



EDITORIAL

Interleukin 12: a new clinical player in cytokine therapy

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Interleukin 12 (IL-12) was identified several years ago, but its potential use in cancer therapy has only been recognised relatively recently. Animal studies have now shown that IL-12 has potent anti-tumour and antimetastatic activity, with less toxicity than that encountered with IL-2. In this editorial, we review the literature concerning the biology of IL-12, focusing on the areas of particular relevance to its possible role in cancer therapy. We propose that an important new cytokine has arrived for use in clinical oncology.

IL-12 (reviewed by Gately, 1993; Brunda, 1994) is a 70–75 kDa glycosylated cytokine which was simultaneously identified and purified from the supernatant of human B-lymphoblastoid cell lines by two independent groups and was initially described on the basis of its actions as 'cytotoxic lymphocyte maturation factor (CLMF)' (Gately *et al.*, 1986; Wong *et al.*, 1988; Stern *et al.*, 1990) and 'natural killer cell stimulatory factor (NKSF)' (Kobayashi *et al.*, 1989). It has an atypical structure for a cytokine, being heterodimeric and consisting of a 35 kDa (p35) subunit and a 40 kDa (p40) subunit linked by a disulphide bond. A mixture of soluble recombinant subunits also has biological activity but only at concentrations several orders of magnitude greater than that of the covalently linked heterodimer (Trinchieri, 1993). The genes for both the p35 and p40 subunits have been cloned (Gubler *et al.*, 1991; Wolf *et al.*, 1991) and mapped in humans to chromosomes 3p12–3q13.2 and 5q31–q33 respectively (Sieburth *et al.*, 1992). The predicted amino acid sequences of the human p35 and p40 IL-12 subunits show 60% and 70% identity with the murine sequences and consist of 20% and 10% carbohydrate respectively (Podlaski *et al.*, 1992; Schoenhaut *et al.*, 1992). Bioactivity studies, however, show species specificity, with human IL-12 exhibiting minimal activity in the murine system but with murine IL-12 being active in human systems. The use of interspecies p35/p40 hybrids suggests that this is determined by the p35 subunits (Schoenhaut *et al.*, 1992). However, as antibodies against the p40 subunit can neutralise IL-12 bioactivity, both subunits may be involved in receptor binding (Chizzonite *et al.*, 1991; D'Andrea *et al.*, 1992), either directly or possibly through one subunit influencing the conformational state of the other.

The main cellular sources of IL-12 are monocytes/macrophages, which secrete IL-12 constitutively and particularly following stimulation with bacterially derived products, and B cells and B-lymphoblastoid cell lines (D'Andrea *et al.*, 1992). Incubation of peripheral blood mononuclear cells (PBMCs) with *Staphylococcus aureus* Cowan I strain or lipopolysaccharide (LPS) increases production of IL-12 but a range of cytokines, including IL-1 α , IL-1 β , IL-2, IL-4, IL-6 and tumour necrosis factor alpha (TNF- α), do not elicit increased production (D'Andrea *et al.*, 1992), and IL-10 has been reported to inhibit synthesis of IL-12 by PBMCs

(D'Andrea *et al.*, 1992; Kubin *et al.*, 1994). Discordant production of the subunits has been demonstrated, indicating independent regulation, with excess uncomplexed p40 being produced by human PBMCs and B-lymphoblastoid cell lines, although production of free p35 has not been observed (Stern *et al.*, 1990; Wolf *et al.*, 1991; D'Andrea *et al.*, 1992). A range of solid tumour-derived, T-cell and myeloid leukaemic cell lines have been found not to secrete IL-12, although many of these cell lines contain mRNA transcripts of the p35 gene alone (D'Andrea *et al.*, 1992). Similarly, in the murine system, p40 mRNA has been found to be restricted to lymphoid tissues, whereas p35 mRNA is detected in both lymphoid and non-lymphoid (lung and brain) tissues (Schoenhaut *et al.*, 1992). However, mRNA for both subunits has been found in two murine thymic epithelial lines and a thymic fibroblastoid clone (Godfrey *et al.*, 1994), although it is not known whether these cell types secrete mature IL-12 protein. It is unclear whether the individual IL-12 subunits can exert as yet unidentified biological effects, but the murine p40 subunit is able to inhibit several biological activities of the IL-12 heterodimer (Mattner *et al.*, 1993). This may be analogous to the IL-1 family, in that IL-1 α and - β can bind to and elicit responses in target cells, but the third member, the IL-1 receptor antagonist, can bind to the receptor but does not induce a response and can competitively inhibit IL-1 α or - β . In effect, the excess of free p40 produced by some cells may serve to regulate the biological activity of IL-12.

