Context-dependent role of IL-18 in cancer biology and counter-regulation by IL-18BP

Marina Fabbì, Grazia Carbotti, and Silvano Ferrini

Laboratory of Biotherapy, Istituto di Ricovero e Cura a Carattere Scientifico Azienda Ospedaliera Universitaria San Martino-Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

RECEIVED JULY 29, 2014; REVISED DECEMBER 2, 2014; ACCEPTED DECEMBER 3, 2014. DOI: 10.1189/jlb.5RU0714-360RR

ABSTRACT
IL-18 is a proinflammatory and immune regulatory cytokine, member of the IL-1 family. IL-18 was initially identified as an IFN-γ-inducing factor in T and NK cells, involved in Th1 responses. IL-18 is produced as an inactive precursor (pro-IL-18) that is enzymatically processed into a mature form by Casp1. Different cells, such as macrophages, DCs, microglial cells, synovial fibroblasts, and epithelial cells, express pro-IL-18, and the production of bioactive IL-18 is mainly regulated at the processing level. PAMP or DAMP molecules activate inflammasomes, which trigger Casp1 activation and IL-18 conversion. The natural inhibitor IL-18BP, whose production is enhanced by IFN-γ and IL-27, further regulates IL-18 activity in the extracellular environment. Inflammasomes and IL-18 represent double-edged swords in cancer, as their activation may promote tumor development and progression or oppositely, enhance anti-tumor immunity and limit tumor growth. IL-18 has shown anti-tumor activity in different preclinical models of cancer immunotherapy through the activation of NK and/or T cell responses and has been tested in clinical studies in cancer patients. However, the dual role of IL-18 in different experimental tumor models and human cancers raises critical issues on its therapeutic use in cancer. This review will summarize the biology of the IL-18/IL-18R/IL-18BP system and will address the role of IL-18 and its inhibitor, IL-18BP, in cancer biology and immunotherapy.

J. Leukoc. Biol. 97: 665–675; 2015.

IL-18 BIOLOGY
IL-18 is a proinflammatory and immune regulatory cytokine, which belongs to the IL-1 family [1]. The IL-18 gene was cloned on the basis of the ability of the encoded protein to promote IFN-γ production by T and NK cells and Th1 differentiation and was initially defined a IFN-γ-inducing factor [2]. IL-18 is produced as an inactive precursor protein of 192 aa (pro-IL-18) that is converted into a mature form of 157 aa by Casp1-mediated cleavage of an N-terminal fragment (Fig. 1A). IL-18 is constitutively expressed by several cell types, including macrophages, DCs, microglial cells, synovial fibroblasts, and epithelial cells. Therefore, different from IL-1β, which is tightly controlled also at the transcription level, the IL-18 biologic activity is mainly regulated by its enzymatic processing [3]. PAMPs (e.g., muramyl dipeptide, bacterial RNA, and viral dsDNA) or DAMPs (e.g., extracellular ATP and uric acid crystals) activate protein complexes, known as inflammasomes, which mediate Casp1 activation and IL-18 processing [4, 5]. The most studied inflammasome, Nlrp3, activates Casp1 through the adapter protein ASC, upon triggering by different PAMPs, DAMPs, or some adjuvants. Several studies indicate that inflammasomes represent a double-edged sword in cancer, as their activation may have tumor-promoting effects or on the other hand, immune-enhancing and anti-tumor activity [4].

Once released from cells, mature IL-18 binds to a heterodimer receptor complex, consisting of IL-18Rα and β-chains. These chains are members of the IL-1R family, characterized by a TIR domain in their intracellular portion [6]. IL-18 binding to the IL-18Rα recruits the IL-18Rβ chain and initiates signal transduction through the adaptor protein MyD88, which binds to the TIR domain. IRAK is then phosphorylated and recruits TRAF-6, which initiates the signaling pathways leading to the activation of the transcription factors NF-κB and AP-1 (Fig. 1A). However, different from IL-1, IL-18 signal transduction primarily involves the MAPK p38 pathway, rather than NF-κB, in human epithelial cells [7]. Therefore, IL-18 does not induce COX-2 and fever, which are typical NF-κB-dependent, IL-1-mediated effects.

