Autologous Activated Natural Killer Cells Suppress Primitive Chronic Myelogenous Leukemia Progenitors in Long-Term Culture

By Francisco Cervantes, Bryce A. Pierson, Philip B. McGlave, Catherine M. Verfaillie, and Jeffrey S. Miller

A graft-versus-leukemia effect has been well documented to prevent relapse in chronic myelogenous leukemia (CML) after allogeneic marrow transplantation. One type of lymphocytes that may contribute to this effect are natural killer cells (NK), which, after activation with interleukin (IL)-2, exhibit a broad range of cytolytic activity against autologous and allogeneic cells. We have previously demonstrated that IL-2-activated NK (ANK) can be generated from blood of patients with CML and are benign in origin. Their proliferation and function, however, diminish with disease progression in CML, suggesting a role in tumor surveillance. We studied the effect of IL-2-activated NK (ANK) on normal and malignant primitive and committed progenitors in a novel long-term bone marrow culture (LTBMC) assay. Because ANK destroy marrow stromal layers, the use of classic stroma-dependent long-term cultures is not possible. Therefore, we used the stroma noncontact LTBMC system developed in our laboratory to analyze the effect of autologous ANK cells on primitive hematopoietic progenitors. Autologous ANK (CD56+/CD3-) were generated from the peripheral blood of 10 patients with chronic phase CML and from six patients with CML who exhibited diminished cytotoxicity against NK-sensitive (K562) and NK-resistant (Raji) tumor targets. The number and functional capacity of activated ANK (NK) of patients with chronic phase CML decrease once CML progresses to the accelerated phase. This suggests that ANK may have a role in the control of the disease progression and raises the possibility of their therapeutic use in chronic phase CML.

CML bone marrow contains a 10-fold increased number of CD34+/HLA-DR+ cells (CD34+/DR+) compared with normal marrow. A majority of these cells are Philadelphia (Ph) chromosome- and BCR/ABL mRNA-positive and contain not only Ph+ colony-forming cells (CFC), but also Ph+ long-term culture-initiating cells (LTIC). In contrast, a smaller, coexistent population of CD34+/HLA-DR- cells contains predominantly Ph+, BCR/ABL-negative LTIC. Although we have previously reported that ANK suppress committed CML progenitors in a 36-hour coculture, studying the effect of ANK on CML primitive progenitors in vitro has been problematic, because these cytotoxic effectors rapidly kill marrow stroma and, thus, cannot be added to classic Dexter-type long-term marrow cultures. The destruction of stroma is mediated by direct ANK contact with stromal components and not by IL-2 alone. Furthermore, addition of ANK to long-term cultures that contain hydrocortisone may result in inhibition of ANK function.

A long-term marrow culture system termed "stroma noncontact culture" has been described by our group. In this culture, plated primitive progenitors are separated from stroma by a microporous membrane that prevents direct progenitor-stroma contact but permits diffusion of stroma-derived soluble factors. This culture system maintains LTIC and induces their differentiation into committed CFC to a
greater extent than when normal CD34+/DR- cells are cultured in classic long-term bone marrow culture (LTBMC), where progenitors are plated in direct contact with stroma. Additional studies have demonstrated that the murine stromal cell line (M2-10B4) can replace primary human marrow stroma used in either classic long-term culture or stroma noncontact culture. We hypothesize that modified long-term culture systems where progenitors are suspended above stroma should be amenable to further modifications to test the effect of ANK and other effector populations on normal and malignant LTCIC. We developed a novel culture system in which ANK are coincubated with primitive progenitors but are separated from the stromal feeder. ANK can be functionally inactivated by adding hydrocortisone back to the culture medium, and the long-term culture can be continued to assess LTCIC. Using this assay, we demonstrate for the first time that autologous ANK can suppress malignant CML LTCIC while sparing normal hematopoiesis.

**MATERIALS AND METHODS**

**Study population.** Peripheral blood and bone marrow from 12 patients with chronic-phase Ph-positive CML were studied. Median duration of the chronic phase was 17 months (range, 4 to 43 months); six patients were studied within 1 year from diagnosis. Two patients had been treated with hydroxyurea, one with alpha interferon, eight with both drugs, and one had received busulfan as initial therapy and then hydroxyurea. Before study, hydroxyurea was discontinued for 5 days and interferon was discontinued for 1.5 to 3 months. White blood cell (WBC) counts ranged from 4.7 × 10^9/L to 4.7 × 10^9/L (median, 13 × 10^9/L) at the time of study. Cytogenetic evaluation of marrow demonstrated 100% Ph^+ metaphases in 10 patients and 23/25 and 9/20 Ph^+ metaphases in the two other patients. Thirteen healthy normal volunteers provided blood and/or marrow as controls. Peripheral blood only from eight normal donors and seven patients with CML was used for hydrocortisone studies and elutriation for ANK generation, respectively. All patient and control samples were obtained using guidelines approved by the Committee on the Use of Human Subjects in Research at the University of Minnesota (Minneapolis, MN).

