A Simple Microabsorption Technique for HL-A Typing[1] (36169)

M. A. PELLEGRINO, S. FERRONE, AND A. PELLEGRINO
(Introduced by R. A. Reisfeld)
Scripps Clinic and Research Foundation, La Jolla, California 92037

Human transplantations performed during the last decade have shown that the success of these procedures depends largely on two genetic systems, the ABO blood group system and the HL-A (human leukocyte locus A) system. Essentially all strong transplantation antigens affecting the survival of allografts belong to these two systems. Progress in successful selection of compatible donors and in a better understanding of the genetics and molecular biology of the HL-A system has been made largely through the extensive application of histocompatibility typing utilizing the lymphocytotoxic technique.

The results of direct typing by specific cytotoxic HL-A alloantisera in the presence of complement, which causes the lysis of leukocytes, can be improved by typing by absorption which overcomes a major disadvantage of the cytotoxic tests, *i.e.*, the CYNAP (cytotoxicity-negative-absorption-positive) reaction responsible for false negative results (1). Moreover, quantitiative absorption of HL-A antisera with human leukocytes permits quantitition of HL-A determinants on these cells and evaluation of gene dose effects (2) to differentiate between homo- and heterozygotes. By quantitative absorption, yields of soluble antigens from cultured cells can be compared meaningfully among different extraction procedures. This is particularly appropriate as cultured cell lines are being used more frequently since they provide the only adequate, uniform genetic source for isolation of large quantities of soluble HL-A antigens (3, 4).

The application of quantitative absorption techniques has, however, been hampered by lack of sufficient amounts of cells and monospecific HL-A alloantisera. To overcome this difficulty, we have developed a simple, reproducible microabsorption technique for HL-A typing of both human cultured cells and peripheral human leukocytes.

Materials and Methods. HL-A alloantisera. Sera were obtained from the serum bank at the National Institute of Allergy and Infectious Diseases and from Professor R. Ceppellini. Each typing serum was titrated against lymphocytes containing the corresponding antigen and was used for the absorption test at a dilution corresponding to 1 cytotoxic unit, *i.e.*, a twofold greater concentration than zero cytotoxic units which, in turn, corresponds to the 95% lytic end point.

Absorbing cells. Peripheral leukocytes were obtained from healthy subjects by sedimentation of heparinized blood with plasmagel. The human cultured lymphoid cell lines were RPMI 1788, RPMI 4098, RPMI 7249, RMPI 8866, and WI-L2. Both peripheral leukocytes and cultured human lymphoid cells were washed three times with PBS (0.005 *M* phosphate buffer, 0.15 *M* NaCl, pH 7.1) and then suspended at the desired concentration.

Cytotoxic test. The slightly modified eosin method of Mittal *et al.* (5) was employed with both peripheral lymphocytes and cultured human lymphoid cells (6, 7). At least three typing sera were used for each HL-A

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specificity.

Microabsorption Technique. The absorption was performed in Beckman microtubes by incubation of cells and alloantisera for 60 min at room temperature with intermittent mixing every 10 min. Five microliters of the HL-A alloantiserum at a dilution corresponding to 1 cytotoxic unit were incubated with 5 μl of the cell suspension. After incubation, the tubes were centrifuged at full speed in a Beckman microfuge for 5 min. The supernatant was then transferred to another tube and was either immediately tested against the selected target cells or stored at —20°.

Results and Discussion. Four HL-A antigens were studied on peripheral leukocytes obtained from four donors and on five cultured human lymphoid cell lines. As shown in Table I, the HL-A phenotypes determined by the microabsorption test and the lymphocytotoxic reactions were identical in each case. In control reactions, cytotoxic alloantisera maintained their specificity when reacted with Hanks' buffered salt solution in place of absorbing cell suspension. When cells lacking the HL-A specificity, against which the alloantibody was directed, were used for absorption, the number of cells required to inhibit cytotoxicity was significantly higher than those of cells possessing HL-A specificity. This increased number of cells average

