IDENTIFICATION OF A SUBPOPULATION OF LYMPHOCYTES IN HUMAN PERIPHERAL BLOOD CYTOTOXIC TO AUTOLOGOUS FIBROBLASTS*

By ROBERTSON PARKMAN‡ AND FRED S. ROSEN

(From the Immunology Division, Department of Medicine, Children's Hospital Medical Center, Boston, Massachusetts 02115)

The clonal theory of immune selection predicts that, during the differentiation of the immune system, clones of lymphocytes capable of recognizing "self," or autoantigens, will be selectively eliminated; that the adult host will have no lymphocytes capable of self-recognition; and that the presence of autocytotoxic cells would not be anticipated (1). Evidence from murine models indicates that autocytotoxic cells do naturally occur, that they produce no demonstrable disease in normal adult animals, and that their reactivity can be demonstrated in vivo and in vitro (2-7). Autocytotoxic cells can be detected after incubating normal spleen cells with irradiated fibroblasts under conditions of thymic deficiency [in vitro in fresh serum from thymectomized (TX)[1] animals (3), in fetal calf serum (FCS) (4), or in heat-inactivated normal fresh mouse serum (3) or in vivo in TX mice (5)].

Human cytotoxic cells have been found in patients with a variety of autoimmune diseases using allogeneic cells as targets (8). Cells spontaneously cytotoxic for a variety of long-term solid tumor and lymphoblast tissue culture lines have been found in normal individuals (9, 10). However, lymphocytes from normal individuals, cytotoxic for short-term cultures of autologous or allogeneic fibroblasts have not been described.

We report here the presence of a naturally occurring subpopulation of human lymphocytes cytotoxic to autologous and allogeneic fibroblasts. Cytotoxicity was determined by measuring the residual radioactivity of prelabeled fibroblasts after overnight incubation with whole and partially purified lymphocyte subpopulations. The cytotoxic cell has characteristics of neither mature T nor B lymphocytes. The tissue distribution and the target specificity of the autocytotoxic cells are presented in this paper.

Materials and Methods

Fibroblast Cultures. Skin fibroblasts were obtained by punch biopsy from normal individuals and HLA identical and mixed leukocyte culture-nonreactive (major histocompatibility region

[MHR] identical) bone marrow transplant donors and recipients. The biopsy was cut into 1-mm sections which were put into 35-mm tissue culture dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) containing 0.05 ml FCS (Microbiological Associates, Bethesda, Md.) and covered with a 20 × 20 mm cover slip. 30 min later, 1 ml of medium RPMI-1640 with 20% FCS and glutamine (2 × 10⁻³ M), and antibiotics (penicillin, 50 U/ml; streptomycin, 50 μg/ml; kanamycin, 50 μg/ml; and microstatin, 50 U/ml) was added. The fibroblast cultures were maintained at 37°C in a 5% CO_2 incubator (National Appliance Co., Portland, Oreg.), and the medium was replaced weekly. Fibroblasts, that had reached confluency, were washed once with Hanks' balanced salt solution (HBBS) and incubated for 10 min in 0.25% trypsin (Grand Island Biological Co., Grand Island, New York). Fibroblasts were aspirated, centrifuged once at 200 g for 10 min, and resuspended in Eagle's minimal essential medium (MEM) with 10% FCS and the aforementioned antibiotics. 2 × 10⁵ fibroblasts were planted in a 100 ml plastic tissue culture dish (Falcon Plastics, Div. of BioQuest). Fibroblasts were also suspended at 1 × 10⁶/ml in MEM with 10% dimethylsulfate (J. T. Baker Chemical Co., Phillipsburg, N. J.) and 20% FCS, slowly frozen, and stored in liquid N_2. All experiments were performed with fibroblasts between the 6th and 12th tissue culture passage.

Separation and Preparation of Lymphocytes.   Peripheral blood was collected in preservative-free heparin (100 U/ml blood). Peripheral blood leukocytes were separated on gradients of Ficoll-Hypaque (11), and the interface cells collected and washed in HBSS. The cells were then resuspended at 1 × 10⁶ cells/ml in RPMI-1640 with 10% heat-inactivated human AB-positive serum with glutamine and antibiotics added (complete medium). Lymphocytes were either used immediately or incubated overnight at 37°C in a 5% CO_2 incubator and used the following day.

