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IL-12 Induces Monocyte IL-18 Binding Protein Expression Via IFN- γ ¹

Korina G. Veenstra,* Zdenka L. Jonak,[†] Stephen Trulli,[†] and Jared A. Gollob^{2*}

IL-18 is a Th1 cytokine that synergizes with IL-12 and IL-2 in the stimulation of lymphocyte IFN- γ production. IL-18 binding protein (IL-18BP) is a recently discovered inhibitor of IL-18 that is distinct from the IL-1 and IL-18 receptor families. In this report we show that IL-18BP_a, the IL-18BP isoform with the highest affinity for IL-18, was strongly induced by IL-12 in human PBMC. Other Th1 cytokines, including IFN- γ , IL-2, IL-15, and IL-18, were also capable of augmenting IL-18BP_a expression. In contrast, IL-1 α , IL-1 β , TNF- α , IFN- γ -inducible protein-10, and Th2 cytokines such as IL-4 and IL-10 did not induce IL-18BP_a. Although monocytes were found to be the primary source of IL-18BP_a, the induction of IL-18BP_a by IL-12 was mediated through IFN- γ derived predominantly from NK cells. IL-18BP_a production was observed in cancer patients receiving recombinant human IL-12 and correlated with the magnitude of IFN- γ production. The IFN- γ /IL-18BP_a negative feedback loop identified in this study may be capable of broadly controlling immune activation by cytokines that synergize with IL-18 to induce IFN- γ and probably plays a key role in the modulation of both innate and adaptive immunity. *The Journal of Immunology*, 2002, 168: 2282–2287.

Interleukin-18, initially termed IFN- γ -inducing factor (1), is a Th1 cytokine that plays a key role in regulating IFN- γ production in response to infectious pathogens (2). Produced in response to endotoxin and LPS stimulation (3, 4), IL-18 synergizes with cytokines such as IL-12 and IL-15 to stimulate IFN- γ production by NK cells and T cells during the early phase of an infection (5). IL-18 also augments T cell activation in conjunction with either IL-2 or CD3 ligation (6, 7) and therefore is capable of influencing the development of adaptive as well as innate immune responses. The antitumor effect of IL-18 has been demonstrated in murine models of breast cancer, renal cell cancer, and melanoma (8–10) and is greatly enhanced by the addition of IL-12 or IL-2 (10). The induction of tumor regression by the combination of IL-18 and IL-12 has been shown to occur through an antiangiogenic mechanism that is IFN- γ dependent (11).

IL-18 binding protein (IL-18BP)³ is a recently discovered inhibitor of IL-18 that is distinct from the IL-1 and IL-18 receptor families (12). A member of the Ig superfamily with significant homology to a family of proteins encoded by several poxviruses, IL-18BP has been shown to block IFN- γ production by LPS and IL-18 or IL-12 and IL-18 in vitro and to block LPS-induced IFN- γ production in vivo (12). Located on chromosome 11q13, the human IL-18BP gene encodes four distinct isoforms derived from mRNA splice variants. The IL-18BP_a and IL-18BP_c isoforms are

neutralizing, with K_d of 400 pM and 2.9 nM, respectively (13). In contrast, IL-18BP_b and IL-18BP_d lack a complete Ig domain and are therefore unable to bind or neutralize IL-18.

It has been postulated that IL-18BP may play a central role in the control of Th1 immune responses by limiting the availability of IL-18 to activate lymphocytes in conjunction with IL-12 or IL-2. However, while IL-18BP_a mRNA has been shown to be constitutively expressed in human spleen and leukocytes (12), nothing is known regarding the control of IL-18BP expression by cytokines or the cell type(s) responsible for IL-18BP synthesis. In this report we show that those cytokines capable of synergizing with IL-18 to stimulate IFN- γ production, most notably IL-12, are the primary inducers of IL-18BP_a in monocytes. In addition, we demonstrate that the induction of IL-18BP_a by IL-12 is IFN- γ dependent, suggesting that IL-18BP_a forms part of a negative feedback loop designed to limit immune activation by IL-12.