 Mature IL-18 is produced by different cell types in response to pathogens and activates host defense mechanisms [8–11]. Indeed, IL-18 augments antimicrobial properties of phagocytes through the up-regulation of reactive oxygen intermediate synthesis, NO production, and cytokine release. IL-18 potentiates the clearance of intracellular bacteria, fungi, and protozoa, and IL18−/− mice show increased susceptibility to several infections [12]. The clearance of different viruses is impaired in IL-18−/−

Abbreviations: /−/− = deficient mouse, ADAM-33 = a disintegrin and metalloproteinase domain-containing protein 33, ADCC = antibody-dependent cellular cytotoxicity, ASC = apoptosis-associated speck-like protein containing a caspase recruitment domain, Casp1 = caspase -1, COX-2 = cyclooxygenase-2, DAMP = damage-associated molecular pattern, DC = dendritic cell, FasL = Fas ligand, HPV = human papillomavirus, (continued on next page)
IL-18 or IL-12, which induce expression of the IL-18R
CCL4 by human NK cells requires the cooperation of IL-18 with
helper differentiation phenotype (CD83+CCR7+CD25+) and
necessary for IL-18 signal transduction in target cells [16].

IFN-γ or PAMPs activate the in
by Casp1 in the mature form (Mat-IL-18). DAMPs
and PAMPs activate the inflammasome, which
triggers Casp1 activation. Mature IL-18 binds to
IL-18Ra, which recruits the β chain to the
complex. The recruitment of MyD88 and IRAK
initiates signal transduction through TRAF6. (B)
IL-18 primes human NK (hNK) cells to acquire
CCR7 expression and migratory capacity to
secondary lymphoid organs. In this environment,
cytokines produced by DCs or Th cells activate “helper” functions of IL-18-primed NK cells,
consisting of high IFN-γ and TNF-α production,
which increases DC activation. In turn, DCs
express higher IL-12 and release chemokines
attracting T cells. IFN-γ, produced by NK cells and
DC-released IL-12, promotes Th1 polarization and
CTL responses.

Importantly, IL-18-primed human NK cells develop a distinct
helper differentiation phenotype (CD83+CCR7+CD25+)
and acquire the ability to migrate to secondary lymphoid tissues
in response to chemokines, such as CCL21 [17] (Fig. 1B).
Helper NK cells show reduced cytotoxic functions but efficiently release
IFN-γ in response to DCs or Th-deriving signals, such as IFN-α,
IL-12, IL-15, or IL-2. They may represent a link between innate
and adaptive immunity, as they stimulate DCs to release IL-12,
which increases DC signal transduction in target cells [16].

Figure 1. IL-18 processing, signaling, and priming
of NK cells. (A) IL-18 is produced as an inactive
precursor protein (Pro-IL-18), which is processed
by Casp1 in the mature form (Mat-IL-18). DAMPs
or PAMPs activate the inflammasome, which
triggers Casp1 activation. Mature IL-18 binds to
IL-18Ra, which recruits the β chain to the
complex. The recruitment of MyD88 and IRAK
initiates signal transduction through TRAF6. (B)
IL-18 primes human NK (hNK) cells to acquire
CCR7 expression and migratory capacity to
secondary lymphoid organs. In this environment,
cytokines produced by DCs or Th cells activate “helper” functions of IL-18-primed NK cells,
consisting of high IFN-γ and TNF-α production,
which increases DC activation. In turn, DCs
express higher IL-12 and release chemokines
attracting T cells. IFN-γ, produced by NK cells and
DC-released IL-12, promotes Th1 polarization and
CTL responses.

nights. Indeed, IL-18 mediates antiviral functions through the
induction of NK [13] or CTL responses [14]. Repeated
administrations of IL-18 restore the impaired immune response
in mice with severe burn injury or splenectomy and improve
survival after bacterial infections [11].

IL-18 acts in concert with other cytokines to modulate immune
system functions. Optimal induction of IFN-γ production, Th1
responses, and NK cell activation in response to pathogen
products requires IL-18 and IL-12, which cooperate in these
activities, as indicated by the study of Il18Δ/Δ, Il12Δ/Δ, and
double-KO mice [15]. IL-18 and IL-12 cooperate for the
induction of IFN-γ, also in human NK cells. In addition,
production of TNF-α, GM-CSF, and the chemokines CCL3 and
CCL4 by human NK cells requires the cooperation of IL-18 with
IL-15 or IL-12, which induce expression of the IL-18Rβ chain,
necessary for IL-18 signal transduction in target cells [16].