Discontinuous gradients of bovine serum albumin (BSA) were prepared following the methods of Dicke et al.(12). BSA (fraction V; Sigma Chemical Co., St. Louis, Mo.) was dissolved in Tris buffer, 0.155 M, pH 7.2, with a final concentration of 35% as determined by a Zeiss refractometer (Carl Zeiss, Inc., New York). The osmolarity, measured on a Fiske osmometer (Fiske Associates, Inc., Uxbridge, Mass.), was corrected to 360 mosmol with NaCl. The 35% BSA solution was diluted with phosphate-buffered saline (PBS), pH 7.1, 300 mosmol. Gradients were prepared in 12 × 75 mm tissue culture tubes (Falcon no. 2003; Falcon Plastics, Div. of BioQuest) by layering 1 ml of 33, 27, and 22% BSA. 10–40 × 10⁶ cells in complete medium were layered on top of the gradient and centrifuged for 30 min at 1,000 g at 4°C. The cells at each interface were collected, washed twice in HBSS, and resuspended at 1 × 10⁶ cells/ml in complete medium.

Human gamma globulin was aggregated by heating at 60°C for 1 h (13). The washed aggregated gamma globulin was incubated at 1 mg/ml with 1 × 10⁶ cells in complete medium for 1 h at 37°C. The cells were washed twice with HBSS and resuspended at 1 × 10⁶ cells/ml in complete medium.

Leucopak nylon (Fenwal Inc., Walter Kidde & Co., Inc., Morton Grove, Ill.) was washed for 1 wk in sterile distilled water with frequent changes. 1 ml of the dried nylon wool was packed into 10-ml syringes. Before use, the nylon wool was washed with 5 ml HBSS and then with 5 ml complete medium. The syringes were prewarmed to 37°C, and 10 × 10⁶ lymphocytes in 1 ml of medium were pipetted on the nylon and incubated at 37°C for 45 min in a 5% CO_2 incubator. The nonadherent cells were then slowly eluted with 10 ml of complete medium over a 5–10 min period (14). In some experiments, Ficoll-Hypaque-separated cells were rosetted with E and EAC3 (15), and rosetted cells were separated on gradients of Ficoll-Hypaque from nonrosetted cells which were resuspended at 1 × 10⁶ cells/ml in complete medium. Surface immunoglobulin was detected on lymphocytes by staining with fluoresceinated monospecific rabbit anti-human IgG, IgM, and IgA (Behring Diagnostics, American Hoechst Corp., Somerville, N. J.) and counting under a Zeiss fluorescent microscope (Carl Zeiss, Inc.).

Fibroblast Cytotoxicity Assay.   Nonconfluent fibroblast cultures were aspirated, washed with HBSS, and then incubated with medium 199 deficient in leucine, isoleucine, valine, and lysine (GIBCO) supplemented with 10% FCS and an equimolar mixture of ¹⁴C-amino acids (leucine, isoleucine, valine, and lysine; sp act, >270 mCi/mmol) (New England Nuclear, Cambridge, Mass.), 1 mCi/100 mm tissue culture dish. After overnight incubation the dishes were washed with HBSS and the fibroblasts were trypsinized. 1,000 fibroblasts in 0.1 ml RPMI-1640 with 10% FCS were added to each well of a 96 well microtiter plate (Linbro Scientific, Hamden, Conn.). The microtiter plates were incubated overnight in the CO_2 incubator to allow fibroblast attachment.

The wells were then aspirated and 0.1 ml of complete medium was added to control wells and 1 × 10⁵ lymphocytes in 0.1 ml of complete medium was added to test wells. Tests were performed in

quadruplicate with two fibroblast lines as targets. The plates were incubated on a rocking platform (Bellco Glass, Inc., Vineland, N. J.) 4–5 cycles/min at 37°C in a 5% CO_2 incubator. After overnight incubation, the plates were emptied by inversion, washed three times with HBSS with inversion, fixed with ice-cold methanol for 10 min, and then air dried. Plates were sprayed; the bottom of the wells were punched into counting vials and assayed for ^{14}C in a liquid scintillation counter (Model 3320; Packard Instrument Co., Downers Grove, Ill.). Results were expressed as a mean percentage ± 1 standard deviation of the residual radioactivity found in control wells incubated with medium alone. Residual radioactivity of less than 80%, when compared to medium controls (100%), was considered significant.