Materials and Methods

Isolation of whole PBMC and cell subsets

Heparinized blood samples were obtained from healthy donors. Blood samples enriched for white blood cells were also obtained from volunteers undergoing platelet pheresis in the Dana-Farber Cancer Institute Blood Bank (Boston, MA) and were provided by Dr. D. Frank. PBMC were isolated from blood samples through density gradient centrifugation using Histopaque-1077 (Sigma-Aldrich, St. Louis, MO). For experiments involving the use of cell subsets, purified populations of monocytes, CD3⁺ T cells, CD4⁺ T cells, CD8⁺ T cells, NK cells, or B cells were isolated from PBMC by negative selection using the MACS system (Miltenyi Biotec, Auburn, CA). The medium for cell culturing consisted of RPMI 1640 containing 5% human AB serum, 2% L-glutamine, 1% sodium pyruvate, 1% gentamicin, and 1% penicillin-streptomycin.

RNA isolation and semiquantitative RT-PCR

Total RNA was extracted using TRIzol reagent (Life Technologies, Grand Island, NY) following the manufacturer's protocol. The resulting RNA precipitate from 5×10^6 cells was resuspended in 10 μ l double distilled (dd)H₂O. RNA (4 μ l) was used for reverse transcription in a total volume of 20 μ l containing 4 μ l 5 \times buffer (Promega, Madison, WI), 2 μ l DTT (0.1 M), 0.8 μ l dNTP (2.5 mM each), 0.5 μ l oligo(dT)₁₅ (50 mM; Promega), 1 μ l Moloney murine leukemia virus reverse transcriptase (Promega), 1 μ l RNasin (Promega), and 6.7 μ l ddH₂O. The reaction was incubated at 65°C for 5 min, at 37°C for 60 min, and at 95°C for 5 min. After the reaction was finished, 80 μ l ddH₂O was added to the mixture. PCR was performed using 10 μ l of this mixture in a total volume of 20 μ l, containing 2 μ l 10 \times buffer,

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³ Abbreviations used in this paper: IL-18BP, IL-18 binding protein; IL-1Ra, IL-1R antagonist; IP-10, IFN- γ -inducible protein-10; rhIL-12, recombinant human IL-12; dd, double distilled.

1.04 μ l MgCl₂ (25 mM), 0.2 μ l dNTP (2.5 mM each), 2 μ l oligonucleotides (10 mM each), 0.2 μ l AmpliTaq Gold (5 U/ μ l; PerkinElmer/Cetus, Norwalk, CT), 0.125 μ l ³²P-labeled dCTP (20 μ Ci/ml; NEN Lifescience, Boston, MA), and 4.435 μ l ddH₂O. PCR conditions included preincubation at 95°C for 10 min and a variable number of cycles (depending on primers used) of the following: 95°C for 1 min, annealing temperature (varied according to primers used) for 2 min, 72°C for 2 min, and an extension at 72°C for 4 min. Four microliters of 6 \times DNA loading buffer was then added, and 4 μ l of that mix was run on a 4% polyacrylamide gel, dried, and exposed to X-OMAT AR film (Eastman Kodak, Rochester, NY). Exposed films were scanned using a Bio-Rad densitometer (Bio-Rad, Hercules, CA) and were analyzed using Molecular Analyst (Bio-Rad).

Primers

The following primers were used: human S9 ribosomal protein: sense, 5'-GAT GAG AAG GAC CCA CGG CGT CTG TTC G-3'; antisense, 5'-GAG ACA ATC CAG CCC AGG AGG GAC A-5'; human IL-18BP: sense, 5'-ACC ATG AGA CAC AAC TGG ACA CCA G-3'; antisense, 5'-TTA ACC CTG CTG CTG TGG ACT GCT G-3' (products: IL-18BP_a, 583 bp; IL-18BP_b, 284 bp; IL-18BP_c, 815 bp); human IFN- γ : sense, 5'-TGG GTT CTC TTG GCT GTT ACT GCC-3'; antisense, 5'-TAC TGG GAT GCT CTT CGA CCT CGA-3'; human IFN- γ -inducible protein-10 (IP-10): sense, 5'-GGA ACC TCC AGT CTC AGC ACC-3'; antisense, 5'-CAG CCT CTG TGT GGT CCA TCC-3'; human IL-18: sense, 5'-GGC AAG CTT GAA TCT AAA TTA TCA GTC-3'; antisense, 5'-GCA TCT TAT TAT CAT GTC CTG GGA CAC-3'; and human IL-12R β 2: sense, 5'-GAC TTG GCA GCC TCC CAG GAA AGA T-3'; antisense, 5'-CAC TCG GGG CTG GCT GTT TAT T-3'.

