

INHIBITION OF BONE MARROW COLONY FORMATION BY HUMAN NATURAL KILLER CELLS AND BY NATURAL KILLER CELL-DERIVED COLONY-INHIBITING ACTIVITY

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A possible role for natural killer (NK)¹ cells in regulating hematopoiesis was originally suggested by Cudkowicz and Hochman (1) and Kiessling et al. (2), in the mouse system. These authors showed that parental hematopoietic or lymphoid grafts do not survive in lethally irradiated F₁ hybrids, even though these animals are universal recipients of grafts of other types of parental tissues. Experiments (3, 4) evaluating the formation of colonies or the proliferation of bone marrow stem cells in the recipient spleen showed that cells with NK characteristics are responsible for the graft rejection. Direct destruction of bone marrow cells in vivo by NK cells has also been observed in experiments in which the clearance of ¹²⁵I-uridine-labeled bone marrow cells after intravenous inoculation was measured (5). In humans, NK cells have been shown to react with cells from different lineages at early stages of differentiation, such as immature thymocytes (6, 7), bone marrow cells (6), and myeloid leukemic cells (8). Lymphocytes with NK cell characteristics are able to inhibit both myeloid and erythroid colony formation in vitro (9–15). There is also evidence for a possible pathogenetic in vivo role of such suppressor lymphocytes in patients with pure red cell aplasia associated with B cell chronic lymphatic leukemia (16, 17), aplastic anemia (18), and, more recently, in patients with NK lymphocytosis, in which the salient clinical feature is neutropenia (19, 20).

Inhibition of hematopoiesis in vitro has been variously ascribed to direct interaction of NK cells with sensitive immature hemopoietic cell targets (6, 8, 11), to release of soluble factors from lymphocytes upon allogeneic or mitogenic stimulation (21, 22), or to both mechanisms operating together. Several soluble cell products, produced by different cell types, have also been shown to mediate suppression of hematopoietic colony formation. Among these are: (a) lactoferrin,

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¹ *Abbreviations used in this paper:* BFU, burst-forming unit; CIA, colony-inhibiting activity; CFU, colony-forming unit; CM, conditioned medium; CSF, colony-stimulating factor; DET-CM, medium conditioned by Detroit 554 cell line; E, erythroid; EPO, erythropoietin; FBS, fetal bovine serum; FcR, receptor for the Fc fragment of IgG; F/H, Ficoll/Hypaque; GEMM, granulocyte, erythrocyte, macrophage, megakaryocyte; GM, granulocyte-macrophage; IFN, interferon; 2-ME, 2-mercaptoethanol; NK, natural killer; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; PHA, phytohemagglutinin.

which acts on macrophages to inhibit the production of colony-stimulating factor (CSF) for granulocyte-monocyte colony-forming units (CFU-GM), and the macrophage-dependent induction of CSF production by T cells (23); (b) isoferritin and acidic isoferritin (or leukemia-inhibiting activity) (24); (c) prostaglandins (25); and (d) interferon (IFN)- α , - β , and - γ (22, 26). Tumor necrosis factor, produced by endotoxin-stimulated macrophages (27, 28) and B cell lines, and lymphotoxins, produced mainly by T and B cells upon antigenic or mitogenic stimulation (29), are two other classes of cytostatic/cytotoxic factors that could affect proliferation of hematopoietic precursors. Upon interaction with NK-sensitive target cells, NK cells release a cytotoxic factor (NKCF) that may be the mediator of NK cell cytotoxicity (30).

To determine whether NK cells participate in hemopoietic homeostasis, either by direct cell-to-cell contact or through release of soluble factors, we studied the effect of highly homogeneous NK cell preparations and soluble factors derived from them on colony formation from bone marrow cells. We report here that human NK cells in vitro inhibit growth of multipotent, early CFU-GM and erythroid precursor cells from allogeneic and autologous bone marrow cells. Upon culture with bone marrow cell preparations enriched for precursor cells and with NK-sensitive target cells, human NK cells release a factor(s) able to inhibit colony growth with the same specificity as the effector NK cells.

Materials and Methods

Cell Lines. The human erythromyeloid leukemic cell line K562, the macrophage line U937, the B cell line Raji, and the mouse mastocytoma line P815y were maintained in culture in RPMI 1640 medium (Flow Laboratories, Inc., Rockville, MD) supplemented with 10% fetal bovine serum (FBS) (Flow Laboratories, Inc.). All cell lines used were repeatedly tested and constantly found free of mycoplasma contamination by Hoechst fluorescent staining for cytoplasmic DNA and by direct anaerobic agar-plate cultivation.

Monoclonal Antibodies. Antibody B73.1 (IgG1) reacts with the receptor for the Fc fragment of IgG (FcR) present on human NK cells and neutrophilic granulocytes, and detects virtually all resting and IFN-induced NK cells (31, 32); antibody B36.1 (IgG2b) reacts with the 69,000 dalton surface molecule (T1 antigen) present on all peripheral blood T cells (32); B116.1 (IgG2a) reacts with the 30,000 and 32,000 dalton chains that constitute the T8 antigen on suppressor/cytotoxic T cells and on a proportion of NK cells (32); B66.6 (IgG1) reacts with the 55,000 dalton protein that constitutes the T4 antigen present on helper T cells. These antibodies, and the anti-HLA-DR common determinant antibody B33.1 (IgG2a) (32, 33) have all been produced in our laboratory. The anti-NK antibody N901 (IgG1) (34) was kindly provided by Dr. J. Griffin (Dana-Farber Cancer Institute, Boston, MA). Antibody HNK-1 (IgM) (35), which reacts with a variable proportion of NK and T cells in the peripheral blood (31, 36), was produced from cell cultures obtained from the American Type Culture Collection (Rockville, MD). Supernatants from the hybrid cell lines were used throughout this study.

Peripheral Blood Lymphocytes (PBL) and PBL Subpopulations. Peripheral blood was obtained by venipuncture from healthy donors and anticoagulated with preservative-free heparin. Mononuclear cells were prepared by density gradient centrifugation on Ficoll/Hypaque (F/H) (1.077 ± 0.01 g/ml) and depleted of monocytes by adherence to plastic for 45 min at 37°C. PBL subpopulations were obtained by indirect rosetting of antibody-sensitized PBL with CrCl₃-treated, anti-mouse Ig-coated sheep erythrocytes (E), according to our previously described technique (31, 33). The goat F(ab')₂ anti-mouse Ig (Cappel Laboratories, Cochranville, PA) used was preabsorbed with human Ig and immunopurified on a mouse Ig-CNBr Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) column as described (31). Both antibody-positive (rosetting) and -negative (non-

rosetting) populations were recovered after F/H density gradient centrifugation, and contaminating E were lysed hypotonically.

Bone Marrow Cell Preparations. Rib bone marrow cells were purified from specimens (kindly provided by Dr. P. Addonizio, University of Pennsylvania, Philadelphia, PA) which were routinely removed during thoracic surgery. The marrow was collected in sterile RPMI 1640–10% FBS and sequentially flushed with medium through 18, 23, and 27 gauge needles, and the cell suspension was layered on an F/H density gradient (1.077 g/ml). Mononuclear cells collected at the interface were washed three times with RPMI 1640 supplemented with 10% FBS. In the indicated experiments, the mononuclear cells were depleted of mature monocytes and other adherent cells by two cycles of adherence to plastic surfaces. The resulting population was further separated into HLA-DR⁺ and HLA-DR⁻ cells by indirect rosetting (as described above) after sensitizing the cells with monoclonal antibody B33.1. Approximately 10–15% of the bone marrow cells were recovered in the HLA-DR⁺ fraction, which contained virtually all CFU-GM.