Results

Fibroblast Cytotoxicity of Whole and Fractionated Human Peripheral Blood Lymphocytes. The capacity of Ficoll-Hypaque-separated peripheral blood lymphocytes to lyse autologous, histo-identical (MHR), and allogeneic skin fibroblasts is presented in Fig. 1. The mean percentage of residual radioactivity of autologous, MHR identical, and allogeneic lymphocyte-fibroblast combinations were 100.8, 101.3, and 96.6%. The standard deviation for control fibroblast wells was 9–10%. Residual radioactivity of less than 80%, a decrease of greater than 2 standard deviations, was considered to represent significant cytotoxicity. No autologous or MHR identical lymphocyte-fibroblast combination demonstrated significant cytotoxicity. Only one allogeneic-lymphocyte fibroblast combination demonstrated a significant decrease in residual radioactivity (69.1%). On repeat testing, this deviant combination fell within the normal range. The allogeneic combinations represent 11 individuals tested against 10 different fibroblast lines over a 9 mo period.

Peripheral blood leukocytes were fractionated on discontinuous gradients of BSA and designated fraction 1, ≤22% BSA; fraction 2, 22–27% BSA; and fraction 3, 27–33% BSA. 10–15% of the lymphocytes were found in fraction 1, 60–80% in fraction 2, and 5–10% in fraction 3. Total cell yield was 60–90%.

Fig. 2 shows the cytotoxicity of unfractionated and BSA-fractionated lymphocytes assayed on autologous fibroblasts. The unfractionated (103.8%) and fraction 1 (88.6%) lymphocytes demonstrate no significant cytotoxicity, whereas fraction 2 (38.1%) and fraction 3 (68.5%) cells produce significant lysis. Cytotoxicity to autologous fibroblasts was detected in fraction 2 lymphocytes from four of six normal individuals, while their unfractionated lymphocytes were not cytotoxic (Table I).

When BSA-fractionated lymphocytes from normal individuals were tested against two or more allogeneic fibroblast lines, fraction 2 lymphocytes from 8 of 11 individuals lysed one or more allogeneic fibroblast lines. Lymphocytes from three individuals lysed only one of two allogeneic fibroblast targets (Table II), whereas five others lysed two or more target cell lines.

A semilogarithmic dose-response curve was obtained with fractionated cytotoxic allogeneic lymphocyte-fibroblast combinations (Fig. 3). Unfractionated lymphocytes at a 100:1 lymphocyte:fibroblast ratio were noncytotoxic (96.7 ± 7.9%) while fraction 2 cells, which represented 94% of the cells recovered after fractionation (total yield, 67%) were cytotoxic at a 100:1 ratio with only 28.1 ± 3.7% of the radioactivity remaining. Significant cytotoxicity was seen at ratios as low as 25:1 (64.1 ± 4.3%) but significant cytotoxicity was not usually detected at a ratio of less than 50:1.
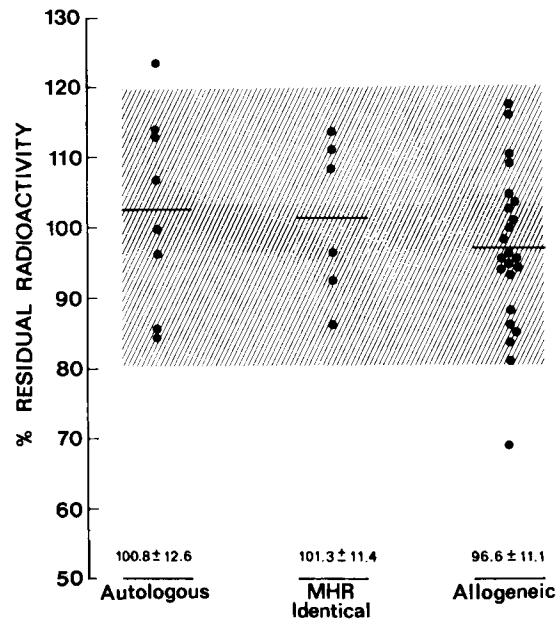
FIG. 1. Residual radioactivity of autologous, MHR identical, and allogeneic lymphocyte-fibroblast combination after overnight incubation. 100% equals residual radioactivity in medium control wells. Shaded area represents normal range.
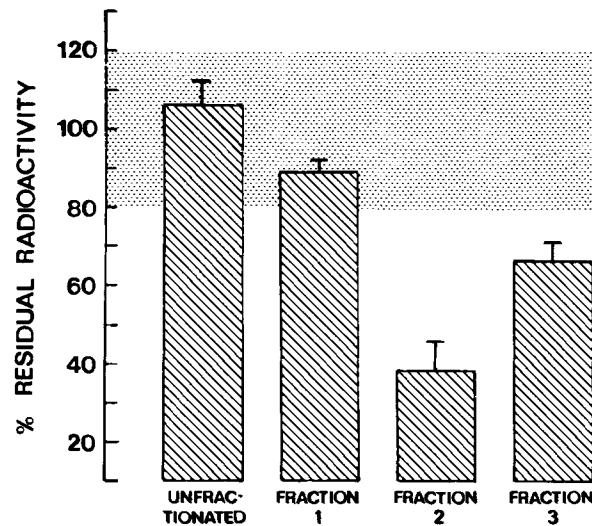

