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ORIGINAL ARTICLE

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Interleukin-13 secretion by normal and posttransplant T lymphocytes; in vitro studies of cellular immune responses in the presence of acute leukaemia blast cells

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Abstract T lymphocyte secretion of interleukin-13 (IL-13) in response to different activation signals was characterized in vitro. IL-13 release was investigated when virus transformed B lymphocytes or acute myelogenous leukaemia (AML) blasts were used as accessory cells during T cell activation. First, a majority of both CD4+ and CD8+ TCRαβ+ T lymphocyte clones, derived from normal individuals and bone marrow transplant recipients, secreted IL-13 in response to a standardized mitogenic activation signal (phytohaemagglutinin+IL-2+ B lymphocyte accessory cells). The CD4+ cells showed significantly higher IL-13 levels than the CD8+ subsets. Second, when leukaemic accessory cells (more than 95% AML blasts) were used during T cell activation, IL-13 was released both during alloactivation of normal T lymphocytes and during mitogen activation of posttransplant T cells. Third, when normal T lymphocytes were stimulated with allogeneic AML blasts, addition of IL-13-neutralizing monoclonal antibodies decreased interferon γ levels. Although addition of IL-13-neutralizing antibodies did not alter granulocyte-colony-stimulating factor secretion by allostimulating AML blasts, altered blast proliferation was detected for certain patients. Thus, most T cell clones can release IL-13, and IL-13 can modulate cytokine responses during T cell recognition of allogeneic AML cells.

Key words T lymphocytes · Acute myelogenous leukaemia · Interleukin-13

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Introduction

Following allogeneic bone marrow transplantation the graft T lymphocytes mediate antileukaemic effects by two major mechanisms: (i) recognition of alloantigens, which may also become clinically apparent as graft versus host disease; and (ii) leukaemia-specific recognition, termed the graft versus leukaemia effect [12, 13, 21, 23]. This antileukaemic reactivity is the basis for treatment of leukaemia relapse after bone marrow transplantation with donor leucocyte transfusions [12]. However, recent studies indicate that even the normal T cell repertoire may encompass leukaemia-specific cells recognising hybrid peptides encoded in the regions of chromosomal translocations [1, 11, 24, 25].

Cytotoxic reactivity against the malignant cells is probably an important effector mechanism for antileukaemic T lymphocytes [21]. However, both cytotoxic and non-cytotoxic T lymphocytes can be stimulated to secrete various cytokines, which can act as either stimulatory or inhibitory signals for leukaemia cells [4–6, 20]. This cytokine response may then be an additional mechanism for interactions between leukaemia cells and T lymphocytes.

Interleukin-13 (IL-13) is a T cell cytokine that can modulate both proliferation and constitutive cytokine secretion by acute myelogenous leukaemia (AML) blasts [2]. The possible function of IL-13 as an antileukaemic T cell effector mechanism was investigated by three different approaches. First, a panel of monoclonal T cell populations (T cell clones) was prepared under defined in vitro conditions, and the ability of individual clones to secrete IL-13 in response to a standardized activation signal was then examined. In the second part of our study we investigated whether IL-13 could be released by T lymphocytes activated in the presence of accessory cells including a major population of AML blasts. In the third part of our study we used IL-13-neutralizing antibodies to characterize the functional effects of IL-13 released by normal T lymphocytes stimulated with allogeneic AML blasts.

Materials and methods

Cell donors

The clinical data of the acute leukaemia patients are presented in Table 1, and the HLA types of patients and healthy controls are included in Table 2.

Reagents

The culture medium was RPMI-1640 with HEPES and glutamine (Gibco, UK) to which was added 10% inactivated fetal calf serum (HiClone, USA) and gentamicin 100 μg/ml. Phytohaemagglutinin (PHA HA16; Wellcome, UK) was used at the concentration 1 μg/ml and IL-2 (R&D Systems Europe, UK) at 10 ng/ml. For IL-13 neutralization we used the mouse IgG1-anti-IL-13 monoclonal antibody mab213 at the concentration 10 μg/ml (R&D Systems Europe; the antibody concentration 1.0–3.0 μg/ml will neutralize 50% of the biological effect of 10 ng/ml IL-13). Control cultures were prepared using an isotypic control antibody.