Bone Marrow Colonies. CFU-GEMM (mixed granulocytes, erythroid, monocyte, and megakaryocyte CFU) were set up using a modification of the method of Fauser and Messner (37). Briefly, bone marrow cells were resuspended (10^5 /ml) in 0.9% methylcellulose (Fisher Scientific Co., Pittsburgh, PA) in RPMI 1640 containing 30% FBS, 5% conditioned medium from phytohemagglutinin (PHA-HA 15; Wellcome Research Laboratories, Beckenham, England)-stimulated (7-d culture) peripheral blood leukocytes (PHA-CM) (10^6 /ml) (38), 5×10^{-5} M 2-mercaptoethanol (2-ME) (Sigma Chemical Co., St. Louis, MO), and human urine erythropoietin (1 IU/ml) (EPO) (lot 1668; Toyobo Co. Ltd., New York). 1-ml aliquots of the cell suspension were seeded into 35-mm culture petri dishes (Lux Scientific, Inc., Newbury Park, CA) and cultured at 37°C in a 5% CO₂ humidified atmosphere in air. The number of colonies was scored on day 14 of culture. Mixed colonies were individually harvested from the dishes, smears were prepared, and morphological analysis was performed after staining with Wright's-Giemsa.

The BFU-E (burst-forming units, erythroid) assay was performed according to Ogawa (39). Bone marrow cells were suspended (10^5 /ml) in 0.8% methylcellulose (Fisher Scientific Co.) in α medium (Gibco Laboratories, Grand Island, NY) containing 30% FBS, 1% bovine serum albumin (BSA) (Calbiochem-Behring Corp., La Jolla, CA), 10^{-4} M 2-ME, and human EPO (2 IU/ml). 1-ml aliquots of the cells were cultured and scored on day 14 of culture as described for CFU-GEMM. Cell aggregates containing >500 cells were scored as colonies.

The CFU-E (CFU, erythroid) assay was performed using the same experimental conditions as for BFU-E, but were scored on day 7 of culture. Cell aggregates containing >10 but <500 cells were scored as colonies.

The CFU-GM assay was performed according to the method of Pike and Robinson (40), with slight modification. Medium conditioned by the Detroit 554 cell line (DET-CM), kindly provided by Dr. R. K. Shaddock (Montefiore Hospital, University of Pittsburgh School of Medicine, Pittsburgh, PA), was used as a source of colony-stimulating factor (CSF) (41). Bone marrow cells were resuspended (10^5 /ml) in 0.3% agar (Difco Laboratories, Inc., Detroit, MI) in supplemented McCoy's medium (Gibco Laboratories), containing 10% CSF. The cells were cultured as described above, and colonies (aggregates containing >50 cells) were scored on days 7 and 14 of culture. Colony growth on day 7 evaluates differentiation and proliferation of more mature GM precursor cells (late CFU-GM); colony growth on day 14 evaluates these parameters in more primitive cells (early CFU-GM). All cultures were performed in triplicate. The results for CFU-GEMM are expressed as number of colonies per 10^6 plated cells; those of BFU-E, CFU-E, and CFU-GM are expressed as number of colonies per 10^5 plated cells. The same lots of FBS, EPO, DET-CM, and PHA-CM were used throughout.

Assay for Inhibition of Colony Growth by PBL and PBL Subsets. To test the direct effect of PBL on bone marrow colony formation, effector cells (PBL or PBL subpopulations) were incubated for 18 h at 37°C in RPMI 1640, 10% FBS in a humidified 5% CO₂ atmosphere in air with bone marrow cells at a 2:1 ratio of PBL to bone marrow cell (final cell concentration, 3×10^6 cells/ml). The cells were then washed three times with RPMI

1640, 10% FBS, and plated (10^5 bone marrow cells per dish) for the different types of colonies as described above. In each experiment, colony formation by precursor cells possibly present in the PBL preparations was tested under the same experimental conditions described above using 2×10^5 PBL/dish. The effect of soluble factors on colony formation was tested by adding supernatants (100 μ l or the quantities indicated in Fig. 1) at the time of addition of the bone marrow cells or at the indicated time after seeding to triplicate dishes.

Production of Supernatant Fluids from PBL Cocultured With Cell Lines or With Bone Marrow Cells. PBL and the different PBL subpopulations were resuspended (5×10^6 cells/ml) in RPMI 1640, 10% FBS with or without 10^3 U/ml partially purified IFN- α (10^6 U/mg; Interferon Sciences, New Brunswick, NJ) and incubated for 18 h at 37°C in a 5% CO₂ humidified atmosphere in tissue culture flasks (Falcon Labware, Becton, Dickinson & Co., Oxnard, CA). After culture, the cells were washed three times with phosphate-buffered saline (PBS) (pH 7.2), and resuspended (3×10^6 /ml) in RPMI 1640, 10% FBS containing K562, Raji, or P815y cells (6×10^5 /ml final concentration). After 6 h culture at 37°C, the cells were centrifuged and cell-free supernatants were harvested. Control supernatants were prepared using either effector or target cells alone under the same experimental conditions as above. Supernatants were also obtained using as inducers either total mononuclear bone marrow cells, nonadherent mononuclear bone marrow cells, or HLA-DR (+ and -) bone marrow subpopulations (3×10^6 cells/ml final concentration). Supernatants were either used immediately or kept frozen at -70°C until used.

[³H]Thymidine ([³H]TdR) Incorporation. Nonadherent mononuclear bone marrow cells, HLA-DR (+ and -) subpopulations obtained therefrom, and cell lines (Raji, U937, P815y) were resuspended in RPMI 1640 10% FBS supplemented or not with DET-CM (10% final concentration) and PBL supernatant prepared as described above (50% final concentration). 200 μ l of the cell suspension (5×10^4 cells) were plated in each well of flat-bottomed microtiter tissue culture plates and cultured for 96 h at 37°C. 6 h before harvesting, the cells were pulsed with [³H]TdR (sp act, 2 Ci/mmol; New England Nuclear, Boston, MA) at 2 μ Ci/well. The cells were collected on glass fiber filters using an automatic cell harvester (Skatron Co., Sterling, VA), and cell-incorporated [³H]TdR was assayed by liquid scintillation counting.

Results

Inhibition of Bone Marrow Hematopoietic Colonies by Human NK Cells. Human bone marrow cells were preincubated for 18 h at 37°C with allogeneic PBL and then washed and plated for colony formation. Growth of early CFU-GM and CFU-E was significantly inhibited, compared with that of bone marrow cells incubated without PBL, whereas late CFU-GM and BFU-E were not affected (Table I). The inhibitory effect exerted by PBL was enhanced when PBL were preincubated with IFN. The number of colonies formed by 2×10^5 total PBL alone was 1.7 ± 1.8 early CFU-GM, 2.0 ± 1.4 BFU-E, and 0.5 ± 0.8 CFU-GEMM. HLA-DR⁺ PBL preparations formed 4.7 ± 1.4 early CFU-GM and 2.0 ± 0.0 BFU-E; no late CFU-GM or CFU-E was ever derived from peripheral blood. PBL were separated into B73.1 (+ and -) cells by indirect rosetting, and allogeneic bone marrow cells were preincubated before plating with the two lymphocyte subsets, each separately. B73.1⁺ cells inhibited formation of CFU-GEMM, CFU-E, and early CFU-GM, but they did not inhibit either BFU-E or late CFU-GM colony growth. The inhibitory effect was increased when the B73.1⁺ cells were pretreated with IFN, whereas no significant inhibition of colony formation was observed after preincubation of bone marrow cells with B73.1⁻ cells, even when they were prestimulated with IFN- α .