FIG. 2. Residual radioactivity of autologous fibroblasts after overnight incubation with unfractionated and BSA-fractionated lymphocytes. Shaded area represents normal range.

Unfractionated lymphocytes incubated with 33% BSA, Tris buffer, or BSA in PBS for 30 min and then processed as usual, displayed no cytotoxicity. To determine if the cytotoxicity was directed toward membrane antigens acquired from the heterologous FCS fibroblasts were grown in human serum for two cell passages (10 days) before use as target cells. The degree of lysis of cells grown in

TABLE I

In Vitro Cytotoxicity of Unfractionated and BSA Gradient Fractionated Peripheral Blood Lymphocytes Tested against Autologous Fibroblasts

Age of do-nor	Unfractionated	Effector cell		
		Fraction 1	Fraction 2	Fraction 3
yr		*% Residual radioactivity ± 1 SD*		
12	99.7 ± 13.4	99.2 ± 2.0	66.0± 7.0	89.9 ± 9.6
15	106.7 ± 6.4	88.5 ± 2.4	38.2 ± 7.6	66.2 ± 3.9
15	86.3 ± 16.6	74.7 ± 2.5	50.6 ± 6.0	101.6 ± 23.5
3	85.5 ± 7.5	105.4 ± 9.4	75.1 ± 7.7	93.1 ± 2.4
9	112.9 ± 7.8	113.0 ± 4.0	82.2 ± 9.4	96.9 ± 12.1
45	113.7 ± 18.4	110.3 ± 6.5	114.4 ± 16.2	98.9 ± 9.4

Ficoll-Hypaque-separated peripheral blood lymphocytes fractionated on discontinuous BSA gradients; fractionated and unfractionated lymphocytes were suspended in RPMI-1640 medium with 10% AB+ serum. Lymphocytes were assayed overnight at a 100:1 ratio with 1×10^3 ^{14}C-amino acid-prelabeled autologous fibroblasts in microtiter tissue culture plates. Plates were incubated at 37°C in a CO_2 incubator on a rocking platform, 4-5 cycles/min. The plates were washed three times in HBSS, fixed, and the residual radioactivity determined. Experiments were performed in quadruplicate and results expressed as mean ± SD. 100% equals residual radioactivity in control wells incubated with medium alone. Unlined values represent significant cytotoxicity.

TABLE II

Cytotoxicity of Unfractionated and Fractionated Lymphocytes against Allogeneic Fibroblasts

Effector cells	Target fibroblasts	
	Allogeneic-1	Allogeneic-2
	% Residual radioactivity ± 1 SD	
Unfractionated	83.5 ± 12.2	120.2 ± 9.6
Fraction 1	107.2 ± 16.9	100.8 ± 7.0
Fraction 2	42.4 ± 5.1	102.4 ± 5.9
Fraction 3	61.8 ± 14.0	105.2 ± 11.3

Ficoll-Hypaque- and BSA-fractionated peripheral blood lymphocytes were incubated at 100:1 ratio with ^{14}C-amino acid prelabeled allogeneic fibroblasts, 1,000 fibroblasts/well in microtiter plates. The plates were incubated overnight at 37°C on a rocking platform, 4-5 cycles/min. The wells were washed three times with HBSS, fixed, and the residual radioactivity determined. Experiments were performed in quadruplicate and results expressed as a mean percentage of control wells ±1 SD. 100% equals residual radioactivity in wells with medium alone.