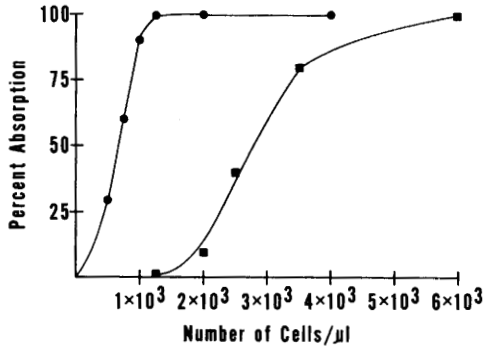

FIG. 1. Relationship between the percentage of absorption of alloantiserum TO-11-03 and the number of cells employed for the absorption: (●) peripheral leukocytes B.P.; (■) human cultured lymphoid cells RPMI 1788. The cultured cell line RPMI 1788 was derived from the peripheral lymphocytes of donor B.P.

TABLE I. HL-A Typing of Peripheral Leukocytes and of Human Cultured Lymphoid Cells by Microabsorption Technique.[a]

HL-A specificity	Typing serum	Serum dilution	Peripheral leukocytes				Human cultured lymphoid cells				
			A.G.	M.P.	B.P.	S.F.	RPMI 1788	RPMI 4098	RPMI 7249	RPMI 8866	WI-L2
HL-A2	TO-11-03	1:32	+	+	+	+	+	−	+	+	+
HL-A3	Storm	1:16	−	−	−	−	−	+	−	+	−
	2-50-6-02-22-01										
HL-A5	D1-03-7-03-21-07	1:4	+	+	−	−	−	−	−	−	+
HL-A7	Cutten	1:8	−	−	+	−	+	−	+	+	−
	2-51-5-01-11-01										
HL-A typing by direct cytotoxic test			2,5	2,5	2,7	2	2,7	3	2,7	2,3,7	2,5

[a] The absorbing peripheral leukocytes were used at a concentration of $1 \times 10^4/\mu$l; the absorbing human cultured lymphoid cells were used at a concentration of $5 \times 10^3/\mu$l.

TABLE II. AD_{50} Values of Peripheral Leukocytes and of Human Cultured Lymphoid Cells for Different HL-A Specificities.

HL-A specificity	Peripheral leukocytes				Human cultured lymphoid cells				
	A.G.	M.P.	B.P.	S.F.	RPMI 1788[a]	RPMI 4098	RPMI 7249	RPMI 8866	WI-L2
HL-A 2	2100	1700	2800	1000	825	56,000	800	400	400
HL-A 3	50,000	50,000	>60,000	>60,000	60,000	600	>60,000	400	8000
HL-A 5	5000	5000	50,000	20,000	48,000	>60,000	>60,000	>60,000	1000
HL-A 7	50,000	50,000	3800	>60,000	1,800	>60,000	3500	350	60,000

[a] The cultured cell line RPMI 1788 was derived from the peripheral lymphocytes of donor B.P.

about 50,000/μl. Quantitative absorption studies were performed by incubating cells at varying concentrations with different HL-A alloantisera. The percentage absorption = 100 —[(% cells killed by absorbed serum/% cells killed by nonabsorbed serum) $\times$ 100]. The relationship between the number of cells used for absorption and the percentage of absorption of a cytotoxic alloantiserum was expressed in sigmoidal fashion when the parameters were plotted on an arithmetic scale (Fig. 1). To compare results from different quantitative absorptions, we adopted the (AD_{50}) parameter, *i.e.*, the number of cells required to inhibit by 50% the cytotoxicity of a selected alloantiserum.