Cytokines and Abs

Recombinant human IL-12 (rhIL-12) and IL-2 were provided by Genetics Institute (Cambridge, MA) and Chiron (Emeryville, CA), respectively. The following cytokines were purchased from R&D Systems (Minneapolis, MN): IL-18, IFN- γ , TNF- α , IL-1 α , IL-1 β , IL-4, IL-10, and IP-10. IL-15 and GM-CSF were purchased from Pierce (Rockford, IL). IFN- α 2b was obtained from Schering-Plough (Kenilworth, NJ). Neutralizing Abs to human IFN- γ , IL-18, and IL-2R β were purchased from R&D Systems. Abs to IFN- α and IFN- β were purchased from BioSource International (Camarillo, CA).

Measurement of plasma IFN- γ and IL-18BP_a in patients receiving rhIL-12

Plasma for measurement of IFN- γ and IL-18BP_a was obtained from patients with advanced renal cell cancer or melanoma treated on a trial of twice weekly i.v. rhIL-12 injections (14). This study was an open-label, nonrandomized, single-center, phase I, dose escalation trial. The treatment protocol was approved by the Cancer Therapy Evaluation Program of the National Cancer Institute (protocol T97-0053) and by the human institutional review board at Beth Israel Deaconess Medical Center (protocol 97-1083), and written informed consent was obtained from each patient. Doses of rhIL-12 were administered as an i.v. bolus 3–4 days apart, with a cycle of therapy lasting 6 wk. Plasma was collected before as well as 12 and 24 h after the first, second, and seventh rhIL-12 doses during cycle 1. An ELISA kit was used to measure plasma IFN- γ (sensitivity, <2 pg/ml; Endogen, Cambridge, MA).

IL-18BP_a was measured using an Origen assay. Two hybridoma cell lines producing anti-human IL-18BP_a Abs were a gift from Hayashibara Biochemical Laboratories (Fujisaki, Japan). One mouse anti-human IL-18BP_a mAb (no. 36) was biotinylated, and the other anti-human IL-18BP_a mAb (no. 13, blocking) was ruthenylated (Igen, Gaithersburg, MD). Biotinylated Ab (25 μ l, 1 μ g/ml) was mixed with ruthenium-labeled Ab (25 μ l, 1 μ g/ml) and the sample to be measured (25 μ l) for 2 h with agitation. Magnetic Dynabeads M-280 (Igen) coated with streptavidin (50 μ l, 300 μ g/ml) were then added and agitated for 20 min. Two hundred microliters of assay buffer (Igen) was added, and the samples were analyzed on the Origen analyzer. The ruthenium and the surrounding tripropylamine assay buffer (Igen) undergo a double oxidation reaction that results in photon release. This resulting chemiluminescence was measured. A standard curve was constructed using rhIL-18BP_a in assay diluent (1% BSA (Sigma-Aldrich) in PBS containing 0.5% Tween 20). The standard curve ranged from 6 to 400 ng/ml.

Results

IL-18BP_a is induced by IL-12 and by other Th1 cytokines

To determine whether IL-18BP gene expression was regulated by cytokines, RNA was isolated from unstimulated or cytokine-stimulated whole PBMC and RT-PCR was performed using primers designed to detect transcripts for either the neutralizing IL-18BP_a and IL-18BP_c isoforms or the nonneutralizing IL-18BP_b isoform. Low-level constitutive expression of IL-18BP_a mRNA was detected in freshly isolated human PBMC (Fig. 1A, lanes 1 and 6) and in PBMC cultured for 24–48 h in medium alone, whereas IL-18BP_c and IL-18BP_b were absent. Following stimulation with IL-12 the expression of IL-18BP_a was strongly up-regulated, increasing 10- to 20-fold over baseline levels (Table I). Peak expression was observed at 12 h and was only modestly attenuated at 48 h (Fig. 1A, lanes 3–5). IFN- γ augmented IL-18BP_a expression to a similar degree, whereas the up-regulation by IL-2 and IL-15 averaged 5- to 10-fold over baseline. Although both IL-18 and IFN- α were also capable of inducing IL-18BP_a, this finding was less consistent (Table I) with PBMC from some healthy donors showing either weak or no up-regulation in response to these cytokines. In addition, as shown in Fig. 1A (lanes 8–10), the expression of IL-18BP_a induced by IL-18 attenuated more rapidly compared with IL-12.

