

BRIEF COMMUNICATION:**Group-specific Human Granulocyte Antigens on a Chronic Myelogenous Leukemia Cell Line With a Philadelphia Chromosome Marker**

By S. Ian Drew, Paul I. Terasaki, Ronald J. Billing, Ole J. Bergh,
Jun Minowada, and Eva Klein

Group-specific human granulocyte antigens are serologically detectable with granulocytotoxic-positive human alloantisera on a cell line, K562, of chronic myelogenous leukemia origin which bears a Philadelphia chromosomal marker. The

same cell line lacks serologically detectable HLA, B₂ microglobulin, and B-lymphocyte antigens. Granulocyte antigens are important cell markers for cell lines of suspected myeloid lineage.

THE OCCURRENCE of non-HLA-associated granulocyte cell surface antigens on normal human polymorphonuclear cells has been documented.^{1,2} Recently, five tentative allelic granulocyte specificities have been defined using granulocytotoxic-positive (GCT+) HLA-negative human antisera and papainized granulocytes as target cells.³ Sera with antigranulocyte activity have been used to identify the presence of normal granulocyte antigens on chronic myelogenous leukemia (CML) cells.⁴ The latter observation prompted the investigation of a CML cell line, K562, which was reported to carry the Philadelphia chromosomal marker.⁵ Using human alloimmune GCT+ antisera, this line has been shown to have group-specific granulocyte cell surface antigens despite its lack of HLA- and Ia-like B-lymphocyte antigens.

MATERIALS AND METHODS

Successful initiation of in vitro cultures and subsequent characterization of cell line K562 was first reported by Lozzio and Lozzio.⁵ The line originated from the pleural effusion aspirate of a patient with CML in terminal blast crisis.

Antisera

Fifty-eight select monospecific HLA antisera, representing the majority of first (A) and second (B) locus HLA antigens, were used for the serologic detection of HLA antigens on K562. Fifty B-lymphocyte alloantisera defining six independent B-lymphocyte specificities were used to type for the presence of these antigens. A total of 40 granulocyte-reactive alloantisera, obtained from multitransfused or multiparous subjects and defining 5 granulocyte antigen specificities, were tested against K562. All GCT+ antisera were free of "contaminating" HLA antibodies, determined by prior screening of the antisera against a random panel of 70 lymphocyte donors. Three heterologous antisera raised in rabbits were tested in dilutions against the cell line: anti-B₂ microglobulin (Dakopatts, Denmark); a non-HLA, B-lymphocyte-reactive heteroantiserum

From the Department of Surgery, School of Medicine, University of California, Los Angeles, Calif.

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Address for reprint requests: S. Ian Drew, M.D., Department of Surgery, School of Medicine, University of California, Los Angeles, Calif. 90024.

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Table 1. Cytotoxic Activity of Various Antisera for K562 and 15 Random Granulocytes

Antisera	No. of Sera	K562	Granulocytes
Human GCT+	40	Positive*	Positive*
Rabbit anti-K562	1	Positive	15/15
Human anti-HLA	58	Negative	Weak positive*
Rabbit anti-B ₂ microglobulin	1	Negative	5/15
Human anti-B lymphocyte	50	Negative	0/15
Rabbit anti-B lymphocyte	1	Negative	0/15

*Positive with antiserum of appropriate specificity.

(9800K), previously described by us,⁶ and negative for mature granulocytes; and an antiserum raised to K562 by three successive intravenous injections of 20×10^6 K562 cells. Serum drawn following the first booster injection was tested.

Cytotoxicity Testing

Serologic reactivity against K562 was tested in a standard two-stage microcytotoxicity assay with an extended 3-hr incubation period. Undiluted rabbit complement, absorbed with human red blood cells, was utilized, and no viability problems were encountered. Positive cytolysis was assessed by eosin dye uptake by the dead cells, and only those reactions with greater than 50% cell kill were recorded as positive. Serologic investigations were performed on at least three different K562 subcultures.

Absorption Studies

Absorption studies were carried out at a ratio of $2-4 \times 10^8$ cells/ml of antiserum. Sera were absorbed undiluted and at dilutions of 1:2 and 1:3 for $\frac{1}{2}$ hr at 37°C.

RESULTS

The cytotoxicity results of a panel of HLA, B-lymphocyte, and GCT+ allo-antisera against cell line K562 as a target cell, are shown in Table 1. The reactivity of the antisera to a random population of 15 granulocyte donors is also given. Uniformly negative results with K562 as the target cell were obtained for all HLA and B-lymphocyte antisera. In addition, the heteroantisera detecting B₂ microglobulin and B-lymphocyte antigens (9800K) were negative at all dilutions tested. However, antisera raised to K562 were positive for both the cell line and random donor granulocytes, whereas the control preimmune rabbit serum failed to show any cytotoxic activity to K562 or purified granulocytes. K562 reacted selectively with the human GCT+ antisera. More specifically, granulocyte groups 1 and 2 antisera were found to be positive for each of the subcultures tested (Table 2). Groups 3 and 4 sera were consistently negative. The recent identification of broader antibody activity in our group 5 antisera re-

Table 2. Cytotoxicity of Group-specific Granulocyte Antisera for Three Subcultures of K562

Number of Sera Per Group	Group 1 Antisera	Group 2 Antisera	Group 3 Antisera	Group 4 Antisera
1	+++ , +++++ , ++	++ , ++ , +	- , - , -	- , - , -
2	+++ , +++++ , +++++	+++ , +++++ , +++	- , - , -	
3	++ , +++++ , ++	++ , +++++ , ++		
4	++ , +++++ , +++			
5	+ , +++++ , +++			

++++ , 80%–100% cytotoxicity; +++ , 60–80%; ++ , 40%–60%; + , 20%–40%; - , < 10%.