**Purification of peripheral blood NK.** Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque (Sigma Diagnostics, St. Louis, MO) density gradient centrifugation (30 minutes at 37°C and 400g). When the WBC count was higher than 10 × 10^9/L in patients with CML, PBMC were subjected to countercflow centrifugation elutriation. PBMC resuspended in phosphate-buffered saline (PBS) supplemented with 0.01% bovine serum albumin (BSA) and 0.01% EDTA were injected into an elutriation system with a standard separation chamber (Beckman Instruments, Palo Alto, CA) primed with PBS-BSA-EDTA. Rotor speed and temperature were maintained at 1,950 rpm and 10°C. After loading, 200 mL of effluent was collected at a flow rate of 6 to 16 mL/min. The NK population was further purified by panning PBMC or elutriated PBMC onto MicroCELLector flasks coated with CD5 and CD8 monoclonal antibodies (provided by Applied Immune Sciences, Inc, Santa Clara, CA) as described. The resultant nonadherent cells were reincubated in a fresh MicroCELLector flask for a second depletion before use. Some ANK populations were prepared by sorting CD56^+CD3^- NK from PBMC after immunomagnetic bead-depletion of PBMC using anti-CD3 (Becton Dickinson, Palo Alto, CA) and anti-CD5 (Becton Dickinson) antibodies with the addition of anti-CD19 (Becton Dickinson), MY8 (Coulter Cytometry, Hialeah, FL), and anti-glycophorin A (AMAC, Westbrook, MD) antibodies for patients with CML.

**Culture of ANK.** Fluorescence-activated cell sorter (FACS)-purified CD56^+CD3^- NK were expanded in direct contact with M2-10B4 accessory cells as previously described. Enriched NK populations from CD5/CD8 panning were suspended at a concentration of 0.5 × 10^6/mL in 5 mL of complete culture medium containing 1,000 U/mL of recombinant IL-2 (a gift from Amgen Inc. Thousand Oaks, CA) in horizontal T-25 flasks (Corning Glassware, Corning, NY) at 37°C in 5% CO2 in a humidified atmosphere. Complete culture medium consisted of a 2:1 vol/vol mixture of Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F12 supplemented with 10% human heat-inactivated AB serum (North American Biologics, Miami, FL), 24 μg/mL 2-mercaptoethanol, 50 μg/mL ethanolamine, 20 mg/L L-ascorbic acid, 5 μg/mL of sodium selenite (Na2SeO3), and 100 U/mL penicillin plus 100 μg/mL streptomycin (GIBCO Laboratories, Grand Island, NY). At day 7 of culture, the culture volume was doubled with fresh NK medium, and thereafter, full medium changes were performed twice weekly.

**Cytotoxicity assays.** ANK were tested at day 14 for cytotoxicity against the NK-sensitive cell line K562 (American Type Culture Collection [ATCC], Rockville, MD) and the NK-resistant cell line Raji (ATCC) in a 4-hour ¹⁵Cr release assay as previously described. Purification of bone marrow primitive progenitors. Bone marrow was aspirated from the posterior iliac crest and bone marrow mononuclear cells (BMMNC) separated using Ficoll-Hypaque centrifugation. An enriched CD34^+ population was obtained as previously described. The resultant cells were labeled with anti-CD34 (phycoerythrin [PE]; Becton Dickinson) antibody for normal individuals and with both anti-CD34 (PE) and anti–HLA-DR fluorescein isothiocyanate (FITC) antibodies for patients with CML. Cells were then sorted on the FACS Star^® laser flow cytometer (Becton Dickinson), selecting CD34^+ cells from normal marrow and CD34^+DR^+ cells from CML marrow as previously described.