Neither IL-12 nor any of the other Th1 cytokines tested was capable of stimulating the expression of IL-18BP_c or IL-18BP_b. Other proinflammatory cytokines, including IL-1 α , IL-1 β , and TNF- α , had no effect on IL-18BP expression. Th2 cytokines, including IL-10 and IL-4, were incapable of inducing IL-18BP, as were GM-CSF and IP-10 (Table I).

Induction of IL-18BP_a by IL-12 is IFN- γ dependent

The ability of IL-12 to eradicate infections and stimulate tumor regression has been linked to the stimulation of IFN- γ and IP-10 production (15–17). To determine whether IL-18BP_a might modulate the induction of IFN- γ and IP-10 in response to IL-12, we compared the time course of IL-12-induced IL-18BP_a gene expression to that of IFN- γ and IP-10 gene expression (Fig. 1B). Both IFN- γ and IP-10 expression peaked earlier than IL-18BP_a in response to IL-12. In addition, the attenuation of IFN- γ and IP-10 expression coincided with the peak of IL-18BP_a induction. These findings suggested not only that IL-18BP_a may have interfered with the induction of IFN- γ and IP-10 by IL-12, but also that the induction of IL-18BP_a by IL-12 may have been indirectly mediated through IFN- γ or IP-10.

As IP-10 itself was unable to induce IL-18BP_a (Table I), it could not have been responsible for the effect of IL-12. To test whether IFN- γ was required for the augmentation of IL-18BP_a expression by IL-12, PBMC were stimulated with IL-12 in the presence of a neutralizing anti-IFN- γ Ab. As shown in Fig. 2, neutralization of IFN- γ almost completely abrogated IL-18BP_a induction by IL-12 (Fig. 2, upper panel, lanes 2 and 4). The anti-IFN- γ Ab had a similar effect on the induction of IL-18BP_a by IL-2 and IL-18 (data not shown). Neutralizing Abs to the IL-2R, IFN- α , and IFN- β had no effect, while a neutralizing IL-18 Ab only modestly diminished the effect of IL-12 on IL-18BP_a induction (data not shown). In addition to its effect on IL-18BP_a, the neutralization of IFN- γ completely abrogated the up-regulation of both IP-10 and IL-12R β 2 expression by IL-12. Although IFN- γ by itself did not stimulate IL-18 production by PBMC, the neutralization of IFN- γ partially inhibited the up-regulation of IL-18 expression by IL-12 (Fig. 2).