Cell preparation

Peripheral blood mononuclear cells (PBMC) were isolated by density-gradient separation (Ficoll-Hypaque, NyCoMed, Norway; specific density 1.077 g cm^{-3}) and stored in liquid nitrogen. Normal PBMC were derived from healthy blood donors. Leukaemic PBMC were prepared from selected patients with high white blood cell counts (above 30×10^9/l; above 85% leukaemia blasts) and contained more than 95% blasts (Table 1). When used in proliferation assays the leukaemic PBMC were γ-irradiated (50 Gy) and are referred to as irradiated leukaemia cells. When leukaemic PBMC were used directly or after depletion of $CD2^+$ and $CD19^+$ cells (see below), they are referred to as non irradiated (native) leukaemia cells.

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

Immunomagnetic beads coated with CD4- or CD8-specific monoclonal antibodies (Dynabeads; Dynal, Norway; final concentration 0.5×10^7 beads/ml) were incubated with PBMC (3 beads/cell) for 30 min at 4 °C under constant rotation. The beads with attached cells were removed using a magnetic field. After incubation with 15 μl Detachabeads (Dynal, Norway) at 18 °C for 1 h under constant shaking, the beads were detached and removed. Light microscopy of isolated cells after incubation with CD4- or CD8-specific beads confirmed the purity of the CD4/CDB cell populations.

$CD2^+$/$CD19^+$-depleted cells

Depletion was performed using CD2/CD19-specific immunomagnetic beads (Dynal, Norway; 3 beads/cell, 1.1×10^7 beads/ml) in a similar procedure to that described for CD4/CD8 enrichment.

Cloned T cells

As described in detail previously [3–6], PBMC derived 4–6 weeks after allogeneic bone marrow transplantation were cultured with IL-2+40-Gy-irradiated host leukaemia cells and, after 7 day, PHA+IL-2+30-Gy-irradiated allogeneic pooled PBMC were added. T cell cloning was performed 7 days later by seeding cells at limiting dilution together with PHA+IL-2+pooled PBMC. Cells were later restimulated weekly by adding PHA+IL-2+pooled PBMC, and IL-2 was also added 3 days after restimulation. Cloned T cells were stored in liquid nitrogen until tested; all those tested were of the phenotype $CD2^+CD3^+TCR\alpha\beta^+\gamma\delta^-$.

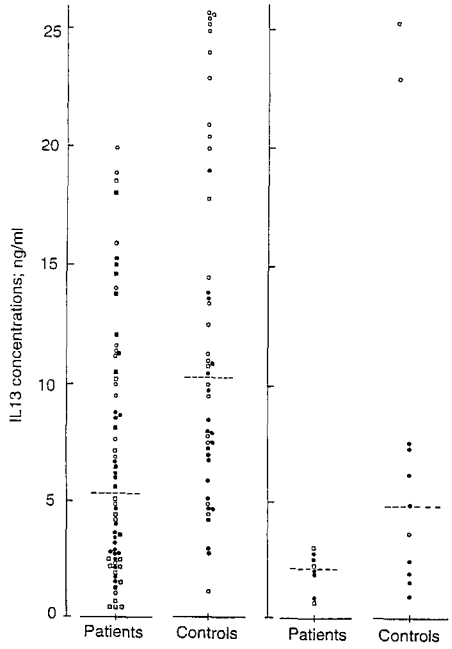

Fig. 1 Interleukin 13 (IL-13) concentrations in culture supernatants derived from $CD4^+TCR\alpha\beta^+$ cloned T cells (*left*) and $CD8^+TCR\alpha\beta^+$ cloned T cells (*right*) activated with phytohaemogglutinin (PHA)+IL-2+AL10 accessory B cells. Posttransplant cloned T cells were derived from four bone marrow transplant recipients (patients UPN-204 □; UPN210 ■; UPN 218 ●, UPN 223 ○) and control cloned T cells from two bone marrow donors (donors for UPN 218 ● and UPN 223 ○). Mean IL-13 concentrations are indicated in the figure (---). IL-13 was not detected for cultures containing PHA+IL-2+B cells (<36 pg/ml)

Virus-transformed B cells

The function of the Epstein-Barr-virus-transformed B cell line AL10 as accessory cells during T cell activation has been characterized in detail previously [4–6]. The B cells were irradiated with 80 Gy before used in the experiments, and the cells then no longer produced detectable amounts of the cytokines IL-3, IL-4, IL-6, IL-7, IL-13, granulocyte/macrophage-colony-stimulating factor (GM-GSF), tumour necrosis factor α and leukaemia-inhibitory factor [4–6].