**Hematopoietic progenitor culture.** LTBMC of hematopoietic progenitors were established in a stroma noncontact system as previously described. This consisted of 24-well plates containing the murine marrow stroma-derived fibroblastic cell line M2-10B4 (a gift from Dr Connie Eaves, Terry Fox Laboratories, Vancouver, Canada) and 5,000 U/mL IL-2. Autologous normal, allogeneic normal, or autologous CML ANK were then added at 6.0 × 10^5/mL. Culture medium consisted of a 2.1 vol/vol mixture of Dulbecco's Modified Eagle's Medium (GIBCO Laboratories, Grand Island, NY). At day 7 of culture, the culture volume was doubled with fresh medium, and thereafter, full medium changes were performed twice weekly.

**Incubation of ANK with hematopoietic progenitors.** On day 14 of culture, LTBMC medium was removed from stroma noncontact cultures and replaced with LTBMC medium without hydrocortisone (HC) but supplemented with 1,000 U/mL IL-2. Autologous normal, allogeneic normal, or autologous CML ANK were then added at effecter-to-target (E:T) ratios ranging from 60:1 to 2:1. The cell cultures were harvested and plated in methylcellulose culture to determine the number of CFU present, as described previously. When allogeneic ANK were used, 5.0 × 10^6/mL HC was added to the methylcellulose culture to inactivate ANK. In the remaining wells, the HC-free, IL-2-containing medium was removed, and HC-containing, IL-2–free LTBMC medium was added to inactivate ANK. Progenitors were maintained in culture for an additional 5 weeks and then plated in methylcellulose culture to determine the number of LTCIC (Fig 1). For the antibody blocking experiments, specific antibodies were added to the Transwell insert containing cultured progenitors 30 minutes before the addition of ANK. The antibodies used were as follows: anti-human interferon (IFN)-γ neutralizing antibody, 50 μg/
Effect of HC on normal ANK function. To determine if the presence of HC in LTBMC medium would affect the cytolytic capacity of ANK, studies were performed with ANK derived from eight healthy volunteers. On day 14 of ANK culture, cells were harvested, and 2 × 10^6 ANK were incubated in LTBMC medium without HC plus 1,000 U/mL IL-2 (LTBMC − HC + IL-2), LTBMC medium with 10^{-5} mol/L HC plus IL-2 (LTBMC + HC + IL-2), or standard LTBMC medium (LTBMC + HC − IL-2). Cytotoxicity against an NK-sensitive target (K562) and NK-resistant target (Raji) was evaluated after 18- and 42-hour exposure. Incubation of ANK with HC for 18 hours significantly reduced the cytotoxic activity against Raji targets and less so against K562 targets (Fig 2). However, 42-hour incubation of ANK in the presence of HC markedly inhibited cytolytic activity against both targets (Fig 2). Addition of IL-2 to LTBMC medium containing HC partially preserved cytotoxic activity of ANK, suggesting that IL-2 can only partially reverse the decrement in ANK function induced by HC. This demonstrates that HC has a significant and rapid inhibitory effect on ANK function. Therefore, evaluation of ANK cytotoxicity for LTCIC in LTBMC should not be performed in the presence of HC.

ANK generation and function. Optimal generation of ANK from normal blood requires monocytes as accessory cells. We have previously demonstrated that a CD5/CD8 depletion leads to an optimal starting population of NK precursors and monocytes. However, these methods are not adequate to generate ANK from patients with CML with high WBC counts, because the blood of patients with CML is flooded with myeloid cells that are inadequately removed by Ficoll Hypaque separation. To further enrich for lymphoid cells in patients with CML, counterflow centrifugation elutriation was used at a constant rotor speed of 1,950 rpm and variable flow rate ranging from 6 to 16 mL/min. A flow rate of 8 mL/min significantly enriched for cells falling within the lymphocyte gate on the forward and side scatter by flow cytometry compared with the starting mononuclear fraction (61.3% ± 4.2% vs 18% ± 5.7%, n = 6; P = .001) and increased the number of cells expressing CD7 (44% ± 4.7% vs 9.5% ± 4.1%, n = 6; P = .003), an antigen found on both NK and T lymphocytes. In five of six experiments, there was less than 1% residual CD7^+ cells found in the rotor off fraction. The resulting fraction 8 cells were then depleted of CD5/CD8-positive cells, and ANK were generated using the same methods as previously described for the generation of ANK from normal blood. There was no difference in function between ANK processed with or without elutriation (data not shown).