A high-affinity 110–135 kDa receptor with a K_d of 100–600 pM has been found on activated human T cells and NK cells but not on B cells, resting PBMCs or several cell lines, including those of T, NK, B, myelomonocytic, epithelial and fibroblast lineage (Chizzonite *et al.*, 1992; Desai *et al.*, 1992). Subsequently, a more detailed analysis has identified three binding sites on phytohaemagglutinin (PHA) activated PBMCs with apparent K_d of 5–20 pM and 2–6 nM (Chua *et al.*, 1994). The extensive homology between the p40 subunit and the extracellular domain of the IL-6 receptor, together with the more distant relationship between the p35 subunit and IL-6 itself (Gearing and Cosman, 1991; Merberg *et al.*, 1992), has provoked speculation that the IL-12 heterodimer may have evolved from a cytokine/soluble receptor complex, such as occurs with IL-6, which then interacts with a cell-bound receptor analogous to gp130 and induces biological activity. This has been given further support by the recent cloning of the cDNA encoding a human IL-12 receptor subunit of approximately 100 kDa which structurally is a member of the haematopoietin receptor family and most closely related to gp130 (Chua *et al.*, 1994). However, unlike the IL-6/soluble IL-6R-induced dimerisation of gp130 and subsequent signal transduction, the oligomerisation of the IL-12R subunit is not dependent on ligand binding and IL-12 only binds dimeric or oligomeric forms of the subunit. Additionally, whereas gp130 expression is widespread, constitutive and converts the IL-6/IL-6R interaction to one of high affinity, the IL-12R subunit is restricted, highly inducible by mitogens or IL-2 and is thought to represent the low-affinity

IL-12 binding site (K_d 2–5 nM) requiring an additional subunit to generate a high affinity IL-12R complex. This may be the receptor-associated protein of 85 kDa previously described (Chizzonite *et al.*, 1992).

It is now recognised that IL-12 has several biological actions including playing a pivotal role in the initiation of cell-mediated immunity via regulation of T_H1 and T_H2 subsets (Romagnani, 1992; Germann *et al.*, 1993; Hsieh *et al.*, 1993; Macatonia *et al.*, 1993; Manetti *et al.*, 1993; Trinchieri, 1993; Wu *et al.*, 1993; McKnight *et al.*, 1994; Romani *et al.*, 1994; Schmitt *et al.*, 1994; Yanagida *et al.*, 1994). In both murine and human systems, it appears that IL-12 is an essential factor for T_H1 generation, with antigenic stimulation in the presence of blocking antibodies to IL-12 preventing the generation of T_H1 cells. This may at least in part be independent of interferon- γ , although studies with neutralising antibodies have produced conflicting results, but may require NK cells (Macatonia *et al.*, 1993; Manetti *et al.*, 1993; Wu *et al.*, 1993; McKnight *et al.*, 1994). Conversely, IL-4 induces the generation of T_H2 cells. Thus, the critical balance of cytokines and particularly IL-4 and IL-12 is essential for determining the T_H1 and T_H2 response. Appropriate negative and positive feedback mechanisms exist, with the T_H1 -derived interferon- γ further increasing the monocytic production of IL-12 and decreasing IL-10 production, whereas the T_H2 cell products, IL-4 and IL-10, inhibit production of IL-12 (Trinchieri, 1993). Activated but not resting T and NK cells, whether isolated from peripheral blood lymphocytes (PBLs), T-cell lines or tumour-infiltrating lymphocytes (TILs), proliferate in response to IL-12 via a mechanism which is in most cases IL-2-independent (Stern *et al.*, 1990; Gately *et al.*, 1991; Wolf *et al.*, 1991; Bertagnoli *et al.*, 1992; Naume *et al.*, 1992; Perussia *et al.*, 1992; Andrews *et al.*, 1993) although additive, synergistic or inhibitory effects of IL-2 have been reported. However, IL-12 has also been reported to inhibit the proliferative response of NK cells, $CD8^+$ cells and a T-cell line to high-dose IL-2 (Perussia *et al.*, 1992; Robertson *et al.*, 1992; Mehrotra *et al.*, 1993). Synergy of IL-12 with the B7/CD28-mediated co-stimulation of proliferation and cytokine production of murine and human T cells has also been shown to occur (Kubin *et al.*, 1994; Murphy *et al.*, 1994), with effective concentrations of IL-12 being lower and inducing greater responses than those of IL-2 (Kubin *et al.*, 1994). IL-12 is not, however, an effective stimulus for anergic T cells (Quill *et al.*, 1994). Whether the IL-12-facilitated induction of MHC-restricted cytotoxic T-lymphocyte activity seen *in vitro* is IL-2 dependent is the subject of dispute (Gately *et al.*, 1992; Mehrotra *et al.*, 1993; Bloom and Horvath, 1994), presumably because of factors such as the nature of the stimulus and the cell populations used. An increase in production of granule proteins such as perforin has been associated with the IL-12-enhanced MHC-restricted and non-restricted cytotoxicity (Cesano *et al.*, 1993; Chehimi *et al.*, 1993; Salcedo *et al.*, 1993; Aste-Amezaga *et al.*, 1994; Bloom and Horvath, 1994; Bonnema *et al.*, 1994).

