Brief Definitive Report

Ia-BEARING T LYMPHOCYTES IN MAN

Their Identification and Role in the Generation of Allogeneic Helper Activity*

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The human Ia-like antigens were recognized initially as a series of HLA-linked alloantigens primarily represented on B lymphocytes with multiple pregnancy sera (1). Later, they were detected by hetero-antisera raised against the isolated protein from cell membranes (2–4). In addition to their presence on B cells and monocytes, they have been detected on the leukemic blast in cases of acute lymphocytic leukemia, acute myelogenous leukemia, and chronic myelogenous leukemia in blastic crisis (4–6). Recently, these Ia antigens were also shown to be present on the surface of precursor cells responsible for colony formation of both the myeloid monocytic and erythroid series (7–9) and on the surface of nonblood cells such as epidermal Langerhans cells (10).

The presence of certain types of Ia antigens on T lymphocytes has been demonstrated in murine systems, although the exact molecular character remains unclear (11). The presence of Ia antigens on human T cells has not been well documented. The present studies demonstrate the presence of Ia antigens on a small population of normal circulating human T lymphocytes, on T lymphocytes grown in long-term cultures, and on certain leukemic T cells. Evidence also is presented showing that cells responsible for the generation of helper activity during a mixed lymphocyte reaction are contained in this Ia-bearing T-cell population.

Materials and Methods

Isolation of Lymphocytes. Mononuclear cells from the peripheral blood of normal individuals and patients with leukemia and various lymphoproliferative states and tonsillar mononuclear cells were isolated as described previously (12). Spontaneous rosette formation between human lymphocytes and sheep erythrocytes (SRBC) were performed with neuraminidase-treated SRBC (E). The rosette-forming cells (E-RFC) were separated from the nonrosette-forming cells by Ficoll-Hypaque (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) gradient centrifugation. The RFC-enriched fraction was purified further by repeated gradients until no significant numbers of cells were observed at the interphase. The E-RFCs were recovered after lysis of SRBC by a Tris-buffered ammonium chloride solution. Tonsillar B cells were obtained by the depletion of E-RFCs from the mononuclear cell preparations.

Detection of Ia Antigens, Surface Immunoglobulin (Ig), Intracellular Ig, and Complement Receptors. A rabbit anti-Ia antiserum was made against the isolated 65,000 dalton bimolecular human Ia complex as described previously (4, 7). 50 mg of rhodamine-conjugated IgG of this antiserum

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TABLE I

Presence of Ia Antigens on Purified Peripheral Blood T Cells

Subject	Condition		E-RFC	Ia	Surface Ig
			%	%	%
A	Normal	Exp. 1	99.0	4.8	0.4
		Exp. 2	99.5	6.0	0.3
B	Normal		99.5	4.3	0.6
C	Normal		96.0	8.0	—
D	Normal		99.5	2.9	0.2
E	Normal		99.5	1.7	0.2
F	Lymphoproliferative disorder		92.0	50.0	—
G	Macroglobulinemia		85.0	31.2	—

in the concentration of 1 mg/ml was absorbed three times with 2 ml of packed cells from four Ia^- T-lymphoblastoid lines. A 1/20 dilution of the absorbed antiserum was used for direct immunofluorescence for the detection of Ia. Surface and intracellular Ig were detected as described previously (13). Purified antibodies with κ- and λ-specificities by affinity column chromatography were used as the anti-Ig reagents. Complement receptors for C3 were detected by ox erythrocytes coated with rabbit IgM and complement. A C5-deficient mouse serum or a C6-deficient rabbit serum was used as the complement source.

Cell Cultures and Plaque-Forming Cell (PFC) Assay. Cell cultures were set up at 37°C with 5% CO_2 in RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 0.2 mM glutamine, 1% trypticase soy broth, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% SRBC-absorbed AB serum with 1×10^6 SRBC as antigens. At day 5, cells were harvested and assayed for PFC against SRBC by a modified Jerne-Nordin PFC assay (12).

Long-term cultures of human T cells were carried out as described by Morgan et al. (14). At the initiation of the cultures, mononuclear cells were either stimulated with irradiated allogeneic cells or untreated.

