separation and Immunofluorescent Staining Techniques. The mononuclear cells and enrichment for B cells by depletion of T cells through binding to sheep erythrocytes was performed as described (18). The isotype of the monoclonal antibody IVD12 was IgG1, 109d6 IgG2a, and 22C6 IgG2a. The monoclonal reagents were used in indirect immunofluorescence as undiluted supernatants containing 20–40 μg of antibody/ml. Control reagents included monoclonal antibodies of the same isotype with irrelevant specificities. 0.020 ml of the supernatant containing the monoclonal antibody was added to $0.5–1 \times 10^5$ cells in a V-bottomed microtiter plate (Cooke Laboratories, Alexandria, VA) and microimmunofluorescence staining was performed as described (19). $F(ab')_2$ fragments of rabbit anti-mouse Ig antibodies that were directly conjugated with tetramethylrhodamine isothiocyanate were used in the second step. Specificity controls to detect interference by the IgG Fc receptor and analysis of the stained cells were performed by established procedures (18). The criterion used for determining positivity was staining of >90% of the lymphocytes stained by the monomorphic anti Ia reagent 22c6 (19). Negativity was defined as staining of <5% of the Ia^+ lymphocytes.

Histocompatibility Typing and Statistical Analysis. HLA DR 1–10 and MT1/MB1, MT2,3, and MB2 typing was performed using the standard National Institutes of Health microlymphocytotoxicity assay with 7th and 8th International Histocompatibility Workshop reagents as well as with locally available sera (20). The chi square with Yates correction was used. The relative risk according to Woolf, or ratio of conditional probabilities of disease in the presence or absence of the marker was used as described (20), except that in the case where fractional values were obtained, the reciprocal of the decimal fraction was used and preceded by a negative sign.

Results

Using alloantisera and allospecific monoclonal reagents, individuals with B type chronic lymphocytic leukemia were found to have similar profiles of allodeterminants (Table I). Of the 29 individuals, 27 reacted positively with the monoclonal reagent IVD12, 26 were MT2+, and 18 expressed the DR5 alloantigen. Of the 11 individuals lacking DR5, seven were DR4+. All individuals with chronic lymphocytic leukemia were either MT2+ or IVD12+, and 24 bore both allodeterminants. Reciprocally, the occurrence of individuals bearing MT1/MB1 or DR2 was infrequent.

Comparison of the frequency of Ia alloantigens with a control group of normal individuals studied at the same time revealed a significant alteration in the frequency of several allodeterminants, as illustrated in Table II. Statistically significant positive associations were found with the antigens detected by the monoclonal antibody IVD12, relative risk 13.5, and with DR5 and MT2 determinants, respective relative risks of 6.0 and 4.8. The use of the lower reported frequency for MT2 among Caucasians (21) resulted in a relative risk of 7.2. In contrast, significant negative associations were found with the presence of MT1/MB1 and DR2, respective relative risks of −8.1 and −7.5. No significant associations were encountered with the DR allospecificities 1, 3, 4, w6, or 7. In both the control and leukemic populations, the presence of MT3 detected by microcytotoxicity paralleled the detection of the determinant by the monoclonal reagent 109d6 using immunofluorescence. However, the frequencies did not significantly differ between the two populations.

Discussion

The principal findings of this study were that individuals with chronic lymphocytic leukemia differed significantly from a control population in the frequency of various Ia allodeterminants, and that strong positive and negative associations were evident with Ia allospecificities that were not alleles of the DR system. These observations suggest the existence of genes mapping in the Ia portion of the major histocompati-

TABLE I

Frequent Occurrence of Certain Ia Alloantigenic Determinants among 29 Individuals with B Cell Type Chronic Lymphocytic Leukemia

Number of individuals with each phenotype	Ia alloantigen phenotype					
	Microcytoxicity with alloantisera				Immunofluorescence with monoclonal antibodies	
	HLA-DR specificities	Non-DR specificities			109d6	IVD12
		MT1/MB1	MT2	MT3		
4	5,	−	+	−	−	+
1	5,	−	+	+	+	+
1	5,1	+	+	−	−	+
2	5,2	+	+	−	−	+
3	5,3	−	+	−	−	+
2	5,4	−	+	+	+	+
4	5,7	−	+	+	+	+
1	5,w8	−	+	−	−	+
1	4,	−	+	+	+	+
1	4,1	+	−	+	+	+
2	4,3	−	+	+	+	+
1	4,w6	−	+	+	+	+
2	4,7	−	−	+	+	+
2	w6,	−	+	−	−	+
1	w6,w10	+	+	−	−	−
1	1,3	+	+	−	−	−