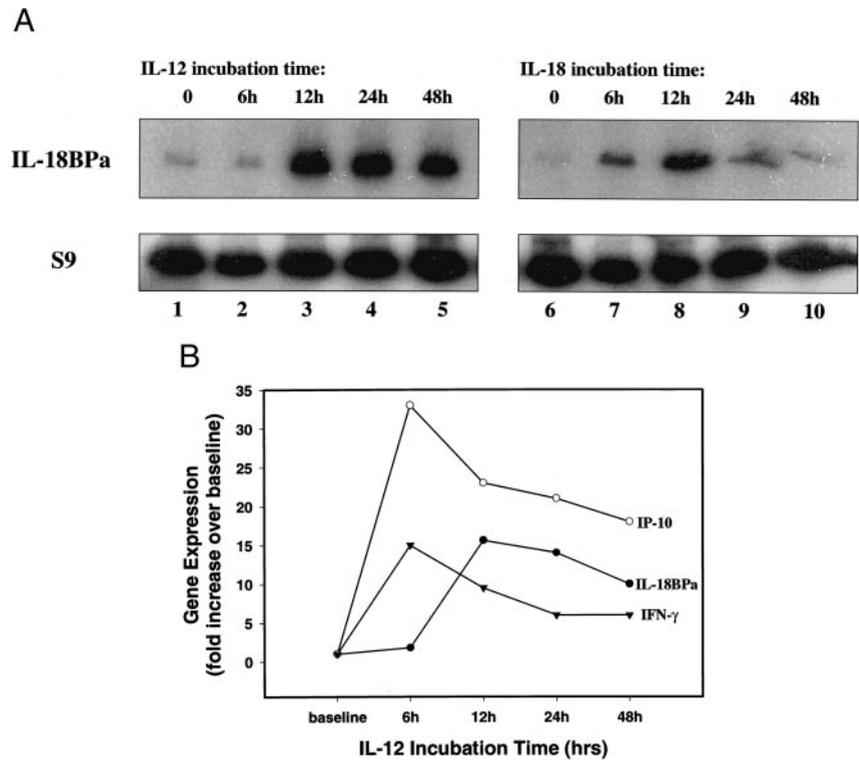


FIGURE 1. Induction of IL-18BP_a gene expression by IL-12. **A**, Whole PBMC were incubated for 0–48 h with either 100 ng/ml IL-12 or 100 ng/ml IL-18. At the indicated times RNA was isolated from the cells and assayed for IL-18BP_a mRNA by RT-PCR. In parallel, RT-PCR for S9 ribosomal protein was performed to determine the ratio of IL-18BP_a/S9 and thereby correct for any differences in the amount of RNA used at each time point. Results are representative of three separate experiments. **B**, Semiquantitative RT-PCR for IL-18BP_a, IFN- γ , and IP-10 was performed on RNA obtained from IL-12-stimulated PBMC at the indicated time points. Values are expressed as the fold increase over expression in unstimulated cells. Results are representative of three separate experiments.

Monocytes are the primary source of IL-12-induced IL-18BP_a

To determine which PBMC subset was producing IL-18BP_a in response to IL-12, PBMC were stimulated for 48 h with medium alone or IL-12 and then separated into purified populations of monocytes, NK cells, and T cells. As shown in Fig. 3, when PBMC were stimulated with IL-12, IL-18BP_a expression was due primarily to its induction in monocytes (Fig. 3, upper panel, lane 4). Monocytes were also the source of low-level constitutive IL-18BP_a expression in unstimulated PBMC (Fig. 3, upper panel, lane 3). Weak induction of IL-18BP_a was observed in NK cells (Fig. 3, upper panel, lane 6), but there was no induction in either CD4⁺ or CD8⁺ T cells (Fig. 3, upper panel, lane 8). The constitutive expression of IP-10 and IL-18 was strongly augmented in monocytes following IL-12 stimulation (Fig. 3). Although there was no expression of IP-10 and very weak expression of IL-18 in NK cells derived from unstimulated PBMC, the stimulation of PBMC with IL-12 also induced the expression of both in NK cells. T cells, in contrast, exhibited weak up-regulation of IP-10 and little

or no up-regulation of IL-18 in response to IL-12. NK cells were the main source of IFN- γ following the activation of PBMC by IL-12 and exhibited the strongest up-regulation of IL-12R β 2 expression. IL-12R β 2 expression was notably absent from monocytes both before and after the activation of PBMC by IL-12 and was only weakly up-regulated in T cells following IL-12 stimulation (Fig. 3).

Table I. Analysis of IL-18BP induction in PBMC by various cytokines^a

	IL-18BP _a	IL-18BP _b	IL-18BP _c
IL-12	+	–	–
IL-2	+	–	–
IL-15	+	–	–
IFN- γ	+	–	–
IFN- α	+/-	–	–
IL-18	+/-	–	–
IL-1 α	–	–	–
IL-1 β	–	–	–
IL-10	–	–	–
IL-4	–	–	–
TNF- α	–	–	–
GM-CSF	–	–	–
IP-10	–	–	–

^a +, Strong, consistent induction; +/-, inconsistent induction; –, no induction.