Inhibition of Bone Marrow Hematopoietic Colonies by NK Cells Supernatants. To

TABLE I
Suppressor Effect of Human PBL on Bone Marrow Hematopoietic Colonies

Effector* PBL	Pre-treatment	Percent colony inhibition (mean \pm SD)				
		Type of colony				
		CFU-GEMM [‡] (8)	BFU-E (4)	CFU-E (4)	CFU-GM (early) (8)	CFU-GM (late) (8)
Unseparated	None	ND [§]	-0.6 \pm 2.4	14.8 \pm 11.1	35.6 \pm 7.3	-0.3 \pm 1.7
	IFN- α	ND	-1.0 \pm 1.4	32.8 \pm 4.0	47.2 \pm 4.8	-0.1 \pm 1.2
B73.1 ⁺	None	84.6 \pm 13.2	1.8 \pm 3.7	55.5 \pm 5.3	65.3 \pm 3.7	0.0 \pm 1.5
	IFN- α	97.4 \pm 5.4	-0.1 \pm 1.9	72.0 \pm 5.8	86.4 \pm 4.4	0.0 \pm 1.3
B73.1 ⁻	None	0.9 \pm 8.4	-0.1 \pm 1.0	1.4 \pm 4.4	0.4 \pm 7.3	-0.1 \pm 1.3
	IFN- α	4.1 \pm 9.6	-1.4 \pm 2.4	12.9 \pm 7.2	18.7 \pm 19.6	0.3 \pm 1.4

* Total PBL or B73.1 (+ and -) PBL subpopulations were used as effector cells against bone marrow cells (2:1 ratio) after 18 h incubation (37°C) in the presence or absence of 10³ U/ml IFN- α . Colony suppressor assay was performed as described in text.

[‡] The number of bone marrow colonies grown in the absence of PBL were: CFU-GEMM, 67 \pm 30 per 10⁶ cells; BFU-E, 47 \pm 7 per 10⁵ cells; CFU-E, 133 \pm 31 per 10⁵ cells; 14-d, early CFU-GM, 84 \pm 2 per 10⁵ cells; 7-d, late CFU-GM, 164 \pm 46 per 10⁵ cells. The number of experiments performed is given in parentheses.

[§] Not done.

TABLE II
Suppressor Effect of Supernatants from Human PBL on Bone Marrow Hematopoietic Colonies

Producer* cells	Treatment [‡]	Percent inhibition (mean \pm SD) (n) of 14-d early CFU-GM				
		Inducer [§]				
		None	K562	Raji	Bone marrow cells	
				HLA-DR ⁺	HLA-DR ⁻	
B73.1 ⁺ PBL	None	0.1 \pm 5.3 (7)	57.6 \pm 7.5 (7)	0.0, 6.7 [¶]	40.8, 43.9	5.2, 4.1
	IFN	-1.3 \pm 6.8 (7)	67.4 \pm 13.5 (7)	1.1, -1.0	55.1, 56.2	7.2, 2.1
B73.1 ⁻ PBL	None	-1.8 \pm 9.2 (7)	7.8 \pm 8.3 (7)	2.2, 0.0	16.3, 2.1	-1.0, 3.1
	IFN	-2.4 \pm 7.5 (7)	8.2 \pm 8.1 (7)	2.2, -1.0	17.4, 18.4	3.1, 3.1

The number of early CFU-GM obtained in the absence of supernatants was 74.1 \pm 11.6 per 10⁵ plated bone marrow cells (seven experiments).

* B73.1 (+ and -) PBL subpopulations were obtained by indirect rosetting technique from nonadherent PBL from different donors.

[‡] The PBL subpopulations were preincubated (18 h at 37°C) with or without 10³ U/ml highly purified IFN- α .

[§] The different cell lines and the HLA-DR (+ and -) subpopulations (prepared by indirect rosetting from nonadherent mononuclear bone marrow cells) were incubated with PBL for 6 h at 37°C.

[¶] When two experiments were performed, individual results are reported.

evaluate the possibility that NK cells, upon contact with bone marrow cells, release a soluble factor(s) that mediates inhibition of early CFU-GM growth, cell-free supernatants from B73.1 (+ and -) cells, preincubated or not with IFN- α and cultured with bone marrow cells and other cell lines, were tested for their effect on colony growth (Table II). Cell-free supernatants from the different PBL subpopulations incubated in the absence of inducer cells did not influence the number of colonies. Instead, supernatants obtained from B73.1⁺ PBL incubated with either NK-sensitive K562 target cells or with allogeneic HLA-DR⁺ bone marrow cells inhibited early CFU-GM colony formation. Greater inhibition

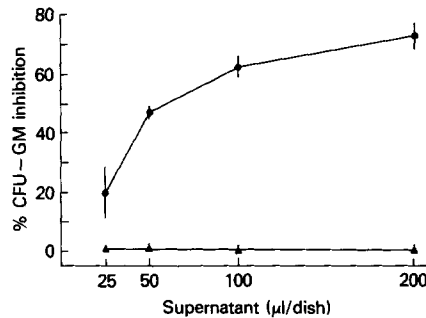


FIGURE 1. Effect of supernatants from cultures of NK cell-enriched PBL populations and K562 cells on bone marrow CFU-GM growth. B73.1⁺ PBL from four donors were prestimulated with purified IFN- α (10^3 U/ml per 5×10^6 cells), and then cultured in the presence of K562 cells. Increasing amounts of cell-free supernatants were added to mononuclear cells from one bone marrow sample during CFU-GM colony growth assay. Colonies were scored on day 7 (▲) and on day 14 (●) of culture. Error bars represent standard deviation. The number of CFU-GM on day 7 was 201 ± 9 per 10^5 cells, and on day 14, 123 ± 9 per 10^5 cells.

was observed with supernatants from IFN- α -treated PBL. No inhibition of colony formation was observed using supernatants obtained from either B73.1⁻ cells or from both B73.1 (+ and -) cells incubated with the NK-insensitive target Raji or with HLA-DR⁻ bone marrow cells, independent of IFN prestimulation. The inhibitory effect of the NK-derived supernatants on early CFU-GM colony formation was dose dependent (Fig. 1) and reached a plateau starting at supernatant concentrations of 10% (100 μ l/dish). By contrast, even 200 μ l supernatant per dish failed to inhibit late CFU-GM. When supernatants produced by PBL cultured with K562 target cells were tested for their effect on the growth of different types of colonies, the results were similar. As shown in Fig. 2, supernatants from total PBL and, to a greater extent, those from B73.1⁺ PBL, significantly inhibited CFU-GEMM, CFU-E, and early CFU-GM colony formation, but not BFU-E and late CFU-GM. This pattern of reactivity is analogous to that observed upon direct incubation of bone marrow cells with PBL subpopulations, without a change in the specificity. Pretreatment of PBL with IFN- α increased the level of inhibition obtained with the supernatants. Supernatants from B73.1⁻ PBL, with or without prestimulation by IFN- α , did not inhibit colony formation.

