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ORIGINAL ARTICLE

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Cellular immune responses in acute leukaemia patients with severe chemotherapy-induced leucopenia; characterization of the cytokine repertoire of clonogenic T cells

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Abstract T lymphocytes are important both for the host defence against infections and probably also as antileukaemic effector cells in patients with acute leukaemia. To investigate the T lymphocyte cytokine repertoire of clonogenic T lymphocytes, CD4+ and CD8+ T lymphocyte clones were prepared from acute leukaemia patients with chemotherapy-induced cytopenia (leucocytes $<0.5\times10^9$/l). A majority of both CD4+ and CD8+ clones secreted detectable interleukin-2 (IL-2), IL-10, IL-13, granulocyte/macrophage-colony-stimulating factor and interferon γ (IFNγ) in response to phytohaemagglutinin + accessory cells (Epstein-Barr-virus-transformed B cell line, 80-Gy-irradiated). The CD4+ clones showed significantly higher levels of IL-10 secretion than the CD8+ clones. Decreased levels of IL-2, IL-13 and IFNγ were observed when acute myelogenous leukaemia (AML) blasts were used instead of cells from the B cell line as accessory cells during phytohaemagglutinin activation, but the differences in IL-13 and IFNγ levels were reversed by addition of exogenous IL-2. On the basis of these results we conclude: (i) the remaining clonogenic T lymphocytes derived from acute leukaemia patients with therapy-induced leucopenia can respond to activation with a broad cytokine response, and T-cell-derived cytokines may then contribute to cytokine responses during complicating infections in these patients; (ii) although T cells can modulate AML blast functions and mediate antileukaemic effects, the leukaemia blasts will also modulate T cell functions and alter the cytokine profile of activated T lymphocytes.

Key words Acute leukaemia · Cytotoxic drugs · Leucopenia · T lymphocytes · Cytokine responses

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Introduction

As more aggressive chemotherapy is employed to increase the rate of long-term survival for patients with malignant diseases, serious infectious complications associated with leucopenia have emerged as a major cause of morbidity and mortality [3, 18, 21, 32]. The intensive chemotherapy for acute leukaemia is followed by a period of severe leucopenia usually lasting for 15–20 days [3, 32], but even during this period increased serum levels of several cytokines can be detected during complicating infections [11, 23]. Thus, a cytokine response can be initiated in leucopenic patients despite the severe impairment of their host defence. Most of these cytokines can be secreted by various immunocompetent cells [14], and it is not known whether T lymphocytes contribute to the responses.

Acute leukaemia patients with chemotherapy-induced leucopenia are susceptible to a wide variety of infections with bacterial infections predominating [3, 18, 27, 32]. Neutropenia is the most important predisposing factor for infections [1, 17, 32], but the severe T lymphopenia represents an additional predisposition especially to infections with fungi, *Pneumocystis carinii* and herpes viruses [15, 20, 26, 27, 29, 32]. In addition to their importance for the host defence against infections, T lymphocytes may also function as antileukaemic effector cells, and cytokines may then be important for these interactions between acute myelogenous leukaemia (AML) blasts and T cells [24]. In this context our present study had two purposes: (i) to characterize the cytokine repertoire of clonogenic T lymphocytes derived from acute leukaemia patients with therapy-induced leucopenia, and (ii) to investigate whether these cytokine responses can be modulated by the presence AML blasts.

Table 1 Clinical data of acute leukaemia patients used as T lymphocyte donors. *AML* acute myelogenous leukaemia, *ALL* acute lymphoblastic leukaemia

Patient	Age (years)	Sex	Diagnosis	Status before chemotherapy
1	32	F	AML-M2	1st complete remission
2	37	F	AML-M5	Untreated AML
3	50	F	AML-M1	Untreated AML
4	70	M	AML-M4	Untreated AML
5	31	F	ALL	1st complete remission
6	45	F	AML-M4	Partial remission
7	28	F	ALL	1st complete remission
8	38	M	AML-M2	2nd complete remission
9	36	F	AML-M2	Untreated AML

Materials and methods

Acute leukaemia patients

AML and acute lymphoblastic leukaemia (ALL) were diagnosed according to generally accepted guidelines [2]. The clinical data of patients used as T lymphocyte donors are presented in Tables 1 and 2. The clinical data of five patients used as AML blast donors are presented in Table 3. Only patient 1 (Table 1) had received previous chemotherapy for a malignant disease (cancer cervicis uteri).