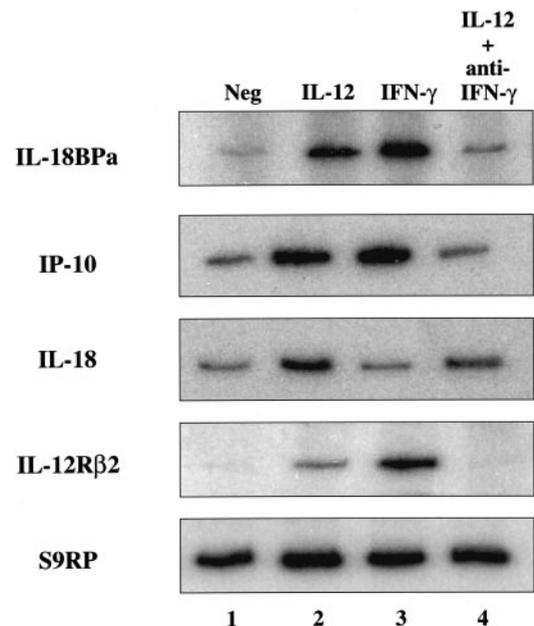
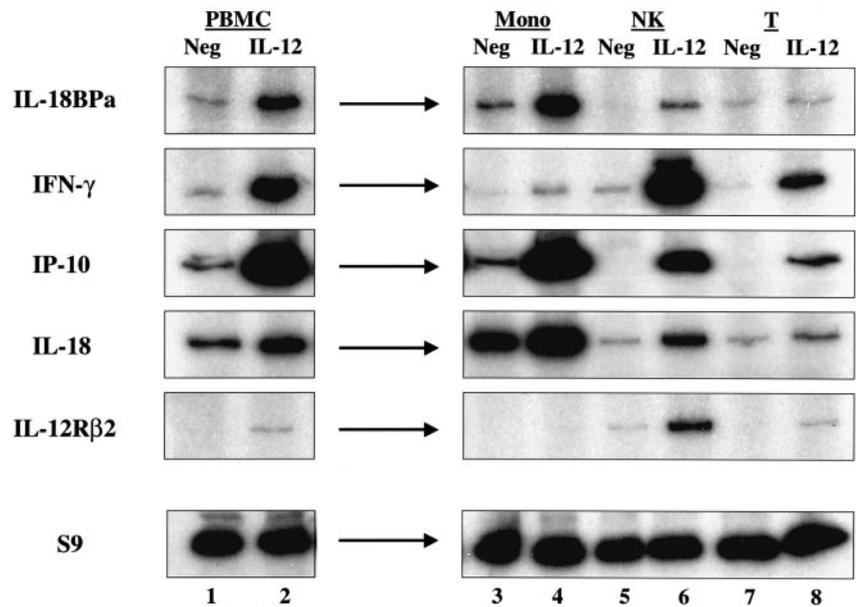


FIGURE 2. IFN- γ mediates the induction of IL-18BP_a, IP-10, and IL-12R β 2 by IL-12. Whole PBMC were stimulated for 48 h with medium alone (lane 1), 100 ng/ml IL-12 (lane 2), 50 ng/ml IFN- γ (lane 3), or IL-12 plus 1 μ g/ml anti-IFN- γ (lane 4). RT-PCR was then performed to assay for the expression of the indicated mRNAs. Results are representative of three separate experiments.

FIGURE 3. Monocytes are the primary source of IL-18BP α in IL-12-stimulated PBMC. Whole PBMC were incubated for 48 h with either medium alone or 100 ng/ml IL-12. RNA was then isolated from a small aliquot of these unstimulated or IL-12-stimulated PBMC, and the expression of the indicated mRNAs was assayed by RT-PCR (lanes 1 and 2). The remaining unstimulated (lanes 3, 5, and 7) or IL-12-stimulated (lanes 4, 6, and 8) cells were used to isolate purified populations of monocytes (lanes 3 and 4), NK cells (lanes 5 and 6), and T cells (lanes 7 and 8). RNA was isolated from these purified cell subsets, and the expression of the indicated mRNAs was assayed by RT-PCR. Identical results were seen in two separate experiments.



As the neutralization of IFN- γ inhibited the induction of IL-18BP α by IL-12 in PBMC, we examined whether IL-18BP α expression in monocytes could be stimulated directly by either IFN- γ or IL-12. Purified populations of monocytes, NK cells, T cells, or B cells were isolated from PBMC and stimulated with either IL-12 or IFN- γ . None of these leukocyte subsets expressed IL-18BP α in response to IL-12. IFN- γ , however, strongly induced IL-18BP α in monocytes but was unable to induce IL-18BP α expression in the other cell types (data not shown). These findings established that the induction of IL-18BP α by IL-12 in monocytes is mediated through IFN- γ .

IL-18BP α is induced in cancer patients treated with rhIL-12

To determine whether IL-12 could induce IL-18BP α protein production as well as gene expression, an Origen assay capable of detecting IL-18BP α concentrations of ≥ 6 ng/ml was used to measure IL-18BP α in cell culture supernatants and in the plasma of rhIL-12-treated cancer patients. In cell culture supernatants from PBMC stimulated with IL-12 for 24–48 h, the IL-18BP α concentration was below the limit of detection (< 6 ng/ml) of the Origen assay. However, in patients receiving a twice weekly schedule of i.v. rhIL-12 at a dose of 500 ng/kg, IL-18BP α was induced 12–24 h following rhIL-12 treatment (Table II). For two patients whose pretreatment IL-18BP α plasma concentration was < 6 ng/ml, IL-18BP α levels subsequently peaked at 32.7–70.6 ng/ml following the first dose of rhIL-12. A third patient, whose baseline IL-18BP α level was 28 ng/ml, had a more modest increase to 44 ng/ml after

the first rhIL-12 dose. These peak levels were fairly well sustained 3 days later, when patients had their IL-18BP α level checked before receiving the second dose of rhIL-12. Following the second dose of rhIL-12, the plasma IL-18BP α concentration again rose within 24 h to peak levels ranging from 26.1 to 101.2 ng/ml (Table II). At the time of the seventh dose of rhIL-12, pretreatment levels had dropped to baseline, and modest induction of IL-18BP α was observed in only one of the three patients.