TABLE II

Contrasting Frequencies of Certain Ia Alloantigenic Determinants between Individuals with Chronic Lymphocytic Leukemia and a Control Population

Ia alloantigen specificity	Individuals with Leukemia $n = 29$	Normal control $n = 28$	Chi square (Yates)	Relative risk
	% positive			
DR1	10.3	21.4		−2.4
DR2	6.9	35.7	5.48 ($P < 0.05$)	−7.5
DR3	20.7	25.0		−1.3
DR4	31.0	25.0		+1.4
DR5	62.1	21.4	8.08 ($P < 0.005$)	+6.0
DRW6	13.8	17.9		−1.4
DR7	20.7	21.4		−1.0
DRW8	3.4	0		—
DRW10	3.4	0		—
MT1	20.7	67.9	11.04 ($P < 0.001$)	−8.1
MT2	89.7	64.3	3.88 ($P < 0.05$)	+4.8
MT3	48.3	50.0		−1.1
109d6	48.3	50.0		−1.1
IVD12	93.1	50.0	11.06 ($P < 0.001$)	+13.5

bility complex that influence the susceptibility or resistance to chronic lymphocytic leukemia.

The two monoclonal antibodies, IVD12 and 109d6, that reacted with Ia gene-encoded determinants were the subject of a separate report.[1] In summary, the pattern of reactivity of 109d6 resembled that of the MT3 specificity (3) with positive reactions given by the B cells of all normal individuals who were $DR4^+$ or $DR7^+$ ($r = 0.943$). The IVD12 determinant was identified on B cells of all $DR4^+$, most $DR5^+$ ($r = 0.820$ for DR4 or DR5), and a subset of $DRw6^+$ individuals in a pattern of reactivity

generally similar to that of the MB3 determinant (2). Evidence has been obtained that each antigen detected by the monoclonal reagents was found on molecules distinct from one another and from those bearing the DR4 or DR5 specificities.[1] The provisional haplotypic association of the putative genes encoding the separate Ia specificities were MT2 and IVD12 in association with DR5, and MT3/109d6 and IVD12 in association with DR4.

Among individuals with chronic lymphocytic leukemia, the most frequent inferred haplotype was that associated with the antigens IVD12, MT2, and DR5. Four individuals appeared to be homozygous for this Ia haplotype. Among the other individuals positive for both IVD12 and MT2 that lacked DR5, the putative genes encoding IVD12 and MT2 were each provided by a different homologous chromosome. It appears most likely that the altered frequency of DR alleles among those with leukemia is attributable to the haplotypic associations with the non-DR Ia determinants.

Further support for this possibility comes from certain earlier studies that reported a decrease in the frequency of a DR2-like specificity (22) and an increase in the frequency of the alleles DR4 and DRw6 (23). These alterations are consistent with the increase in the frequency of haplotypes containing either MT2 or IVD12 among those with leukemia. This finding of a primary association of disease susceptibility with alleles of the MT and MB series provides further support for the separate genetic control of these determinants and suggests the possibility that these or related gene products have functions distinct from those of the DR molecules.

Possible additional evidence favoring an influence of genes in the Ia system on the susceptibility identified in this report can be inferred from the lower percentage of leukemic deaths attributed to chronic lymphocytic leukemia among Japanese, 2.9%, compared with 31.5% among American Caucasians (24). Although the frequency of IVD12 is not defined in different populations, the frequency of DR5, the DR alloantigen associated with IVD12 and MT2, is 4.3% among the Japanese and 18.8% among American Caucasians (21).

Summary

The Ia antigen allospecificities of individuals with B type chronic lymphocytic leukemia differed significantly from those of a control population. A monoclonal antibody, IVD12, directed to a MB3-like determinant, reacted with 92.5% of the individuals with leukemia and yielded the greatest positive relative risk, 13.5. A lower degree of positive association was found with the presence of the MT2 determinant. In contrast, the low observed frequency of the MT1/MB1 determinant among leukemic individuals was associated with the most significant negative relative risk, −8.1. Among HLA-DR specificities, the relative risk associated with the presence of DR5 was positive while that with DR2 was negative.

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