IL-12 markedly stimulates production of interferon- γ from resting or activated PBLs and T and NK cells (Kobayashi *et al.*, 1989; Chan *et al.*, 1991; Wolf *et al.*, 1991; Naume *et al.*, 1993; Wu *et al.*, 1993), acting synergistically with IL-2, although resting cells also require accessory cells. The action of IL-12 in enhancing the cytolytic activity of NK cells and induction of LAK activity (Kobayashi *et al.*, 1989; Stern *et al.*, 1990; Gubler *et al.*, 1991; Wolf *et al.*, 1991; Gately *et al.*, 1992; Naume *et al.*, 1992; Robertson *et al.*, 1992; Chehimi *et al.*, 1993) is not thought to be mediated by IL-2 or interferon- γ . However, production of endogenous TNF- α has been implicated in the IL-12-mediated generation of lymphokine-activated killer cell (LAK) activity (Gately *et al.*, 1992; Naume *et al.*, 1992), but whether it is involved in the augmentation of NK cytotoxic activity in short-term cultures may be dependent on whether mature or immature NK cells are used (Chehimi *et al.*, 1993; Jewett and Bonavida, 1994). IL-12 has been reported to both inhibit and act synergistically with IL-2-induced LAK activity depending on the

experimental conditions (Gately *et al.*, 1992; Zeh *et al.*, 1993) and, indeed, endogenous IL-12 may be a partial mediator of the IL-2 response, with antibodies to IL-12 producing a 50% inhibition of IL-2-induced interferon- γ production by PBLs *in vitro* (D'Andrea *et al.*, 1992). Interestingly, in view of IL-10's ability to inhibit IL-12 synthesis (D'Andrea *et al.*, 1993), following IL-12 injection in mice, a decrease in splenic IL-3 and IL-4 production was noted together with an increase in IL-10 production (Morris *et al.*, 1994), possibly indicating a negative feedback mechanism. Other biological actions of IL-12 include the up-regulation of HLA-DR, the adhesion molecules ICAM-1, and LFA-1 and receptors for the cytokines IL-2, IL-12, IL-4 and TNF on NK cells (Naume *et al.*, 1992, 1993; Robertson *et al.*, 1992; Rabinowich *et al.*, 1993; Jewett and Bonavida, 1994), the inhibition of IgE production (Kiniwa *et al.*, 1992; Morris *et al.*, 1994), and actions as a growth modulator for murine and human haemopoietic stem cells (Jacobsen *et al.*, 1993; Ploemacher *et al.*, 1993a,b; Bellone and Trinchieri, 1994).