At day 14 of culture, morphologic examination of Wright-Giemsa-stained cells demonstrated a marked predominance of cells with the appearance of large granular lymphocytes. Phenotypic analysis of ANK demonstrated a similar number of CD56^+/CD3^- cells from normal donors (95% ± 1.6%) and patients with CML (88% ± 2%). However, the number of CD56^+/CD3^- cells was significantly lower in patients with CML (57.4% ± 4.5%) than in normal donors (79.5%...
Fig 2. Effect of HC on ANK function. ANK from eight normal donors were harvested after 14 days of culture and preincubated in LTBMC medium without $10^{-6}$ mol/L HC but with 1,000 U/mL IL-2 (LTBMC − HC + IL-2) for 42 hours to serve as the baseline function for each panel (○). The effect of HC on ANK was tested by incubating effectors in medium containing LTBMC + HC + IL-2 (open symbols in A and C) or LTBMC + HC − IL-2 (open symbols in B and D) for 18 (□) or 42 hours (□). All data represent cytotoxicity tested in C⁹¹ assays against the NK-sensitive K562 target (A and B) and NK-resistant Raji target (C and D). Data are presented as the mean ± SEM at six different E:T ratios.

Hematopoietic progenitor studies. Because HC rapidly inhibits ANK function and ANK rapidly lose activity if not maintained in IL-2,¹⁹ we designed a novel long-term culture assay to test the effect of ANK on primitive progenitor populations (Fig 1). CD34⁺ cells from normal donors (containing both CFC and LTCIC) and CD34⁺/DR⁺ cells from patients with CML (containing Ph⁺ CFC and LTCIC) were plated in Transwell inserts placed above M2-10B4 stromal monolayers (Fig 1, step 1, 2). After 14 days, HC-containing LTBMC medium was replaced by IL-2−containing, HC-free LTBMC medium, and autologous ANK were added to the Transwell insert (Fig 1, step 3). After 48 hours, the culture medium was removed and replaced with HC-containing LTBMC medium free of IL-2 to inactivate residual ANK function, and long-term culture was continued (Fig 1, steps 4 and 5).

In control experiments, IL-2 alone or IL-2 plus ANK placed in Transwell inserts above M2-10B4 had no detrimen-
nal effect on M2-10B4 stromal layers over a 5-week period. The number of absolute colonies derived from the initially plated normal CD34+ cells compared with CML CD34+/DR− cells maintained in LTBM alone was not significantly different for either CFC (201 ± 12 v 189 ± 29) or LTCIC secondary colonies (89 ± 8 v 96 ± 24). The number of CFC and LTCIC was not affected by the 48-hour removal of HC within the culture. Expansion; n

control CFC; 102% ± 11% of control LTCIC) or CML CD34+/DR− cells (93% ± 6% of control CFC; 97% ± 3% of control LTCIC). Finally, addition of autologous CML ANK without removal of HC and without addition of IL-2 did not result in suppression of either CML CFC or LTCIC.

Coculture of normal CD34+ cells with autologous ANK did not affect the numbers of CFC or LTCIC at any of the E:T ratios tested. In marked contrast, when ANK from the seven patients with CML, which exhibited significant killing of K562 and Raji tumor target cells, were incubated with CD34+/DR− cells, a dose-dependent suppression of the growth of both CFC and LTCIC was observed (Fig 5). Although the data are presented as the E:T ratio based on the number of initial CD34+/DR− cells plated on day 0 to standardize experiments, there was significant expansion in the Transwell before addition of ANK (12.7-± 4.1-fold expansion; n = 7). Therefore, the true 60:1 E:T ratio in the assay is significantly lower (approximately 5:1) and conceivably more physiologic. To further support the observation that suppression induced by CML ANK on progenitors correlated with ANK functional capacity, we demonstrate that ANK from the two patients with CML that killed K562 and Raji poorly also did not suppress the growth of autologous CFC and LTCIC (Fig 5).

CML progenitor killing by ANK is major histocompatibility complex (MHC)-unrestricted and mediated by direct cell-to-cell contact. As the effect of autologous ANK on CFC and LTC-IC was essentially the same, the shorter readout CFC assay was used in subsequent experiments. The mechanism of NK killing is presumed to be MHC-unrestricted. Therefore, allogeneic normal NK should have the same effect on CML progenitors as autologous NK. This was tested directly using a cultured population of allogeneic normal ANK (greater than 90% CD56+bright) devoid of T-lymphocytes (less than 1%) prepared by sorting, which resulted in significant suppression of CML progenitors (Fig 5). The increased killing observed with allogeneic normal ANK