Lymphocyte Subset that Inhibits Early CFU-GM Growth Also Produces Colony-inhibiting Activity (CIA). To test whether the PBL subset able to directly inhibit colony formation was also responsible for the production of CIA, the effect of preincubation of the bone marrow cells with the different lymphocyte subsets on early CFU-GM colony formation was compared with that of supernatants from the same PBL subsets cultured with K562 cells. The large majority of cells that directly inhibited CFU-GM and that produced CIA were recovered in the B73.1⁺ and in the N901⁺ population (Table III), which also contained the cells with NK activity (not shown). Cells bearing HLA-DR antigens or T cell-specific B36.1 (Leu-1) or B66.6 (T4) antigens were unable to mediate either activity even after IFN induction. Cells suppressing colony formation as well as those producing CIA-containing supernatants were almost equally distributed among the HNK-1 (+ and -) and the B116.1 (T8) (+ and -) subsets. However, the B116.1⁺ subset

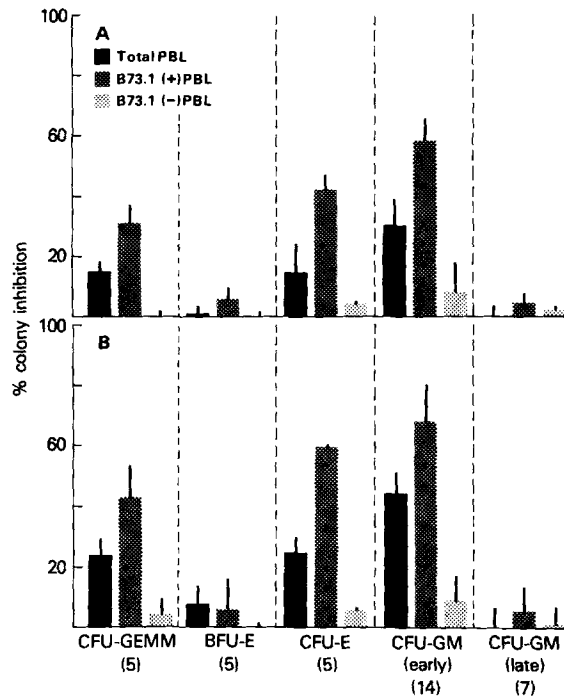


FIGURE 2. Effect of supernatants from cultures of PBL and K562 cells on allogeneic bone marrow colony growth. Supernatants from PBL or B73.1 (+ and -) subpopulations cultured with K562 cells were added to allogeneic bone marrow cells in colony assays (100 μ l per plate). PBL used for supernatant production were pretreated (B) or not (A) with IFN- α as described. The number of colonies grown in the absence of supernatant was: 72.5 ± 17.0 per 10^6 cells (CFU-GEMM); 44 ± 7 per 10^5 cells (BFU-E); 148 ± 24 per 10^5 cells (CFU-E); 75 ± 13 per 10^5 cells (CFU-GM day 14); and 201 ± 25 per 10^5 cells (CFU-GM day 7). Bars and error bars represent the mean \pm SD of the percent colony inhibition observed (number of experiments in parentheses).

purified from B73.1⁻ cells was unable to suppress colony formation. IFN- α treatment increased the efficiency of the cell subsets able to mediate both activities.

CIA-containing Supernatants Inhibit Colony Formation by Autologous Bone Marrow. Supernatants from B73.1 (+ and -) cells were tested for CIA on autologous bone marrow cells (Table IV). Supernatants obtained from both subpopulations cultured in the absence of inducer did not have any effect on either CFU-GM (early and late) or BFU-E. Supernatants produced by B73.1⁺ cells (but not by the respective B73.1⁻ population), upon culture with K562 cells, were able to inhibit early CFU-GM but not BFU-E colony formation when tested on autologous bone marrow cells. Slight inhibition was also observed on late CFU-GM colony growth.

NK-CIA-containing Supernatants Inhibit [³H]TdR Incorporation by CSF-induced Bone Marrow Cells. To test whether the NK-CIA-containing supernatants affected CSF-induced bone marrow cell proliferation, we incubated bone marrow cells, stimulated and unstimulated with CSF, for 4 d in the presence of CIA-

TABLE III
Surface Markers of the PBL Subpopulation that Suppresses 14-d Early CFU-GM and Produces CIA

Exp.	Effector [§] cells	Cell-mediated colony suppression*		CIA (supernatant) [‡]	
		Untreated PBL [†]	IFN-treated PBL [†]	Untreated PBL	IFN-treated PBL
A	Total PBL	36.9 [†]	48.0	25.3	39.0
	B73.1 ⁺	63.2	91.6	53.7	75.8
	B73.1 ⁻	4.3	22.2	11.6	21.1
	HNK-1 ⁺	23.2	48.5	11.6	25.3
	HNK-1 ⁻	23.2	51.6	8.5	23.2
	B116.1 ⁺	45.3	67.4	26.4	34.8
	B116.1 ⁻	52.7	74.8	14.8	34.8
	B73.1 ⁻ /B116.1 ⁺	-3.1	14.8	ND**	ND
	B73.1 ⁻ /B116.1 ⁻	-1.0	0.0	5.3	11.6
B	Total PBL	16.9	34.8	36.9	50.6
	B33.1 ⁺	6.4	6.4	5.3	8.5
	B33.1 ⁻	17.9	37.9	34.8	47.4
	B36.1 ⁺	6.4	5.3	7.4	11.6
	B36.1 ⁻	73.7	91.6	56.9	69.5
	B66.6 ⁺	5.3	8.5	9.5	9.5
	B66.6 ⁻	20.0	42.1	35.8	52.7
	N901 ⁺	81.1	91.6	65.3	74.8
	N901 ⁻	8.5	12.7	7.4	10.6

* PBL or PBL subsets were added at a 2:1 ratio to bone marrow cells before the latter were assayed for colony formation.

[‡] 100 μ l of supernatant from PBL or PBL subsets, cultured with K562 cells as described, was added to each dish in the colony growth assay.

[§] Effector cells were separated by the indirect rosetting method using monoclonal antibodies.

[†] PBL and PBL subsets were preincubated for 18 h at 37°C in medium with or without 10³ U/ml of purified IFN- α and used as effector cells in cell-mediated inhibition assays or for supernatant production.

[†] Percent inhibition of 14-d (early) CFU-GM colony formation (average of results obtained with two lymphocyte donors). The number of early CFU-GM obtained with the bone marrow preparations used in these experiments was 95.0 \pm 7.8 per 10⁵ plated cells.

** Not done.

containing supernatant (Table V). Total bone marrow cells and HLA-DR (+ and -) subsets did not incorporate significant amounts of [³H]TdR when cultured in medium, with or without CIA, in the absence of CSF induction. CSF induced [³H]TdR uptake in total bone marrow and in both HLA-DR⁺ and HLA-DR⁻ cells. In the presence of CSF, HLA-DR⁺ bone marrow cells incorporated significantly more [³H]TdR than did total or HLA-DR⁻ bone marrow cells. The presence of NK-CIA in the culture significantly inhibited the CSF-induced [³H]TdR uptake by total and HLA-DR⁺ bone marrow cells, but not that by HLA-DR⁻ cells. The same supernatants inhibited almost completely the proliferation of NK-sensitive U937 cells, but not of the two NK-insensitive cell lines, Raji and P815y.

Kinetics of NK-CIA Inhibition of Early CFU-GM. The effect of pretreatment of bone marrow cells with NK-CIA-containing supernatants (for 6 h at room

TABLE IV
Effect of NK-CIA on Autologous Bone Marrow

Producer PBL [‡]	Inducer	Percent inhibition of colony formation*		
		Type of colonies		
		CFU-GM [§] (late)	CFU-GM (early)	BFU-E
B73.1 ⁺	None	9.4 ± 8.0	-3.0 ± 3.0	2.2 ± 12.7
B73.1 ⁺	K562	18.2 ± 4.2	49.3 ± 3.0	+2.1 ± 14.8
B73.1 ⁻	None	-0.5 ± 3.2	0.0 ± 7.6	0.0 ± 2.5
B73.1 ⁻	K562	2.7 ± 3.7	7.7 ± 7.6	+2.1 ± 14.8

* Data are mean ± SD of percent inhibition of colony formation in the presence of supernatants obtained from the indicated cultures.