Reagents

The culture medium was RPMI-1640 with glutamine and HEPES (Gibco; UK) to which was added 100 µg/ml gentamicin and 10% inactivated fetal calf serum (HiClone; USA). Interleukin-2 (IL-2; R&D Systems, UK) was used at the concentration of 10 ng/ml and phytohaemagglutinin (PHA HA16; Wellcome, UK) at 1 µg/ml [7, 8, 16].

Cell preparation

Peripheral blood mononuclear cells (PBMC)

PBMC were prepared by density-gradient separation (Lymphoprep, Nycomed, Norway; specific density 1.077 g cm^{-3}) from the peripheral blood of AML patients with high blast counts. All leukaemic PBMC contained at least 95% blasts.

Enriched $CD4^+$ and $CD8^+$ T lymphocytes

CD4- or CD8-specific immunomagnetic beads (Dynabeads; Dynal, Norway) were added to blood samples (citric acid anticoagulant; 1.1×10^7 beads/ml) and attached cells removed by a magnetic field after 30 min incubation at 4 °C under constant rotation. Cells were washed twice before being resuspended in 0.3 ml RPMI medium with 1% fetal calf serum and 15 µl Detachabeads (Dynal, Norway). After incubation for 60 min at 18 °C under constant shaking, the detached beads were removed. As a control of the separation procedures, on day 10 immunomagnetic beads were incubated with ten T cell cultures prepared from $CD4^+$- or $CD8^+$-enriched populations (see below), and the $CD4^+CD8^-/CD4^-CD8^+$ phenotypes were verified by light microscopy.

Preparation of T lymphocyte clones

As described previously [7, 8, 16], $CD4^+/CD8^+$ T cells were seeded at limiting dilution in 60-microwell plates (20 µl medium/well). Each well contained T lymphocytes together with pooled allogeneic 30-Gy-irradiated peripheral blood mononuclear cells (PBMC; 10^4 cells/well) + PHA + IL-2. Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 10 days before restimulation.
All T cell populations studied were derived from microcultures where the number of seeded T cells resulted in fewer than 30% of the cultures containing proliferating cells. According to Poisson's equation, fewer than 5% of these wells contained more than one proliferating cell originally [19]. The cells are therefore referred to as cloned T cells (CTC).

Analysis of cytokine secretion by T lymphocyte clones

Restimulation of cloned T lymphocytes

After 10 days, T lymphocyte cultures that contained proliferating cells, as judged by light microscopy, were transferred to separate microtitre wells. Each clone (1.5×10^4 CTC/well) was restimulated with PHA + IL-2 + accessory cells; 5×10^4/well, 250 µl medium/well). The accessory cells were either (i) 80-Gy-irradiated cells from the Epstein-Barr-virus-transformed B cell line (BCL) AL-10 [8, 16] or (ii) 50-Gy-irradiated leukaemic PBMC (Table 3). Culture supernatants were harvested after 48 hours and stored frozen until analysed.

To minimize the problem of clonal selection during in vitro culture, the CTC were tested after 10 days of expansion. Each T cell clone

Table 2 Chemotherapy, duration of cytopenia and number of clones derived from acute leukaemia patients. The overall numbers of clones included in the study, are shown. The duration of cytopenia is presented as the duration of chemotherapy-induced cytopenia before investigation (*first number*) and the overall duration of cytopenia (*second number*). RD resistent disease

Patient	Chemotherapy	Duration of cytopenia (days)	Number of T lymphocyte clones	
			$CD4^+$ clones	$CD8^+$ clones
1	Cytarabine 3 g/m^2 × 2; d 1, 3, 5	2/14	13	11
2	Cytarabine 200 mg/m^2 × 2; d 1–7 Daunorubicin 50 mg/m^2; d 1–3	21/RD	15	17
3	Cytarabine 200 mg/m^2 × 2; d 1–7 Daunorubicin 50 mg/m^2; d 1–3	6/8	21	24
4	Cytarabine 200 mg/m^2 × 2; d 1–7 Daunorubicin 50 mg/m^2; d 1–3	3/20	8	21
5	ALL induction therapy[a]	11/RD	22	21
6	Cytarabine 3 g/m^2 × 2; d 1, 3, 5	18/30	17	6
7	ALL induction therapy[a]	13/20	2	29
8	Cytarabine 3 g/m^2 × 2; d 1, 3, 5	10/17	2	4
9	Cytarabine 200 mg/m^2 × 2; d 1–7 Daunorubicin 50 mg/m^2; d 1–3	18/23	13	6