AB+ serum and medium was equal to, or greater than, cells grown in FCS. Human AB+ serum from nontransfused normal blood donors, predominantly males, was routinely used in the cytotoxic assay. The use of different lots of AB+ serum, or autologous serum, did not alter the fraction 2 lymphocyte cytotoxicity.

Relation of Fibroblast Cytotoxicity to the MHR. To determine if the target fibroblast antigen was linked to the MHR, fibroblasts were established from MHR identical siblings; whole and fractionated lymphocytes were tested. Table III shows the results with the lymphocytes of one of three MHR identical brothers tested against autologous, MHR identical, and one allogeneic fibroblast
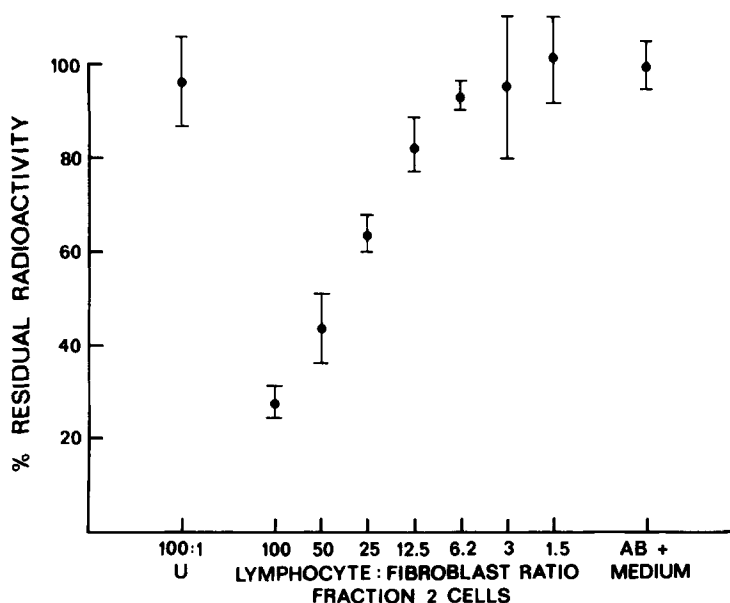
FIG. 3. Dose-response curve of unfractionated (U) and fraction 2 lymphocytes tested against allogeneic fibroblasts.

TABLE III
Relationship of Fibroblast Cytotoxicity to MHR of Target Cells

Effector cells	Target fibroblast			
	Autologous	MHR identical-1	MHR identical-2	Allogeneic
	% Residual radioactivity ± SD			
Unfractionated	99.7 ± 13.4	86.0 ± 14.2	92.4 ± 15.1	96.3 ± 13.8
Fraction 1	99.2 ± 2.0	96.3 ± 7.7	96.7 ± 3.0	108.7 ± 29.3
Fraction 2	66.0 ± 7.0	81.7 ± 5.2	74.4 ± 11.0	59.6 ± 12.3
Fraction 3	89.9 ± 9.6	100.7 ± 14.1	105.7 ± 9.9	96.9 ± 16.4
BSA Control	97.2 ± 13.3	105.3 ± 12.5	103.9 ± 5.4	103.7 ± 14.4

Peripheral blood leukocytes were separated in Ficoll-Hypaque gradients. They were further fractionated on discontinuous BSA gradients or mixed with 33% BSA for 45 min. The unfractionated, BSA fractionated, and BSA treated were resuspended in RPMI 1640 with 10% heat-inactivated human AB and serum at 1×10^6 cells/ml and assayed at a 100:1 ratio with 1,000 ^{14}C-amino acid-prelabeled fibroblasts in microtiter II plates. Experiments were performed in triplicate; the plates were incubated on a rocking platform, 4-5 cycles/min in a 5% CO_2 incubator at 37°C overnight, washed three times with HBSS, and fixed with cold methanol. The residual radioactivity determined in a liquid scintillation counter. 100% equals the residual radioactivity present in wells incubated with medium alone. Results expressed as the mean percentage of control wells ±1 SD.Unlined values represent significant cytotoxicity.

line. Fraction 2 lymphocytes significantly lysed autologous (66.0%), allogeneic (59.6%) and one MHR identical (MHR-2) fibroblast line (74.4%). A second MHR identical line (MHR-1) was not significantly lysed (81.7%).

