DETECTION OF ANTIGENS SPECIFIC FOR B-LYMPHOID CULTURED CELL LINES WITH HUMAN ALLOANTISERA

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The human histocompatibility antigens (HL-A) which can be readily defined by serological procedures appear to form part of the larger histocompatibility complex which is designated HL-1 (1, 2). The HL-1 complex is believed to include determinants which control the ability to stimulate in mixed lymphocyte culture (MLR)[1] and which map separately from the *HL-A* loci (3). While the HL-A antigens are expressed on all lymphocytes, the ability to stimulate in MLR appears to be limited to a lymphoid subpopulation generally classified as "B" cells (4).

In an analogous histocompatibility system (*H-2*) of the mouse a number of antigens (Ia) controlled by the *I* region of the *H-2* complex have been described that have specific expression on lymphoid B cells (5–7). These Ia antigens are allotypic and appear to be controlled by genes linked to *H-2* (8). The capacity to stimulate in mouse MLR is predominantly expressed by B cells (9). Studies in mice have progressed quite rapidly due in large part to the availability of inbred strains which allow production of specific antisera detecting *I*-region cell membrane antigens. In man, however, allotypic reagents specifically detecting non-HL-A antigens associated with subpopulations of human lymphoid cells have not been described. We have attempted to identify serologic reagents which might react specifically with subpopulations of B cells, using long-term tissue-cultured lymphoid cell lines derived from the same individuals that have B and "T"-cell characteristics (10, 11). (These cell lines were kindly made available by Dr. Richard Smith.) We have used two sources of antisera, one consisting of HL-A-typing sera, in anticipation that these sera may include non-HL-A antibodies; and secondly, maternal sera obtained from a relatively homogeneous population of people, the Amish.

Materials and Methods

Six long-term tissue-cultured cell lines were used primarily for these studies. These cell lines were paired, two lines having been derived from each of the same three individuals. Each of the individual cell lines from the pair had either T- or B-cell characteristics. Each individual cell line of the pair was found to possess the same HL-A alloantigens as determined by complement (C)-dependent cytotoxicity (12). Tissue culture cell lines were labeled with ^{51}Cr (300 μCi/10^7 cells) for 45 min at

[1] *Abbreviations used in this paper:* BSS-FCS, balanced salt solution with 10% fetal calf serum; MLR, mixed lymphocyte culture.

37°C. The cells were washed twice with balanced salt solution with 10% fetal bovine serum (BSS-FCS), 100 volumes on each occasion. 10^4 viable cells were distributed into microtiter plates and 10 μl of the appropriate antisera added, as well as 10 μl of the rabbit sera as a source of C. The mixture was allowed to incubate at room temperature for 1 h. 150 μl of BSS-FCS was added to stop the reaction, the cells sedimented by centrifugation, and 100 μl of the supernate counted for the presence of ^{51}Cr. The percent ^{51}Cr released was calculated on the basis of the maximum amount of ^{51}Cr taken up by 10^4 cells.

The source of C was found to be important in that normal rabbit serum contains natural cytotoxic antibody for tissue culture cell lines. This high C background was obviated by using sera from 19–21-day old baby rabbits and absorbing with the target cell for 30 min in the cold (4°C) before use.

All tests were performed in triplicate. Standard errors were calculated on each experiment and ranged from ±0.01 to ±5.69. Controls included sera known to cause cytolysis of the target cells (positive control) and C alone in the presence of normal human sera (background control). Reactions were considered positive when ^{51}Cr release was greater than 20% above the background control. In most of the tests 40–70% of the ^{51}Cr was released.

Sera from the Amish multiparous (at least four pregnancies) were assayed for reactivity against a panel of peripheral lymphocytes containing all the known HL-A antigens. These tests were performed using the dye exclusion test as described by Kosty, et al. (13). These sera showed no reactivity against this panel of peripheral blood lymphocytes. Other sera used were HL-A-typing sera obtained from the Transplantation and Immunology Branch of the National Institute of Allergy and Infectious Disease, Bethesda, Md. When cytotoxicity was seen against the B-cell line and not the T-cell line of the appropriate pair, the serum was absorbed with the corresponding T-cell line at concentrations of 5 x 10^7 cells/0.5 ml sera.

