10. Van der Berg HW, Leahey WJ, Lynch M, Clark R, Nelson J. Recombinant human interferon alpha increases oestrogen receptor expression in human breast cancer cells (ZR-75-1) and sensitises them to the anti-proliferative effects of tamoxifen. *Br J Cancer* 1987, 55, 255–257.
11. Hakes T, Menendez-Botet C, Moore M, Osborne M. Modulation of estrogen (ER) and progesterone (PR) receptors in human breast cancers by alpha-2b interferon. *Proc ASCO* 1990, **9**, 44.
12. Zoon KC, Karasaki Y, Zur Nedden DL, Hu R, Arnheiter H. Modulation of epidermal growth factor receptors by human a-interferon. *Proc Natl Acad Sci USA* 1986, **83**, 8226–8230.
13. Battaglia F, Scambia G, Benedetti Panici P, Baiocchi G, Perrone L, Iacobelli S. Epidermal growth factor receptor expression in gynecological malignancies. *Gynecol Obstet Invest* 1989, **27**, 42–44.
14. Bauknecht T, Kohler M, Janz I, Pfleider A. The occurrence of epidermal growth factor receptors and the characterization of EGF-like factors in human ovarian, endometrial, cervical, and breast cancer. *J Cancer Res Clin Oncol* 1989, **115**, 193–199.
15. Dimitrov MV, Meyer CJ, Strander H, Heihorn S, Cantell K. Interferon as a modifier of estrogen receptors. *Ann Clin Lab Sci* 1984, **14**, 32–39.
16. Marth Ch, Mayer I, Bock G, Gastl G, Huber CH, Flener R. Effects of human interferon a-2 and gamma on proliferation, estrogen receptor content, and sensitivity to anti-estrogens of cultured breast cancer cells. In: Dianzani F. and Rossi GB eds. *The Interferon System*. 1981, New York: Raven Press, Vol 24, 367–371.
17. Goldstein D, Busshmeyer S, Witt PL, Jordan VC, Borden EC. Effects of type I and II interferons on cultured human breast cells: interactions with estrogen receptors and with tamoxifen. *Cancer Res* 1989, **49**, 2698–2702.
18. Pfeffer LM, Donner DB, Tamm I. Interferon-a down-regulates insulin receptors in lymphoblastoid (Daudi) cells. *J Biol Chem* 1987, **262**, 3665–3670.
19. Korc M, Haussler CA, Trookman NS. Divergent effects of epidermal growth factor and transforming growth factors on a human endometrial carcinoma cell line. *Cancer Res* 1987, **47**, 4909–4914.
20. Fisher PB, Miranda AF, Babiss LE, Pestka S, Weinstein WB, Opposing effects of interferon produced in bacteria and of tumor promoters on myogenesis in human myoblast cultures. *Proc Natl Acad Sci USA* 1983, **80**, 2961–2965.
21. Ball ED, Guyre PM, Shen JM, Glynn GR, Maliszewski CR, Baker PE. Gamma interferon induces monocytoid differentiation in the HL-60 cell line. *J Clin Invest* 1984, **73**, 1072–1077.
22. Kameyama K, Tanaka S, Ishida Y, Hearing VJ. Interferons modulate the expression of hormone receptors on the surface of murine melanoma cells. *J Clin Invest* 1989 **83**, 213–221.
23. Dickson RB, Lippmann ME. Estrogenic regulation of growth and polypeptide growth factor secretion in human breast carcinoma *Endocr Rev* 1987, **8**, 29–43.
24. Sica G, Natoli V. The mechanism of action of anti-oestrogens. In: Pannuti F, ed. *Anti-oestrogenes in Oncology. Past, present and prospects*. Current clinical practice series 31, Amsterdam, Excepta Medica, 1985, 54–61.
25. Iacobelli S, Natoli C, Arno E, Sbarigia G, Gaggini C. An anti-estrogenic action of IFNs in human breast cancer cells. *Anticancer Res* 1986, **6**, 1391–1394.
26. Kangas L, Nieminen AL, Cantell K. Additive and sinergistics effect of a novel anti-estrogen toremifene (Fc-1157a) and human interferons on estrogens responsive MCF7 cells *in vitro*. *Med Biol* 1985, **63**, 187–190.