[a] Acute lymphoblastic leukaemia (ALL) induction therapy lasting 5 weeks: prednisone 40 mg/m^2 daily for 4 weeks and reduced doses during the last week; 2 mg vincristine on days 1, 8, 15, 22 and 29; 10000 U/m^2 asparaginase on days 8–21; 30 mg/m^2 doxorubicin on days 8, 15, 22; 750 mg/m^2 cyclophosphamide on days 15, 22, 29; 15 mg intrathecal methotrexate on days 15, 29, 36

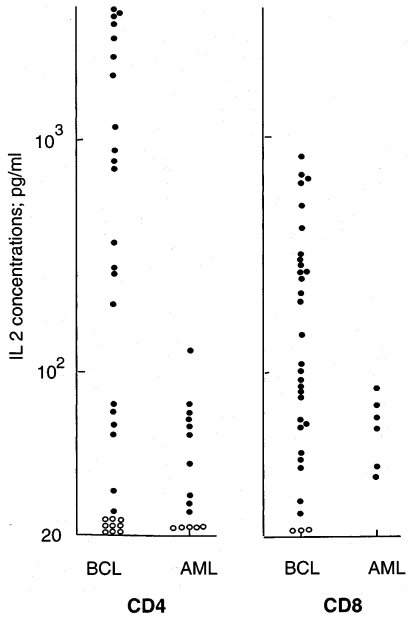

Fig. 1 Interleukin-2 (*IL-2*) levels in culture supernatants for CD4+ (*left*) and CD8+ (*right*) T lymphocyte clones derived from acute leukaemia patients with chemotherapy-induced cytopenia. Clones were activated with accessory cells + phytohaemagglutinin (PHA), and the results are presented as the IL-2 concentration (pg/ml) for each clone. As accessory cells, either AL-10 B cells (*BCL*) or leukaemic peripheral blood mononuclear cells (*PBMC*) (*AML*) were used. Control cultures with PHA + BCL did not contain detectable IL-2 (less than 30 pg/ml). ○ Non-detectable cytokine levels

could therefore be tested in combination with only one type of accessory cell (either AL10-BCL or leukaemic PBMC derived from one patient and either with PHA alone or PHA + IL-2 as the activation signal). For each individual clone the accessory cells and the activation signal were randomly selected.

Analysis of cytokine concentrations

Enzyme-linked immunosorbent assay (ELISA) methods were used to determine cytokine concentrations (Quantikine ELISA kits; R&D Systems Europe, UK). All assays were performed strictly according to the manufacturer's instructions. Standard curves were determined using the mean of duplicate determinations, differences between duplicates being less than $<10\%$ of the mean. Supernatants were analysed at dilutions resulting in measured concentrations within the range of the standard curve. All supernatants were diluted 1:5 to allow the analysis of several cytokines for each individual clone. Minimal detectable cytokine levels were then 30 pg/ml IL-2, 0.45 pg/ml IL-4, 7.5 pg/ml IL-10, 160 pg/ml IL13, 15 pg/ml interferon γ (IFNγ), 7.5 pg/ml granulocyte/macrophage-colony-stimulating factor (GM-CSF).

Control cultures containing unstimulated T cells showed no detectable cytokine secretion.

Statistical analysis

For statistical comparisons Wilcoxon's rank-sum test was used. The χ^2 test was used for categorized data, and Kendall's test for correlation analysis. Differences were regarded as statistically significant when P was less than 0.05.

Results

Secretion of IL-2 by clonogenic T lymphocytes derived during chemotherapy-induced lymphopenia

IL-2 secretion in response to PHA+BCL was investigated for 30 CD4+ and 32 CD8+ T lymphocyte clones (derived from patients 1–7, 9). IL-2 was detected for a majority of both CD4+ and CD8+ clones without any significant difference between the two subsets (Fig. 1).

IL-2 concentrations were also determined for 15 CD4+ and 6 CD8+ T cell clones activated with PHA + leukaemic PBMC accessory cells (Table 3). A majority of both CD4+ and CD8+ clones secreted detectable IL-2 in response to this activation signal (Fig. 1). For CD4+ clones IL-2 levels were significantly lower in the presence of leukaemic PBMC than with BCL accessory cells ($P = 0.01$). Although decreased IL-2 levels were also detected for the 6 CD8+ T cell clones activated with PHA + leukaemic PBMC, this difference was not statistically significant.

IL-2 could not be detected in control cultures containing PHA+accessory cells or PHA + IL-2+accessory cells cultured together with necrotic cells from non-proliferating cultures.