Results

42 of the HL-A-typing sera were screened for reactivity against the T- and B-cell lines. These results are shown in Table I. 20 sera were positive against the SB (B cell) line and not against the paired HBS (T cell) line. Of these 20, reactivity to the B-cell line could be removed by absorption with the T-cell line with 15 sera, leaving 5 sera which, after absorption reacted exclusively with the B-cell line. In testing the CEM (T cell) and PA-3 (B cell) lines, 16 sera showed reactivity against the B-cell lines. Absorption with the corresponding T-cell line removed the reactivity of 12 of these sera against the corresponding B-cell line. Thus four T-cell-absorbed sera reacted only with the B cells. Of the 17 sera cytotoxic to the 8392 (B) cell line, the reactivity of 8 sera could be removed by absorption with the T (8402)-cell line. Several sera reacted with more than one cell line. Serum A14 was positive against the SB and 8392 cell lines and sera A25 and A33 were cytotoxic to the PA-3 and 8392 cell lines. The remaining 15 sera

TABLE I

Reactions of HL-A Typing Sera to T and B-Lymphoid-Cell Lines

"T" cell	"B" cell	Total sera sisted	No. of sera reacting with "B" cells*	No. of sera absorption positive‡	No. of sera absorption negative§
HSB	SB	42	20	15	5
CEM	PA-3	42	16	12	4
8402	8392	42	17	8	9

* Criteria for positive reaction defined in text.

‡ Cytotoxicity to B cell removed by absorption with T cell.

§ Total number of sera reacting with B cells.

were absorbed with the two nonreactive T- and B-cell lines and tested against the cell line that demonstrated the original cytotoxicity. No appreciable decrease in titers of cytotoxicity reactivity was seen.

15 sera obtained from the Amish exclusively showed positive reactivity against one or several of the B-cell lines. These sera demonstrated more restricted activity than the typing sera as no direct cytotoxicity could be demonstrated against T-cell lines.

Table II shows the results of the reactivity of these and other sera positive for the SB cell line. Also shown in this table are the results of the cytotoxic reactivity of these sera after absorption with the T-cell lines and other B-cell lines. Sera 9, 640, and 359 were exclusively cytotoxic to the SB line. This cytotoxicity could not be removed by absorption with the corresponding T-cell line or other T- or B-cell lines.

Absorption of serum 657 with another B-cell line, PA-3, removed cytotoxicity to the SB line, indicating common antigenic determinants. The positive reactivity of serum 76 to SB could be removed by absorption with both of the other B-cell lines, suggesting shared antigens on the three B-cell lines. A summary of the results of the reactivity of these results are shown in Table III. Also shown in this table are the HL-A types of the tissue culture cell lines as well as the potential antibody in these sera that could be predicted by knowing the HL-A phenotype of the immunizing partner (husband). None of these serum donors had received transfusion and, therefore, the antibody was presumably produced by pregnancy. Several sera could potentially be detecting the HL-A antigens expressed on the B-cell lines. This is probably not the case, as absorption with the corresponding T-cell lines (possessing the same HL-A antigens as the B-cell lines) did not remove cytotoxic activity. Absorption of the sera with the nonreacting B- and T-cell line did not remove the reactivity to the primary target cell. The results suggest that several sera may be detecting an antigen or antigens shared by more than one B-cell line while others are detecting an antigen or antigens restricted to an individual B-cell line.

TABLE II

Cytotoxicity of Amish Sera to SB Lymphoid Cell Line

	Serum designation						
	9	640	359	657	76	Io*	C‡
	% cytotoxicity ± SE of mean						
Preabsorption	56.1±3.2	68.5±2.6	65.1±3.6	58.2±4.3	69.2±4.5	70.6±4.2	8.1±2.1
Absorption§ HSB‖	59.6±4.1	70.2±1.8	62.1±3.1	65.1±3.2	66.1±2.1	6.5±2.1	5.9±0.1
Absorption PA-3¶	54.2±2.1	64.7±3.1	70.1±4.8	7.8±0.3	4.5±0.8	3.3±1.9	4.7±1.2
Absorption CEM‖	61.6±3.8	69.2±1.8	69.2±3.6	65.1±1.9	61.5±2.1	4.5±0.8	5.1±1.2
Absorption 8392¶	66.1±5.1	70.1±4.2	62.1±1.9	66.4±1.8	6.1±1.2	3.9±1.2	3.2±2.2
Absorption 8402‖	59.8±3.2	72.6±4.2	65.3±3.6	63.1±3.4	59.1±3.4	4.6±2.1	6.1±1.1

* Io, positive serum control.