Eur J Cancer, Vol. 27, No. 1, pp. 53–57, 1991.
Printed in Great Britain
0277–5379/91 $3.00 + 0.00

Potentiation of Retinoid-induced Differentiation of HL-60 and U937 Cell Lines by Cytokines

Richard Peck and Werner Bollag

Retinoids varied in their capacity to induce differentiation in HL-60 cells in this order: Ro 13-6307, tretinoin, isotretinoin, acitretin and Ro 13-7410 (high to low). In contrast, retinoids lacking a polar carboxylic acid, such as temarotene and Ro 14-6113, were inactive. Various cytokines had no differentiation-inducing effect by themselves. However, the addition of cytokines to retinoids increased differentiation. Combined with tretinoin, cytokines increased differentiation in this order: interferon (IFN) gamma, granulocyte colony-stimulating factor, interleukin-1α (IL-1α), IL-4, tumour necrosis factor alpha and IFN-α. Combination of cytokines with isotretinoin, acitretin, Ro 13-7410, and Ro 13-6307 showed a similar pattern of potentiation to that of tretinoin. Temarotene or Ro 14-6113 did not induce differentiation, alone or with cytokines. Combinations of cytokines were not synergistic in the presence of retinoids; antagonism was even observed. In U937 cells, lower levels of differentiation-induction were observed. Potentiation of the differentiation-inducing effect of retinoids by cytokines might indicate a clinical differentiation therapy of tumours.
Eur J Cancer, Vol. 27, No. 1, pp. 53–57, 1991.

INTRODUCTION

RETINOIDS ARE structurally related to vitamin A and are active, experimentally as well as clinically, in prevention and therapy of a variety of neoplastic diseases [1–3]. The mechanism of action is not understood and includes: inhibition of proliferation, induction of differentiation or immune stimulation. Complete clinical remissions have been described in acute promyelocytic leukaemia after treatment with tretinoin (all-trans retinoic acid) [4, 5]. Since tretinoin induces differentiation, these results have aroused new interest in differentiation therapy. The differentiating effect of tretinoin on HL-60 cells, a human promyelocytic leukaemia cell line, was first reported by Breitman *et al.* [6]. Differentiation of tumour cells has been described in a variety of cell lineages, including U937, a human histiocytic lymphoma [7], murine embryonal carcinoma [8], murine teratocarcinoma [9] and human neuroblastoma [10]. Retinoids modulate tumour

Correspondence to W. Bollag.
The authors are at Pharmaceutical Research, F. Hoffmann–La Roche Ltd, CH-4002 Basle, Switzerland.
Revised and accepted 24 Oct. 1990.

cell proliferation and differentiation via a family of well characterised nuclear retinoic acid receptors that regulate gene expression [11–13].

Cytokines are active on a wide variety of immunocompetent, haematopoietic and other cell types via induction of proliferation or differentiation [14]. The overlapping activities of retinoids and cytokines on regulation of cell growth and function via a network of receptors and gene induction makes the investigation of possible interactions of these molecules attractive. Cytokines do not appear to be potent inducers of differentiation in transformed cell lines [15]. However, in combination with retinoids, interferon (IFN) alpha [15–18], beta [16, 19] and gamma (17, 20], tumour necrosis factor (TNF) [20] and granulocyte colony-stimulating factor (G-CSF) [21, 22] potentiate differentiation.

Here we extend previous studies to include a panel of human recombinant cytokines in combination with a variety of retinoids of divergent chemical structure.