Secretion of IL-10 by clonogenic T lymphocytes derived during chemotherapy-induced cytopenia

IL-10 levels were determined for the 30 CD4+ and 32 CD8+ CTC stimulated with PHA+BCL, as described for IL-2. IL-10 was detected both for CD4+ (19/30) and CD8+ (15/32) clones after activation with PHA + BCL (Fig. 2), but with significantly higher IL-10 levels for CD4+ than for CD8+

Table 3 Clinical and biological characteristics of AML patients used as accessory cell donors. Membrane molecule expression was analysed by flow-cytometric analysis and judged to be positive when expressed by more than 20% of the blasts. The percentage of blasts was determined by light microscopy of May-Grünewald-Giemsa-stained preparations. *PBMC* peripheral blood mononuclear cells

Patient	Age (years)	Sex	FAB class	AML blast phenotype							AML blast cytogenetics	Blasts among PBMC (%)
				CD2	CD13	CD14	CD15	CD19	CD33	CD34		
I	64	M	M1	–	+	–	–	–	+	–	Normal	98
II	38	F	M4	–	+	+	+	–	+	+	Normal	97
III	55	F	M4	–	+	–	–	–	+	+	t(3;3) (q21;q26) [12]	96
IV	83	F	M5	–	+	+	+	–	+	–	Normal	95
V	48	M	M4	–	+	–	+	–	+	–	Normal	95

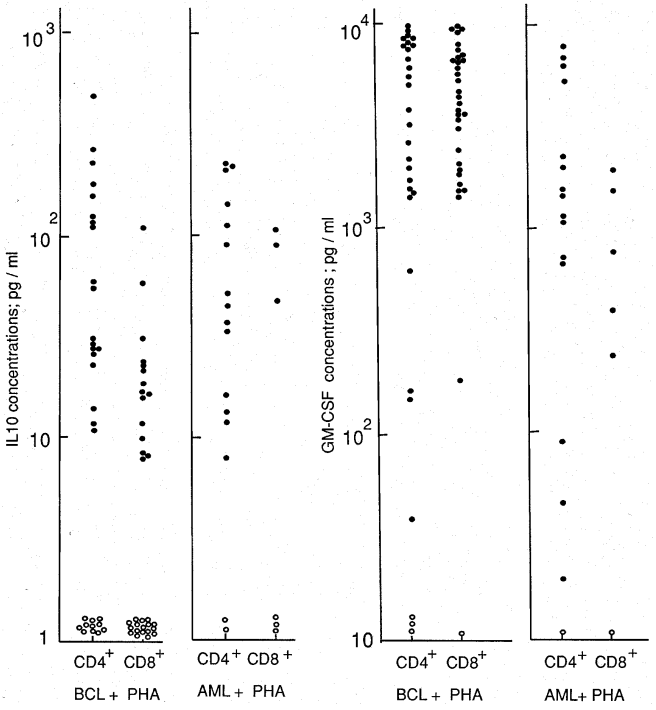

Fig. 2 IL-10 (*left*) and granulocyte/macrophage-colony-stimulating factor (GM-CSF, *right*) concentrations in culture supernatants for CD4+ and CD8+ T lymphocyte clones derived during chemotherapy-induced cytopenia in patients with acute leukaemia. Each clone was activated with accessory cells+PHA and the results are presented as the cytokine concentration (pg/ml) for each clone tested. As accessory cells, AL-10 BCL (BCL + PHA) or leukaemic PBMC (AML + PHA) were used. Control cultures with PHA + BCL + non-proliferating cells did not contain detectable IL-10 and GM-CSF (<30 pg/ml). ○ Non-detectable cytokine levels are indicated in the figure

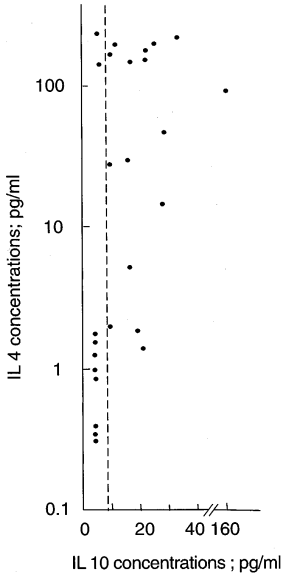

Fig. 3 Concentrations of IL-4 and IL-10 in culture supernatants. A total of 26 T cell clones (10 CD4+ and 16 CD8+ clones) derived during leukopenia were activated with PHA + BCL, and cytokine concentrations determined in the supernatants. – – – The lowest detectable IL-10 level. All T cell clones secreted detectable IL-4

clones (Fig. 2; Wilcoxon's test, $P = 0.035$). IL-4 was detected for 26 of these clones (10 CD4+ and 16 CD8+ clones derived from patients A–D), and a significant correlation between IL-10 and IL-4 levels was observed (Fig. 3, Kendall's correlation test, $P = 0.0031$). IL-10 levels showed no correlation to IL-2 levels.