[‡] PBL from one donor were separated into B73.1 (+ and -) cells by indirect rosetting and were cultured for 6 h in the presence or absence of K562 cells.

[§] The number of colonies obtained from 10⁵ autologous bone marrow cells in the absence of supernatants was: 187 ± 10 (late CFU-GM, day 7); 65 ± 10 (early CFU-GM, day 14); 42 ± 4 (BFU-E).

TABLE V
Effect of Supernatants Containing NK-CIA on [³H]TdR Uptake by CSF-induced Bone Marrow Cells and by Cell Lines

Target cells	[³ H]TdR uptake (mean cpm ± SD) per 50,000 cells (n = 3)			
	Culture conditions*			
	Medium	NK-CIA	CSF	CSF + NK-CIA
Bone marrow [‡] Total	105 ± 47	130 ± 28	634 ± 171	381 ± 102 (45.1, P < 0.05) [§]
HLA-DR ⁺	340 ± 80	318 ± 54	8,409 ± 1,674	2,602 ± 122 (69.1, P < 0.01)
HLA-DR ⁻	164 ± 49	221 ± 142	944 ± 237	737 ± 78
Cell lines				
U937 cells	116,076 ± 13,293	4,856 ± 1,170 (95.8, P < 0.01)	ND [¶]	ND
Raji cells	132,926 ± 15,610	130,069 ± 13,542	ND	ND
P815 cells	219,863 ± 11,309	220,966 ± 14,863		

* Bone marrow cells were cultured for 4 d in the presence of medium alone or with NK-CIA (supernatant from IFN- α -stimulated B73.1⁺ PBL cultured with K562 cells; 50% final concentration) or CSF (DET-CM, 10% final concentration) or both and pulsed with [³H]TdR (2 μ Ci/well) for 6 h before harvesting.

[‡] F/H-separated nonadherent bone marrow cells or their HLA-DR (+ and -) subpopulations obtained by indirect rosetting with antibody B33.1.

[§] Percent inhibition of [³H]TdR incorporation is given when the difference between cultures in the presence or absence of NK-CIA was significant (Student's *t* test).

[¶] Not done.

temperature) was tested on adherent cell-depleted bone marrow preparations or on HLA-DR (+ and -) populations derived from them. The cells were then washed and plated for colony growth. All late and early CFU-GM precursors were contained and enriched in the HLA-DR⁺ subset (in both cases a 10-fold enrichment of CFU-GM was observed), whereas almost no CFU-GM colony of either late or early type grew from the HLA-DR⁻ subset (Table VI). Late CFU-GM colony growth was unaffected by pretreatment of either nonadherent total or HLA-DR⁺ bone marrow cells. On the contrary, 50 and 65% inhibition of early CFU-GM colony growth was observed within both total and HLA-DR⁺ bone marrow populations, respectively, after their pretreatment with NK-CIA-

TABLE VI
Inhibition of CFU-GM Colonies by Pretreatment of Bone Marrow Cells with NK-CIA-containing Supernatant

Bone marrow cells*	CFU-GM (late) per 10 ⁵ cells		CFU-GM (early) per 10 ⁵ cells	
	Untreated [‡]	NK-CIA-treated [‡]	Untreated	NK-CIA-treated
Nonadherent	182.7 ± 36.6 [§]	176.1 ± 34.4	96.6 ± 30.0	43.9 ± 13.2
HLA-DR ⁺	1,581.7 ± 77.0	1,596.6 ± 70.9	751.7 ± 179.9	250.0 ± 50.2
HLA-DR ⁻	19.7 ± 5.0	17.0 ± 4.3	4.7 ± 2.2	2.0 ± 1.7

* Bone marrow cells, separated by F/H gradient centrifugation and depleted of adherent cells by incubation on plastic, were separated into HLA-DR (+) and (-) subsets by indirect rosetting using antibody B33.1.

[‡] The three cell populations were incubated (5×10^6 /ml, 6 h at 20°C) with or without supernatant from B73.1⁺ PBL induced with K562 cells. The cells were then washed three times and plated for colony formation.

[§] Data are mean ± standard deviation of the number of colonies grown from four independent experiments.

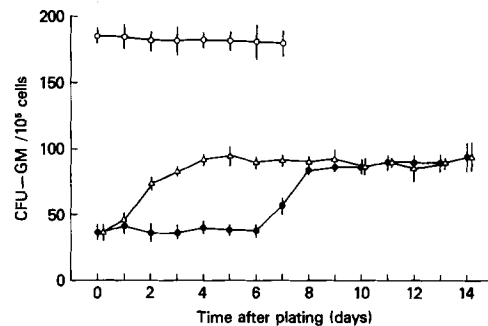


FIGURE 3. Kinetics of the effect of NK-CIA on CFU-GM colonies. NK-CIA-containing supernatant was produced upon culture of IFN- α -treated B73.1⁺ PBL with K562 target cells. Allogeneic mononuclear bone marrow cells were cultured for CFU-GM. The supernatant was added (100 μ l per plate) to the cultures at different days after seeding, and colonies were scored on days 7 and 14 of culture. (O) Bone marrow late CFU-GM, scored on day 7 and (●) early CFU-GM, scored on day 14; supernatant, kept frozen, was added to the cultures on the indicated days. (Δ) Early CFU-GM, scored on day 14; supernatant was kept in culture at 37°C for the indicated number of days, and added to the cultures on the indicated days. Error bars indicate standard deviations of triplicate cultures.

containing supernatants and subsequent culture of the washed cells in the absence of supernatant.

Supernatants, aliquoted and kept either frozen or at 37°C, were added to separate bone marrow cell cultures every day from the start of the culture. Late and early CFU-GM colony growth from dishes to which supernatants were added at different days were scored on days 7 and 14, respectively (Fig. 3). Late (7-d) CFU-GM were unaffected by the addition of CIA-containing supernatants at any of the days of culture. The same supernatants were able to inhibit early (14-d) CFU-GM colony formation only if added to the bone marrow culture on or before day 6. The same supernatants lost the inhibitory effect on early CFU-GM after a 2-d incubation at 37°C when added to either 2-d-old cultures (Fig. 3) or fresh bone marrow cultures (not shown).

Discussion

We have shown that human NK cells exert an inhibitory effect on hematopoietic cells and that, when cultured with either HLA-DR⁺ bone marrow cells or NK-sensitive target cells, they produce a soluble factor(s) that inhibits *in vitro* colony formation from allogeneic and autologous bone marrow cells.