MATERIALS AND METHODS

Chemicals

The retinoids tretinoin, isotretinoin (13-cis retinoic acid), the active metabolite of etretinate, acitretin, [all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid]; Ro 13-7410 [*p*-<(E)-2(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)propenyl>benzoic acid]; temarotene [1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-6-<(E)-alpha-methylstyryl>naphthalene]; Ro 14-6113 [*p*-<(E)-2-(5,5,8,8-tetramethyl-2-naphthyl)propenyl>phenol] and Ro 13-6307 [(all-E)-3-methyl-7-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-2,4,6-octatrienoic acid] were synthesised at F. Hoffmann–La Roche Ltd, Basle. The compounds were dissolved in dimethylsulphoxide (DMSO) at 10^{-2} mol/l and diluted in culture medium to final concentration. The stock solutions were stored at room temperature and protected from light and oxygen.

Nitroblue tetrazolium (NBT) was purchased from Sigma. Human recombinant cytokines interleukin-1 alpha (IL-1α), IL-1β, IL-2, IL-4, IFN-α, IFN-β, IFN-γ and TNF-α were from Hoffmann–La Roche. G-CSF was from Amgen. Epidermal growth factor (EGF), transforming growth factor beta-1 (TGF-β1) and TGF-β2 were from Boehringer Mannheim.

Cell culture and differentiation assay

The effect of cytokines and/or retinoids on induction of differentiation was determined in four independent experiments by the capacity to reduce NBT as follows. Triplicate cultures of 10^4 HL-60 or U937 cells were incubated in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Amimed), 2 mmol/l L-glutamine, 1 mmol/l sodium pyruvate, 1% non-essential aminoacids, 10 mmol/l HEPES, 100 U/ml penicillin and 100 μg/ml streptomycin (all Gibco) containing the indicated concentration of each retinoid or cytokine or their appropriate vehicle controls. An HL-60 variant which is relatively insensitive to retinoids was chosen to assess potentiation with cytokines. Cytokine concentrations that yielded a 50% level of activity were used as follows: IFN-α and IFN-β, inhibition of the proliferation of vesicular stomatitis virus when assayed on human WISH cell lines; IFN-γ, activation of human monocytes to tumoricidal activity against K562 target cells; IL-2 and IL-4, induction of proliferation of CTLL thymoma cells. TNF-α, induction of cytotoxicity against murine WEHI 164 cells; G-CSF, induction of progranulocytic colonies from bone marrow stem cells; IL-1, induction of murine thymocyte proliferation in the presence of sub-stimulatory concentrations of mitogens; TGF-β, inhibition of fibroblast proliferation; and EGF, maintenance of keratinocyte proliferation. After 2 days incubation of HL-60 or U937 cells with retinoids and/or cytokines, the medium was removed from the wells and replaced with 100 μl per 1 mg/ml NBT containing 200 nmol/l PMA per well [23]. After an additional 1 h incubation, the NBT solution was removed and 100 μl 0.04 mol/l HCl containing 10% sodium dodecyl sulphate was added. Colour development (change in optical density [OD] at 540 nm) was read 24 h later, and the data are presented as change in OD per h, above a background of zero in undifferentiated cells. Cell viability determined by exclusion of propidium iodide, after 2 days' incubation was over 85%. Retinoids were not cytotoxic in the concentrations we used. Our method proved useful since in an investigation comparing this method with that of counting the percentage of NBT-positive cells, we came to virtually identical results [24].

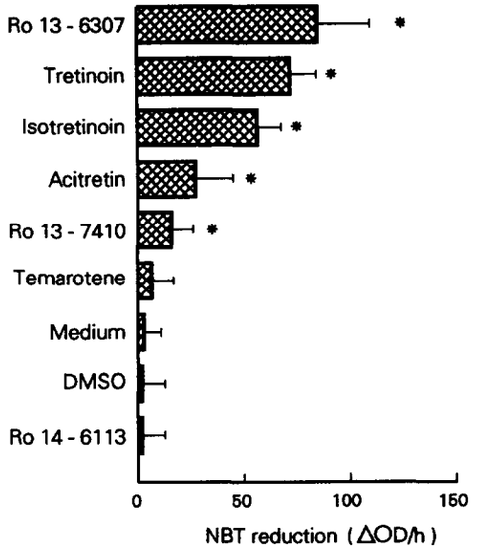

Fig. 1. Induction of differentiation in HL-60 cells by retinoids. *$P < 0.005$ compared with DMSO control.