IL-10 secretion was also detected for a majority of the CD4+ (14/16) and CD8+ (3/6) clones (derived from patients 1–9) activated with PHA + leukaemic PBMC, and the IL-10 levels did not differ significantly from the levels reached after activation with PHA + BCL.

IL-10 was not detected for control cultures containing nonproliferating cells.

Secretion of GM-CSF by clonogenic T lymphocytes derived during chemotherapy-induced cytopenia

GM-CSF levels in response to the activation signal PHA+BCL were determined for 29 CD4+ and 32 CD8+ clones as described for IL-2. High GM-CSF levels were detected with no significant difference between CD4+ and CD8+ CTC (Fig. 2). GM-CSF was also detected for a majority of the CD4+ (15/16) and CD8+ (5/6) clones activated with PHA + leukaemic PBMC, and these GM-CSF levels did not differ significantly from the levels detected after activation with PHA + BCL (Fig. 2). GM-CSF was not detected in control cultures containing non-proliferating cells.

Secretion of IL-13 by clonogenic CD4+ and CD8+ T lymphocytes derived during cytopenia

IL-13 secretion in response to PHA+BCL was analysed for 28 CD4+ and 32 CD8+ clones. A majority of CD4+ and CD8+ CTC secreted IL-13. These results were then compared with IL-13 levels reached for 16 CD4+ and 6 CD8+ clones activated with PHA + leukaemic PBMC (Fig. 4). For CD4+ CTC, a large proportion of non-secreting CTC were detected when both activation signals were tested. When the IL-13-secreting subsets were compared, significantly lower IL-13 levels were detected in response to PHA + leukaemic PBMC than with PHA + BCL (Wilcoxon's test, $P = 0.026$). For the CD8+ subset the fraction of non-secreting CTC was significantly higher for CTC activated with PHA + leukaemic PBMC than for clones activated with PHA + BCL (χ^2, $P < 0.002$), but because of the low number of available CD8+ CTC these data must be interpreted with caution.

IL-13 secretion was also investigated for 48 CD4+ clones and 55 CD8+ clones (derived from patients 1–9) activated with PHA+IL-2+BCL. All these CD4+ and CD8+ clones released detectable IL-13 in response to PHA + IL-2 + BCL (Fig. 4), but IL-13 levels were significantly higher for CD4+ than for CD8+ clones (Wilcoxon's test, $P = 0.0041$). The IL-13 levels for clones stimulated with

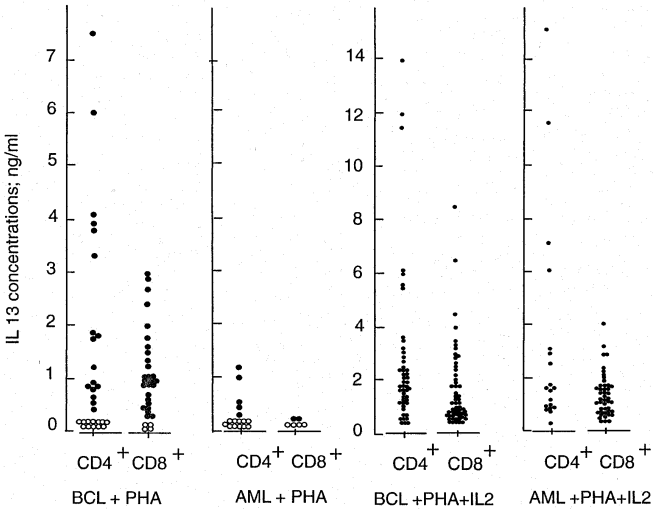

Fig. 4 IL-13 concentrations in culture supernatants for CD4+ and CD8+ T lymphocyte clones derived from acute leukaemia patients with chemotherapy-induced cytopenia. The results are presented as the cytokine concentration (ng/ml) for each clone tested. IL-13 secretion was examined after activation with AL10-BCL + 1 μg/ml PHA (*BCL + PHA*), leukaemic PBMC + PHA (*AML + PHA*), AL10-BCL + PHA + 10 ng/ml IL-2 (*BCL + PHA + IL-2*) or leukaemic PBMC + PHA + IL-2 (*AML + PHA + IL-2*). ○ Non-detectable cytokine levels