Colony growth of CFU-GEMM, CFU-E, and early CFU-GM was significantly inhibited when bone marrow cells were preincubated with PBL, and this inhibition was enhanced in IFN-treated PBL. No effect on the number of BFU-E or late CFU-GM was observed. Our results from experiments using purified lymphocyte preparations show that inhibition with identical specificity is obtained when B73.1⁺ NK cells are incubated with bone marrow cells. These data confirm and extend previous reports showing that cell preparations enriched for NK cells on the basis of their physical properties (density) (9) or surface phenotype (e.g., HNK-1⁺ cells [15], FcR⁺ [11, 16, 18], E-rosetting and -nonrosetting cells [13, 14]) suppress CFU-E, BFU-E, and CFU-GM bone marrow colonies. We now report that proliferation and differentiation of multipotent CFU-GEMM precursor cells are also efficiently inhibited by NK cells. These data, showing that the most primitive precursor cells identifiable in humans are inhibited by NK cells *in vitro*, are similar to the data reported in the murine model *in vivo*, in which NK cells appear to affect pluripotent stem cells (1, 2). Our data contrast with previous studies which indicated that 7-d CFU-GM are inhibited by NK cell-containing PBL preparations (9–15). There are two likely possibilities to account for this discrepancy. First, in those studies, IFN- γ might have been produced during the culture that acted synergistically with NK-CIA to inhibit late CFU-GM growth. We did not detect significant amounts of IFN in our supernatants; however, particular batches of FBS or CSF preparations may either induce or contain different quantities of IFN- γ , contributing to the observed effect. Alternatively, different CSF preparations may drive myeloid differentiation differently and thus the difference in results may only be superficial. The inability of NK cells to suppress BFU-E colonies is consistent with findings in other studies (15, 16), which have all shown that this type of colony is much more resistant to the inhibitory effect of NK cells than are CFU-E or CFU-GM. In our experiments, the number of precursor cells present in the PBL preparations added to the bone marrow cells was not sufficient to affect the measured extent of inhibition, due to the low PBL-to-bone marrow cell ratio used. However, the possibility remains that some of the PBL preparations produced factors that enhanced colony formation, thereby partially or completely masking the inhibitory effects. Due to the difficulty in obtaining peripheral blood and bone marrow samples from the same donors, all the experiments of NK cell-mediated inhibition were done in allogeneic combinations. However, previous studies (13, 18) have shown that the effect of NK cells on colony formation is identical in allogeneic and autologous combinations and that a genetic restriction, as described for bone marrow rejection in the murine system (1, 2), can not be readily demonstrated *in vitro* with human cells.

Previous studies on the effect of human NK cells on hematopoiesis did not unambiguously characterize effector cells as NK cells on the basis of specific surface markers; thus, a role for effector cells other than NK was not excluded.

Using NK cells purified on the basis of two independent NK-specific surface antigens, i.e., the FcR for immune complexes detected by antibody B73.1 (31, 32) and the antigen detected by antibody N901 (34), we determined that the cells responsible for both spontaneous and IFN-induced cell-mediated colony suppression were NK cells. Moreover, the results of independent experiments with cell populations enriched for or depleted of NK cells, using a series of monoclonal antibodies reacting with antigens specifically present on different lymphocyte subsets, confirm that the cells inhibiting colony formation bear only, and all, those markers known to be present on fresh NK cells (32, 36). The absence of colony inhibition with HLA-DR⁺ or T1 antigen (B36.1⁺) lymphocytes excludes a role for monocytes and B cells, or for T cells, respectively, in mediating the inhibitory effect. Like NK cells, the cells responsible for colony inhibition are distributed among both B116.1 (T8) (+ and -) (36, 42) and HNK-1 (+ and -) (36, 43) lymphocytes. Alloantigen-stimulated cytotoxic T8⁺ T lymphocytes have been shown to suppress CFU-C colony formation (45). Under our experimental conditions, only cells with the NK phenotype, within the T8⁺ population, and not T8⁺ T cells, are responsible for the inhibition of colonies, because T8⁺ cells purified from cells previously depleted of B73.1⁺ NK cells are not inhibitory. In all cell preparations depleted of NK cells, including B73.1⁻ cells, production of a modest, but significant, level of CIA was occasionally observed, especially after IFN- α pretreatment of the cells. In the latter case, the supernatants have been shown to contain small titers of IFN, always <10 U/ml. This level of IFN- α , in control experiments, induced only minimal inhibition of colony formation, but may contribute to the observed inhibition by these supernatants. We also cannot exclude the possibility of a small contamination, by a few NK cells or by other cells with IFN-induced, NK-like activity, of the NK cell-depleted preparation.

The inhibitory effect exerted by NK cells could be mediated through direct cell-to-cell contact that results in killing of the precursor cells, or through release of cytotoxic or cytostatic factors, either acting at distance or concentrated in the milieu surrounding the target cells. Because immature hemopoietic (lymphoid or myeloid) cells had been shown to be targets for NK cell-dependent killing (4, 6-8), and because NK cells can be induced to produce several soluble factors upon culture with NK-sensitive target cells (30) or when stimulated by several inducers (45), we tested supernatants from NK cells cocultured with NK-sensitive and -insensitive targets or with allogeneic bone marrow cells for their effect on colony formation. Supernatants from 6-h cultures of NK cells with NK-sensitive K562 or HLA-DR⁺ bone marrow cells, but not with the NK-insensitive Raji or with HLA-DR⁻ bone marrow cells, contained a factor (NK-CIA) that inhibited colonies with the same pattern of specificity as NK cells. Further, the population with NK cell characteristics responsible for cell-mediated colony suppression was also responsible for the NK-CIA production induced by K562 and by bone marrow cells. NK-CIA suppresses colony formation from bone marrow cells that are both autologous and allogeneic to the producer lymphocytes. NK cells produce NK cytotoxic factor (NKCF) (30) under all the experimental conditions in which NK-CIA is produced. Mycoplasma contamination does not account for NK-CIA and NKCF production because (a) the bone marrow cells used for the

induction were freshly obtained, and (b) all the cell lines used (both inducers and noninducers of NK-CIA) were constantly found to be free of mycoplasma contamination. The production of NK-CIA during incubation of NK cells with bone marrow cells and the identical specificity of colony inhibition mediated directly by NK cells or by NK-CIA strongly suggest that the effect of NK cells on hematopoietic progenitors is partially or completely mediated through the release of NK-CIA. The concentration of NK-CIA that was reached in the medium during the preincubation of NK cells with bone marrow cells in the experiments of direct cell-mediated inhibition is sufficient to account for the inhibitory effect of NK cells. The fact that both the hematopoietic precursor cells affected by NK cells and the cells inducing NK-CIA (and NKCF) production are contained in the HLA-DR⁺ fraction of bone marrow cells suggests that the same cells that act as target cells for NK cells, also induce production of NK-CIA. Similarly, a good, although not absolute, correlation has been shown (30) between sensitivity of cell lines to NK cells and their ability to induce NKCF production. Several pieces of correlative evidence suggest that NKCF and NK-CIA activity might be mediated by the same factor, and studies are in progress for the biochemical identification of this factor. Neither α nor γ IFN was present at a significant level in the NK-CIA preparation, and antisera or monoclonal antibodies against the two types of IFN did not block the activity of NK-CIA.² However, IFN- γ acts synergistically with NK-CIA, and the simultaneous addition to the colony assay of IFN- γ and NK-CIA resulted in significant inhibition of late (day 7) CFU-GM colonies that are not inhibited by either factor separately.²

The effect of NK-CIA on the bone marrow precursor cells may be cytotoxic or cytostatic and it is difficult, at present, to distinguish between these two possibilities. Massive toxicity in bone marrow preparations incubated with NK-CIA was not evidenced in the dye exclusion assay. A modest cytotoxicity on bone marrow cells, mediated by NK cell-enriched PBL, was demonstrated in ⁵¹Cr release assay, but we could not demonstrate a significant cytotoxicity of NK-CIA-containing supernatants, even when HLA-DR⁺ bone marrow cells were used as targets, in the same types of assays. These findings may depend on the fact that the proportion of target cells lysed by NK-CIA is low within bone marrow cells, and only the colony inhibition test is sensitive for NK-CIA detection. Alternatively, NK-CIA might affect proliferation, as suggested by the observation that NK-CIA-containing supernatants inhibited [³H]TdR incorporation in 4-d culture of CSF-induced HLA-DR⁺ bone marrow cells. Significant [³H]TdR incorporation was observed in the HLA-DR⁺ cells and also in the HLA-DR⁻ population upon CSF induction, probably due to relatively mature cells (e.g., promyelocytes) in the latter subset still able to proliferate in response to CSF. NK-CIA inhibited [³H]TdR incorporation only from proliferating HLA-DR⁺ and not from HLA-DR⁻ cells. This may depend on lack of NK-CIA receptors or on resistance of more mature cells to the cytotoxic or cytostatic effect of NK-CIA. The effect of NK-CIA on the precursor cells occurred in the first 2 d of culture; after that time, at 37°C, the activity of NK-CIA was completely lost.

