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Specific human B lymphocyte alloantigens linked to HL-A

(lymphocyte subpopulations/non-HL-A alloantigens/genetic control)

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ABSTRACT Sera, previously found to react specifically with B lymphoid cultured cells, were tested on isolated T and B peripheral blood lymphocytes in a microcytotoxicity assay. Studies were performed on lymphocytes obtained from several large Amish families. The sera used in these studies were cytotoxic to peripheral blood, B lymphocytes, but not cytotoxic to T lymphocytes. The antigens detected followed the inheritance pattern of HL-A haplotypes. The strong linkage disequilibrium with HL-A antigens suggests that genes controlling the expression of B lymphocyte antigens are linked to genes controlling HL-A alloantigens.

Human histocompatibility antigens (HL-A) are controlled by genes in an apparently large complex that has been designated HL-1 (1). The major HL-A antigens are readily identified by serologic techniques. There is ample evidence that the expression of other lymphoid cell surface antigens are controlled by genes in this complex (2–4).

Such antigens have heretofore been defined by mixed lymphocyte reactions and have been designated LD, or lymphocyte determined antigens. Evidence, therefore, indicates that the lymphocyte determined antigens are expressed only on B lymphocytes, in contrast to the serologically defined antigens (HL-A), which are found on both T and B lymphocytes. Several reports have demonstrated serologic detection of single antigens expressed on B cells, one of which appears to be lymphocyte determined antigen (5, 6).

Winchester *et al.* (7) and Mann *et al.* (8) independently reported the detection of antigens specific for human "B" lymphoid tissue cultured cell lines. Our observation was made using nonabsorbed multiparous sera containing no antibodies against HL-A. These sera were obtained from women of a religous sect, the Amish. (This sect, because of religous and cultural preference, live in relatively isolated communities, intermarry, and have large families.) Using these sera, we explored the possibility of serologically identifying antigens specific for human "B" lymphoid cells in two relatively large families. A number of specific B lymphoid cell antigens apparently genetically linked to HL-A antigens were found.

METHODS AND MATERIALS

Sera were prepared from whole blood obtained from multiparous women and stored frozen (−20°) until use. Peripheral blood lymphocytes were separated from other formed blood elements by Ficoll-Hypaque (Bionetics) sedimentation. Human "B" lymphocytes were isolated by a technique that takes advantage of the expression of receptors for the Fc portion of complexed immunoglobulin on B lymphocytes (7). The characteristics of the separated cell populations are described in detail elsewhere (Henkart and Alexander, submitted for publication). In brief, this technique separates the Fc positive, Ig positive, and complement receptor cell positive cells (adherent population) from those peripheral blood lymphocytes not bearing the above cell surface markers. In this manuscript we are calling the adherent cells B lymphocytes and the nonadherent, T lymphocytes. The separation and testing procedures are as follows. Polystyrene tissue culture flasks were coated with antigen–antibody complexes by incubating their lower surface with fetal calf serum, washing, modifying the absorbed protein with trinitrobenzene sulfonate, washing, and finally incubating with rabbit antibody against dinitrophenyl (Dnp)-bovine serum albumin. (Antibodies to Dnp and trinitrobenzene sulfonate are cross-reactive.) After 20 min at 37° the plates were again washed and the prepared lymphocytes were added. The cells were allowed to settle for ½ hr at 37°. Nonadherent cells (T lymphocytes) were removed by pouring the supernatant from the plates and centrifugation. The adherent cells were removed by adding a solution of 1 mM Dnp-L-lysine in phosphate-buffered saline containing 0.01 M EDTA and incubating for ½ hr at 37°. Seven to twenty percent of the total population of lymphocytes adhered to the antigen–antibody coated plastic surfaces consistent with reported numbers of human peripheral blood "B" lymphocytes.

Three thousand T or B lymphocytes were added to 1 μl of serum in microtiter plates and incubated for 1 hr at room temperature. The cells were washed once and 5 μl of whole rabbit serum was added to provide a source of complement. (The rabbit serum was absorbed for 1 hr at 4° with a "B" lymphoid cultured cell line in proportions of 1 ml of sera to 2×10^7 cells.)

The complement was removed by "flicking" the microtiter plate. Trypan blue containing 1.4% EDTA was added. Cell death was determined by incorporation of trypan blue dye.