![Fig 3. ANK function from patients with CML and normal donors. ANK from six normal donors (□), seven patients with CML with good ANK function (●), and two patients with CML with poor ANK function (■) were tested in cytotoxicity assays against K562 (left) and Raji targets (right). The differences between normal donors and patients with CML with good ANK function (n = 7) and CML with poor ANK function (n = 2) are statistically significant for K562 at E:T ratios of 0.75:1 and 0.25:1 for the K562 target and at all E:T ratios for Raji targets. The differences between CML with good ANK function (n = 7) and CML with poor ANK function (n = 2) are statistically significant for K562 at E:T ratios of 20:1 (P < .05) and 6.6:1 (P < .03) and for Raji at E:T ratios of 20:1 (P < .03) and 6.6:1 (P < .02).](https://www.bloodjournal.org/content/111/24/2480/F5)

![Fig 4. Cytotoxicity correlates with the number of CD56+bright cells within the ANK population. The percent of cells within the ANK population expressing the CD56+bright/CD3− phenotype is plotted on the X axis versus the percent specific lysis in Cr51 release assays against Raji targets at an E:T ratio of 6.6:1 on the Y axis. ANK derived from normal donors (□), patients with CML generating ANK with good function (●), and patients with CML generating ANK with poor function (■) were included in this analysis. The best-fit line was determined using simple regression analysis, which demonstrated a good correlation between function and CD56+bright expression (correlation coefficient R = .937; P < .001).](https://www.bloodjournal.org/content/111/24/2480/F4)
Fig 5. ANK suppress autologous malignant progenitors but not benign progenitors. ANK were incubated with normal CD34+ (●) or CML CD34+/DR+ progenitors (open symbols) cultured for 14 days in stroma noncontact culture as described in Fig 1. ANK were added to autologous progenitors in medium without HC and with IL-2 for 48 hours. To evaluate the effect of ANK on CFC, CD34+ and CD34+/DR+ progeny were evaluated after the 48-hour incubation with ANK by plating in methylcellulose colony assay. To evaluate the effect of ANK on LTCIC, LTBMIC medium (+ HC - IL-2) was added after the 48-hour incubation and was maintained for 5 weeks and then evaluated for the number of secondary CFC. CFC (left) and LTCIC (right) were analyzed in an autologous setting from normal donors (●, n = 8), patients with CML with good ANK function (▲, n = 6), and patients with CML with poor ANK function (□, n = 2). CFC were also evaluated after incubation of CML progenitors with allogeneic normal ANK (○, n = 7). CML ANK with good function significantly suppressed autologous CFC at all but the lowest E:T ratio (P < 0.01 for the three highest E:T ratios) and significantly suppressed autologous LTCIC at all E:T ratios (P < 0.001 for each E:T ratio) compared with normal ANK and autologous progenitors. CML ANK with poor function did not suppress CFC or LTCIC similarly to normal ANK and autologous progenitors. There was a significant difference in suppression between CML ANK with good function and CML ANK with poor function for autologous CFC (P < 0.05 at three highest E:T ratios) and LTCIC (P < 0.015 at all but the E:T ratios of 5:1, where P = 0.9).

Compared with autologous CML ANK was not significant and could be explained by the higher number of CD56+ cells in the normal allogeneic population and corresponding increased cytotoxicity of the normal ANK population compared with ANK from patients with CML (similar to data in Fig 3, data not shown).

The importance of direct contact on the observed ANK suppression of CML progenitors was further evaluated. CML CD34+/DR+ progenitors were first initiated into long-term culture in Transwells as described above for 14 days and then transferred into four different conditions (Fig 6). In condition A, Transwells containing CML progenitors were transferred to a well containing M2-10B4 conditioned medium (+ IL-2) alone in the bottom compartment, and after 48 hours, progenitors were harvested from the Transwell and plated into methylcellulose culture without IL-2. There was no difference in CFC outgrowth from CML progenitors incubated above viable stroma or progenitors incubated in conditioned medium (110 ± 46 CFC v 95 ± 40 CFC, n = 4; P = not significant [NS]). In condition B, Transwells containing CML progenitors were transferred to a well containing M2-10B4 conditioned medium (+ IL-2) and ANK (E:T, 60:1) in the bottom compartment. After 48 hours, progenitors were harvested from the Transwell and plated into methylcellulose culture without IL-2. There was no suppression of CML progenitors by soluble factors produced by ANK (Fig 6, condition B).

