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In vivo effects of interleukin-11 and stem cell factor in combination with erythropoietin in the regulation of erythropoiesis

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Summary. In this study we evaluated the *in vivo* effects of interleukin-11 (IL-11) and stem cell factor (SCF), in combination with erythropoietin (EPO) on murine erythropoiesis. Mice were treated for 7 d with IL-11, SCF and EPO, each at three dose levels. In total, 27 different dose combinations were tested. IL-11 as well as SCF could only marginally stimulate erythroid progenitor cell numbers, but IL-11 in combination with SCF was able to increase BFU-E and CFU-E numbers 4-fold, in the absence of exogenous EPO. This resulted in an increased reticulocyte count. In contrast with the stimulatory effect on immature erythroid cell stages, IL-11 treatment induced a mild anaemia, which probably resulted from a plasma volume expansion. The additional treatment with EPO resulted in strong synergistic effects on CFU-E numbers. The combination of high-dose IL-11 and high-dose SCF was able to increase the overall efficiency of EPO-induced erythroid amplification, which was reflected by a left-shift of the *in vivo* EPO dose–response curve. The stimulating effects of IL-11 and SCF were further demonstrated when the effects on the reticulocyte count of a single high-dose EPO injection were assessed in normal and SCF + IL-11 treated mice. Whereas a single EPO dose increased the reticulocyte count by a factor of 3, IL-11 + SCF pretreatment increased this to a factor of 7. This study shows that *in vivo* SCF and IL-11 are important modulators of red blood cell production. First, these factors probably increase the input from the stem cell compartment into the erythroid lineage, where subsequently EPO is required for further amplification. Additionally, however, IL-11 and SCF increase the overall efficiency of EPO-induced amplification, probably due to a stimulatory effect on late-stage erythroid cells and to a redistribution of cells from marrow to spleen.

Keywords: erythropoiesis, IL-11, SCF, EPO.

Haemopoietic cell production is governed by a complex network of regulatory molecules which can synergize or antagonize with each other. Some of these factors have very restricted potencies, whereas others have more pleiotropic effects. One of these multifunctional factors is interleukin-11 (IL-11), a recently discovered cytokine (Paul *et al*, 1990). IL-11 is produced by stromal cells and has been reported to stimulate primitive multipotent and myeloid (Mushashi *et al*, 1991a, b; Leary *et al*, 1992; Tsuji *et al*, 1992), megakaryocytic (Teramura *et al*, 1992; Bruno *et al*, 1991) and erythroid (Quesniaux *et al*, 1992) progenitor cells *in vitro* (Du & Williams, 1994). As a further illustration of its pleiotropic nature, it has also been demonstrated that IL-11 increases the recovery of intestinal epithelial damage after chemotherapy of mice (Du *et al*, 1994). Clinically one of its most promising applications is considered to be its ability to shorten the period of thrombocytopenia resulting from irradiation or chemotherapy. IL-11 administration to normal and myelosuppressed mice has shown to stimulate platelet production (Neben *et al*, 1993; Hangoc *et al*, 1993; Du *et al*, 1993b; Leonard *et al*, 1994). In agreement with *in vitro* results, *in vivo* IL-11 administration also affects other lineages. Within 24 h after injection of normal mice, the cycling activity and the frequency of early myeloid and erythroid progenitors was increased (Hangoc *et al*, 1993). In normal mice, IL-11 induced a mild neutrophilia (Neben *et al*, 1993; Hangoc *et al*, 1993) which resulted in slightly reduced neutropenic periods when administered to myelosuppressed mice (Hangoc *et al*, 1993; Du *et al*, 1993b). The effects of

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IL-11 on *in vivo* erythropoiesis are ambiguous; transplantation of lethally irradiated mice with bone marrow cells, transfected with the IL-11 gene and expressing high levels of IL-11, reduced the degree and duration of the anaemia (Paul *et al*, 1994). Also IL-11 administration to irradiated and carboplatin-treated mice significantly improved the recovery of the haematocrit (Leonard *et al*, 1994). On the other hand, IL-11 administration to normal mice did not result in changes of the haematocrit or reticulocyte count (Neben *et al*, 1993). Also, no effect of IL-11 treatment could be demonstrated on CFU-E and BFU-E numbers of mice which had undergone total body irradiation and a bone marrow transplantation (Du *et al*, 1993a). When IL-11 was administered to cynomolgus monkeys (Mason *et al*, 1993) or humans (Gordon *et al*, 1993), however, a moderate anaemia was observed.

In vitro and *in vivo*, optimal blood cell production is achieved by combinations of different cytokines. Therefore in the therapeutic application of growth factors the most favourable effects can be expected when cocktails of factors are administered. Also the *in vivo* effects of IL-11 can be expected to be most pronounced and most physiological when combined with other factors. In fact, this may be the reason for the observed discrepancies of the *in vivo* erythroid effects of IL-11. IL-11 administration to normal mice analyses the effects of IL-11 alone, whereas IL-11 administration to myelosuppressed mice determines the effects of IL-11 in combination with an array of endogenously produced cytokines. To test this hypothesis and to assess the role of IL-11 in erythropoiesis, we determined in the present study the effect of IL-11 in combination with stem cell factor (SCF) and erythropoietin (EPO) on the regulation of *in vivo* erythropoiesis. SCF, like IL-11, is a growth factor produced by stromal cells. Similar to IL-11, SCF has been demonstrated to stimulate primitive (de Vries *et al*, 1991), megakaryoid (Briddel *et al*, 1991), erythroid (Dai *et al*, 1991) and granuloid (Metcalf & Nicola, 1991) progenitors *in vitro*. In normal mice we have shown that SCF transiently increases the number of BFU-E, CFU-E and reticulocytes. We also demonstrated *in vivo* synergistic interactions between SCF and EPO, especially in the spleen (de Haan *et al*, 1995). Whereas IL-11 and SCF are locally produced in the bone marrow environment, EPO, being regarded as the main systemic regulator of erythrocyte formation, is predominantly produced in the kidneys. Little is known about the regulation of SCF and IL-11 concentration in the serum, or locally within the microenvironment. The regulation of EPO levels, in contrast, has been relatively well established. This level can be continuously elevated in severe anaemic situations, but also sharp transient rises can occur after certain insults (Nijhof *et al*, 1993b).