IFN- γ was also measured in the plasma of these patients receiving rhIL-12. In the three patients tested, the magnitude of IFN- γ induction over the 24-h period following each dose of rhIL-12 correlated with the magnitude of IL-18BP α induction. In most patients treated with twice-weekly i.v. rhIL-12, IFN- γ induction was usually strongest after the second rhIL-12 dose and then attenuated significantly with subsequent doses, becoming quite weak by the seventh dose (14). This pattern of IFN- γ induction was for the most part observed in the three rhIL-12-treated patients shown in Table II and was accompanied by a similar pattern of IL-18BP α induction.

Discussion

In this report we have shown that IFN- γ plays a key role in regulating the expression of IL-18BP α , the IL-18BP isoform most capable of neutralizing the activity of IL-18 by virtue of its high binding affinity. As one of the primary functions of IL-18 is the stimulation of IFN- γ production, this link between IFN- γ and IL-18BP α provides a mechanism by which immune activation by a

Table II. Induction of IL-18BP in patients treated with rhIL-12^a

Patient No./Assay	Dose 1			Dose 2			Dose 7		
	Pre	+12 h	+24 h	Pre	+12 h	+24 h	Pre	+12 h	+24 h
26/IL-18BP α	<6	70.6	58.9	64	66	101.2	<6	21.8	24.1
26/IFN- γ	0	610	2040	0	6508	2792	0	240	90
40/IL-18BP α	<6	<6	32.7	18.1	20.5	26.1	<6	<6	<6
40/IFN- γ	0	2186	2390	0	1704	1121	0	399	220
30/IL-18BP α	28	33	44	50	50	95	30	34	34
30/IFN- γ	0	377	1559	0	5918	1601	0	63	11

^a Pre, Just prior to rhIL-12 dose; +12 h, 12 h after rhIL-12 dose; +24 h, 24 h after rhIL-12 dose. IL-18BP was measured in nanograms per milliliter; IFN- γ was measured in picograms per milliliter.

cytokine can be contained by a negative feedback loop initiated through the primary by-product of that activation. The finding that IL-12 is one of the most potent inducers of IL-18BP α , acting indirectly through IFN- γ , underscores the important role that IL-18 probably plays in modulating T and NK cell activation by IL-12 in vivo. This modulatory function of IL-18 probably applies to lymphocyte activation by IL-2 and IL-15 as well, for they were also capable of inducing IL-18BP α via IFN- γ . In fact, one of the unique aspects of an IFN- γ /IL-18BP α negative feedback loop is that it is capable of inhibiting the activity of any cytokine that synergizes with IL-18, thereby facilitating broad control over stimuli of IFN- γ production. This function of IL-18BP α resembles that of the IL-1R antagonist (IL-1Ra), the expression of which is also induced by IFN- γ (18). Although there have been no studies of the ability of IL-12 to induce IL-1Ra in vitro, the in vivo induction of IL-1Ra by i.v. IL-12 has been demonstrated in primates (19). As IFN- γ production by IL-12 and IL-2 is augmented by IL-1 (20, 21), there is a clear parallel between the IFN- γ /IL-18BP α and IFN- γ /IL-1Ra negative feedback loops with regard to their broad inhibitory effects on cytokine-mediated IFN- γ production. While IFN- α is also a strong inducer of IL-1Ra (18), we found that the induction of IL-18BP α by IFN- α was relatively weak and inconsistent compared with that by IFN- γ .