Analysis of cytokine secretion

PHA-stimulated cytokine secretion by T cell clones

Cloned T cells at 2.5×10^5/ml were cultured with 2.5×10^5/ml accessory cells in medium containing PHA+IL-2. In these experiments, the accessory cells were either 50-Gy-irradiated leukaemic PBMC or 80-Gy-irradiated cells from the B cell line AL10. Supernatants were collected after 48 h and levels of IL-13 and IL-4 determined by enzyme-linked immunosorbent assay (ELISA).

Analysis of cytokine release during allorecognition of leukaemic PBMC

Non-irradiated leukaemic PBMC stimulator cells (5×10^5/ml) were cultured with normal allogeneic responder cells (2.5×10^5/ml). Supernatants were collected after 6 days and cytokine levels then determined by ELISA. Because the stimulator cells contain only a minor contamination of normal cells, interferon γ (IFNγ) is mainly derived from the responder cells [7, 14] . This was further confirmed by the investigation of control cultures only containing leukaemic PBMC+PHA, and IFNγ levels were then minimal or undetectable (see also [7]). When enriched T cell populations are used as responder cells in the allostimulated cultures, granulocyte-colony-stimulating factor (G-CSF) will

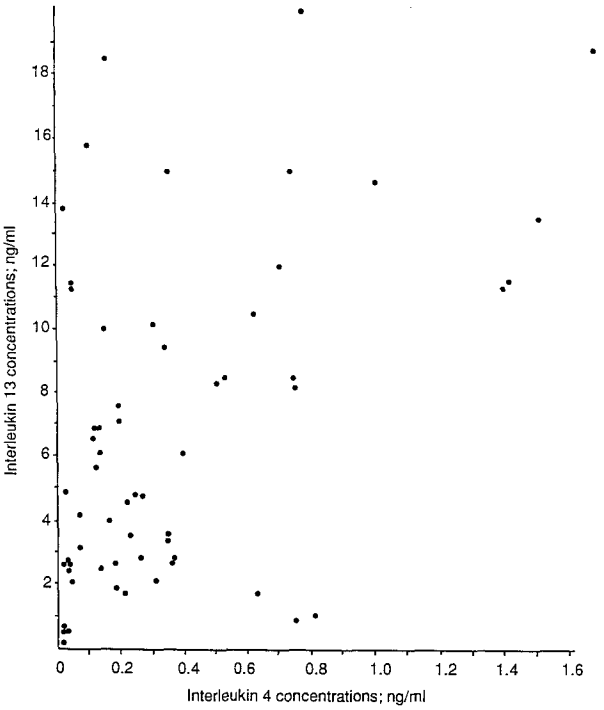

Fig. 2 The correlation between IL-4 and IL-13 concentrations in culture supernatants. Cytokine concentrations were determined in the supernatants 48 h after clonal activation with PHA+IL-2+AL10 B cells. The figure shows cytokine levels for 58 posttransplant CD4+ T cell clones

mainly be derived from the leukaemia blasts among the stimulator cells [7, 8].

ELISA analysis

Supernatants were stored frozen before cytokine concentrations were analyzed. ELISA (Quantikine ELISA kits, R&D Systems Europe) was used to determine concentrations of IL-4, IL-13, IFNγ and G-CSF. All assays were performed strictly according to the manufacturers' instructions. Briefly, standards and sample dilutions were prepared in medium. Standard curves were determined using the mean of duplicate determinations, the variation between duplicates being less than 10% of the mean. Samples were analyzed at a dilution resulting in a measured concentration within the range of the standard curve. The minimal detectable concentrations were 4.1 pg/ml IL-4, 36 pg/ml IL-13, 3.0 pg/ml IFNγ and 7.2 pg/ml G-CSF.

Proliferation assays

AML blast proliferation

Non-irradiated leukaemic PBMC (2.5×10^5/ml) were cultured together with 2.5×10^5/ml normal PBMC responder cells. [^{3}H]thymidine (Amersham, UK; TRA 310, final concentration 185 kBq/ml) was added on day 6 and, 18 h later, depletion of adherent cells, CD2+ and CD19+ cells was performed. The remaining non-adherent CD2-CD19- cells consisted mainly of AML blasts, and the nuclear radioactivity was then determined for this population [7].