Statistical analysis

The 12 observations (4 triplicates) in each group are assumed to be independent. Means (S.D.) of these 12 values were calculated for each cytokine/retinoid combination and for retinoids alone. For each retinoid an overall analysis of variance (ANOVA) was done to examine the impact of the cytokines. If this ANOVA led to a significant result at $\alpha = 0.05$, then each cytokine/retinoid combination was separately compared with retinoid alone. If the variable could be accepted as normally distributed, a two-sample t test was used. Otherwise the Mann–Whitney rank-sum test was used. The P values were not adjusted because of the explorative nature of this experiment.

RESULTS

HL-60 cell differentiation induced by retinoids

Tretinoin induced differentiation of HL-60 cells as demonstrated by an increased reduction of NBT (Fig. 1). Isotretinoin was less effective, followed in turn by acitretin and Ro 13-7410. Ro 13-6307 was the most active. In contrast to these active retinoids, which all contain a carboxylic acid, retinoids lacking a carboxylic acid, such as temarotene and Ro 14-6113, failed to

Table 1. Induction of HL-60 cell differentiation by retinoids and cytokines alone and in combination

Cytokines*	Retinoids†							
	Tretinoin	Isotretinoin	Acitretin	Ro 13-7410	Ro 14-6113	Ro 13-6307	DMSO (0.1%)	Medium
IFN-α	85 (20)‡	104 (28)§	88 (35)§	69 (29)§	0 (9)	76 (19)	0 (7)	3 (9)
IFN-β	71 (10)	53 (6)	65 (10)§	30 (8)‡	4 (6)	96 (15)	0 (6)	1 (12)
IFN-γ	289 (81)§	165 (43)§	161 (85)§	130 (23)§	12 (21)	247 (31)§	2 (10)	5 (9)
IL-1α	121 (21)§	114 (6)§	72 (31)§	74 (50)§	8 (9)	136 (32)§	8 (10)	6 (5)
IL-1β	77 (6)	75 (10)‡	41 (4)	15 (16)	9 (1)	92 (20)	10 (4)	5 (3)
TNF-α	94 (37)‡	104 (26)§	100 (44)§	78 (40)§	6 (4)	148 (76)‡	5 (8)	5 (8)
G-CSF	161 (23)§	134 (31)§	67 (34)§	62 (24)§	0 (10)	150 (55)§	0 (8)	0 (4)
EGF	77 (16)	56 (15)	18 (7)	15 (16)	30 (35)‡	86 (6)	14 (28)	0 (12)
TGF-β1	65 (7)	65 (20)	20 (11)	12 (6)	8 (9)	85 (32)	0 (1)	0 (5)
TGF-β2	57 (6)	64 (3)	17 (2)	14 (11)	12 (8)	86 (9)	0 (8)	3 (15)
IL-2	75 (13)	52 (13)	19 (16)	11 (8)	10 (9)	96 (14)	2 (22)	0 (9)
IL-4	108 (11)§	86 (12)§	25 (23)	29 (30)	6 (8)	141 (35)§	4 (12)	0 (7)
None	71 (13)	55 (12)	26 (18)	15 (10)	1 (11)	85 (27)	0 (11)	2 (8)

NBT reduction in Δ OD/h, mean (S.D.).
*Concentrations as follows: IFN-α and IFN-β, 1000 U/ml; IFN-γ, 100 U/ml; IL-1α and IL-1β, 3 ng/ml; TNF-α, 2 ng/ml; G-CSF, 30 ng/ml; EGF, 20 ng/ml; TGF-β1 and TGF-β2, 3 ng/ml; and IL-2 and IL-4, 100 U/ml.
†Retinoids at 10^{-5} mol/l.
‡$P < 0.05$ and §$P < 0.005$ compared with controls (retinoids without cytokines).

induce differentiation. The DMSO and medium controls had no activity.

Potentiation of retinoid activity by cytokines

In the absence of retinoids, all cytokines in medium or DMSO failed to induce NBT reduction in HL-60 cells (Table 1).