Clearly, the above actions of IL-12 indicate its potential usefulness as an anti-tumour agent, and this is borne out by recent experimental work. *In vitro* IL-12 has been shown to augment significantly the NK activity of human PBMCs against a variety of tumour-derived cell lines, including colon and neuroblastoma (Lieberman *et al.*, 1991; Rossi *et al.*, 1994) and to correct the defect of NK activity of PBMCs seen in patients with various solid tumours (Soiffer *et al.*, 1993; Kusher *et al.*, 1994). In those patients receiving IL-2, co-culture of PBMCs with IL-12 produced a dramatic increase in cytolytic activity against NK-sensitive and NK-resistant tumour targets (Soiffer *et al.*, 1993). The cytolytic activity of TILs against autologous tumours including melanoma, breast and ovary (Andrews *et al.*, 1993; Zeh *et al.*, 1993) is also enhanced by IL-12.

Early animal studies have now shown that IL-12 administered either systemically, directly into the tumour or locally by fibroblasts genetically engineered to produce IL-12 has potent anti-tumour and antimetastatic activity in a number of tumour models, including carcinomas, sarcomas, melanomas and lymphomas (Brunda *et al.*, 1993; O'Toole *et al.*, 1993; Tahara *et al.*, 1994; Mayor *et al.*, 1994; Nastala *et al.*, 1994; Stern *et al.*, 1994). Most importantly, IL-12 was not only able to inhibit growth of new tumours but also caused regression of existing extensive tumours. Whether IL-12 is having any direct effects on the tumours is not clear, although *in vitro* it has no effect on the proliferation of several murine tumour cell lines (Brunda *et al.*, 1993; O'Toole *et al.*, 1993; Mayor *et al.*, 1994). Both increased NK lytic activity and specific allogeneic cytotoxic T-lymphocyte (CTL) responses have been seen in normal mice injected with IL-12 (Gately *et al.*, 1994), although NK activity was low and declined within 2 days. As IL-12 retains its anti-tumour activity in mice depleted of NK cells but not in nude mice, it has been suggested that activation of T cells rather than of NK cells is the predominant mechanism of its anti-tumour activity (Brunda *et al.*, 1993). This is supported by the reduction of efficacy of IL-12 with depletion of $CD8^+$ cells but not with $CD4^+$ cells, the latter finding being somewhat surprising given the actions of IL-12 on $CD4^+$ T_H1 cells (Brunda *et al.*, 1993). However, a further study has found that the elimination of both $CD4^+$ and $CD8^+$ subsets is necessary before the effect of IL-12 is lost (Nastala *et al.*, 1994). In addition, other studies have found that the anti-tumour effect is present in severe combined immunodeficient (SCID) mice which lack B or T cells, implicating NK cells (O'Toole *et al.*, 1993). Co-injection of NK cells with IL-12 produced no appreciable anti-tumour effect on human melanoma xenografts in SCID mice, but the co-injection of IL-12 with IL-2 and NK cells significantly increased the anti-tumour effect of IL-2 and NK cells alone (Hill *et al.*, 1994).

The extent to which the anti-tumour activity of IL-12 is mediated by the secondary induction of interferon- γ is not clear, but dramatically increased serum levels of interferon- γ are seen in mice following IL-12 administration (Gately *et al.*, 1994; Hendrzak *et al.*, 1994; Nastala *et al.*, 1994) with

antibodies to interferon- γ nearly completely abrogating the anti-tumour, and partially reducing the antimetastatic, effects of IL-12 (Hendrzak *et al.*, 1994; Nastala *et al.*, 1994). Administration of interferon- γ alone, however, fails to produce the anti-tumour effects, suggesting that, although production of interferon- γ is essential for the anti-tumour action of IL-12, other cytokine cascades/actions of IL-12 are also required. Only low levels of TNF- α are found following IL-12 administration and antibodies to TNF- α have no effect on the anti-tumour activity of IL-12 (Nastala *et al.*, 1994). Initial reports indicate that, in contrast to IL-2, little toxicity has so far been associated with administration of IL-12 to mice at effective doses ($1 \mu\text{g day}^{-1}$) (Brunda *et al.*, 1993; Gately *et al.*, 1994; Nastala *et al.*, 1994; Stern *et al.*, 1994).

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