Autocrine clonal proliferation

Cloned T cells (2×10^4/well) were cultured with 5×10^4 AL10 accessory cells and 1 μg/ml PHA in 200 μl medium, and [^{3}H]thymidine incorporation was then assayed on day 3. Cloned T cells showing a proliferative response exceeding 1000 cpm were regarded as showing autocrine proliferation [4].

Presentation of the data

Proliferation assays were performed in triplicates and median responses used for calculations. Proliferation was regarded as significant when exceeding the negative control by (i) at least 1000 cpm and (ii) at least three standard deviations (SD). The Willcoxon test for paired samples was used for statistical comparisons, and the Kendall test was used for correlation analysis. Differences were regarded as statistically significant when P was less than 0.05 after correction for the number of comparisons.

Results

PHA-stimulated IL-13 secretion by posttransplant CD4+TCRαβ+ cloned T cells when B lymphocyte accessory cells were used

IL-13 concentrations in response to PHA+AL10 virus-transformed B cells were determined for 16 T cell clones exhibiting autocrine proliferation and 10 clones not capable of autocrine proliferation (for a definition of autocrine proliferation, see Materials and methods) IL-13 levels were significantly higher for T cell clones exhibiting autocrine proliferation (median IL-13 concentration 2.7 ng/ml, range 0.25–7.0 ng/ml) than for T cell clones not capable of autocrine proliferation (median 0.8 ng/ml, range 0.15–4.5 ng/ml; Willcoxon's test, P=0.01). Thus, IL-13 release is highest when the activation signal results in clonal proliferation. Because all our clones could undergo IL-2-dependent proliferation [3], we investigated IL-13 release in response to the activation signal PHA+IL-2+accessory cells in the following experiments.

PHA-IL-2-stimulated IL-13 secretion by normal and posttransplant CD4+ TCRαβ+ cloned T cells when B lymphocyte accessory cells were used

To investigate IL-13 secretion independently of the capacity for autocrine proliferation, IL-13 levels were determined after cloned T cell activation with PHA+IL-2+AL10 B cells [3–6] . IL-13 concentrations were determined for 62 posttransplant T cells clones derived from four leukaemia patients and 44 normal T cell clones derived from two of the bone marrow donors. IL-13 was detected in the supernatants for all T cell clones (Fig. 1). Despite a wide variation in IL-13 concentrations, IL-13 levels were significantly higher for the normal than for the posttransplant cloned T cells (Willcoxon's test, P=0.0051). IL-4 and IFNγ levels were also determined for 58 posttransplant and 32 control clones. IL-13 and IL-4 levels were significantly correlated both for posttransplant (Fig. 2, Kendall's test, P=0.003) and control clones (P<0.002; data not shown). No correlation was detected between IL-13 and IFNγ levels (data not shown).

Table 1 Clinical and biological characteristics of acute leukaemia patients included in the study. AML blasts were regarded as positive when more than 20% of the cells stained positive in flow-cytometric analysis. *NT* not tested, *PBMC* peripheral blood mononuclear cells, *ALL* acute lymphoblastic leukaemia, *AML* acute myelogenous leukaemia

Patient	Sex	Age (years)	Cytogenetic analysis	FAB classification	Membrane molecule expression								Blasts among PBMC (%)	HLA-class-II-positive blasts (%)
					CD3	CD13	CD14	CD15	CD19	CD20	CD33	CD34		
1	M	21	Normal	ALL	–	–	–	–	+	+	–	+	97	97
2	M	30	Normal	AML-M2	–	–	–	NT	–	–	+	–	95	–
3	F	36	Normal	AML-M5	–	+	+	+	–	–	+	–	97	30
4	M	27	Normal	AML-M2	–	+	–	+	–	–	+	+	95	86
5	F	61	t(2;3), (q37;q21)(q13;q21;q21) der(11q)19q+	AML-M5	–	–	+	+	–	–	+	+	98	94
6	M	47	Normal	AML-M4	–	+	+	NT	–	–	–	–	>99	43
7	M	50	Normal	AML-M4	–	+	–	+	–	–	+	–	95	56
8	M	21	+8	AML-M2	–	+	–	+	–	–	–	–	93	96
9	F	41	der(13)t(1;13)(1pter→1p13::13p12→13qter)	AML-M1	–	–	–	+	–	–	+	–	99	18
10	M	82	Normal	AML-M4	–	+	+	NT	–	–	–	–	>99	–