Moderate potentiation of tretinoin-induced HL-60 cell differentiation was observed in the presence of IFN-α and TNF-α. A marked increase was seen by the addition of IL-4 or IL-1α. The highest degree of potentiation was achieved with G-CSF and particularly IFN-γ. No increase was obtained with IFN-β, IL-1β, IL-2, EGF, TGF-β1 and TGF-β2. Isotretinoin showed a similar, but not identical pattern of potentiation to that of tretinoin. IFN-α and TNF-α showed greater potentiation with isotretinoin than with tretinoin. Acitretin alone induced only a moderate increase in differentiation. Nevertheless, a two to six fold increase in levels of NBT reduction was achieved with (in order of efficacy) IFN-γ, TNF-α, IFN-α, IL-1α, G-CSF and IFN-β. In contrast to tretinoin and isotretinoin, the acitretin-induced differentiation was potentiated by IFN-β, but not by IL-4.

Ro 13-7410 alone induced a low but significant increase in differentiation. Marked increases, up to nine fold, in levels of NBT reduction were achieved with (in order of efficacy) IFN-γ, TNF-α, IL-1α, IFN-α, G-CSF and IFN-β. Ro 14-6113 did not induce differentiation by itself. Only in the presence of one cytokine—EGF—was a marginal but statistically significant increase observed. Ro 13-6307 induced the strongest differentiation when given alone, and this differentiation was potentiated by IFN-γ, G-CSF, TNF-α, IL-4 and IL-1α. In contrast to the other retinoids with a carboxylic acid, IFN-α and IFN-β had no potentiating effect.

Interaction between cytokines in the presence of retinoids

The values in Table 2 are derived from the same experiments as those in Table 1, allowing a direct comparison between the values in the two tables. When two cytokines were combined in the presence of retinoids, the level of differentiation observed

Table 2. Induction of HL-60 cell differentiation by combinations of cytokines in presence or absence of retinoids

Cytokines*	Retinoids*							
	Tretinoin	Isotretinoin	Acitretin	Ro 13-7410	Ro 14-6113	Ro 13-6307	DMSO (0.1%)	Medium
IFN-α+IFN-γ	77 (21)	77 (19)†	88 (29)‡	84 (59)‡	0 (3)	95 (24)	0 (4)	0 (3)
IL-1α+IL-1β	73 (20)	38 (9)†	15 (10)	13 (17)	12 (2)	108 (3)	0 (11)	2 (12)
TNF-α+IFN-γ	212 (25)‡	98 (12)‡	92 (25)‡	83 (17)‡	10 (12)	214 (23)‡	26 (5)‡	20 (5)‡
G-CSF+IFN-γ	218 (28)‡	150 (45)‡	99 (9)‡	88 (20)‡	0 (3)	273 (10)‡	0 (1)	3 (7)
G-CSF+TNF-α	105 (19)‡	108 (21)‡	110 (6)‡	96 (10)‡	0 (4)	113 (26)	7 (21)	0 (1)
TFG-β1+β2	64 (2)	52 (5)	30 (6)	18 (10)	8 (29)	97 (32)	0 (6)	1 (9)
None	71 (13)	55 (12)	26 (18)	15 (10)	1 (29)	85 (27)	0 (11)	2 (8)

*Concentrations as in Table 1.
†$P < 0.05$ and ‡$P < 0.005$ compared with controls.