Table 3. Absorption of Group-specific Granulocyte Antisera With K562

Granulocyte Antiserum Specificity	Absorbing Cell	No. of Target Cells Tested	Target Cell Specificity	Per Cent Cytotoxicity Preabsorption	Per Cent Cytotoxicity Postabsorption
Group 1	—	Granulocyte (2)	Group 1	70, 100	—, —
Group 1	—	K562 (2)	Group 1	80, 100	—, —
Group 1	K562	Granulocyte (2)	Group 1	70, 100	2, 10
Group 3	K562	Granulocyte (2)	Group 3	70, 70	70, 50

quired exclusion of these sera from the present analysis. On three occasions, a single group 1 and 2 antiserum was less than 50% cytotoxic for a subculture tested. Variation in the cell surface antigen expression during the log phase of cell growth was investigated by initiating a subculture of the cell line and testing K562 daily for eight consecutive days. Whereas group 1 antisera remained positively cytolytic against the daily samples, group 3 antisera were consistently negative. The specificity of the GCT+ antisera for K562 is shown in Table 3. K562 was able to absorb granulocyte antisera positive for two random granulocyte donors of group 1 specificity. However, no loss in cytotoxicity was noted for two granulocyte donors following the absorption of group 3 GCT+ antisera with the cell line.

DISCUSSION

A number of characteristics of K562 have recently been documented:^{5,7} When comparing K562 to B and T lymphocytes and cell lines, typical B-lymphocyte and B-cell line markers were notably absent, as evidenced by the failure to detect Epstein-Barr virus genome and nuclear antigen as well as surface immunoglobulin. Rosetting of sheep red blood cells, a characteristic of T lymphocytes and cell lines, was markedly decreased. However, similar to T lymphocytes, K562 was a poor stimulator in mixed leukocyte cultures. Although Fc receptors could be detected, K562 lacked the ability to mediate antibody-dependent cellular cytotoxicity or phagocytosis of antibody-coated target erythrocytes. Whereas intracellular acid phosphatase was present, N-APase, an alkaline phosphatase with substrate specificity, and terminal deoxynucleotidyl transferase,⁸ appeared absent. Karyotype analysis established the presence of a Philadelphia chromosome, possibly existing as a ring form, r(22), and a near triploid genetic complement with a modal number of 70 chromosomes. On scanning electron microscopy, K562 bore similar surface characteristics to granulocytic leukemia cells.

Similar to T and B lymphocytes, the majority of established T- and B-cell lines possess polymorphic cell surface HLA antigens. One exception, however, is cell line Daudi, which bears no detectable HLA antigens, or the molecular subunit component of HLA, B₂ microglobulin. Furthermore, B lymphocytes carry non-HLA B-lymphocyte antigens, which, like HLA antigens, exist as alleles of a polymorphic antigenic system.⁹ These B-lymphocyte antigens have been shown to occur on B-cell lines, which are consequently susceptible to complement-mediated lysis by B-lymphocyte antisera. Failure to detect HLA antigens on K562 has been evidenced by the uniformly negative cytotoxic reactions obtained on exposure of the cell line to monospecific HLA antisera, repre-

senting the majority of known HLA antigens. In addition, a heterologous B₂ microglobulin antiserum failed to lyse the cell line in the presence of complement. Negative results were also obtained with a typing panel of human B-lymphocyte alloantisera as well as with a heterologous B-lymphocyte reactive antiserum, which is strongly cytotoxic for all B-cell lines tested to date.⁶ In contrast, K562 reacted extensively against a panel of GCT+ typing antisera and confirmed the presence of granulocyte antigens on this myeloid leukemia cell line. Five GCT+ antisera defining group 1 and all three antisera defining group 2 were found to be positive. The gene frequencies of these two antigens in a random panel of whites has been estimated recently at 0.3 and 0.09, respectively. The specificity of the granulocyte antigen groups was further substantiated by the selective absorption studies that were carried out (Table 3). Contrary to the results of others,⁸ it was found that a heterologous antiserum raised in rabbits to K562 lysed purified populations of granulocytes from random donors. The specificity of the serologic activity of this antiserum is presently being assessed.

In conclusion, the occurrence of normal granulocyte antigens on a cell line of CML origin has the following implications: (1) it stresses the importance of positively establishing the absence of GCT+ antibodies in sera defining leukemia-associated antigens; (2) it confirms the evidence favoring the categorizing of K562 as a cell line of myeloid origin; (3) it enables granulocyte antigens to provide a serologically detectable cell marker for the identification of cell cultures believed to originate from myeloid elements, yet exposed to the possibility of cellular overgrowth by other tissues;¹⁰ and (4) it invites investigation into the association of leukemia-associated granulocyte antigen markers and disease susceptibility.

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