² Degliantoni, G., B. Perussia, M. Kobayashi, and G. Trinchieri. Relationship of natural killer cell-derived hematopoietic colony-inhibiting activity with NK cytotoxic factor and synergism with immune interferon. Manuscript in preparation.

Several lines of evidence suggest that NK-CIA acts directly on the precursor cells and that its action is not mediated through activation of other cell types. The nonadherent HLA-DR⁺ bone marrow cells that we used were depleted of mature monocytes/macrophages, T cells, and NK cells, as well as of cells of myeloid or erythroid lineage more mature than blasts; colony growth was still inhibited by NK-CIA. These cell preparations can also effectively absorb NK-CIA activity, whereas HLA-DR⁻ cells cannot.² Inhibition of colony formation was observed when total or HLA-DR⁺ bone marrow cells were incubated for 6 h at 20°C with NK-CIA-containing supernatants, washed, and cultured in standard medium. Thus, the initial binding of NK-CIA is sufficient to determine inhibition of proliferation of precursor cells. However, inhibition of early CFU-GM was still observed when the NK-CIA was added at up to the 6th d of culture. After day 7, no inhibition could be induced by NK-CIA, suggesting that GM precursor cells have matured *in vitro* to an NK-CIA-resistant stage, possibly analogous to that of the day 7 late CFU-GM present in the bone marrow. The differential sensitivity of early and late CFU-GM to NK-CIA does not seem to be only quantitative, because doses of NK-CIA up to 10-fold higher than those effective on early CFU-GM were still inactive on late colonies. The possibility remains that different types of CSF, inducing differentiation along slightly different myeloid, monocytic, or mixed pathways of differentiation, may affect in different ways the ability to respond to NK-CIA.

The results reported here, showing that human NK cells and their soluble products are effective *in vitro* in controlling hematopoiesis, may be important to understanding both physiological and pathological conditions in which alterations of the hematopoietic system are observed. The data support the hypothesis that the NK cell subset, which in normal donors constitutes ~15% of the total peripheral blood mononuclear cell population, plays a primary role in the homeostasis of the hematopoietic system.

Summary

Incubation of human peripheral blood lymphocytes with bone marrow cells resulted in significant inhibition of colony formation by committed myeloid and erythroid cells. Using positively selected homogeneous natural killer (NK) cell preparations and lymphocyte subpopulations depleted of or enriched for NK cells, we definitively characterize as NK cells the cells in normal peripheral blood that are responsible for inhibition of bone marrow colony growth. The inhibitory effect of NK cells on hematopoiesis can be mediated by a soluble factor that is produced only by NK cells upon culture with HLA-DR⁺ hematopoietic cells and with NK-sensitive cell lines. Both NK cells and the NK-produced, colony-inhibiting activity (NK-CIA) are suppressive for allogeneic and autologous bone marrow CFU-GEMM (colony-forming units, granulocyte, erythroid, monocyte, megakaryocyte), CFU-E (CFU, erythroid), and early CFU-GM (CFU, granulocyte, monocyte), but not for either BFU-E (burst-forming units, erythroid) or late CFU-GM. [³H]Thymidine incorporation was inhibited by NK-CIA-containing supernatants in HLA-DR⁺ but not HLA-DR⁻ bone marrow cell populations stimulated to proliferative by colony-stimulating factor (CSF). These data suggest that the NK cell-mediated inhibitory effect on proliferation and differentiation

of hematopoietic precursor cells is mediated in part or completely by the secreted NK-CIA. The concentration of NK-CIA reached in the supernatant of the mixture of NK cell-containing lymphocyte populations with bone marrow cells is sufficient to account for the inhibitory effect mediated by NK cells. Our data support the hypothesis that human NK cells play a major role in the control of hematopoiesis, down-regulating it under conditions in which the NK cells are functionally activated.

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References

1. Cudkowicz, G., and P. S. Hochman. 1979. Do natural killer cells engage in regulated reaction against self to ensure homeostasis? *Immunol. Rev.* 44:13.
2. Kiessling, R., P. S. Hochman, O. Haller, G. M. Shearer, H. Wigzell, and G. Cudkowicz. 1977. Evidence for a similar or common mechanism for natural killer cell activity and resistance to hemopoietic grafts. *Eur. J. Immunol.* 7:655.
3. Gallagher, M. T., S. K. Datta, and J. J. Trentin. 1980. Genetic resistance to bone marrow transplantation as a leukemia-lymphoma defense mechanism: correlation of "in vivo" lymphoma resistance and in vitro NK activity. *Biomedicine (Paris)*. 33:73.
4. Kumar, V., E. Luevano, and M. Bennett. 1979. Hybrid resistance to EL-4 lymphoma cells. I. Characterization of natural killer cells that lyse EL-4 cells and their distinction from marrow-dependent natural killer cells. *J. Exp. Med.* 150:531.
5. Riccardi, C., A. Santoni, T. Barlozzari, and R. B. Herberman. 1981. In vivo reactivity of mouse natural killer (NK) cells against normal bone marrow cells. *Cell. Immunol.* 60:136.
6. Hansson, M., R. Kiessling, and B. Andersson. 1981. Human fetal thymus and bone marrow contain target cells for natural killer cells. *Eur. J. Immunol.* 11:8.
7. Hansson, M., R. Kiessling, B. Andersson, K. Karre, and J. Roder. 1979. Natural killer (NK)-sensitive T-cell subpopulation in the thymus: inverse correlation to NK activity of the host. *Nature (Lond.)*. 278:174.
8. Gidlund, M., M. Nose, I. Axberg, H. Wigzell, T. Totterman, K. Nilsson. 1982. Analysis of differentiation events causing changes in NK cell tumor-target sensitivity. *In NK Cells and Other Natural Effector Cells*. R. B. Herberman, editor. Academic Press, Inc., New York. 733-741.
9. Hansson, M., M. Miroslav, B. Andersson, and R. Kiessling. 1982. Inhibition of in vitro granulopoiesis by autologous allogeneic human NK cells. *J. Immunol.* 129:126.
10. Barr, R. D., and C. A. Stevens. 1982. The role of autologous helper and suppressor T cells in the regulation of human granulopoiesis. *Am. J. Hematol.* 12:323.
11. Spitzer, G., and D. S. Verma. 1982. Cells with Fc γ receptors from normal donors suppress granulocytic macrophage-colony formation. *Blood*. 60:758.
12. Torok-Storb, B., and J. A. Hansen. 1982. Modulation of in vitro BFU-E growth by normal Ia-positive T cells is restricted by HLA-DR. *Nature (Lond.)*. 298:473.
13. Bacigalupo, A., M. Podesta, M. C. Mingari, L. Moretta, G. Piaggio, M. T. Van Lint, A. Durando, and A. M. Marmont. 1981. Generation of CFU-C/suppressor T cells in vitro: an experimental model for immune-mediated marrow failure. *Blood*. 57:491.
14. Morris, T. C. M., P. C. Vincent, R. Sutherland, and P. Hersey. 1980. Inhibition of normal human granulopoiesis in vitro by non-B non-T lymphocytes. *Br. J. Haematol.* 45:541.