‡ Complement control.

§ ABsorption of 0.5 ml of sera with 5×10^7 cells.

‖ T-cell line.

¶ B-cell line.

TABLE III

Reactions of Sera from Amish Multiparous to B-Lymphoid-Cell Lines

Cell line and HL-A phenotype	Sera reactivity* and potential anti-HL-A antibody														
	9‡ (11)§	640 (1.8) (W10)	359 (2.12) (W10)	657 (3.7) (W10)	76 (W5)	590 (W5)	192 (1.3) (7,W27)	35 (2,x) (W10)	301 (3,W10)	43 (1,2) (12,5)	52 (1,3) (W10,W27)	386 (W10) (W32)	669 (2.7) (3,W10)	50 (2.7) (3,W10)	124 (1.8) (3,W10)
SB															
1,2	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
12,17															
PA-3															
1,10	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−
8,W10															
8392															
1,10	−	−	−	−	+	+	+	−	+	+	+	+	+	+	+
7,W17															

* Criteria for positive (+) and negative (−) reactions given in text.

‡ Serum designation.

§ Potential anti HL-A antibody.

Discussion

The results presented above suggest that human non-HL-A alloantigens may be expressed exclusively on B-lymphoid cells. The most convincing evidence is provided by the reactions of the Amish sera to the B-cell lines and lack of reactivity of these sera (by direct cytotoxicity and absorption) to the T cells having the same HL-A antigens.

The ability to serologically detect antigens restricted to a subpopulation of human lymphocytes offers the possibility of identifying genes within or outside the HL-A complex that control the expression of non-HL-A cell membrane antigens. The results presented in this communication strongly suggest that such antigens do exist and that the appropriate reagents can be identified. If these non-HL-A antigens are similar to the Ia antigens of the mouse, one might expect the antigens to be expressed as allotypes. The selected reactivity of the sera, particularly those obtained from the Amish, to one or more of the B-cell lines suggests that this might also be the situation in man.

For reference purposes we propose to give a provisional designation of antigens on the B-cell lines. Antigen B_1 is expressed on line SB; B_2 on PA-3; and B_3 on 8392. Sera 9, 640, and 359 appear to react with B_1; serum 35 appears to be anti-B_2; and sera 301, 43, 52, 386, 669, 50, and 124 anti-B_3.

Analysis of the reactivity of these sera to peripheral blood lymphocytes, with particular attention to the reactivity to subpopulations of cells, remains the critical pursuit. Preliminary investigation has shown these sera to be cytotoxic to a subpopulation of peripheral blood lymphocytes having B-cell characteristics. These studies have been performed with lymphocytes from random blood donors as well as with lymphocytes from three families of the Amish sect. Reactivity to B lymphocytes with certain sera do not appear to correspond to a particular HL-A phenotype, however, in the family studies, reactions appear to be associated with certain HL-A haplotypes. The determination of genetic linkage of these antigens to HL-A is being pursued. Access to cells and serum from the Amish religious sect offers a distinct advantage in these studies. This population of individuals, because of religious and cultural preference, have intermarried within the isolated community(s) for over 100 years. Studies by Kostyn et al. (13) have shown limited expression of HL-A antigens in this population with rather restricted haplotypes. With these constraints, and because of the consistently large families, genetic analysis of the serologic reactions observed will be greatly facilitated.

Summary

Human sera were tested for cytotoxicity to pairs of long-term tissue-cultured cell lines. Each pair had been derived from the same individual and one of the pairs possessed the characteristics of either "T" or "B" cells. The alloantisera used were HL-A-typing reagents or sera obtained from Amish multiparas. Selected cytotoxicity was found against the B-cell lines by direct testing. Cytotoxicity was abolished by absorption with B-cell line but not by absorption with the T-cell lines. The results suggest that a group of allotypic antigens may be expressed exclusively on human B cells.

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