Absorption studies were undertaken to determine the relationship between the antigens expressed by autologous and MHR identical fibroblasts and allogeneic fibroblasts. Fraction 2 lymphocytes were incubated for 6 h on MHR-1 and

TABLE IV

Effect of Fibroblast Absorption on Cytotoxicity of Fraction 2 Lymphocytes

Fraction 2 lymphocyte treatment	Target fibroblast			
	Autologous	MHR identical-1	MHR identical-2	Allogeneic
	% Residual radioactivity ± 1 SD			
None	66.0 ± 7.0	81.7 ± 5.2	74.4 ± 11.0	59.6 ± 12.3
Absorption on MHR-1 fibroblasts	89.0 ± 7.9	90.6 ± 1.4	84.6 ± 14.7	65.3 ± 20.0
Absorption on MHR-2 fibroblasts	95.8 ± 7.1	96.2 ± 12.9	108.4 ± 2.0	64.4 ± 20.6

Fraction 2 lymphocytes in AB+ medium incubated on confluent fibroblast monolayers for 6 h. The nonadherent cells were collected, washed, and assayed in the standard fibroblast cytotoxicity assay. Unlined values represent significant cytotoxicity.

MHR-2 fibroblasts; the nonadherent cells were collected and assayed on the target fibroblasts (Table IV). After absorption on MHR-2 fibroblasts, the nonadherent cells exhibited no cytotoxicity toward autologous (95.8%) and the MHR-2 (108.4%) fibroblasts but still lysed the allogeneic fibroblasts (64.4%). The MHR-1 fibroblasts, which were not significantly lysed initially, also removed the cytotoxicity directed towards the autologous (89.0%) and MHR-2 (84.6%) fibroblast lines. The decrease in cytoxicity after fibroblast absorption was not a nonspecific effect since significant cytotoxicity of the allogeneic fibroblasts was still present in both cases. Both histo-identical fibroblast lines express antigens that specifically remove lymphocytes cytotoxic to themselves and the autologous fibroblasts but not to the allogeneic fibroblasts.

Surface Characteristics of the Fibroblast Cytotoxic Cell. To determine if the lymphocytes responsible for fibroblast cytotoxicity were either mature T or B lymphocytes, Ficoll-Hypaque-separated lymphocytes were rosetted with E or EAC3 cells and the nonrosette-forming cells fractionated on BSA gradients. Control and rosette-depleted lymphocytes were then assayed on the same allogeneic target fibroblasts (Table V). Removal of EAC3 rosette-forming cells significantly reduced the cytotoxicity of fraction 2 lymphocytes (72.5 → 106.9) whereas removal of E-rosetting lymphocytes had no significant effect on the cytotoxicity of fraction 2 cells. Passage of reactive fraction 2 cells through nylon wool (yield 60%) increased the reactivity of the passaged cells (Table VI, exps. 1 and 2). The increase in cytotoxicity was specific since the passage of noncytotoxic fraction 2 cells did not increase their cytotoxicity (exp. 3). The nylon-passed cells had a markedly reduced number of immunoglobulin-bearing cells (<3%). Preincubation of cytotoxic fraction 2 lymphocytes with 1 mg/ml of aggregated gamma globulin before assay completely inhibited their cytotoxicity for fibroblasts.

Tissue Distribution of Fibroblast Cytotoxic Cells. Tonsils from normal children (2- to 5-yr old) demonstrated no fibroblast cytotoxicity even after fractionation; thymocytes from children, 6-mo to 2-yr old, were also noncytotoxic (Table VII).

The peripheral blood leukocytes of three patients with X-linked agammaglobulinemia were assayed on allogeneic target fibroblast lines. These patients have an absence of mature B cells in both their peripheral blood and bone marrow. Neither whole nor BSA-fractionated cells demonstrated any cytotoxicity.

TABLE V
Effect of Rosette Depletion on Fibroblast Cytotoxicity

	E-rosette formation			EAC3-rosette formation		
	Control	E Depleted		Control	EAC3-Depleted	
Unfractionated	87.1 ± 10.2	84.0 ± 9.4	NS*	85.6 ± 17.0	123.1 ± 7.6	P = 0.05
Fraction 1	81.7 ± 13.4	78.1 ± 5.0	NS	104.3 ± 2.7	ND‡	–
Fraction 2	50.9 ± 2.4	55.7 ± 5.7	NS	72.5 ± 11.1	106.9 ± 2.8	P = 0.02
Fraction 3	60.4 ± 6.1	80.6 ± 8.5	P = 0.05	84.8 ± 7.4	108.1	NS

Unfractionated peripheral blood lymphocytes rosetted with E or EAC3 cells, and the nonrosetted and control cells fractionated on the standard BSA gradients. Unfractionated and fractionated cells were then assayed against allogeneic fibroblast targets.
* NS, not significant.
‡ ND, not done.

