

DISEASE ASSOCIATIONS OF THE Ia-LIKE  
HUMAN ALLOANTIGENS  
Contrasting Patterns in Rheumatoid Arthritis and  
Systemic Lupus Erythematosus\*

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Studies of the molecular systems determined by genes that map in the major histocompatibility complex (MHC) have led to the recognition of an alloantigenic system preferentially expressed on B lymphocytes (1). This B-cell system has extensive biological and chemical homologies with the I-region antigens of the murine histocompatibility system and has been termed Ia-like or simply Ia. These Ia alloantigens were recognized with alloantibodies that developed as a result of immunization with paternal antigens during pregnancy, or in the sera of renal transplant recipients who became immunized against nonmatching antigens present on the homograft. These human Ia determinants are highly polymorphic, with certain alloantigenic specificities relating closely to HLA-D alleles defined by the mixed lymphocyte culture reaction (2); for this reason, particular Ia phenotypes were designated HLA-DR (3).

The relevance of the B-cell alloantigens to the immunogenetics of disease was first shown for multiple sclerosis (4), where a very high percentage of patients reacted with a particular serum with a specificity related to what is now termed DRw2. These studies suggested that an important application of the B-cell alloantisera might be their potential for the recognition of MHC-related disease, even in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) where no clear MHC associations had been evident by classical HLA-A and B locus typing.

The preliminary observation that one B-cell alloantiserum reacted with a high percentage of patients with RA (5), suggested that patients with SLE would also show a relationship to alleles of the MHC, because these two diseases while clinically distinct, are nonetheless thought to be related. In the present study, further evidence for the value of Ia alloantigen typing was obtained in patients with RA or SLE; however, markedly contrasting MHC relationships were observed for these two diseases, suggesting that they have very different etiologies.

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TABLE I  
*Contrasting B-Cell Alloantigen Profiles in Patients with Rheumatoid Arthritis and Systemic Lupus Erythematosus*

Reagent	HLA-DRw Specificity	Normal (n = 40) Frequency	Rheumatoid arthritis (n = 44)			Systemic lupus erythematosus (n = 24)		
			Frequency	$\chi^2$ <sup>a</sup>	RR <sup>b</sup>	Frequency	$\chi^2$	RR
7Am	1	31	9	4.9*	0.2	30	0.0	1.0
1239	2	25	13	1.2	0.5	50	3.9*	3.0
1066	2 + 3	34	34	0.0	1.0	75	9.4*	5.7
1033	3	20	14	0.2	0.6	54	6.5*	4.7
1283	4 × 7 × 10	31	80	16.9*	9.1	12	1.7	0.3
1995	5	11	4	0.5	0.4	8	0.0	0.7

a = chi square, corrected (Yates).

b = relative risk.

\* Statistically significant values: for  $\chi^2 > 3.8$ ,  $P < 0.05$ ; 6.6,  $P < 0.01$ ; 7.9,  $P < 0.005$ .

## Materials and Methods

*Patient Selection and Cell Preparation.* Mononuclear cells were isolated by Ficoll-Hypaque centrifugation from blood samples obtained from normal volunteers and from patients meeting the American Rheumatism Association criteria for seropositive RA or SLE. T cells were removed by depletion of the lymphocytes forming rosettes with sheep erythrocytes previously treated with neuraminidase as described elsewhere (6). The enriched preparations of B cells were incubated overnight in medium RPMI-1640 supplemented with 10% fetal calf serum in plastic T flasks (BioQuest, BBL & Falcon Products, Becton, Dickinson, & Co., Cockeysville, Md.) to remove adherent cells. B-cell lymphoid lines were maintained in medium RPMI-1640 supplemented with 10% fetal calf serum. Included among the 40 cell lines used for reference purposes were those derived from individuals who were homozygous for mixed lymphocyte culture specificities HLA-DW1, DW2, DW3, DW4, DW5, DW6, DW7, and DW10.

*Preparation of Ia Antisera Typing Panel.* Over 200 sera from multiparous women and renal transplant recipients characterized as having B-cell alloantibodies (7) were absorbed twice, volume-for volume, with T-lymphocytes and packed pooled platelets, for 1 h at 4°C. The resulting sera were free of anti-HLA A, B, and C activity when retested on peripheral blood T lymphocytes.

*B-Cell Alloantigen Assay.* A modified Amos two-stage microcytotoxicity assay was performed on the purified B cells and reference B-cell lines, in accord with standard methods (8). In the first step, the cell-serum mixture was incubated for 20 min at 37°C. Prescreened rabbit serum found to be free of naturally occurring cytotoxic activity against human lymphocytes was added as a source of complement, the mixture incubated for 30 min at 37°C. Each reaction was performed in duplicate, and was considered positive if 50% or greater number of cells above control stained with trypan blue dye. Relative risk, calculated according to Woolf (9), estimates the risk of developing the disease if the alloantigen is present. If no association exists, the relative risk = 1.0.

## Results

Table I illustrates contrasting B-cell alloantigen profiles in patients with RA and SLE detected by the selected reagent alloantisera. The most significant association in patients with RA was the high frequency of alloantigens detected by serum 1283. This serum reacted with 80% of patients with RA (relative risk = 9.1), 31% of normals, but only 12% of patients with SLE. Two alloantisera 259 and 1038 not illustrated in the Table gave essentially the same pattern of reaction as serum 1283. An additional two antisera 924 and 191 which were very similar to each other gave reactions that partially resembled the pattern of the previous three alloantisera in

that they reacted with 60% of patients with RA, 22% of normal subjects and only 4% of patients with SLE, relative risk 0.2. The alloantigen detected by serum 7Am was detected in only 9% of patients with RA but in 31% of the normal control population as well as in 30% of patients with SLE.