Results

Ia Antigens on Purified Peripheral Blood T Cells and on Cultured T Cells. Cell preparations with >99% E-RFCs were obtained from isolated peripheral blood mononuclear cells by repeated Ficoll-Hypaque gradient centrifugation. After erythrocyte lysis, the cells were stained with an absorbed rhodamine-conjugated rabbit anti-Ia antiserum. A small population of the purified T cells were positive for Ia antigens. Representative cases are shown in Table I. As high as 6% of the E-RFCs were stained with the antiserum as shown in subject A. Similar results were obtained in two additional experiments on this individual. In general, 2–4% of the E-RFCs were shown to bear Ia antigens in the normal donors studied. However, as few as 1.7% of E-RFCs and as high as 8% were shown to be Ia^+ as shown in Table I. Only a negligible percentage (0.2–0.6%) of the isolated T cells stained for surface Ig.

In three cases, mononuclear cells isolated from peripheral blood were stained with the anti-Ia antiserum and then incubated with neuraminidase-treated SRBCs. 5.8, 4.2, and 2.6% of the E-RFCs were found to bear Ia antigens, respectively. The staining intensities of these purified T cells for Ia antigens varied from cell to cell. In general, the intensity was much weaker than that seen when B cells were stained with the same reagent. Nevertheless, the staining was distinct.

In a number of pathological states, higher percentages of Ia^+ T cells were found. T cells from two patients with lymphoproliferative disorders are shown in Table I. In patient F, 50% of the cells were Ia bearing. These cells were clearly distinct from a few contaminating leukemic B cells.

Long-term cultures of human T cells were maintained with conditioned medium

TABLE II

*Lack of Allogeneic T-Cell Helper Activity of Purified T-Cell Preparations After Elimination of Ia-Bearing T Lymphocytes**

Tonsillar B cells	Allogeneic T cells	Treatment of T cells	PFC against SRBC/culture
Exp. 1			
1×10^6	None	—	5
1×10^6	1×10^6	None	400
1×10^6	1×10^6	Normal rab. serum + comp.	385
1×10^6	1×10^6	Anti-Ia antiserum + comp.	25
Exp. 2			
1×10^6	None	—	5
1×10^6	1×10^6	None	845
1×10^6	1×10^6	Normal rabbit serum + comp.	815
1×10^6	1×10^6	Anti-Ia antiserum + comp.	15

* T cells were incubated with either normal rabbit serum or anti-Ia antiserum for 30 min at 25°C. Rabbit serum complement was then added. After a further incubation for 30 min at 37°C, cells were washed three times and cultured overnight. Cells were then added to the tonsillar B cells.

generated by phytohemagglutinin-stimulated lymphocytes. In a 15-day-old culture to which irradiated allogeneic cells were added at its initiation, 96% of the viable cells were E-RFCs and 92% stained with the anti-Ia antiserum. In a separate culture, which was not stimulated at its initiation and was maintained for 28 days, 95% of the cells were E-RFCs and 96% stained for the Ia antigens. None of the cells in these two cultures had surface Ig detectable with the anti-Ig reagents. The staining with the anti-Ia antiserum was bright in both experiments.

Sheep Erythrocyte Rosette Formation by Ia^+ Leukemic Cells. In two cases of leukemia, Ia-bearing leukemic cells were found to form E rosettes. The first patient had marked leukocytosis (82,000/mm^3) with proliferating cells morphologically closely related to Sézary cells. 99% of the mononuclear cells stained with the anti-Ia antiserum and 71.5% of these cells formed E rosettes. In the second patient, the diagnosis was acute lymphocytic leukemia with 33,500 leukocytes/mm^3. Whereas 88% of the mononuclear cells stained for Ia, 37.5% of the cells also formed E rosettes. Thus, the vast majority of the blasts were Ia^+ and substantial numbers of them also formed E rosettes. Very few cells formed EAC rosettes or stained for surface Ig. Less than 0.1% of the cells stained for intracellular Ig.

Loss of Allogeneic Helper Activity of Purified T-Cell Preparations after the Elimination of Ia-Bearing Lymphocytes. Considerable experience has been obtained recently in assaying the helper activity of a T-cell population by its effect on the generation of sheep erythrocyte-specific PFCs by a tonsillar B-cell preparation (13). Representative experiments with this system are shown in Table II. 1×10^6 tonsillar B cells cultured only with SRBCs as stimulators produced few plaques. With the addition of 1×10^6 allogeneic T cells from a normal individual, 400 PFCs were generated as shown in Exp. 1 (Table II). The elimination of Ia-bearing lymphocytes from the T-cell preparations (99.5% E-RFCs) had marked effects on this system. Only 25 PFCs/culture were seen when the T cells were treated with the anti-Ia antiserum and complement. In the control culture containing T cells treated with a normal rabbit serum and complement, 385 PFCs were detected. Similar results were obtained in the second experiment. In both experiments, the treated T cells gave slightly reduced proliferative responses (<30% reduction) as measured by [^{3}H]thymidine incorporation due to allogeneic cell stimulation in comparison to the control T cells.