TABLE VI
Effect of Nylon Passage on the Fibroblast Cytotoxicity of Fraction 2 Lymphocytes

Treatment	Exp. 1	Exp. 2	Exp. 3
	% Residual radioactivity ± 1 SD		
None	75.5 ± 9.2	77.3 ± 6.9	83.9 ± 16.9
Nylon passed	20.3 ± 5.8	17.9 ± 2.2	90.5 ± 4.7

10×10^6 Fraction 2 lymphocytes were incubated with 1 ml of washed nylon for 45 min at 37°C. The eluted and control cells were then assayed on allogeneic fibroblast targets.

TABLE VII
Fibroblast Cytotoxicity of Lymphocytes from Thymus and Tonsils

	Fibroblast-1	Fibroblast-2
	% Residual radioactivity	
Thymocytes		
Unfractionated	119.0 ± 20.0	121.8 ± 6.0
Fraction 1	102.8 ± 6.6	108.6 ± 4.8
Fraction 2	107.8 ± 24.4	105.7 ± 5.3
Fraction 3	108.2 ± 3.6	107.2 ± 3.0
Tonsillar leukocytes		
Unfractionated	92.0 ± 7.6	100.6 ± 6.0
Fraction 1	108.1 ± 19.6	100.3 ± 8.0
Fraction 2	98.0 ± 15.5	94.7 ± 9.6
Fraction 3	103.9 ± 12.4	85.3 ± 10.0

Single cell suspensions of tonsils and thymus were fractionated on BSA gradients; fractionated and unfractionated lymphocytes were then assayed on allogeneic fibroblast targets.

Discussion

Unfractionated peripheral blood leukocytes from normal individuals were noncytotoxic to established human fibroblast lines of autologous or isologous origin and rarely cytotoxic to allogeneic cells. After fractionation on discontinuous gradients of BSA, a fraction of medium density cells (fraction 2) from 8 of 11 individuals were cytotoxic for autologous, MHR identical, and some allogeneic fibroblasts. The cytotoxicity was not due to monocytes nonspecifically activated by the separation procedures since fractionated peripheral blood leukocytes from some normal individuals and from patients with X-linked agammaglobulinemia were noncytotoxic. The specificity of target destruction displayed by fraction 2 cells from normal individuals (Table III) minimizes the significance of alteration

of the targets by the acquisition of infectious agents (pleuropneumonia-like organisms or viruses) or by the adsorption of heterologous serum antigens. The use of AB+ serum from untransfused male donors excludes the possibility that the cytotoxicity is due to the presence of antibodies to histocompatible antigens.

Naturally occurring human cells, spontaneously cytotoxic for long-term lymphoblast and solid tumor tissue culture lines, have been described (9, 10) and may, in some cases, be due to acquired heterologous serum antigens (16). Experiments with short-term allogeneic or autologous fibroblast cultures have shown no cytotoxicity and have been used as control for tumor-specific cytotoxicity (17). The presence in peripheral blood of lymphocytes that are cytotoxic to autologous and allogeneic fibroblasts have not been previously reported.

The absorption experiments demonstrate that the target specificity of the autocytotoxic cells are antigens broader than those expressed on the autologous fibroblasts. The antigens expressed on autologous fibroblasts are present on histo-identical fibroblasts (Table IV); however, it is not possible to determine if they are linked to the MHR. Removal of autologous reactivity by histo-identical fibroblasts does not effect the lysis of allogeneic fibroblasts. Thus, the cytotoxicity cell is "sensitized" to alloantigens as well as to autoantigens.