Serologic reactions were performed in triplicate. A positive control was added to each cell preparation. This consisted of a human serum containing antibodies to human peripheral blood lymphocytes which by previous determination was cytotoxic to lymphocytes from approximately 95% of a normal population. Background controls consisted of complement plus heat-inactivated sera from male donor red blood cell type AB. Each well was scored for numbers of cells incorporating trypan blue (cell death) in the following manner: 1 = 0–10%; 2 = 10–20%; 3 = 20–30%; 4 = 30–50%; 5 = 50–70%; and 6 = 70–100%. Serologic reactions were called positive when numbers were at least 2 greater than the complement controls. These controls were generally 1 or 2 and occasionally 3. Those tests in which the complement controls were greater than 3 were not considered in the computation of the data. An example of the triplicate scores given to serologic reaction in a father and mother of one of the families studied is presented in Table 1. Sera reacting

Table 1. Cytotoxic reaction of sera to isolated T and B lymphocytes from wife and husband in Le Bouf family

Family member	HL-A antigens	Cell type	Serum designations and test results*														
			9	76	359	52	289	177	189	35	192	244	590	196	107	C'†	Io‡
Husband	2,7	T	1	2	2	1	2	2	1	2	2	1	2	1	1	2	6
			2	2	2	1	1	1	2	1	1	2	1	2	2	2	6
			1	2	1	1	1	1	2	2	1	1	1	1	1	1	6
	W29,W10	B	1	2	2	5	5	2	2	4	6	6	4	1	2	2	6
			2	1	2	3	4	1	2	4	4	5	4	2	1	2	5
			3	2	2	6	5	2	2	4	5	5	6	1	2	2	6
Wife	1,W5	T	1	1	1	1	1	1	1	1	2	1	1	1	1	1	6
			2	1	1	1	1	1	2	1	2	2	1	1	1	2	6
			2	1	1	1	1	1	2	1	1	2	1	1	1	1	6
	9,W15	B	4	5	4	2	2	4	3	2	1	1	2	5	4	2	6
			5	5	4	1	2	5	6	1	2	1	2	4	4	2	5
			4	3	4	1	1	4	4	2	2	1	1	5	5	1	4

* Numerical scores represent % cells killed in cytotoxicity assays. 1 = 0–10%; 2 = 10–20%; 3 = 20–30%; 4 = 30–50%; 5 = 50–70%; and 6 = 70–100%.
† Complement control.
‡ Positive serum control.

specifically with B lymphocytes (shown in boxes) from the husband were 52, 289, 35, 192, 244, and 590. Those reacting with the wife were 9, 76, 359, 177, 189, 196, and 107. All reactions were scored in a similar fashion and are recorded by plus (+) for a positive reaction and negative (−) for nonreactive sera. In some instances in each of the families studied, reactions were not present where expected or cytotoxic reactions occurred that did not fit the haplotype association. These were considered to be false negative or false positive reactions. The frequency of these reactions was within the error of the test system (11%), which was determined on repeated testing of one individual. HL-A phenotyping was performed on the lymphocytes from the individuals studied using the dye exclusion technique as described by Kostyu *et al.* (10).

RESULTS

Two families were studied to determine if the serologic reactions to B lymphocytes were associated with or linked to an HL-A haplotype. These results are summarized in Table 2. A (+) indicates a positive reaction of the sera to B lymphocytes using the criteria given above and exemplified in Table 1. Due to technical problems, the mother in Family 1 was not typed for B cell antigens. However, her HL-A type had previously been determined, allowing assignment of B cell reactivity to maternal HL-A haplotypes. Nine sera (43, 124, 386, 116, 35, 76, 1, 189, and 207) reacted with B cells from the father and all of the children expressing the HL-A 1,8 haplotype. Sera 289 and 52 reacted with B cells from those individuals possessing the paternal HL-A 2,7 haplotype. Another 2,7 haplotype was inherited from the mother by the offspring in this family. The sera (177, 176, and 590) reacting in association with the maternal 2,7 haplotype were different from those reacting with cells from offspring possessing the paternal HL-A 2,7 haplotype.

In Family 2, the serologic reactions again followed a pattern indicating HL-A haplotype association. Sera 9, 76, and 359 reacted with maternal and offspring cells bearing the HL-A 1, W5 haplotype. The other maternal haplotype, HL-A 9, W15, was identified in mother and children by the serologic reactions of 177 and 189. Sera 107 and 196 reacted with B lymphocytes from mother and all of the offspring, thus not differentiating the maternal HL-A haplotypes. Sera 35, 192, 244, and 250 reacted in association with the paternal HL-A W29, W10 haplotype. Sera 52 and 289 reacted with B cells from the father and all of the children and thus did not discriminate between the HL-A 2,7 and W29, W10 haplotypes. Thus, in both families the inheritance of B cell alloantigens closely follows a pattern of linkage disequilibrium with genes controlling the HL-A antigens.

DISCUSSION

There is ample evidence indicating that the serologic reactions seen were not detecting HL-A activity. All lymphocytes (T and B) express HL-A antigens. The sera used in these assays had been previously screened for HL-A reactivity on preparations of peripheral blood lymphocytes and were found to be nonreactive. These studies were performed by a dye exclusion assay (as described and used in the present study). Since B cells represent only 6–20% of the total population of peripheral blood lymphocytes, positive reactions to B cells only could have been easily missed. The separation of the B subpopulation from T cells and the exclusive reactivity of these sera to the B cells and not T cells indicates non-HL-A reactivity.