In this study we investigated in detail *in vivo* erythroid cell production in normal mice treated with different dose combinations of three growth factors: IL-11, SCF and EPO. This study reveals that IL-11 and SCF have major effects on *in vivo* erythropoiesis. In combination, these stromal factors are very potent in optimizing the efficacy of EPO, thus enabling increased erythroid amplification.

MATERIALS AND METHODS

Mice. In all experiments female C57Bl/6 mice, 12–16 weeks old, weighing 20–25 g were used. Each data point in the figures was obtained by analysing individually two to five mice per group.

Haemopoietic growth factors. Recombinant rat pegylated SCF was donated by Amgen (Thousand Oaks, Calif.). Recombinant human IL-11 was a gift from Genetics Institute (Cambridge, Mass.). Recombinant human EPO was a donation from Boehringer Mannheim (Almere, the Netherlands).

Administration of growth factors. Growth factors were appropriately diluted, mixed, and administered by subcutaneously implanted osmotic mini pumps (type Alzet 1007D or 2002, Alza Corporation, Palo Alto, Calif.) to avoid high variation in serum cytokine levels. To test whether the implantation of the pump itself affected haematological values, we assessed the effect of a 7 and a 14 d implantation of a pump filled with saline. No changes compared to normal untreated mice were observed (data not shown). Mice were treated with 0, 0·5 or 2·0 μg IL-11/d in combination with 0, 1 or 2·5 μg SCF/d, and in combination with 0, 2.5 or 25 U EPO/d. In total, $3 \times 3 \times 3 = 27$ different dose combinations were thus tested. The dosages were carefully selected, according to previously published or preliminary experiments done in our group (Nijhof *et al*, 1993a; de Haan *et al*, 1995). For each factor, one dose was chosen with medium activity and one with high activity. Thus the complete dose–response range of individual, exogenously administered, growth factors was covered. Since SCF and IL-11 have been reported to act on early cells, and we wanted to evaluate the effects on the entire erythroid pathway, a prolonged, 7 d treatment protocol was used.

Progenitor cell assays and calculation of total body cell numbers. Femur and spleen single-cell suspensions were made according to standard procedures. BFU-E and CFU-E were grown in α-medium in 1·2% and 0·8% methylcellulose respectively, supplemented with 10 mM HEPES, 25 mM $NaHCO_3$ and 30% FCS. In addition, BFU-E were stimulated with 100 ng rrSCF/ml, 10 ng rmGM-CSF/ml (supplied by Behringwerke, Marburg, Germany) and 2 U rhEPO/ml. CFU-E were stimulated with 500 mU/ml rhEPO. These culturing conditions resulted in optimal colony growth. Since the aim of this study was to evaluate the effect of growth factors on the total, whole animal, production of erythroid cell stages, the number of the progenitors in marrow and spleen were added and shown in the figures as total cell numbers. This also facilitates the comparison with the reticulocyte and haematocrit data. Total body cell numbers were calculated as we have reported before (Nijhof *et al*, 1993a; de Haan *et al*, 1992). Femur cellularity was assumed to represent 6% of total marrow cellularity (Chervenick *et al*, 1968; Briganti *et al*, 1970). Total body haemopoietic cellularity was calculated by adding total marrow cellularity and spleen cellularity. Thus changes in femur and spleen cellularity are reflected in this calculation.

Reticulocytes. The percentage of reticulocytes was microscopically determined after staining the cells with brilliant-

cresyl-blue. A minimum of 500 cells were evaluated. In the combination study, mice were bled from the orbital plexus, before they were killed. In the time-course study, 10 μl of blood was obtained from the tip of the tail by a micropipet.

Haematocrit. The haematocrit was determined with haematocrit capillaries according to standard procedures.

RESULTS

Effects of a combined IL-11, SCF and EPO treatment during 7 d

Effects on total BFU-E numbers. SCF alone dose-dependently increased the number of BFU-E approximately two-fold. IL-11 as a single factor had hardly any affect on BFU-E (Fig 1a). In mice which were treated with combinations of SCF + IL-11, additive effects were observed. Maximal BFU-E numbers, 3–4-fold normal values, were obtained with 2·5 μg SCF in combination with 0·5 or 2 μg IL-11. When the additional effect of EPO was evaluated, it appeared that EPO, both 2·5 U and 25 U, alone or in any combination, only marginally further increased BFU-E numbers (Figs 1b and 1c). The slope and the magnitude of the three dose–response surfaces shown in Fig 1 were very similar, whether EPO was present or not.

Effects on total CFU-E numbers. SCF and IL-11 influenced the numbers of CFU-E similarly to BFU-E numbers (Fig 2a). Alone, both factors had mild stimulatory activity. In combination, SCF and IL-11 had additive effects, resulting in a 4-fold increased total CFU-E number at the highest SCF and medium or high IL-11 dose combination. It should be stressed that this is achieved without exogenously administered EPO. In contrast to the response seen in BFU-E numbers, however, the additional inclusion of EPO in the treatment protocol further augmented CFU-E numbers. The addition of only 2·5 U EPO, which by itself had hardly any effect on CFU-E numbers, strongly stimulated the production of this cell type, but almost exclusively when combined with 2·5 μg SCF + 2·0 μg IL-11 (Fig 2b). Remarkably, the number of CFU-E detected in this group was far higher than all other combinations, reaching values of approximately 17 times normal. Most of these CFU-E (95%) were located in the spleen (data not shown). This highly synergistic phenomenon was also observed when the EPO dose was increased to 25 U (Fig 2c). This EPO dose alone increased CFU-E numbers by a factor of 9. In mice receiving 2·5 μg SCF 2 μg IL-11 + 25 U EPO, CFU-E numbers exceeded values of 60 times normal.