Our analysis of IL-18BP induction in PBMC focused on gene expression. As IL-18BP α does not require post-translational modification and is produced only in a secreted form (12, 13), it is likely that the mRNA detected by RT-PCR will correlate with the production of biologically active IL-18BP α available for binding to IL-18. This is further suggested by our observation that IL-12-induced IFN- γ and IP-10 gene expression declined at the time of peak IL-18BP α expression, which may also point to the role that endogenous IL-18 plays in modulating IFN- γ production by IL-12. The inability of our IL-18BP α Origen assay to detect protein in supernatants from IL-12-stimulated PBMC may have been largely due to the low sensitivity of the assay, which can only detect IL-18BP α concentrations ≥ 6 ng/ml. While we were able to detect high concentrations of IL-18BP α in the plasma of rhIL-12-treated patients, the in vitro conditions may not have been suitable for the generation of high concentrations of IL-18BP α by IL-12. Our failure to detect IL-18BP α , the other neutralizing human IL-18BP isoform (12, 13), in either resting or cytokine-stimulated PBMC suggests that this mRNA splice variant may not be physiologically relevant. Alternatively, it may be under the control of other stimuli that remain undefined.

When PBMC were stimulated with IL-12, we found that IL-18BP α was produced almost exclusively by monocytes. NK cells did produce a small amount of IL-18BP α when PBMC were stimulated with IL-12, although purified NK cells did not produce IL-18BP α when stimulated directly with either IL-12 or IFN- γ . This suggests that a cytokine other than IFN- γ may mediate the weak IL-18BP α induction by IL-12 in NK cells. While monocytes were the primary source of IL-18BP α in IL-12-stimulated PBMC, most of the IFN- γ was produced by NK cells. The IFN- γ /IL-18BP α negative feedback loop thus provides a functional link between NK cells and monocytes and may therefore be especially relevant to the control of the innate immune response. While IL-12 is central to adaptive immune responses through its role in Th1 development, it is produced by monocytes early in the course of an infection and therefore is also crucial to the activation of innate immunity. We have shown that IL-12 augments IL-18 gene expression in both monocytes and NK cells. This corroborates the finding in both humans and primates that i.v. IL-12 induces the production of IL-18 (14, 19). Interestingly, this stimulation of IL-18 gene expression is only partially dependent on IFN- γ and

may therefore also be mediated directly by IL-12. The induction of IL-18 by IL-12 is thus another early event in the development of an innate immune response, leading to the synergistic activation of NK cells and T cells that results in IFN- γ production. We have shown that IFN- γ , in turn, up-regulates IL-12R β 2 expression on NK cells and, to a lesser extent, T cells, which would further sensitize these lymphocytes to IL-12 (22). In addition, IL-12 is known to up-regulate IL-18R expression on T and NK cells (23), which would heighten their responsiveness to IL-18. If left unchecked, the ensuing augmentation of IFN- γ production and amplification of the innate immune response would probably result in prohibitive toxicity and activation-induced lymphocyte apoptosis. By disarming IL-18, the IFN- γ /IL-18BP α negative feedback loop between NK cells and monocytes may play an essential role, not necessarily in shutting off an immune response, but in preventing the excessive activation of innate immunity from interfering with the subsequent development of an adaptive immune response.

The demonstration that IL-18BP α is induced in vivo in cancer patients treated with rhIL-12 corroborates the in vitro effect of IL-12 on IL-18BP α gene expression. Furthermore, the finding that the magnitude of IL-18BP α induction parallels the magnitude of IFN- γ induction by rhIL-12 lends further support to the important role that IFN- γ plays in mediating IL-18BP α induction by IL-12. As plasma levels of IL-18 are augmented in patients treated twice weekly with rhIL-12 (14), it is likely that this endogenous IL-18 works in conjunction with rhIL-12 to stimulate IFN- γ production. In patients treated with rhIL-12, it is therefore possible that IL-18BP α serves to limit the magnitude and duration of IFN- γ induction occurring with each rhIL-12 dose. However, it is important to note that the marked attenuation of IFN- γ production occurring at the time of the seventh rhIL-12 dose in patients treated twice weekly with rhIL-12 (Table II) was not accompanied by an increase in IL-18BP α induction but, instead, was associated with a similar attenuation of IL-18BP α induction. Therefore, it appears that IL-18BP α is not responsible for the sharp down-regulation of rhIL-12-induced IFN- γ production observed after 3.5 wk of rhIL-12 therapy. While the IFN- γ /IL-18BP α negative feedback loop is likely to have an important impact on the modulation of cellular immunity by cytokine-based antitumor therapies, other mechanisms, including alterations in lymphocyte responsiveness to cytokines (14), are probably operative in the tachyphylaxis observed during chronic therapy with rhIL-12.

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