Table 3. Induction of U937 cell differentiation by retinoids and cytokines alone and in combination

	Retinoids*							
Cytokines*	Tretinoin	Isotretinoin	Acitretin	Ro 13-7410	Ro 14-6113	Ro 13-6307	DMSO (0.1%)	Medium
IFN-α	25 (8)	4 (2)	0 (1)	0 (1)	0 (2)	62 (1)	0 (2)	3 (9)
IFN-β	31 (7)	11 (2)	1 (1)	0 (0)	1 (0)	74 (4)	1 (0)	1 (12)
IFN-γ	56 (1)‡	29 (1)‡	21 (5)‡	20 (3)‡	9 (5)	100 (2)‡	6 (5)	5 (9)
IL-1α	24 (9)	3 (4)	0 (2)	0 (3)	1 (3)	64 (3)	0 (3)	6 (5)
IL-1β	21 (10)	1 (3)	0 (1)	0 (2)	2 (2)	67 (4)	0 (2)	5 (3)
TNF-α	38 (5)†	24 (8)†	0 (3)	0 (4)	6 (2)	102 (4)‡	2 (2)	5 (8)
G-CSF	48 (10)‡	5 (1)	0 (1)	2 (2)	4 (7)	85 (5)‡	0 (7)	0 (4)
EGF	11 (3)	4 (1)	0 (2)	0 (2)	2 (2)	46 (12)	0 (2)	0 (12)
TGF-β1	27 (2)	8 (5)	7 (3)	15 (13)	3 (1)	37 (7)	0 (1)	0 (5)
TGF-β2	25 (3)	9 (4)	9 (3)	10 (5)	2 (2)	43 (8)	0 (2)	3 (15)
IL-2	25 (1)	8 (2)	0 (2)	0 (4)	0 (2)	48 (6)	0 (2)	0 (9)
IL-4	16 (9)	15 (2)	0 (5)	0 (4)	0 (2)	44 (10)	0 (2)	0 (7)
None	20 (12)	13 (2)	0 (3)	0 (5)	1 (4)	61 (1)	0 (4)	2 (8)

*Concentrations as in Table 1.
†$P < 0.05$ and ‡$P < 0.005$ compared with controls.

never exceeded, at a statistically significant level, those obtained with a single cytokine. In fact, the combination of tretinoin, isotretinoin, acitretin or Ro 13-6307 with IFN-α and IFN-γ together resulted in an NBT reduction less than that obtained with IFN-γ alone, suggesting an antagonistic effect between IFN-α and IFN-γ. Similarly, IL-1β antagonised the ability of IL-1α to potentiate differentiation. The combination of IFN-γ or TNF-α with G-CSF in the presence or absence of retinoids resulted in no significantly enhanced differentiation compared with each cytokine by itself. No synergistic effect was observed between IFN-γ and TNF-α in the presence of retinoids. However, a significant increase in NBT reduction could be seen when these cytokines were combined in the absence of retinoids.

U937 cell differentiation

The human histiocytic lymphoma cell line U937 is less sensitive to retinoids alone as well as to combinations of retinoids and cytokines than is HL-60 (Table 3). Significant induction of differentiation with retinoids alone was obtained only with tretinoin, isotretinoin and, particularly, with Ro 13-6307. Combinations of retinoids with IFN-γ, G-CSF or TNF-α were effective as potentiators of differentiation induced by tretinoin, isotretinoin and Ro 13-6307. In addition, IFN-γ induced differentiation in the presence of acitretin or Ro 13-7410, which alone were inactive.

DISCUSSION

In HL-60 cells, tretinoin is a potent inducer of terminal granulocytic differentiation [6], and in U937 cells, as an inducer of terminal monocytic differentiation [7]. Myeloid or monocytic differentiation can be measured, among other indices, by the generation of an oxidative burst potential with the attendant production of superoxide radicals known to reduce NBT [23]. The present report demonstrates that individual retinoids differ in their capacity to induce differentiation (Fig. 1), which is consistent with previous reports [24, 25]. In addition, our data show that particular cytokines combined with certain retinoids potentiate the induction of differentiation of a myeloid leukaemia and a lymphoma cell line.