Effects on the CFU-E/BFU-E ratio. To determine the efficiency of EPO-induced *in vivo* erythroid amplification, a correction should be made for the altered input into the CFU-E compartment from the BFU-E stages. Therefore the ratio of the total number of CFU-E to BFU-E as a function of the EPO dose was calculated for mice which had been treated with EPO alone, and mice which had been treated with EPO + 2·5 μg SCF + 2μg IL-11. Fig 3 shows the obtained EPO dose–response curves and demonstrates that, particularly at low EPO doses, the erythroid amplification was increased by SCF + IL-11. The number of CFU-E produced per BFU-E at 2·5 U EPO was not different from normal values in control mice. In SCF + IL-11-treated mice, this EPO dose,

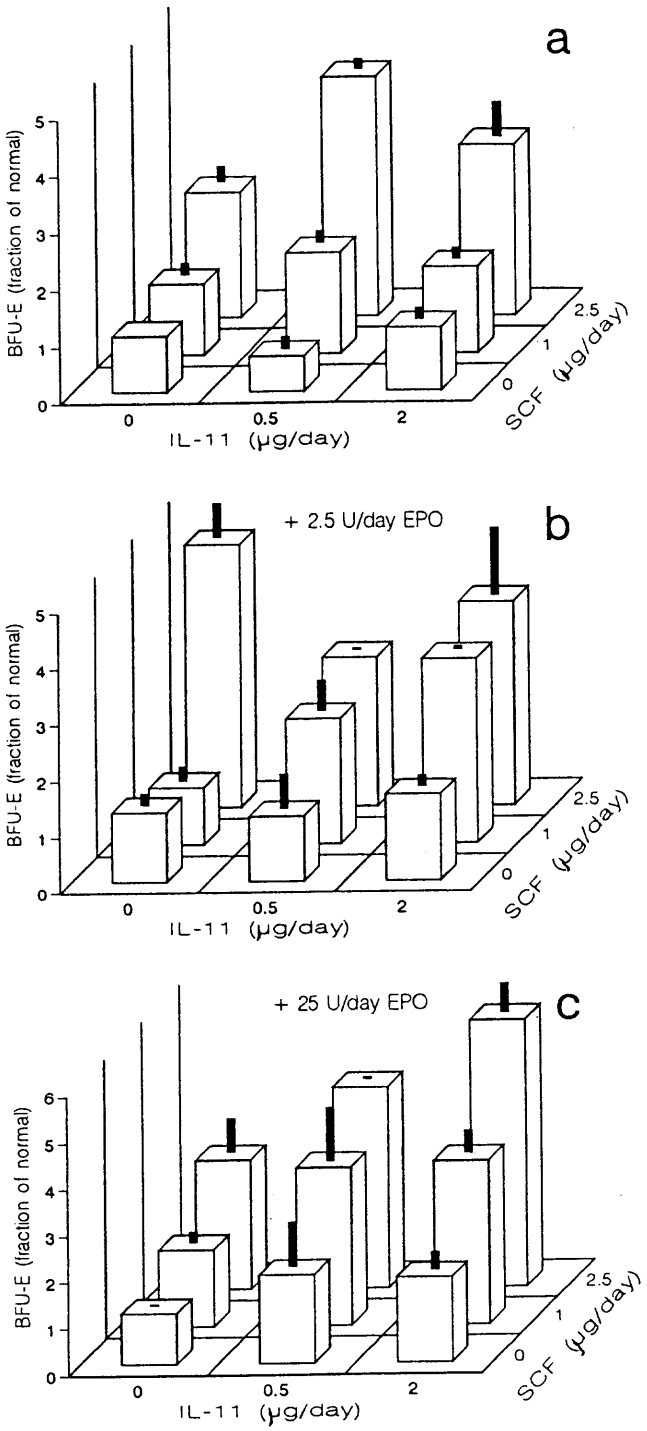

Fig 1. Dose–response surface showing the effect on total animal (marrow + spleen) BFU-E numbers of a 7 d treatment with combinations of SCF and IL-11, either alone (a) or in combination with 2·5 (b) or 25 (c) U EPO/d. BFU-E numbers are shown as fraction of normal, untreated control mice, +1 SEM. Normal BFU-E values were $79{\cdot}9 \pm 10{\cdot}2 \times 10^3$ ($n = 4$).

however, resulted in a 5-fold increase in the number of CFU-E per BFU-E. At high EPO doses, these effects were less pronounced, because EPO alone already resulted in strong stimulation.

Effects on reticulocytes. SCF alone had a mild stimulatory effect on the percentage of reticulocytes in the peripheral