Studies in mice have demonstrated the presence of non-H-2 antigens, associated with B lymphocytes and designated Ia (11). Genes controlling these antigens map between the H-2d and the H-2k regions of the H-2 histocompatibility complex. Ia-like antigens may be expressed on the peripheral blood lymphocytes of rhesus monkeys (12). Human B cell antigens may be analogous to the Ia antigens in mice.

There are several recent reports of the serologic detection of non-HL-A antigens on human peripheral blood lymphocytes. Legrand and Dausett (5) described sera that caused cytotoxicity to a subpopulation of peripheral blood lymphocytes. The pattern of reactivity did not associate with the inheritance of HL-A in family studies. van Rood *et al.* (13) studied a serum by immunofluorescence, cytotoxicity, and inhibition of mixed lymphocyte reactivity. The antigen detected by this serum was inherited with HL-A haplotypes and showed an association with the MLR locus in a family where crossover occurred between MLR and the four series

Table 2. Summary of results of specific B lymphocyte serologic reactions in two families

Family 1	HL-A type	Serum designations and test results													
		43	124	386	116	35	76	1	189	207	289	52	177	176	590
Ez (father)	1,8 2,7	+	+	+	+	+	+	+	+	+	+	+	—	—	—
Er	1,8 2,7	+	+	+	+	+	+	+	+	+	—	—	+	+	+
Id	1,8 2,7	+	+	+	+	+	+	+	—	+	—	—	+	+	+
Rb	1,8 2,7	+	+	+	+	+	+	+	+	+	—	—	+	+	+
Gr	1,8 2,7	+	+	+	+	+	+	+	+	+	—	—	+	+	+
Ar	1,8 2,*x*	+	+	+	+	+	+	—	+	+	—	—	—	—	—
Le	2,7 2,7	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Wi	2,7 2,7	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Rt	2,7 2,7	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Ol	2,7 2,7	—	—	—	—	—	—	—	—	—	+	+	+	+	+
HL-A haplotype association						1,8					2,7 (paternal)			2,7 (maternal)	

Family 2	Cell type	9	76	359	52	289	177	189	35	192	244	590	107	196
Le (father)	2,5 W29,W10	—	—	—	+	+	—	—	+	+	+	+	—	—
Rb (mother)	1,W5 9,W15	+	+	+	—	—	+	+	—	—	—	—	+	+
Ma	W29,W10 9,W15	—	—	—	+	+	+	+	—	+	+	+	+	+
Ve	W29,W10 9,W15	—	—	—	+	+	+	+	+	+	+	+	+	+
Le	W29,W10 9,W15	—	—	—	+	+	+	+	+	+	+	—	+	+
Ab	1,W5 W29,W10	+	+	+	+	+	—	—	+	—	—	+	+	+
Fa	2,7 1,W5	+	+	+	+	+	—	+	—	—	—	—	+	+
Ed	2,7 1,W5	+	+	+	+	+	—	—	—	—	—	+	+	+
Ez	2,7 9, W15	—	+	—	+	+	—	+	—	—	—	—	+	+
Wi	1,W5 W29,W10	+	+	+	+	+	—	—	+	+	—	+	+	+
Hl-A haplotype association			1,W5		2,7 W29,W10		9,W15			W29,W10			1,W5 9,W15	

of HL- alloantigens. This antigen appeared to be present only on B lymphocytes.

The results of the studies presented here expand the observation of van Rood and present evidence for the existence of a number of specific B cell antigens. A minimum of seven different B lymphocyte antigens were detected with the sera studied. However, several sera may be detecting more than one antigen. Serum 35 reacts with those individuals in Family 1 where the HL-A 1,8 haplotype is expressed, and in Family 2 this serum reacts with B cells from those individuals possessing the W29, W10 HL-A haplotype. Other sera reacting in association with these haplotypes do not share this pattern of reactivity. Sera 76 and 189 reacted with B cells from those individuals with the 1,8 HL-A haplotype in Family 1 but had different reactivity patterns in Family 2. It appears that there are several antibodies detecting different antigens in these sera and that B lymphocytes may express several specific antigens.

The large family studies provide evidence for strong linkage disequilibrium of genes controlling the B lymphocyte antigen expression with genes controlling the expression of HL-A. The B cell antigen reported by van Rood also appears to be in linkage disequilibrium with HL-A and is probably an antigen responsible for the stimulation seen in mixed lymphocyte reactions. Whether or not the sera used in our studies are detecting the antigen or antigens responsible for

mixed lymphocyte reactivity remains to be determined. Preliminary results demonstrate blocking of the mixed lymphocyte reactions by some but not all of the sera. The relationship of the antigens described in this report to the lymphocyte determined (LD) antigens remains to be determined.

More precise definition of the numbers of B cell antigen specificities, their allelic association, and genetic mapping is needed. Until this is accomplished, we would propose that these antigens be called L-B antigens.

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