Recent clinical findings have added importance to the role of retinoid-induced differentiation. Two clinical investigators have independently reported dramatic successes in the treatment of acute promyelocytic leukaemia with tretinoin resulting in complete remissions [4, 5]. The possible application of *in vitro* results in the clinic has increased interest in a wider use of differentiation therapy for a variety of oncological diseases [26]. In contrast to these dramatic results in promyelocytic leukaemia, remissions in other neoplasms have been observed with tretinoin as well as with other retinoids, such as isotretinoin and etretinate, but the remissions were in most cases much less satisfactory [3]. With the hypothesis that clinical remissions with retinoids are at least in part due to differentiation induction, an improvement of this therapeutic approach might be obtained by the use of more effective retinoids or, alternatively, by a combination of retinoids with cytokines. Until now, only combinations of a limited number of cytokines with a single retinoid, tretinoin, have been reported [15–22]. The present report expands this approach to include a large panel of cytokines interacting with various retinoids. The results showed that only those retinoids containing a carboxylic acid, such as Ro 13-6307, tretinoin, isotretinoin, acitretin and Ro 13-7410, could induce differentiation. Retinoids lacking a carboxylic acid, such as temarotene and Ro 14-6113, were inactive. This is consistent with recent observations that binding of these retinoids to the retinoic acid receptors α and β, or activation of these receptors, requires the presence of a carboxylic acid (M. Crettaz Hoffman–La Roche, Basle). The HL-60 cell line used in these experiments was relatively insensitive to retinoids. The use of a more sensitive line [24] revealed that the concentrations needed for differentiation induction *in vitro* (10^{-7}–10^{-6} mol/l) correspond to easily achievable plasma levels in man (30–300 ng/ml).

Cytokines alone had no differentiation-inducing effect in HL-60 or U937 cells even at higher concentrations than those reported (data not shown). However, the combination of retinoids with cytokines resulted in a substantial increase in differentiation, depending on the use of a specific retinoid with a specific cytokine. Since cytokines alone are inactive, the observed increases in retinoid-induced differentiation reflect a potentiation or eventually a sensitisation rather than synergy.

When combined with tretinoin, IFN-α, IFN-β, IFN-γ, G-CSF and TNF-α have been reported to potentiate differentiation of HL-60 cells. The present data show that IL-1α, IL-

1β and IL-4 also potentiate differentiation when combined with retinoids. Even retinoids such as acitretin, which alone has little differentiation-inducing capability, were potentiated by IFN-γ, TNF-α, IFNα, IL-1α, G-CSF and IFN-β to reach two to six fold increases in levels of NBT reduction.

In the absence of retinoids, combinations of cytokines showed no synergistic effects with one another, with the exception of TNF-α and IFN-γ. In the presence of retinoids, combinations of cytokines even resulted in antagonistic activities. IFN-α inhibited differentiation induced by IFN-γ in combination with retinoids; similarly, IL-1β inhibited IL-1α activity.

These *in vitro* observations have been made in two transformed haematopoietic cell lines. Further investigations are underway to determine whether these results might be applicable to non-haematopoietic transformed cell lineages. It will be of interest to know whether these *in vitro* results are of predictive value for the treatment of human neoplastic disease. Clinical studies will determine if this is the case, and if so, which retinoid combined with which cytokine is most effective in a specific malignant disease.