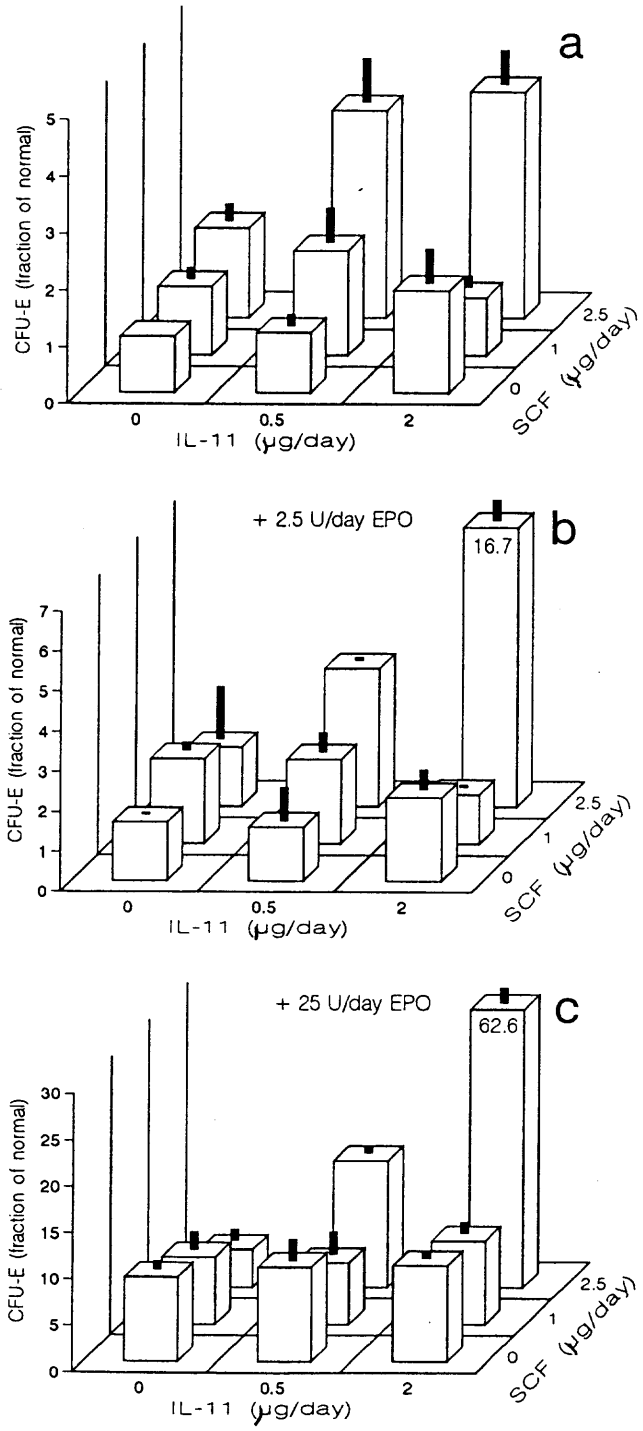

Fig 2. Dose–response surface showing the effect on total animal (marrow + spleen) CFU-E numbers of a 7 d treatment with combinations of SCF and IL-11, either alone (a) or in combination with 2·5 (b) or 25 (c) U EPO/d. CFU-E numbers are shown as fraction of normal, untreated control mice, +1 SEM. Normal CFU-E values were $264{\cdot}3 \pm 33 \times 10^3$ ($n = 4$). Note that the y-axis differs in each figure, and that in (b) and (c) the values obtained for the highest dose combinations are beyond the y-axis scaling and are therefore printed in the bars.

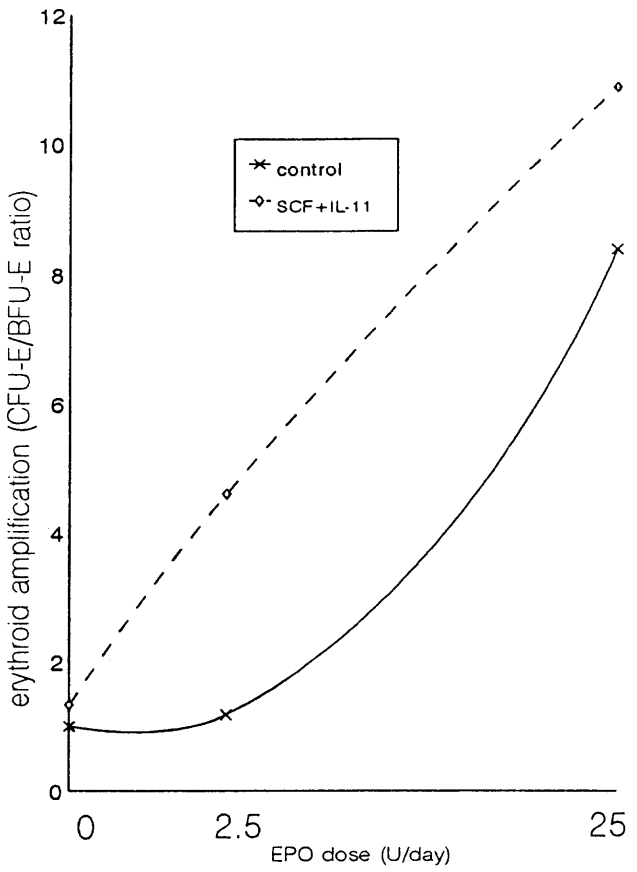

Fig 3. Erythroid amplification after 7 d of EPO treatment, given as the ratio of normalized total CFU-E numbers to normalized total BFU-E numbers, of control and 2·5 μg SCF +2·0 μg IL-11-treated mice.

blood. IL-11, however, dose-dependently induced a marked reticulocytosis. No additive effects of SCF + IL-11 were observed in the reticulocyte count (Fig 4a). This additive effect became apparent however when 2·5 and 25 U EPO were coadministered (Figs 4b and 4c). The highest reticulocyte counts were obtained consistently in mice which were treated with high dose SCF and IL-11. The exceptional increases in CFU-E numbers in the highest dose combinations were, however, not translated in the reticulocyte count. Also, it is evident from Fig 4(c) that high EPO doses overruled to some extent the effects of SCF + IL-11 on the reticulocytes.

Effects on haematocrit. In contrast to the stimulatory activity of IL-11 on all previous erythroid parameters, IL-11 induced a mild anaemia (Fig 5a). This anaemia was dose-dependent, reaching values of 38% after 7 d of 2 μg IL-11. SCF was able to reduce the degree of anaemia to some extent, but only a high EPO dose was capable of preventing it (Figs 5b and 5c). At the lowest EPO dose also, SCF was necessary for this prevention. Even if the anaemia could completely be prevented at 25 U EPO doses, the suppressive effect of IL-11 on the haematocrit was still clearly demonstrated. The highest haematocrits were consistently obtained with the combination of only SCF + EPO.

Time course study of the effects on reticulocytes of a single injection with high-dose EPO in normal or SCF + IL-11-treated mice