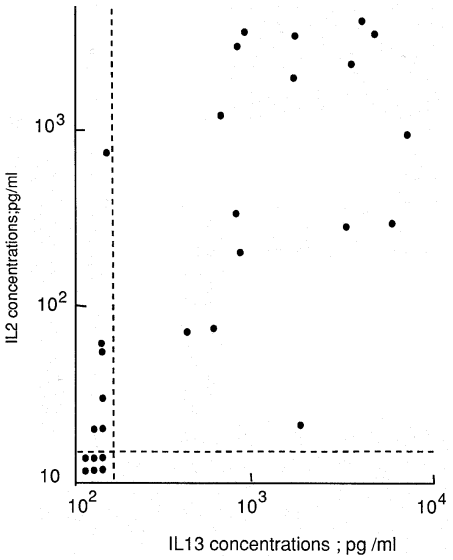

Fig. 5 Concentrations of IL-2 and IL-13 in culture supernatants. A total of 28 CD4+ T cell clones derived during leukopenia were activated with PHA + BCL, and cytokine concentrations determined in the supernatants. – – – The lowest detectable cytokine levels

PHA + IL-2 + BCL were then compared with the results for 19 CD4+ and 46 CD8+ clones activated with PHA + IL-2 + leukaemic PBMC derived from five AML patients (Table 3). IL-13 levels did not differ between CTC activated with BCL and those treated with leukaemic PBMC accessory cells (Fig. 4). Fewer CTC were available for analysis of IFNγ levels (see below), and similar differences in IL-13 levels were also detected when the statistical analysis was limited to the CTC included in both the IL-13 and IFNγ studies.

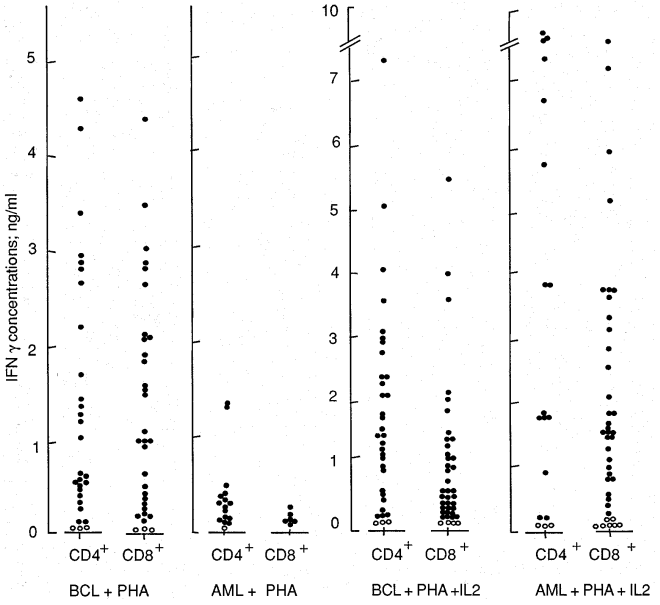

Fig. 6 Interferon γ (IFNγ) concentrations in culture supernatants for CD4+ and CD8+ T lymphocyte clones derived from acute leukaemia patients with chemotherapy-induced cytopenia. The results are presented as the cytokine concentration (ng/ml) for each clone tested. IFNγ secretion was examined after activation with AL10-BCL + 1 μg/ml PHA (*BCL + PHA*), leukaemic PBMC + PHA (*AML + PHA*), AL10-BCL + PHA+10 ng/ml IL-2 (*BCL + PHA + IL-2*) or leukaemic PBMC + PHA + IL-2 (*AML + PHA+IL-2*). ○ Non-detectable cytokine levels

Secretion of IL-2 and IL-13 in response to PHA + BCL was compared for the CD4+ CTC, and the levels of these two cytokines showed a significant correlation (Fig. 5; $n = 28$, Kendall's test, $P = 0.0004$). For the CD8+ subset ($n = 30$) no such correlation was detected.

IL-4 (see Fig. 3) and IL-13 levels were compared for 26 T cell clones activated with PHA+BCL, and IL-13 levels showed no correlation to the IL-4 levels. IL-4 levels were also determined for 30 CD4+ (median IL-4 levels 20.5 pg/ml; range 0.7–3100 pg/ml) and 30 CD8+ (median 1.8 pg/ml, range less than 0.15–240 ng/ml) T cell clones activated with PHA + IL-2 + BCL. High IL-13 levels were detected for CD4+ and CD8+ clones independent of their ability to secrete IL-4 (data not shown).

When IL-13 and IL-10 levels were compared for CD4+ ($n = 29$) and CD8+ ($n = 32$) clones activated with PHA + BCL, high IL-13 concentrations were detected independently of the clonal ability to secrete IL-10 (data not shown).