1. Bollag W. Vitamin A and retinoids: from nutrition to pharmacotherapy in dermatology and oncology. *Lancet* 1983, **i**, 860–863.
2. Moon RC, Itri LM. Retinoids and cancer. In: Sporn MB, Roberts AB, Goodman DS, eds. *The Retinoids*. Orlando, Academic Press, 1984, Vol. 2, 327–371.
3. Lippman SM, Kessler JF, Meyskens FL Jr. Retinoids as preventive and therapeutic anticancer agents (Part II). *Cancer Treat Rep* 1987, **71**, 493–515.
4. Huang ME, Ye YC, Chen SR, *et al.* Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* 1988, **72**, 567–572.
5. Degos L, Shroot B, de Thé H, Chomienne C, Wang Z, Castaigne S. Retinoic acid in hematopoietic differentiation. *Nouv Rev Fr Hematol* 1990, **32**, 25–38.
6. Breitman TR, Selonick SE, Collins SJ. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc Natl Acad Sci USA* 1980, **77**, 2936–2940.
7. Breitman TR, Keene BR, Hemmi H. Retinoic acid-induced differentiation of fresh human leukaemia cells and the human myelomonocytic leukaemia cell lines, HL-60, U-937 and THP-1. *Cancer Surv* 1983, **2**, 263–291.
8. Sherman MI, Matthaei KI, Schindler J. Studies on the mechanism of induction of embryonal carcinoma cell differentiation by retinoic acid. *Ann NY Acad Sci* 1981, **359**, 192–199.
9. Strickland S, Mahdavi V. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. *Cell* 1978, **15**, 393–403.
10. Sidell N. Retinoic acid-induced growth inhibition and morphologic differentiation of human neuroblastoma cells *in vitro*. *J Natl Cancer Inst* 1982, **68**, 589–593.
11. Petkovich M, Brand NJ, Krust A, Chambon P. A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature* 1987, **330**, 444–450.
12. Darmon M, Rocher M, Cavey MT, *et al.* Biological activity of retinoids correlates with affinity for nuclear receptors but not for cytosolic binding protein. *Skin Pharmacol* 1988, **1**, 161–175.
13. Zelent A, Krust A, Petkovich M, Kastner Ph, Chambon P. Cloning of murine α and β retinoic acid receptors and a novel receptor γ predominantly expressed in skin. *Nature* 1989, **339**, 714–717.
14. Balkwill FR, Burke F. The cytokine network. *Immunol Today* 1989, **10**, 299–303.
15. Fisher PB, Grant S. Effects of interferon on differentiation of normal and tumor cells. *Pharmacol Ther* 1985, **27**, 143–166.
16. Tomida M, Yamamoto Y, Hozumi M. Stimulation by interferon of induction of differentiation of human promyelocytic leukemia cells. *Biochem Biophys Res Commun* 1982, **104**, 30–37.
17. Hemmi H, Breitman TR. Combinations of recombinant human interferons and retinoic acid synergistically induce differentiation of the human promyelocytic leukemia cell line HL-60. *Blood* 1987, **69**, 501–507.
18. Kohlhepp EA, Condon ME, Hamburger AW. Recombinant human interferon α enhancement of retinoic acid-induced differentiation of HL-60 cells. *Exp Hematol* 1987, **15**, 414–418.
19. Ho CK. Synergistic anticellular effect of a combination of β-interferon and retinoic acid against U937 cells. *Cancer Res* 1985, **45**, 5348–5351.
20. Trinchieri G, Rosen M, Perussia B. Retinoic acid cooperates with tumor necrosis factor and immune interferon in inducing differentiation and growth inhibition of the human promyelocytic leukemic cell line HL-60. *Blood* 1987, **69**, 1218–1224.
21. Santini V, Delwel R, Löwenberg B. Terminal granulocytic maturation in acute myeloid leukemia (AML) following granulocyte colony stimulating factor (G-CSF) and retinoic acid (RA) stimulation. *Exp Hematol* 1989, **17**, 646.
22. Nakamaki T, Sakashita A, Sano M, *et al.* Granulocyte-colony stimulating factor and retinoic acid cooperatively induce granulocytic differentiation of acute promyelocytic leukemia cells *in vitro*. *Jpn J Cancer Res* 1989, **80**, 1077–1082.
23. Pick E, Charon J, Mizel D. A rapid densitometric microassay for nitroblue tetrazolium reduction and application of the microassay to macrophages. *J Reticuloendothel Soc* 1981, **30**, 581–593.
24. Bollag W. New retinoids with potential use in humans. In: Saurat JH, ed. *Retinoids: New Trends in Research and Therapy*. Basel, Karger, 1985, 274–288.
25. Chomienne C, Balitrand N, Cost H, Degos L, Abita JP. Structure-activity relationships of aromatic retinoids on the differentiation of the human histiocytic lymphoma cell line U-937. *Leukemia Res* 1986, **10**, 1301–1305.
26. Francis GE, Pinsky CM. Clinical trials of differentiation therapy: current trials and future prospects. In: Waxman S, Rossi GB, Takatu F, eds. *The Status of Differentiation Therapy of Cancer*. New York, Raven Press, 1988, 331–347.