In order to mimic an erythroid insult, and in addition to

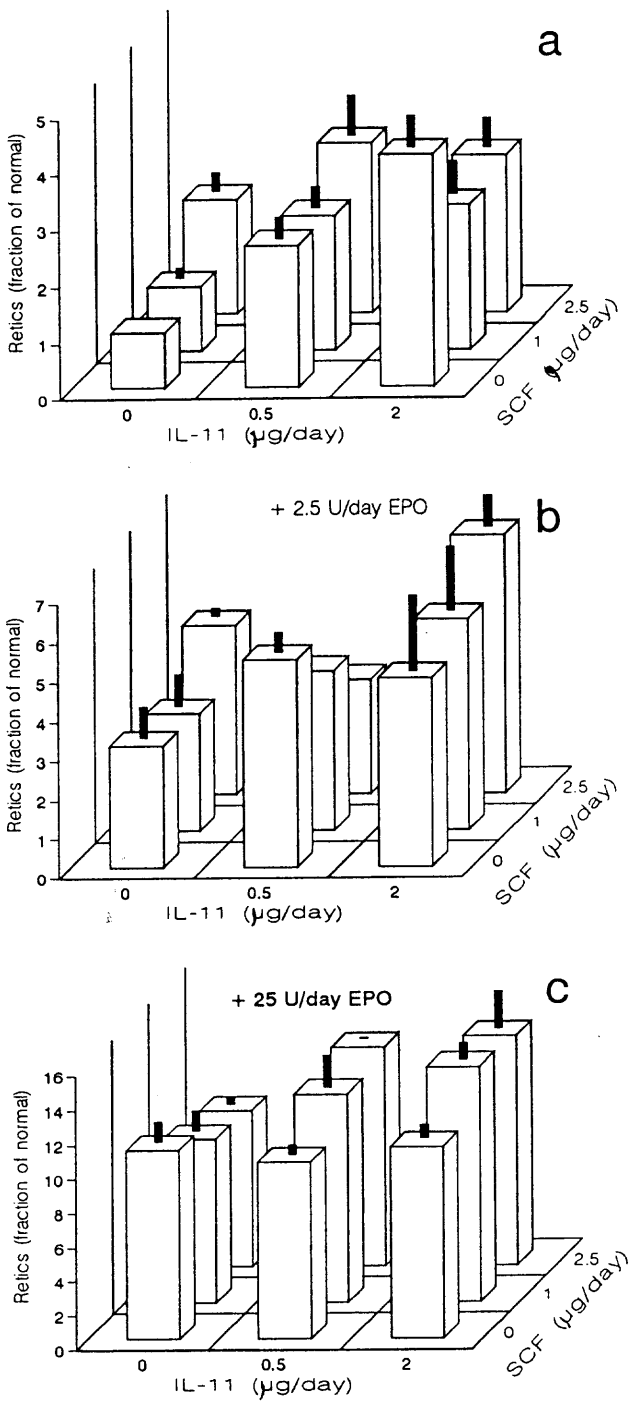

Fig 4. Dose–response surface showing the effect on the percentage of reticulocytes in the peripheral blood of a 7 d treatment with combinations of SCF and IL-11, either alone (a) or in combination with 2·5 (b) or 25 (c) U EPO/d. Reticulocyte values are shown as fraction of normal, untreated control mice, +1 SEM. Normal reticulocytes values were 1·73 ± 0·6% ($n = 4$).

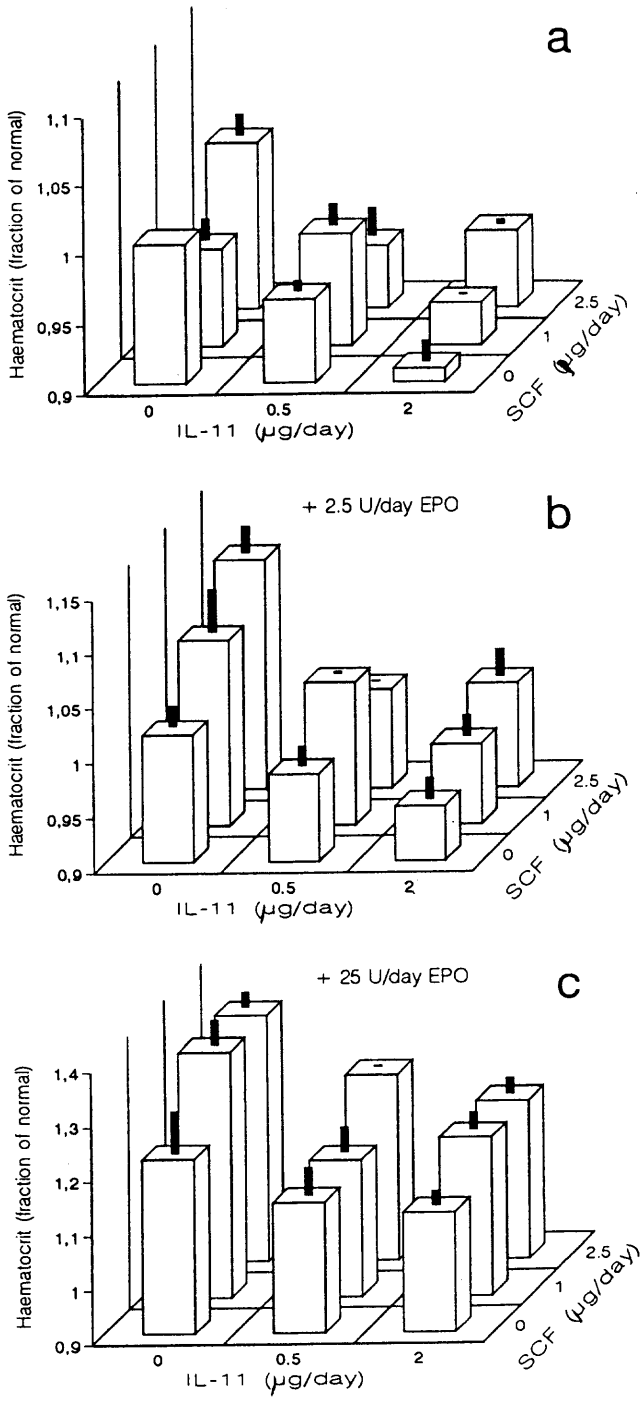

Fig 5. Dose–response surface of the effect on haematocrit of a 7 d treatment with combinations of SCF and IL-11, either alone (a) or in combination with 2·5 (b) or 25 (c) U EPO/d. Haematocrit values are shown as fraction of normal, untreated control mice, +1 SEM. Normal haematocrit values were 44·5 ± 0·6% ($n = 4$).

determine the time dependence of the effects of SCF + IL-11, the effects of a single i.v. injection with high-dose (50 U) EPO on the reticulocyte count was determined in normal and continuously (12 d) 2·5 μg SCF + 2·0 μg IL-11-treated mice (Fig 6). IL-11 + SCF administration resulted in a rapid reticulocytosis. Within 4 d the effects appeared to be maximal and a new steady state was reached. Upon injection of 50 U EPO, normal mice responded with increases in the percentage of reticulocytes to approximately 3-fold at day 3 after injection. Mice which were continuously treated with SCF + IL-11 responded to this challenge much stronger in absolute terms. 3 d after i.v. EPO injection the reticulocyte count had increased 7-fold.