Secretion of IFNγ by CD4+ and CD8+ T cell clones derived during chemotherapy-induced leucopenia

IFNγ secretion in response to PHA+BCL was examined for 29 CD4+ and 32 CD8+ T cell clones. A majority of the clones secreted IFNγ in response to this activation signal (Fig. 6). High IFNγ levels were detected both for CD4+ and CD8+ clones, and IFNγ levels showed a significant corre-

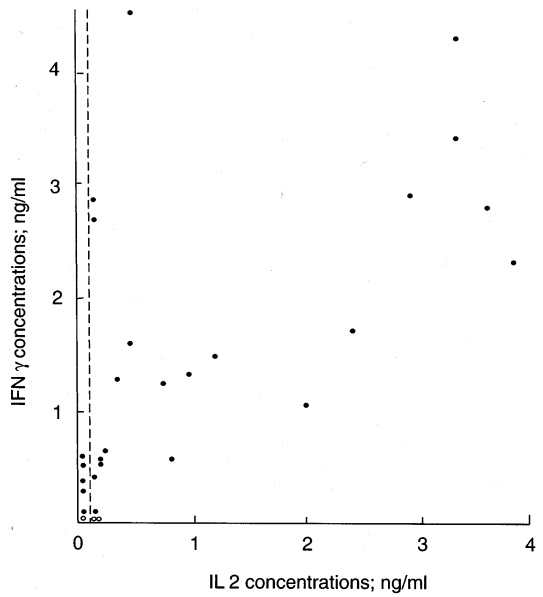

Fig. 7 Concentrations of IL-2 (ng/ml) and IFNγ (ng/ml) in culture supernatants for 29 CD4+ CTC activated with BCL + PHA. – – – The lowest detectable IL-2 concentration, ○ non-detectable IFNγ levels

lation to IL-2 levels both for the CD4+ (Fig. 7; $n = 29$, $P = 0.003$) and the CD8+ subset ($n = 30$, $P = 0.019$, data not shown). IFNγ secretion in response to PHA + leukaemic PBMC (16 CD4+ and 6 CD8+ clones tested) was significantly decreased both for the CD4+ (Fig. 6; Willcoxon's test, $P = 0.003$) and CD8+ ($P = 0.005$) subset compared with the levels detected with PHA+BCL.

The IFNγ levels detected after activation with PHA + IL-2 + BCL (34 CD4+ and 40 CD8+ clones tested) did not differ significantly from the levels observed after activation with PHA+BCL (Fig. 6). IFNγ levels were also compared for clones activated with PHA + IL-2 + leukaemic PBMC (18 CD4+ and 39 CD8+ clones tested) and PHA + IL-2 + BCL, and no significant differences in IFNγ levels could be detected. For the CD8+ subset increased IFNγ levels were even detected for PHA + IL-2 + leukaemic PBMC compared with PHA + IL-2 + BCL (Fig. 6, $P = 0.013$).

Analysis of the cytokine profile for clones with undetectable PHA-stimulated cytokine secretion

Among the 84 PHA-stimulated clones, 54 had undetectable levels of at least one cytokine (IL-2, IL-10, IL-13, GM-CSF, IFNγ). However, all clones showed detectable levels of at least one cytokine, and 75 clones showed detectable levels of at least three cytokines.

The commonest cytokine defect was an inability to release detectable amounts of IL-10 (33/54), and this was observed as the only defect in the cytokine profile for 21 of these T cell clones. Undetectable IL-13 levels were also common (31/54); for 10 T cell clones undetectable IL-13 levels were the single defect and for 14 T cell clones undetectable IL-13 levels were observed in combination with undetectable IL-2 and/or IFNγ. The combination of undetectable IL-10 and IL-13 levels was only observed for 6 clones. The remaining minority of these CTC showed various defects in the cytokine profile (data not shown). Thus, for a majority of these CTC, undetectable cytokine levels were observed as a defect in either Th1- or Th2-associated cytokines [22, 25].

Discussion

T lymphocytes seem to be important, both as a part of the host defence against infections and as antileukemic effector cells in AML patients. Bacterial infections will induce a broad cytokine response even in acute leukaemia patients with chemotherapy-induced leucopenia [11], and our present results indicate that T cells contribute to this cytokine response. The function of T lymphocytes as antileukaemic effector cells is less well characterized. However, our results demonstrate that T cell cytokine release can take place in the presence of native AML blasts, although the leukaemia cells seem to have an immunomodulatory function.