15. Mangan, K. F., M. E. Hartnett, S. A. Matis, A. Winkelstein, and T. Abo. 1984. Natural killer cells suppress human erythroid stem cell proliferation in vitro. *Blood*. 63:260.
16. Mangan, K. F., G. Chikkappa, and P. C. Farley. 1982. T gamma (T γ) cells suppress growth of erythroid colony-forming units in vitro in the pure red cell aplasia of B-cell chronic lymphocytic leukemia. *J. Clin. Invest.* 70:1148.
17. Nagasawa, T., T. Abe, and T. Nakagawa. 1981. Pure red cell aplasia and hypogammaglobulinemia associated with T γ -cell chronic lymphocytic leukemia. *Blood*. 57:1025.
18. Bacigalupo, A., M. Podesta, M. C. Mingari, L. Moretta, M. T. Van Lint, and A. Marmont. 1980. Immune suppression of hematopoiesis in aplastic anemia: activity of T- γ lymphocytes. *J. Immunol.* 125:1449.
19. Bom-Van Noorloos, A. A., H. G. Pegels, R. H. J. Van Oers, J. Silverbush, T. M. Feltkamp-Vroom, R. Gondsmid, W. P. Zeijlemaker, A. E. G. K. Von Dem Borne, and C. J. M. Melief. 1980. Proliferation of T cells with killer-cell activity in two patients with neutropenia and recurrent infections. *N. Engl. J. Med.* 17:933.
20. Pandolfi, F., G. Semenzato, G. de Rossi, D. M. Strong, L. Quinti, A. Pezzutto, F. Mandelli, and F. Aiuti. 1982. Heterogeneity of T-CLL defined by monoclonal antibodies in nine patients. *Clin. Immunol. Immunopathol.* 24:330.
21. Spitzer, G., D. S. Verma, M. Beran, A. R. Zander, K. B. McCredie, and K. A. Dicke. 1982. Human adherent cells contact-mediated modulation of normal myeloid colony formation. *J. Natl. Cancer Inst.* 68:35.
22. Zombos, N. C., J. Y. Djeu, and N. S. Young. 1984. Interferon is the suppressor of hematopoiesis generated by stimulated lymphocytes in vitro. *J. Immunol.* 133:769.
23. Broxmeyer, H. E., and E. Platzer. 1984. Lactoferrin acts on I-A and I-E/C antigen⁺ subpopulations of mouse peritoneal macrophages in the absence of T lymphocytes and other cell types to inhibit production of granulocyte-macrophage colony stimulatory factors in vitro. *J. Immunol.* 133:306.
24. Broxmeyer, H. E., J. Bognacki, M. H. Dormer, and M. de Sousa. 1981. Identification of leukemia-associated inhibitory activity as acid isoferritins. A regulatory role for acidic isoferritins in production of granulocytes and macrophages. *J. Exp. Med.* 153:1426.
25. Kurland, J. I., H. E. Broxmeyer, L. M. Pelus, R. S. Bockman, and M. A. S. Moore. 1978. Role for monocyte-macrophage-derived colony-stimulating factor and prostaglandin E in the positive and negative feedback control of myeloid stem cell proliferation. *Blood*. 52:388.
26. Broxmeyer, H. E., L. Lu, E. Platzer, C. Feit, L. Juliano, and B. Y. Rubin. 1983. Comparative analysis of the influences of human gamma, alpha and beta interferons on human multipotential (CFU-GEMM), erythroid (BFU-E) and granulocyte-macrophage (CFU-GM) progenitor cells. *J. Immunol.* 131:1300.
27. Hoffman, M. K., H. F. Oettgen, L. J. Old, R. S. Mittler, and U. Hammerling. 1978. Induction and immunologic properties of tumor necrosis factor. *J. Reticuloendothel. Soc.* 23:307.
28. Butler, R. C., A. M. Abdelnoor, and A. Nowotny. 1978. Bone marrow colony-stimulating factor and tumor resistance-enhancing activity of postendotoxic mouse sera. *Proc. Natl. Acad. Sci. USA.* 75:2893.
29. Hiserodt, J. C., A.-M. Prieur, and G. A. Granger. 1976. In vitro lymphocyte cytotoxicity. I. Evidence of multiple cytotoxic molecules secreted by mitogen activated human lymphoid cells in vitro. *Cell. Immunol.* 24:277.
30. Wright, S., M. L. Weitzen, R. Kahle, G. A. Granger, and B. Bonavida. 1983. Studies on the mechanism of natural killer cytotoxicity. II. Coculture of human PBL with NK-sensitive or resistant cell lines stimulates release of natural killer cytotoxic factors (NKCF) selectively cytotoxic to NK-sensitive target cells. *J. Immunol.* 130:2479.

31. Perussia, B., S. Starr, S. Abraham, V. Fanning, and G. Trinchieri. 1983. Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. I. Characterization of the lymphocyte subset reactive with B73.1. *J. Immunol.* 130:2133.
32. Perussia, B., O. Acuto, C. Terhorst, J. Faust, R. Lazarus, V. Fanning, and G. Trinchieri. 1983. Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. II. Studies of B73.1 antibody-antigen interaction on the lymphocyte membrane. *J. Immunol.* 130:2142.
33. Perussia, B., G. Trinchieri, D. Leberman, J. Jankiewicz, B. Lange, and G. Rovera. 1982. Monoclonal antibodies that detect differentiation surface antigens on human myelomonocytic cells. *Blood.* 59:382.
34. Griffin, J. D., T. Hercend, R. Beveridge, and S. F. Schlossman. 1983. Characterization of an antigen expressed on human natural killer cells. *J. Immunol.* 130:2947.
35. Abo, T., and C. M. Balch. 1981. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J. Immunol.* 127:1024.
36. Perussia, B., G. Trinchieri, A. Jackson, N. L. Warner, J. Faust, H. Rumpold, D. Kraft, and L. L. Lanier. 1984. The Fc receptor for IgG on human natural killer cells: phenotypic, functional and comparative studies with monoclonal antibodies. *J. Immunol.* 133:180.
37. Fauser, A. A., and H. A. Messner. 1978. Granuloerythropoietic colonies in human bone marrow, peripheral blood, and cord blood. *Blood.* 52:1243.
38. Aye, M. T., Y. Niho, J. E. Till, and E. A. Culloch. 1974. Studies of leukemic cell population in culture. *Blood.* 44:205.
39. Ogawa, M., R. T. Parmley, H. L. Bank, and S. S. Spicer. 1976. Human marrow erythropoiesis in culture. I. Characterization of methylcellulose colony assay. *Blood.* 48:407.
40. Pike, B. L., and W. A. Robinson. 1970. Human bone marrow colony growth in vitro. *J. Cell. Physiol.* 76:77.
41. Inoue, S., and M. J. Ottenbreit. 1978. Heterogeneity of human colony-forming cells. *Blood.* 51:195.
42. Perussia, B., V. Fanning, and G. Trinchieri. 1983. A human NK and K cell subset shares with cytotoxic T cells expression of the antigen recognized by antibody OKT8. *J. Immunol.* 131:223.
43. Mangan, K. F., G. Chikkappa, L. Z. Bieler, W. B. Scharfman, and D. R. Parkinson. 1982. Regulation of human blood erythroid burst-forming unit (BFU-E) proliferation by T lymphocyte subpopulations defined by Fc receptors and monoclonal antibodies. *Blood.* 59:990.
44. Nakao, S., M. Harada, K. Kondo, K. Odaka, M. Ueda, K. Matsue, T. Mori, and K.-I. Hattori. 1984. Effect of activated lymphocytes on the regulation of hematopoiesis: suppression of in vitro granulopoiesis by OKT8⁺Ia⁺ T cells induced by alloantigen stimulation. *J. Immunol.* 132:160.
45. Trinchieri, G., M. Matsumoto-Kobayashi, S. Clark, J. Sehra, L. London, and B. Perussia. 1984. Response of resting human peripheral blood natural killer cells to interleukin 2. *J. Exp. Med.* 160:1147.