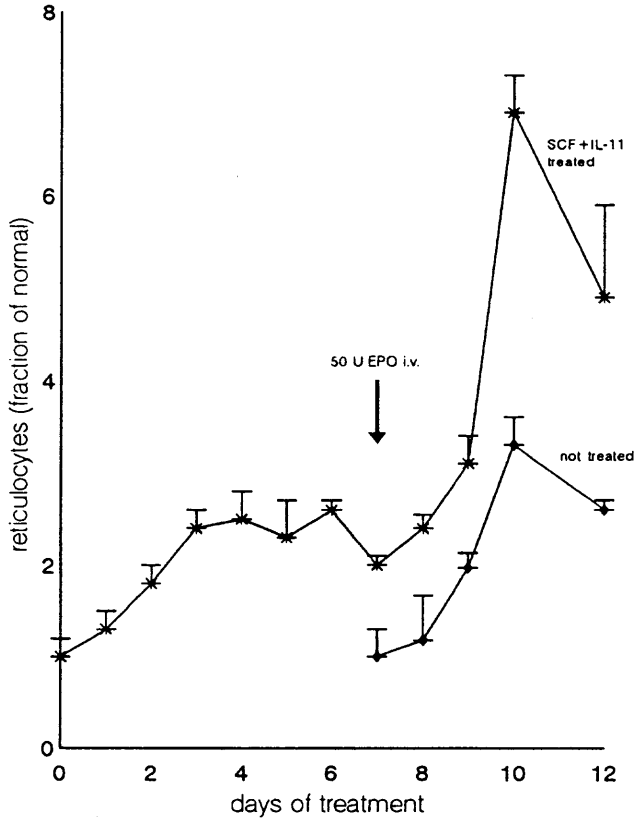

Fig 6. The effect of a single i.v. EPO injection (50 U) at day 7, on reticulocytes, in normal (◆) or continuously (12 d) 2·5 μg SCF + 2·0 μg IL-11 (*) treated mice. Reticulocytes are expressed as fraction of normal +1 SEM.

Relatively, however, this was also about a 3-fold increase, because the steady-state erythropoiesis (measured as reticulocyte percentage) of these mice was already 2-fold above normal.

DISCUSSION

Although it has been clearly established that EPO is a major regulatory growth factor for *in vivo* erythrocyte production, many other cytokines also have erythroid stimulatory activity (Quesniaux *et al*, 1992; Kurtz *et al*, 1988; Muta & Krantz, 1993; Donahue *et al*, 1990). Some of this stimulatory activity may be attributed to effects on early cell compartments; increasing the input into the erythroid (erythropoietin-sensitive) pathway will increase erythrocyte production, particularly when such an early-acting factor is combined with EPO. Also, some of the stimulatory activity of several cytokines on erythropoiesis may, however, be caused by effects on late-stage erythropoietin-sensitive cells.

In this study we demonstrate that *in vivo* SCF and IL-11 are important co-regulators of erythropoiesis. Optimal high doses of both stromal factors increased the efficacy of EPO-induced amplification, which resulted in a left shift of the *in vivo* EPO dose–response curve. This was revealed by the pronounced stimulating effect of low-dose, 2·5 U, EPO on CFU-E numbers. The finding that SCF and IL-11 interfere with the *in vivo* EPO dose–response curve may imply that some of the observed effects of these factors take place in late-stage EPO-sensitive cells. Also, however, part of this observation may be attributed to a redistribution of cells from marrow to spleen. We have demonstrated that erythroid amplification is more efficient in spleen than in marrow (Nijhof *et al*, 1993a).

In addition, our data show that the increase of CFU-E numbers at a high EPO dose (25 U) could be further potentiated by simultaneous SCF + IL-11 treatment. The CFU-E/BFU-E ratio calculation shows that at this dose, EPO, as a single factor, is capable of stimulating erythroid amplification close to optimal (which is in agreement with our previously published data (Nijhof *et al*, 1993a)). This suggests that the enhanced erythroid response in terms of CFU-E numbers is also due to an increased input into the erythroid lineage by SCF and IL-11. This implies that both factors also act on more primitive cells, which is in agreement with the stimulating effects on BFU-E numbers that we observed.

Since an important effect of EPO is to prevent apoptosis of EPO-sensitive cells (Koury & Bondurant, 1990), the increased input into the erythroid pathway induced by SCF + IL-11 may only be exploited optimally under elevated EPO levels. If SCF + IL-11 alone are administered, many CFU-E *in vivo* may in fact die, assuming that the normal serum EPO concentration is not sufficiently high to prevent apoptosis of all CFU-E (Nijhof *et al*, 1995).

A discrepancy was observed between the magnitude of CFU-E expansion, which increased 60-fold in response to high EPO doses, and the rise of peripheral reticulocytes which rose only 16-fold. This may be partly explained by the fact that we have assessed both cell types at the same day, whereas the progeny of CFU-E measured on day 7 may only appear in the peripheral blood 2–3 d later. In addition, however, it is obvious that the recticulocyte percentage, which is normally 1–2%, cannot increase 60-fold, as we have observed for total CFU-E numbers. It should be realized that the reticulocyte percentage is only an indication of activity of proliferation of cells before that stage. Quantitatively, this parameter is difficult to interpret, because of possible changes in maturation times, bone marrow release times, and, more importantly, the differences in lifespan between reticulocytes and erythrocytes.

The time-course study was aimed to test the system's rapid dynamics. SCF + IL-11 were able to shift erythropoiesis to a new steady rate within 4–6 d. These mice were able to respond much stronger in absolute numbers to a single high-dose EPO injection than normal mice.

In the literature, evidence is available showing that *in vitro* IL-11 and SCF are able to act on both primitive cells and late-stage erythroid cells. First, both factors have been shown to stimulate multipotent cells (Mushashi *et al*, 1991a, b; Tsuji *et al*, 1992; de Vries *et al*, 1991). Thus, when combined with the lineage-restricted growth factor EPO, an increased input into the erythroid pathway will be multiplicated. In fact, we have demonstrated this *in vivo* synergistic phenomenon previously for SCF and EPO (de Haan *et al*, 1995). Also,

however, both IL-11 and SCF have been reported to act on late-stage erythropoiesis. Quesniaux *et al* (1992) have clearly demonstrated that a fraction of murine or fetal CFU-E only requires IL-11 (no EPO) for full amplification and haemoglobinization. Interestingly, in their study, optimal CFU-E seeding efficiency was obtained with high doses of EPO; the addition of IL-11 did not further increase CFU-E growth at high EPO doses. At low EPO doses, IL-11 and EPO acted additively; thus, like in our *in vivo* situation, left-shifting the EPO dose–response curve.

Similarly, SCF has been shown to be able to reduce, to some extent, the percentage of apoptotic, EPO-derived CFU-E (Muta & Krantz, 1993). Thus there are indications that SCF and IL-11 act throughout the erythroid development.