Among patients with SLE, the greatest frequency of reactivity was found with serum 1066, accounting for 75% of the population (relative risk = 5.7). Serums 1239 and 1033 gave lesser but still significantly increased frequencies of reaction among patients with SLE. The positive reactions of serum 1066 included those patients with SLE who were positive with either serum 1239 or 1033. Not illustrated in the Table are four additional sera that gave nearly identical patterns of reaction to either serum 1239 or serum 1033. Problems were encountered in typing a few of the SLE patients, presumably due to high concentrations of monocytes in the typing preparations. These patients were excluded from the study.

Analysis of the reactivity of the reagent sera with a panel of reference B-cell lymphoblastoid lines derived from individuals homozygous for HLA-D alleles provided a means of describing the relationship of the alloantigens detected by the sera to certain known markers of the histocompatibility complex, as shown in the second column of the Table. Sera 1283, 259, and 1038 reacted with each of four B-cell lines derived from individuals homozygous for DW4, all three from individuals homozygous for DW7, and all three from individuals homozygous for DW10. Sera 924 and 191 not illustrated in the Table reacted with all cell lines derived from individuals who were either DW4 or DW10 but not with lines derived from individuals who had any other D locus specificities including DW7. Serum 1066 reacted with each of five cell lines from individuals who were homozygous for DW2 and with lines from five individuals homozygous for DW3. Serum 1239 reacted only with the DW2 cell lines and serum 1033 reacted only with the lines derived from the DW3 positive individuals. Sera 7Am and 1995 reacted respectively with cell lines derived from DW1 or DW5 positive individuals.

Absorption experiments with the prototype alloantisera, 1066 and 1283, gave different results. Absorption of serum 1066 with B-cell lines derived from individuals positive for either DW2 or DW3 left intact an alloantibody reacting with the cell line of the other specificity. For this reason, the specificity of serum 1066 was designated DRw2+3. Absorption of serum 1283 with B-cell lines derived from any individuals homozygous for either DW4, DW7, or DW10 removed antibody reactivity against all three. The specificity defined by this alloantiserum was designated DRw4x7x10.

### Discussion

The present study demonstrated distinct B-cell alloantigen profiles in patients with SLE or RA, two rheumatic diseases frequently considered related. Two alloantisera were identified that discriminated between the disease groups particularly well. Serum 1066 reacted with 75% of the SLE patients. In contrast, serum 1283 reacted with 80% of patients with RA, but only 12% of patients with SLE.

Sera in this study were defined by their disease association. This was of some novelty in that it afforded insights into the nature of genetic associations that might not otherwise have been as readily apparent. In this context, the relationship of a particular alloantigen defined by a B-cell alloantiserum to individual alleles of a single MHC locus, might be of lesser significance than the relationship to an as yet

undefined disease susceptibility gene with which it is in linkage disequilibrium. It will remain for future work to delineate the exact fine specificities of these antisera.

Also of interest is the observation of a low fractional relative risk for a specific alloantigen in a given patient population. Examples of this are the decreased frequency of the reactivity with allosera 924, 191, and 1283 among patients with SLE, and the decreased frequency of the reactivity of serum 7AM in patients with RA. This suggests that although the inheritance of particular alloantigens was associated with susceptibility to certain diseases, the possibility exists that they may also confer resistance to others.

The use of reference B-cell lines permitted a description of the relationship of the alloantigens detected by these sera to certain defined markers of the HLA system. Serum 1066 contained two antibody specificities that in part related to DRw2 and DRw3. Absorption of this antiserum with B cells homozygous for either specificity did not remove reactivity against the other, indicating that these two antibodies were distinct and not cross-reactive. The association between susceptibility to SLE and DRw2 and DRw3 has also been recognized in another population by Reinartsen et al. (10) as well as in a preliminary report on the present patients (11). Serum 1283 and the related sera 259 and 1038 detected an alloantigen shared by individuals positive for either DRw4, DRw7, or DRw10. A lower association between DRw4 alone and susceptibility to RA has been reported by Stastny (12). Absorption of serum 1283 with B cells homozygous for either specificity completely and reciprocally removed antibody activity against any single one. In this instance, partial analysis indicated that the serum detected a public specificity which was, in turn, associated with susceptibility to RA.

### Summary

Increasing evidence has been obtained of the special value of Ia-like B-cell alloantisera for demonstrating disease associations with histocompatibility antigens. This was particularly evident for the study of the immunogenetics of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), two conditions frequently considered related. The profiles of antigens recognized by the alloantisera in patients from each disease group was distinctive. Two types of alloantisera were obtained that illustrated the divergence between the two diseases. One type showed a higher than normal incidence in RA but lower than normal in SLE; the other showed a higher incidence in SLE. While these sera were not totally defined, evidence was obtained that the SLE-reactive alloantiserum related to two alleles of the major histocompatibility complex DRw2 and DRw3, while the RA-reactive alloantiserum related to a common specificity shared by cells positive for either DRw4, DRw7, or DRw10. The data indicate that immunogenetic factors are relevant to the development of both RA and SLE, but that these are distinct for each disease.

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