Discussion

In the present investigation, a small population of normal circulating T lymphocytes was shown to bear Ia-like antigens. Although previous studies from both this and other laboratories (1, 6) were not able to demonstrate Ia presence on human T lymphocytes, the ability to obtain highly purified T cells, optical improvement of immunofluorescence microscopy, and the high potency of the heterologous anti-Ia antiserum contributed to the identification of this small population. The existence of 2–6% Ia-positive lymphocytes in a T-cell population which contained 99.5% sheep erythrocyte rosette-forming cells was detected. The lack of surface Ig-bearing cells in these preparations excluded the possibility that these Ia-bearing lymphocytes were B cells binding sheep erythrocytes by surface immunoglobulins specific for sheep RBC. Furthermore, no intracellular Ig positive cells were seen in the T-cell preparations. The existence of this population was also demonstrated by double-marker experiments with the identification of Ia staining on the rosetting lymphocytes.

The vast majority of the cultured T cells, maintained with conditioned medium, formed E rosettes and had Ia antigens on their surface. The possibility that these antigens were acquired from the conditioned medium requires consideration. Some evidence against this was obtained with Ia alloantisera. However, this work is continuing to totally rule out this possibility. The finding that cultured T cells bear Ia antigens is in agreement with that reported by Gillis et al. (15).

The heteroantiserum used in this study was raised against isolated Ia molecules. Previous studies (4, 7) have shown it to be highly specific for the 28,000, 37,000 dalton bimolecular complex typical for the known human and certain murine Ia antigens (16). Absorption of the antiserum with Ia^- T-cell lymphoid lines failed to remove this specificity; absorption with Ia^+ B-cell lines removed it. Thus, the accumulated evidence indicated that staining on T cells was due to the presence of the bimolecular type Ia antigens.

The intensity of Ia staining on the circulating T cells was much less than that on B cells. Whether this is due to less Ia antigens on T cells or only a distinct subset of Ia molecules expressed on these T cells remains to be clarified. The present studies were limited to peripheral blood lymphocytes. Preliminary studies also suggest the presence of Ia antigens on a small population of tonsillar T cells. The size of this T-lymphocyte population in various other human central lymphoid organs remains to be determined.

Although only a small population of circulating T cells bear Ia antigens in normal individuals, the expansion of this population in certain patients was encountered. In addition, two patients with leukemia were shown to have clonal proliferation of a leukemia cell which formed sheep erythrocyte rosettes and stained for Ia antigens. The bimolecular Ia-like structure was precipitated from lymphoblasts isolated from the first patient (unpublished observation). These two patients appeared to represent instances of the leukemic transformation of Ia-bearing T lymphocytes.

Allogeneic helper function of murine T cells has been studied extensively (17). Recently, a culture system of human tonsillar B cells has been utilized to assay T-cell helper activity generated during the mixed lymphocyte reaction. The T cells responsible for the generation of human allogeneic helper activity have been characterized recently (13). They are radiation sensitive and do not belong to the population with Fc receptor for IgG. The present studies further document that these cells bear Ia antigens on their surface.

Summary

The presence of Ia-like antigens was demonstrated on a small population (2–6%) of highly purified human circulating T lymphocytes by immunofluorescence with a rabbit anti-Ia serum raised against the isolated bimolecular Ia structure. The Ia^+ T lymphocytes have no surface or intracellular immunoglobulins. The expansion of this Ia^+ T-cell population was encountered in certain patients. Ia antigens were also found on T blasts grown in long-term cultures with conditioned medium generated by phytohemagglutinin-stimulated lymphocytes. In addition, leukemia blasts which stained for Ia antigens and formed E rosettes were identified in the peripheral blood of two leukemic patients. This evidence further supports the existence of Ia-bearing T cells in man.

The Ia^+ T-lymphocyte population was shown to contain cells responsible for the generation of allogeneic helper activity. Elimination of Ia^+ lymphocytes from a purified T-cell population by the anti-Ia antiserum and complement abolished its ability to help an allogeneic B-cell preparation to generate plaque-forming cells against sheep erythrocytes in vitro in the presence of the antigen.

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