Although our study suggests that for optimal erythroid stimulation SCF, IL-11 and EPO are required, we consistently found that IL-11 had a negative effect on the haematocrit. EPO and SCF were capable of restoring this anaemia, but in all dose combinations tested IL-11 dose-dependently reduced the haematocrit. Recently it has been demonstrated that IL-11 administration to normal human subjects increased the plasma volume (Ault *et al*, 1994). It is likely that this explains the anaemia that we observed in our study, given the stimulatory effects of IL-11 on each erythroid cell stage. A similar effect has also been encountered for IL-6 (Herodin *et al*, 1992). IL-11 and IL-6 have many biological effects in common, due to a shared receptor subunit (Yin *et al*, 1993). At present, IL-11 has only been reported to induce an anaemia in primates (Mason *et al*, 1993; Gordon *et al*, 1993; Ault *et al*. 1994). It is not clear to us why this effect was not observed by Neben *et al*, 1993). It cannot be attributed to mice strain differences, because both studies use C57Bl/6. Also dosages were comparable. The only possibility may be the mode of administration. Instead of injecting twice daily, we prefer administration by osmotic minipumps, in order to avoid major serum level changes. The effects on platelet counts that we observed in this study were slightly more pronounced than described by Neben *et al* (1993) (data not shown), indicating that our IL-11 administration may be more effective. This was confirmed in recent studies done in their laboratory (Samuel Goldman, personal communication). This may also explain why we observed an IL-11 dose-dependent increase in the reticulocyte count, which was also not reported by Neben. We believe that this is a direct stimulating effect of IL-11 and is not caused indirectly by endogenous EPO, which could have been produced in response to the observed anaemia. First, we measured EPO-levels with an appropriate EPO-ELISA (Medac, Hamburg, Germany) but failed to demonstrate any increase (data not shown). Also, the anaemia is relatively mild and may not be sufficient to stimulate EPO production. A further hint that IL-11 is directly stimulating reticulocyte production comes from the observations in the time-course study. Here it was demonstrated that during the first 4 d of IL-11 and SCF treatment the reticulocytes increased already.

Our data are not in contrast with studies showing an increase in the recovery of the haematocrit after irradiation or chemotherapy induced by IL-11 (Leonard *et al*, 1994; Paul *et al*, 1994). The effect of IL-11 on the plasma volume may become most apparent in normal mice. If mice are myelosuppressed and thus already anaemic, the stimulating effects of IL-11 on erythropoiesis may dominate, especially since in these mice IL-11 will interact with many endogenously produced cytokines. Du *et al* (1993a) have reported on the effects of SCF + IL-11 administration after irradiation and bone marrow transplantation. Our data are in agreement with their findings that SCF alone increased the haematocrit pronouncedly. In conclusion, our data indicate that SCF and IL-11 in combination play a major role in determining the *in vivo* EPO dose–response. These stromal factors seem to be able to shift erythropoiesis to a higher steady state level, where subsequently renal EPO is required for optimal exploitation of erythroid amplification.

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REFERENCES

Ault, K.A., Mitchell, J., Knowles, C., Clendamiel, A., Egan, A., Loewy, J., Garnick, M.B. & Kaye, J.A. (1994) Interleukin-11 (neumega rhIL-11 growth factor) increases plasma volume and decreases urinary sodium excretion in normal human subjects. *Blood*, **84**, 276a.

Briddel, R., Bruno, E., Looper, R.J., Brandt, J.E. & Hoffman, R. (1991) Effect of c-kit ligand on *in vitro* human megakaryocytopoiesis. *Blood*, **78**, 2854–2859.

Briganti, G., Covelli, V., Silini, G. & Srivastava, P.N. (1970) The distribution of erythropoietic bone marrow in the mouse. *Acta Haematologica*, **44**, 355–360.

Bruno, E., Briddel, R. & Hoffman, R. (1991) Effects of recombinant interleukin 11 on human megakaryocyte progenitor cells. *Experimental Hematology*, **19**, 378–381.

Chervenick, P.A., Boggs, D.R., Marsh, J.C., Cartwright, G.E. & Wintrobe, M.M. (1968) Quantitative studies of blood and bone marrow neutrophils in normal mice. *American Journal of Physiology*, **215**, 353–359.

Dai, C.H., Krantz, S.B. & Zsebo, K.M. (1991) Human burst forming units-erythroid need direct interaction with stem cell factor for further development. *Blood*, **78**, 2493–2497.

de Haan, G., Dontje, B., Nijhof, W. & Loeffler, M. (1995) Effects of continuous stem cell factor administration on normal and erythropoietin-stimulated murine hemopoiesis: experimental results and model analysis. *Stem Cells*, **13**, 65–76.

de Haan, G., Loeffler, M. & Nijohf, W. (1992) Long-term recombinant human granulocyte colony-stimulating factor (rhG-CSF) treatment severely depresses murine marrow erythropoiesis without causing an anemia. *Experimental Hematology*, **20**, 600–604.

de Vries, P., Brasel, K.A., Eisenman, J.R., Alpert, A.R. & Williams, D.E. (1991) The effect of recombinant mast cell growth factor in purified murine hematopoietic stem cells. *Journal of Experimental Medicine*, **173**, 1205–1211.

Donahue, R.E., Yang, Y.-C. & Clark, S.C. (1990) Human P40 T-cell growth factor (interleukin-9) supports erythroid colony formation. *Blood*, **75**, 2271–2275.

Du, X.X., Doerschuk, C.M., Orazi, A. & Williams, D.A. (1994) A bone marrow stromal-derived growth factor, interleukin-11, stimulates recovery of small intestinal mucosal cells after cytoablative therapy. *Blood*, **83**, 33–37.

Du, X.X., Keller, D., Maze, R. & Williams, D.A. (1993a) Comparative effects of *in vivo* treatment using interleukin-11 and stem cell

factor on reconstitution in mice after bone marrow transplantation. *Blood*, **82**, 1016–1022.

Du, X.X., Neben, T., Goldman, S. & Williams, D.A. (1993b) Effects of recombinant human interleukin-11 on hematopoietic reconstitution in transplant mice: acceleration of recovery of peripheral blood neutrophils and platelets. *Blood*, **81**, 27–34.

Du, X.X. & Williams, D.A. (1994) Interleukin-11: a multifunctional growth factor derived from the hematopoietic mircoenvironment. *Blood*, **83**, 2023–2030.