Quantitative absorption tests made it possible to compare the same HL-A specificities located on different cells as well as different HL-A specificities on the same cells (Table

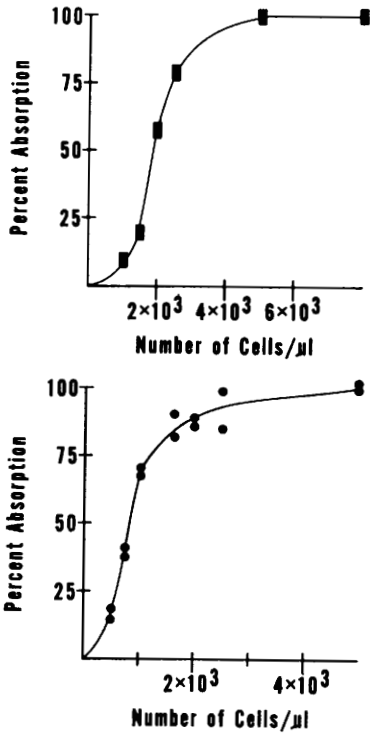

FIG. 2. Reproducibility of the quantitative microabsorption procedure carried out on two samples of the alloantisera on the same day: (upper) alloantiserum Cutten 2-51-5-01-11-01; absorbing cells: cultured cells RPMI 1788. (lower) alloantiserum TO-11-03; absorbing cells: cultured cells RPMI 1788.

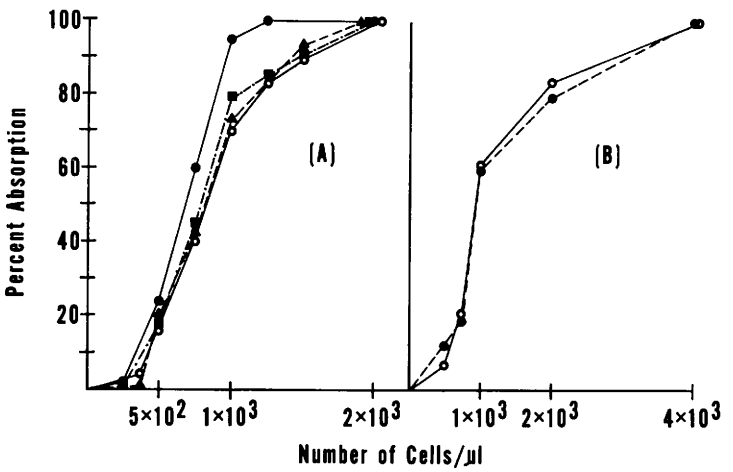

FIG. 3. Reproducibility of the quantitative microabsorption procedure carried out on different days: (A) alloantiserum TO-11-03; absorbing cells: cultured cells RPMI 1788; (B) alloantiserum D 1-03-7-03-21-07; absorbing cells: cultured cells WI-L2. Different symbols denote separate days on which tests were carried out.

II). In agreement with results obtained with the ^{51}Cr release technique (8), human cultured lymphoid cells showed a density of HL-A determinants higher than that of autologous peripheral leukocytes.

In order to assess the reproducibility of the quantitative absorption procedure, some alloantisera were absorbed with a given cell on different occasions and then tested against the target cell; no significant variability was observed (Fig. 2 and 3).

The method described is simple, rapid, and highly reproducible. Since it is performed on a microscale, the technique requires only few cells for absorption. In addition, HL-A alloantisera, usually in critically short supply, are utilized in the most economic fashion as they are used at various dilutions on a microscale. Because the density of antigenic determinants can vary from cell to cell, absorption with different numbers of cells seems advisable in order to avoid false negative results caused by use of insufficient numbers of cells. A standardized system, including alloantiserum diluted at chosen cytotoxic units and selected target cells, will eliminate the source of variability provided by the difference in titer of the same alloantiserum with different target cells.

Although other microabsorption techniques have been described (9, 10), they require numerous precautions as well as special equipment. The simple microabsorption technique described here overcomes some difficulties which have limited routine application of this method in HL-A typing.

Summary. A simple, reproducible microabsorption method for HL-A typing of peripheral leukocytes and of human cultured lymphoid cells is described. The test is economical as it requires only few cells and small amounts of diluted typing HL-A alloantisera. The method can be used to quantitate different HL-A determinants on the cell surface.

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