The autocytotoxic cells producing fibroblast lysis have the characteristics of neither mature T nor B lymphocytes. Although the cytotoxic cells are recovered from fraction 2 of the BSA gradients, which is composed primarily of mature T lymphocytes, they are not removed by E-rosette formation nor are they present in thymus; thus, they are not T lymphocytes. The autocytotoxic cells also appear not to be mature B lymphocytes since they are not found primarily in BSA gradient fraction 3, where B lymphocytes predominate (18), nor are they present in normal tonsils which contain significant numbers of B lymphocytes. Further passage of reactive fraction 2 cells through nylon (Table VI), which significantly reduces the number of immunoglobulin-bearing cells (10% → <3%), rather than decreasing the fibroblast cytotoxicity, which would be expected if the reactive cell were a B lymphocyte, actually increased fibroblast cytotoxicity. They do have a receptor for C3. The possibility that the cells are nonadherent cells of the monocyte-macrophage series appears remote due to the target specificity displayed. Experiments with aggregated human gamma globulin have shown inhibition of cytotoxicity, suggesting that the cell expresses an Fc receptor.

The absence of cytotoxic cells in the peripheral blood of three patients with X-linked agammaglobulinemia and their absence of mature B lymphocytes may indicate that the autocytotoxic cell is a precursor B lymphocyte, missing in the patients (18). Thus, the cell responsible for the fibroblast cytotoxicity appears to be a lymphocyte but cannot be classified as a mature T or B lymphocyte.

Autocytotoxic cells were found in the peripheral blood of the majority of normal adults. They were not found in the thymus or tonsils assayed; their absence may indicate that the cytotoxic cells are found predominantly in the peripheral circulation and not in the fixed lymphoid organs.

The cytotoxicity displayed by fraction 2 lymphocytes can not be due to concentration effects alone. Fraction 2 represents 60–80% of the total recovered cells; however, the increase in cytotoxicity cannot be explained by the minor increase (20–50%) in the concentration of fraction 2 cells. Doubling of the concentration of unfractionated cells has never produced any cytotoxicity.

The possibility was considered that the fractionation procedures separated the autocytotoxic cells from a minor population of lymphocytes that "regulated" the cytotoxic cells. Experiments in which cytotoxic fraction 2 cells were incubated overnight with fraction 1, fraction 3, and unfractionated cells demonstrated varying degrees of inhibition when fraction 1, but not fraction 3 or unfractionated cells, were added. Not all normal individuals gave reproducible inhibition, however. Further work is underway concerning the nature of the "regulator" cells.

The surface characteristics of the murine cells, autocytotoxic to syngeneic fibroblasts, have not been well described. Preliminary evidence (M. Osband and R. Parkman, unpublished observations) indicate that the murine autocytotoxic cell has a medium density in BSA gradients, expresses no θ, and is nylon wool nonadherent. The human and murine autocytotoxic cells, therefore, have many surface characteristics in common.

Cytotoxicity of syngeneic murine target cells by unfractionated spleen cells has been described in mice after viral infection (19). If the "regulator" and cytotoxic cells are in balance in the normal spleen and if the virus challenge selectively and temporarily decreases the number of regulator cells, the relative excess of the autocytotoxic cells would be detected by the in vitro lysis of syngeneic fibroblasts.

Because of the similarities between the human autocytotoxic lymphocytes and the murine models, active thymic humoral factor (THF) extracted from calf thymus, which blocked the sensitization of murine autocytotoxic cells (4), was added to human fraction 2 lymphocytes during the fibroblast assay. No decrease in fibroblast cytotoxicity was observed. THF does not inhibit murine autocytotoxic cells once they have become "sensitized." The human autocytotoxic cell may not be sensitive to the action of THF or may be already sensitized when the THF was added to the cultures. Experiments are underway to define the possible role of thymic hormones in the control of the human autocytotoxic cells. If thymic humoral control is important, increasing host age with its concomitant decrease in thymic function may be a possible explanation for the observed increase in human "autoimmune" diseases seen with advancing age.

Summary

A naturally occurring subpopulation of human peripheral blood lymphocytes is cytotoxic to autologous and/or allogeneic fibroblasts. The autocytotoxic lymphocytes have a receptor for the third component of complement and for aggregated gamma globulin, do not form rosettes with sheep red blood cells, and are not removed by passage through nylon. The autocytotoxic subpopulation is not present in the thymus and tonsils of normal children or in the peripheral blood of individuals with X-linked agammaglobulinemia. Fibroblast absorption experiments demonstrate that the autocytotoxic cells are "sensitized" to antigens expressed on allogeneic fibroblasts in addition to the antigens expressed on autologous cells. Some normal individuals have a second subpopulation of lymphocytes that may "regulate" the autocytotoxic cells. The relevance of these observations to the murine autocytotoxic cells is discussed.

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