Gordon, M.S., Battiato, L., Hoffman, R., Breeden, C., McCaskill-Stevens, W.J., Kuca, B., Kaye, J. & Sledge, G.W. (1993) Subcutaneously (SC) administered recombinant human interleukin-11 (neumegaTM rhIL-11 growth factor; rhIL-11) prevents thrombocytopenia following chemotherapy (CT) with cyclophosphamide (C) and doxorubicin (A) in women with breast cancer. *Blood*, **82**, 318a (abstr. suppl.1).

Hangoc, G., Yin, T., Cooper, S., Schendel, P., Yang, Y.-C. & Broxmeyer, H.E. (1993) *In vivo* effects of recombinant interleukin-11 on myelopoiesis in mice. *Blood*, **81**, 965–972.

Herodin, F., Mestries, J.-C., Janodet, D., Martin, S., Mathieu, J., Gascon, M.-P., Pernin, M.-O. & Ythier, A. (1992) Recombinant glycosylated human interleukin-6 accelerates peripheral blood platelet count recovery in radiation-induced bone marrow depression in baboons. *Blood*, **80**, 688–995.

Koury, M.J. & Bondurant, M.C. (1990) Erythropoietin retards DNA breakdown and prevents programmed cell death in erythroid progenitor cells. *Science*, **248**, 378–382.

Kurtz, A., Zapf, J., Eckardt, K.U., Clemons, G., Froesch, E.R. & Bauer, C. (1988) Insulin-like growth factor I stimulates erythropoiesis in hypophysectomized rats. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 7825–7829.

Leary, A.G., Zeng, H.Q., Clark, S.C. & Ogawa, M. (1992) Growth factor requirements for survival in Go and entry into the cell cycle of primitive human hemopoietic progenitors. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 4013–4017.

Leonard, J.P., Quinto, C.M., Kozitza, M.K., Neben, T.Y. & Goldman, S.J. (1994) Recombinant human interleukin-11 stimulates multilineage hematopoietic recovery in mice after a myelosuppressive regimen of sublethal irradiation and carboplatin. *Blood*, **83**, 1499–1506.

Mason, L., Timony, G., Perkin, C., Badalone, V., Bree, A., Goldman, S., Hayes, L. & Schaub, R. (1993) Human recombinant interleukin-11 promotes maturation of bone marrow megakaryocytes in non-human primates: an electron microscopic study. *Blood*, **82**, 69a (abstr. suppl. 1).

Metcalf, D. & Nicola, N.A. (1991) Direct proliferative action of stem cell factor on murine bone marrow cells *in vitro*: effects of combinations with colony stimulating factors. *Proceedings of the National Academy of Sciences of the United States of America*, **88**, 6239–6243.

Musashi, M., Clark, S.C., Sudo, T., Urdal, D.L. & Ogawa, M. (1991a) Synergistic interactions between interleukin-11 and interleukin-4 in support of proliferation of primitive hematopoietic progenitors of mice. *Blood*, **78**, 1448–1451.

Musashi, M., Yang, Y.-C., Paul, S.R., Clark, S.C., Sudo, T. & Ogawa, M. (1991b) Direct and synergistic effects of interleukin-11 on murine hemopoiesis in culture. *Proceedings of the National Academy of Sciences of the United States of America*, **88**, 765–769.

Muta, K. & Krantz, S.B. (1993) Apoptosis of human erythroid colony-forming cells is decreased by stem cell factor and insulin-like growth factor I as well as erythropoietin. *Journal of Cell Physiology*, **156**, 264–271.

Neben, T.Y., Loebelenz, J., Hayes, L., McCarthy, K., Stoudemire, J., Schaub, R. & Goldman, S.J. (1993) Recombinant human interleukin-11 stimulates megakaryocytopoiesis and increases peripheral platelets in normal and spelenectomized mice. *Blood*, **81**, 901–908.

Nijhof, W., de Haan, G., Pietens, J. & Dontje, B. (1995) Mechanistic options of erythropoietin-stimulated erythropoiesis. *Experimental Hematology*, **23**, 369–375.

Nijhof, W., Goris, H. Dontje, B., Dresz, J. & Loeffler, M. (1993a) Optimalization of murine erythroid cell production occurs by exploiting the splenic microenvironment. *Experimental Haematology*, **21**, 496–501.

Nijhof, W., Goris, H. & Hartsuiker, H. (1993b) Production of erythropoietin during and after thiamphenicol treatment occurs independently of peripheral blood cell numbers. *Pathophysiology and Pharmacology of Erythropoietin* (Pagel/Weiss/Jelkmann eds), pp. 253–259. Springer, Berlin.

Paul, S.R., Bennett, F., Calvetti, J.A., Kelleher, K., Wood, C.R., O'Hara, R.M., Leary, A.C., Sibley, B., Clark, S.C., Williams, D.A. & Yang, Y.-C. (1990) Molecular cloning of a cDNA encoding interleukin-11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 7512–7516.

Paul, S.R., Haynes, L.L., Palmer, R., Morris, G.E., Neben, T.Y., Loebelenz, J., Pedneault, G., Brooks, J., Blue, I., Moore, M.A.S., Muench, M., Turner, K.J., Schaub, R., Goldman, S.J. & Wood, C.R. (1994) Interleukin-11 expression in donor bone marrow cells improves hematological reconstitution in lethally irradiated recipient mice. *Experimental Hematology*, **22**, 295–301.

Quesniaux, V.F.J., Clark, S.C., Turner, K. & Fagg, B. (1992) Interleukin-11 stimulates multiple phases of erythropoiesis in vitro. *Blood*, **80**, 1218–1223.

Teramura, M., Kobayashi, S., Hoshino, S., Oshimi, K. & Mizoguchi, H. (1992) Interleukin-11 enhances human megakaryocytopoiesis in vitro. *Blood*, **79**, 327–331.

Tsuji, K., Lyman, S.D., Sudo, T., Clark, S.C. & Ogawa, M., (1992) Enhancement of murine hematopoiesis by synergistic interactions between steel factor (ligand for c-kit), interleukin-11, and other early acting factors in culture. *Blood*, **79**, 2855–2860.

Yin, T., Taga, T., Tsang, M.L., Yasukawa, K., Kishimoto, T. & Yang, Y.-C. (1993) Involvement of IL-6 signal transducer gp130 in IL-11-mediated signal transduction. *Journal of Immunology*, **15**, 2555–2561.