We used the technique of T lymphocyte cloning to obtain sufficient cells for functional studies. During the long-term culture several T cell clones will stop growing before a cell number sufficient for functional studies is reached [16]. To minimize this problem of clonal selection, all CTC were tested during the first in vitro restimulation. However, after this short period of expansion the cell number for each clone was sufficient for testing in combination with only one type of accessory cell. Neither the BCL AL10 [8] nor any of the AML blast accessory cells secreted any of the cytokines examined and, for both types of accessory cells, a wide variation in cytokine levels was detected when individual clones were compared. Similar wide variation has also been described both for normal CTC and for CTC derived after allogeneic bone marrow transplantation [8, 31].

The majority of CD4+ and CD8+ clones secreted IL-2 in response to both PHA + BCL and PHA + leukaemic PBMC accessory cells. All CTC were derived from cultures with excess IL-2 and should be regarded as being IL-2-responsive [7]. Thus, most clones are capable of autocrine IL-2-dependent proliferation in the presence of both types of accessory cell.

High GM-CSF levels were detected for the majority of CD4+ and CD8+ CTC. GM-CSF can be a growth factor for AML blasts [4–6, 9, 10], and our results thus indicate that a T cell cytokine response may enhance blast proliferation. However, other T-cell-derived cytokines may inhibit (e.g. IL-4, IL-10, IL-13, IFNγ) AML blast functions [5, 9, 10, 28]. The final effect of a T cell cytokine response will thus depend on both (i) the balance between enhancing and inhibitory T cell cytokines and (ii) differences in cytokine responsiveness between blasts derived from various patients [5].

IL-4, IL-10 and IL-13 have common anti-inflammatory effects and inhibit constitutive cytokine secretion by human AML blasts [5, 9, 10, 28]. Most CD4+ and CD8+ CTC derived during cytopenia secreted all three cytokines, and these cytokines may therefore have additive effects during cellular immune responses.

Two different IL-4 receptors have been detected, and IL-13 can bind to one of these [30, 33]. The generally higher levels of IL-13 than of IL-4 (Figs. 3, 4) indicate that IL-13 is most important for the cytokine effects mediated via this common receptor, whereas additional IL-4 effects may be mediated via the other IL-4 receptor.

On the basis of their cytokine profile, T helper cells are sometimes divided into Th1 and Th2 subsets. The Th1 subset is characterized by IL-2 and IFNγ secretion, whereas the Th2 cells secrete IL-4, IL-10 and IL-13 [22, 25]. The majority of our clones secreted both Th1- and Th2-associated cytokines. However, the levels of the Th2 cytokines IL-10 and IL-4 were significantly correlated, and for the CD4+ subset the levels of the Th1 cytokines IL-2 and IFNγ were correlated. IL-13 is usually regarded as a Th2-associated cytokine but, for our CD4+ clones, IL-13 levels were correlated with the levels of IL-2 and not with Th2-associated cytokines.

A dominance of either Th1- or Th2-associated cytokine secretion was detected when CTC with undetectable cytokine levels were analysed, and the commonest phenotypes for these CTC were either a single negativity for IL-10 as the only defect or negativity for IL-13 alone or in combination with IL-2/IFNγ.

Although no qualitative differences in the cytokine profile were detected between CD4+ and CD8+ CTC, quantitative differences were observed with decreased levels of IL-10 (PHA activation) and IL-13 (PHA + IL-2 activation) for CD8+ clones. We have previously described decreased release of the Th2-associated cytokine IL-4 from CD8+ clones derived after chemotherapy [12].

IL-2/IL-13/IFNγ levels were higher after activation of CD4+ and CD8+ CTC with PHA + BCL than after stimulation with PHA+leukaemic PBMC, whereas no difference was detected for GM-CSF and IL-10. Addition of exogenous IL-2 increased IL-13 and IFNγ levels, and no difference was then detected between normal and leukaemic accessory cells. For the Th2 cytokine IL-13, this was observed both when investigating the same CTC as for IFNγ and when including additional CTC in the statistical analysis. Thus, the use of leukaemic PBMC accessory cells caused a modulation of the clonal cytokine profile. This modulation of IL-13 and IFNγ release seems to be secondary to decreased IL-2 secretion, because the difference was not detected in the presence of exogenous IL-2.

Previous studies have demonstrated that leukaemic accessory cells can modulate T cell responses and induce T cell anergy [13]. However, our present results demonstrate that AML blasts could only induce a quantitative difference and not a qualitative difference like T cell anergy.

To conclude, clonogenic CD4+ and CD8+ T lymphocytes, derived from acute leukaemia patients with chemotherapy-induced cytopenia, show a broad cytokine response after mitogenic activation. However, the T cell cytokine profile can be modulated by the presence of AML blasts during T cell activation, and this modulation may become important during T cell targeting immunotherapy in AML patients.

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