PHA+IL-2-stimulated IL-13 secretion by normal and posttransplant CD8+ TCRαβ+ cloned T cells when B lymphocyte accessory cells were used

IL-13 concentrations were compared for 8 posttransplant and 11 normal T cells clones. IL-13 was detected for all CD8+ cloned T cells, but IL-13 levels were significantly lower for posttransplant than for normal T cell clones (Fig. 1; Willcoxon's test, P=0.04). IL-13 concentrations were significantly lower for CD8+ than for CD4+ cloned T cells when both posttransplant (P=0.003) and normal T cell clones were compared (P=0.005). IL-4 and IFNγ levels were also determined for the clones. A significant correlation was detected between IL-13 and IL-4 levels for the posttransplant clones (P=0.04), whereas no correlation was detected between IL-13 and IFNγ levels (data not shown).

PHA+IL-2-stimulated IL-13 secretion by posttransplant cloned T cells when leukaemic PBMC accessory cells were used

IL-13 release in response to PHA+IL-2+leukaemic PBMC (patients 6, 10) was investigated for 10 posttransplant CD4+TCRαβ+ T cell clones. When leukaemic PBMC derived from patient 6 were tested, IL-13 was not detected (below 36 pg/ml) in control cultures containing only cloned T cells + leukaemic PBMC for 9 out of the 10 T cell clones (IL-13 concentration 1.5 ng/ml for 1 T cell clone), whereas high IL-13 levels were detected for all cloned T cells when PHA was added together with IL-2 and leukaemic PBMC (range of incremental IL-13 concentrations 460–6350 pg/ml). Significant PHA-stimulated IL-13 secretion was also detected when leukaemic PBMC from patient 10 were used

Table 2 Interleukin-13 concentrations in allostimulated cultures using normal PBMC stimulator cells and non-irradiated leukaemic PBMC as stimulator cells. Allostimulated cultures were prepared using 1×10^6 non-irradiated leukaemic PBMC as stimulator cells and 5×10^5 normal PBMC as responder cells. The cells were cultured in 2 ml culture medium for 6 days before supernatants were harvested and concentrations of IL-13 determined. All concentrations are expressed as the mean ± standard deviation of duplicate determinations. *ND* not detectable (<36 pg/ml)

Patient/stimulator cells	Interleukin-13 (pg/ml)				
	Healthy responder I A1,9; B12,27; DR2,4; DQ1,3	Healthy responder II A2,11; B40; DR6,8; Dq1,4	Healthy responder III A1,2; B8,15; DR2,4; DQ1,3	Healthy responder IV A2,3; B8,15; DR2,4	Healthy responder V A1,3; B7,8; DR3,4
1. A1,2;B8,15;DR3,4; DQ2,3	ND	ND	34± 7	ND	48± 7
2. A11,28;B35,60;DR1,4; DQ1,3	ND	ND	ND	ND	ND
3. A2;B8,40;DR3,6; DQ1,2	ND	ND	ND	ND	ND
4. A11,19;B5,27;DR1,8; DQ1,4	120±31	510±98	115±19	400±69	320±85
5. A10,19;B18	ND	ND	–	–	ND
6. A2,30;B57,60;DR2,13; DQ1	84±15	90±10	165±84	240±46	940±79