Direct contact between ANK and CML progenitors was tested by adding ANK to CML progenitor-containing Transwells that were then transferred to a well over viable M2-10B4 (+ IL-2). After 48 hours, the ANK-progenitor mixture was harvested from the Transwell and plated into methylcellulose culture without IL-2. In contrast to culture with conditioned medium (condition A) and ANK soluble factors (condition B), when ANK were incubated in direct contact with CML progenitors, near complete CML progenitor suppression is observed (Fig 6, condition C). Finally, CML progenitor containing Transwells were transferred to a well containing conditioned medium (+ IL-2) and ANK in the bottom compartment. After 48 hours, progenitors were harvested from the Transwell, mixed with ANK harvested from the bottom compartment, and plated into methylcellulose culture without IL-2. Minimal suppression was observed when contact was prohibited during the 48-hour incubation, but progenitors and ANK were mixed back together before methylcellulose culture (Fig 6, condition D). This observation validates the design of this model where the effects of ANK on progenitors primarily occur during the 48-hour incubation period and not when the ANK-progenitor mixture is plated in methylcellulose culture.

The suppression of CML progenitors by ANK was further evaluated using blocking antibodies against soluble factors (IFN-γ, TNF-α, TGF-β) and ligands/receptors with known importance in cell recognition (Table 1). There was no effect...
on suppression by blocking antibodies used against soluble factors or the corresponding control antibodies during the
48-hour incubation of ANK in direct contact with CML progenitors. However, antibodies against the β2 integrin,
with known importance in NK recognition, significantly blocked suppression compared with control antibodies (con-
trol IgG, antibody: 73% ± 16% suppression v anti-β2: 37% ± 11% suppression, n = 3; P = .021). These blocking experi-
ments further corroborate the above studies, demonstrating that suppression of CML progenitors is mediated through
direct cell-to-cell contact and soluble factors are not involved under the conditions tested here.

**DISCUSSION**

The effect of autologous ANK cells on primitive normal and CML hematopoietic progenitors (LTCIC) has been dif-
cult to study in the classic stroma contact Dexter culture, mainly because direct contact between ANK and stroma
results in destruction of stroma. Therefore, we developed a novel in vitro assay based on further modifications of the
stroma noncontact culture described by our laboratory. In this assay, hematopoietic progenitors and ANK are separated
from stromal layers by a 0.4-μm microporous membrane preventing direct contact with the stromal feeder while
allowing diffusion of stroma-derived factors sufficient for the in vitro induction of progenitor proliferation and differen-
tiation. A second limitation on the coinoculation of ANK cells and primitive hematopoietic progenitors in classical
LTBMC is that the HC present in LTBMC medium and required for optimal growth of LTCIC and CFC suppresses
ANK function. In agreement with previous studies, we demonstrate that HC significantly inhibits ANK killing of
K562 and especially Raji targets. The time course of this interaction is rapid, as an effect is seen as early as 18 hours
after incubation, and becomes more pronounced after 42 hours. This finding differs from data reported by Klingemann
et al that shows no inhibition of cytotoxicity was observed when fresh Percoll-separated marrow cells were cultured for
7 days with simultaneous addition of HC-containing LTBMC medium and IL-2. We demonstrate that sequential
addition of HC to IL-2-activated NK suppresses function. Although IL-2 can partially protect against this loss of func-
tion, HC was still significantly suppressive under the conditions tested here. This discrepancy may be explained by
differing effects of HC on mature blood NK (used here) versus marrow NK or by differing actions of HC when added
to previously cultured, IL-2-activated NK (used here) versus simultaneous addition of IL-2 and HC to resting marrow
NK.

Several studies have addressed the effects of various killer cell populations on autologous hematopoietic colony growth
using various experimental conditions. Results vary from significant suppression to stimulation of different colony
types. These divergent results may be explained by differences in type of effector cell populations studied, such as
NK clones, purified CD16+/CD3- cells, Percoll-enriched NK, HNK1+ NK, and conventional lymphokine-activated killer (LAK) cells. Results may also vary be-
Table 1. Effects of Antibody Blocking on CFC Generation During
48-Hour Coincubation of ANK and CML Progenitors (n = 3)

<table>
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<tr>
<th>Antibody (isotype)</th>
<th>% Suppression of CFC</th>
<th>P*</th>
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<tr>
<td>No antibody</td>
<td>72 ± 16</td>
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<tr>
<td>Anti-IFN-γ (Goat IgG)</td>
<td>66 ± 19</td>
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<tr>
<td>Control goat IgG</td>
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<td>NS</td>
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<td>Anti-TNF-α (rabbit anti-goat IgG)</td>
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* P value compared with no antibody control.

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Autologous activated natural killer cells suppress primitive chronic myelogenous leukemia progenitors in long-term culture

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