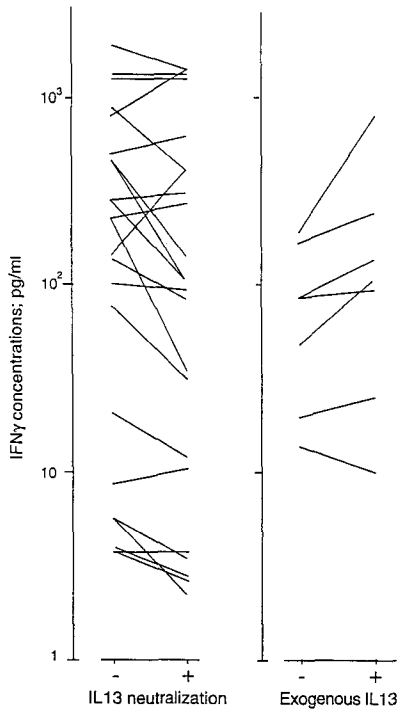

Fig. 3 *Left* interferon γ (*IFNγ*) concentrations in culture supernatants derived from allostimulated cultures containing normal responder peripheral blood mononuclear cells (PBMC) and non-irradiated allogeneic leukaemic PBMC as stimulator cells. The figure presents the results for 23 responder/stimulator combinations prepared either with IL-13-neutralizing (+) or isotypic control antibodies (–). *Right* IFNγ concentrations in allostimulated cultures (7 responder/simulator combinations) prepared with (+) and without (–) exogenous IL-13 (50 ng/ml). IL-13 could not be detected in the detected in the cultures without exogenous cytokine (<36 pg/ml)

as accessory cells (data not shown). Thus, T cells can be activated to secrete IL-13 also when AML blasts are the major population among the accessory cells.

IL-13 secretion by normal PBMC in response to allostimulation with leukaemic PBMC

Normal PBMC responder cells derived from five healthy individuals were cultured with non-irradiated leukaemic PBMC stimulators derived from one patient with acute lymphoblastic leukaemia and 5 AML patients (Table 1, patients 1–6). IL-13 was not detected (below 36 pg/ml) in control cultures containing responder or stimulator cells alone or leukaemic PBMC+PHA, whereas all responder cells secreted IL-13 in response to PHA stimulation (range 110–340 pg/ml). IL-13 could be detected in the supernatants for 12 out of 28 responder/stimulator combinations tested (Table 2). High IL-13 levels were detected for stimulator cells derived from patients 4 and 6, whereas IL-13 was not detected after allostimulation with blasts derived from patients 2 and 3. A significant proliferative response was detected for all these responder/stimulator combinations when 50-Gy-irradiated leukaemia PBMC were used as stimulator cells (data not shown).

IFNγ and IL-4 levels were also determined for the allostimulated cultures. IFNγ was detected for all stimulator/responder combinations investigated, and IFNγ and IL-13 levels were significantly correlated (Kendall's test, P=0.001). IL-4 was not detected in any culture (data not shown).

Allostimulated secretion of IL-13; effects on IFNγ release from the normal PBMC responder cells

PBMC derived from four healthy individuals (responders I–IV, Table 2) were cultured with non-irradiated leukaemic PBMC derived from patients 2–9 and IFNγ concentrations determined in the supernatants. Cultures were prepared with either anti-IL-13 or isotypic control antibodies, and IFNγ was detected for 23 responder stimulator combinations. For the majority of responder/stimulator combinations investigated, IL-13 neutralization decreased IFNγ levels (Fig. 3; Willcoxon's test, P=0.002). Thus, IL-13 neutralization inhibits IFNγ responses during allostimulation. IFNγ levels were also determined for cultures containing normal responder PBMC together with allogeneic AML cells when cultures were prepared without or with exogenous 50 ng/ml IL-13. For these experiments we selected seven responder/stimulator combinations that showed low or undetectable endogenous IL-13 release, and increased IFNγ levels were then detected *in* the presence of exogenous IL-13 (Fig. 3; P=0.023).

Table 3 Proliferation of non-adherent CD2⁻CD19⁻ cells in allostimulated cultures containing normal responder PBMC derived from individual 2 and native leukaemic PBMC stimulator cells derived from four different AML patients. Cultures were prepared using 2.5×10^5 cells/ml of both responder and stimulator cells, antibodies were used at the concentration of 10 μg/ml and [³H]thymidine incorporation was assayed after 7 days of in vitro culture. Proliferation of the non-adherent CD2⁻CD19⁻ cells is presented as the mean radioactivity of triplicate cultures ± standard deviation

Leukaemic PBMC donor	Proliferation of non-adherent CD2⁻CD19⁻ cells (cpm)		Proliferation of AML blasts + control antibody (cpm)
	Anti-IL-13 antibodies	Control antibodies	
AML patient 4	7044± 255	8270± 174	11427±2989
AML patient 5	184± 16	254± 59	777± 384
AML patient 6	14620±1893	13465±2135	21218±1622
AML patient 7	24095±1753	17024±1396	30112±2496

Allostimupated secretion of IL-13; effects on proliferation and G-CFS secretion by the AML blast stimulator cells

Normal responder PBMC (individuals I and II) and non-irradiated leukaemic PBMC stimulators (patients 2–7) were cultured together. [^{3}H]thymidine was added on day 6, and 24 h later cultures were depleted of adherent cells and CD2$^+$ and CD19$^+$ cells. The majority of the non-adherent CD2$^-$ CD19$^-$ cells will have been AML blasts (see Materials and methods), and [^{3}H]thymidine incorporation of these cells was then compared for cultures containing either anti-IL-13 or control antibodies. The results for 4 of the 12 responder/stimulator combinations are presented in Table 3. Although the presence of alloreactive T cells modulated the proliferation of non-adherent CD2$^-$CD19$^-$ cells, IL-13 neutralization had no effect on their [^{3}H]thymidine incorporation for the majority of the responder/stimulator combinations. IL-13 neutralization increased proliferation only when leukaemic stimulator PBMC from patient 7 were tested (Table 3), and this was observed in repeated experiments.

CD4$^+$- and CD8$^+$-enriched responder T lymphocytes derived from individuals I–IV (Table 2) were cultured with leukaemic PBMC stimulators derived from patients 2–7. Cultures were prepared with either anti-IL-13 or control antibodies. The enriched responder cells cannot secrete detectable G-CSF, and G-CSF is released mainly by the AML blast stimulator cells [7]. IL-13 neutralization did not alter G-CSF levels when either CD4$^+$ or CD8$^+$ T lymphocytes were tested (data not shown).

Discussion

In our present study we used three different in vitro approaches to characterize IL-13 as a T cell effector function. First, we investigated the capability of individual T cell clones to secrete IL-13 under defined in vitro conditions. Second, we investigated whether IL-13 could be released when normal T cells were activated in the presence of leukaemic accessory cells. Third, the functional effects of IL-13 released in the presence of allostimulating leukaemia cells were investigated using IL-13 neutralizing antibodies. Our results indicate that secretion of IL-13 is functionally important during in vitro alloresponses.

To investigate the capability of individual T cell clones to secrete IL-13 we prepared monoclonal T cell populations and compared IL-13 levels for different clonal subsets. We investigated T cell responses to a well-defined in vitro activation signal (PHA+IL-2) and, as accessory cells, we used the B cell line AL10. This is a well-characterized cell line used as accessory cells for T cell activation in several previous studies [4–6]. Although virus-transformed B cells can secrete low levels of IL-13 [9, 15], irradiated AL10 cells could not secrete detectable amounts of this cytokines.

Certain T lymphocyte clones can undergo autocrine proliferation after PHA activation, whereas other clones cannot release the growth factors needed for autocrine proliferation. When individual clones were stimulated with PHA+accessory cells (the AL10 B cell line), IL-13 secretion could be detected both for clones undergoing autocrine proliferation and for non-proliferating clones. However, significantly higher IL-13 levels were detected for clones undergoing autocrine proliferation.

Our clones were prepared in the presence of exogenous IL-2 and are IL-2-responsive [3]. To study clonal IL-13 secretion independently of the ability to undergo autocrine proliferation, we investigated IL-13 levels after clonal activation with PHA+IL-2+B cells. Both normal and post-transplant CD4$^+$ and CD8$^+$TCR$\alpha\beta^+$ cloned T cells were capable of IL-13 secretion (Fig. 1).

Significantly lower IL-13 levels were detected for post-transplant CD4$^+$ and CD8$^+$ clones than for their normal counterparts, derived from bone marrow donors. Both for normal and posttransplant clones, IL-13 levels were higher for the CD4$^+$ than for the CD8$^+$ subset.

IFNγ is a Th1-associated cytokine, whereas IL-4 is a Th2-associated cytokine [5, 26]. Clonal secretion of these two cytokines has been characterized in detail previously [4, 5]. A majority of clones secreted both IL-13 and IL-4. IL-13 levels were significantly correlated to the levels of the Th2 cytokine IL-4, although IL-13 levels were generally higher than IL-4 levels (Fig. 2). IL-13 concentrations were also higher than the IL-4 levels when investigating polyclonal alloresponses (Table 2), and similar difference between IL-13 and IL-4 levels has also been described previously for allergen-specific T cell clones [9].

In the second part of our study we investigated whether T lymphocytes could be activated to secrete IL-13 in the presence of AML blast accessory cells. In contrast to the B cell line AL10, AML blasts are characterized by constitutive secretion of several cytokines that can modulate T cell activation [2, 4–7, 10, 27, 28]. Our results demonstrate that T lymphocytes can undergo both mitogen- and alloantigen-stimulated activation in the presence of AML blast accessory cells. However, to avoid induction of cytokine gene expression by the cell separation procedure [10, 28], leukaemia blasts were prepared by density-gradient separation from the peripheral blood of patients with high blast counts in the blood [2, 7]. The blast count seems to be a prognostic factor in acute leukaemia and may thus reflect intrinsic properties of the blasts [16], and these results may therefore be representative only for this selected subset of patients.

In the third part of our study we used IL-13-neutralizing antibodies to evaluate functional effects of endogenous IL-13 released during alloactivation. We investigated possible effects of IL-13 on both normal responder PBMC and AML blast stimulator cells. Leukaemic PBMC contained at least 95% leukaemia blasts with only a minimal contamination of normal lymphocytes. When leukaemic PBMC are used as stimulator cells for normal responder PBMC, IFNγ will be derived from either activated T cells or natural killer (NK) cells among the responder cells [14, 19]. The decreased IFNγ levels detected in the presence of IL-13-neutralizing antibodies indicate that IL-13 is an enhancing factor for IFNγ release during allorecognition of leukaemic PBMC. This is further supported by the observation that

exogenous IL-13 increased IFNγ levels when added to cultures with low endogenous IL-13 release. It is likely that this enhancement of IFNγ release is at least partly caused by an effect on NK cells, because (i) IL-13 does not seem to have an effect directly on T lymphocytes [15, 18, 19]; (ii) IL-12 is secreted by alloactivated T cells, and IL-13 may then enhance the IL-2-stimulated release of IFNγ by NK cells [7, 15, 19].

We used two different approaches to evaluate the effects of IL-13 on the allostimulating AML blasts. First, when enriched $CD4^+$ or $CD8^+$ responder T lymphocytes were used in allostimulated cultures, G-CSF levels in supernatants could be used as a marker of AML blast cytokine secretion [7, 8]. Second, [^{3}H]thymidine incorporation of the non-adherent $CD2^-CD19^-$ cell population (depletion of adherent monocytes, B and T lymphocytes) will reflect proliferation of AML blasts in the allostimulated cultures [7]. IL-13 neutralization had no effect on G-CSF release during allostimulation, and an effect on blast proliferation was detected only for a minority of responder/stimulator combinations. Thus, in most cases, endogenous IL-13 release did-not alter AML blast functions. However, in a previous report we described how exogenous IL-13 inhibited constitutive cytokine secretion (including G-CSF release) by AML blasts [2] . The most likely explanation for this difference between the effects of endogenous and exogenous IL-13 on the AML blasts is that the IL-13 concentrations reached after alloactivation were generally lower than the concentrations needed to alter AML blast functions (more than 5 ng/ml, see [2]). Other possible explanations for this difference are that (i) the direct inhibitory effect of IL-13 on AML blasts may be counteracted by a stimulatory effect of the increased IFNγ levels [7]; (ii) in vitro effects of IL-13 on AML blast proliferation can be modulated by addition of exogenous cytokines [2], and a similar modulation of G-CSF release may then occur in the presence of alloactivated T lymphocytes. This last hypothesis is supported by the observation that the inhibitory effect of IL-4 on AML blasts is modulated by the cytokine environment [30], and a similar modulation of the IL-13 effect may then be mediated via the common receptors for IL-4 and IL-13 [17, 22, 29, 31–33].

From our present in vitro study we conclude that (i) most T cell clones can secrete IL-13; (ii) IL-13 can be released by T lymphocytes activated in the presence of AML blast accessory cells; and (iii) IL-13 released in response to allostimulating AML blasts has a modulatory effect on cytokine responses. However, further studies are needed to clarify whether IL-13 is important for interactions between AML blasts and immunocompetent cells in vivo.

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