IL-18 and IL-12 induce a distinct helper T cell phenotype
(CD83+CCR7+CD25+) and acquire the ability to migrate to secondary lymphoid tissues
in response to chemokines, such as CCL21 [17] (Fig. 1B).
Helper NK cells show reduced cytotoxic functions but efficiently release
IFN-γ in response to DCs or Th-deriving signals, such as IFN-α,
IL-12, IL-15, or IL-2. They may represent a link between innate
and adaptive immunity, as they stimulate DCs to release IL-12,
which induces Th1 responses, and chemokines, such as CXCL9,
CXCL10, and CCL5 attracting CD8+ T cells [18]. Indeed, helper
NK cells have been shown to prime tumor-specific Th1 and CTL
responses via IFN-γ and TNF-α DC activation [19].

In addition, a recent study showed that a subset of M-CSF-
activated human macrophages expresses a membrane form of
processed IL-18, which is released in a soluble form upon LPS
stimulation. This soluble IL-18 induces the expression of CCR7
and the production of IFN-γ in resting NK cells [20]. A similar
membrane-bound form of IL-18 is also present in tumor-
associated macrophages [21].

Further studies indicate a role of IL-18 also in Th2 and Th17
responses, in relationship to the different cytokine milieu. In
the absence of IL-15 or IL-12, IL-18 induces naïve T cells to
polarize into Th2 cells and mediates IL-13 and/or IL-4 pro-
duction also by NK cells, mast cells, and basophils [22–24]. In mouse
basophils, IL-18-mediated Th2 cytokine production is dependent
on MyD88 and MAPK-p88x signaling [24]. Therefore, IL-18 may play
a role in allergic diseases, including asthma [25]. The combination of
IL-18 or IL-1 with IL-23 stimulates IL-17 production by CD4+ or
Th2-β” T cells in the absence of TCR engagement, suggesting
a role, not only for IL-1 but also for IL-18 in Th17 responses [26].

IL-18 may also have pathogenic effects in autoimmune
disorders. For example, processing of IL-18 and IL-1 and the
consequent IL-17 production is essential in experimental autoimmune
encephalomyelitis induced by DCs primed with Mycobacterium tuberculosis and myelin antigen [27]. In fact, the administration
of a Casp1 inhibitor reduces disease severity in this model. Several
other studies indicate a role for IL-18 in human autoimmune
disorders, such as lupus erythematosus [28, 29], arthritis [30], and
Crohn’s disease [31]. Altogether, these data suggest that IL-18-
blocking agents represent novel, potential therapeutic approaches
in inflammatory disorders or autoimmunity.

A natural inhibitor of IL-18 biologic activity is IL-18BP, which
has high affinity for mature IL-18 and blocks its interaction with
the IL-18Ra, thus preventing receptor dimerization. Therefore IL-18BP is considered a therapeutic tool to limit IL-18-based inflammation [32]. IL-18BP is a member of the Ig superfamily [33]. Alternative splicing of the IL18BP transcript leads to the generation of different isoforms, among which, IL-18BPa is the most widely expressed [34]. High levels of IL-18BPa in IL18BP-transgenic mice limit IL-18-mediated tissue injuries in response to different inflammatory stimuli, such as bacterial endotoxin or Con A [35]. IL-18BP accumulation may inhibit IL-18-mediated responses also in humans, as in the case of renal failure, where high levels of IL-18BP contribute to the defective immune response [36]. IL-18BP is constitutively produced by monocytes and macrophages and is present in the systemic circulation of healthy donors in molar excess relative to IL-18 [37]. However, IFN-γ up-regulates IL-18BP expression in myeloid cells [38] and induces de novo expression in nonleukocytic cells, such as normal keratinocytes and mesangial cells [39]. Therefore, IL-18BP secretion is part of a negative-feedback loop, which proceeds from IL-18-triggered IFN-γ production to IFN-γ-induced IL18BP gene expression to prevent exaggerated Th1 responses. STAT1 signaling induced by IFN-γ and STAT1 sites in the IL18BP promoter region are crucial for IL18BP expression [40] (Fig. 2). Recent data showed that another STAT1-activating cytokine, IL-27, induces IL-18BPa expression in human keratinocytes in vitro [41]. This finding suggests a possible anti-inflammatory role of IL-27-induced IL-18BP in skin diseases, such as psoriasis, where keratinocyte-produced IL-18 may play a pathogenic role.

In view of its Th1-enhancing properties, IL-18 has been considered as a molecule with potential anticancer activity. However, as a result of the complexity of IL-18 biologic functions, which are dependent on the context (e.g., cytokine milieu, different tissues, and counter-regulation by IL-18BP), IL-18 may play divergent roles in cancer [42, 43]. We will review the anti-cancer and the tumor-promoting activities of IL-18 in experimental tumor models and human tumors.

**ANTI-CANCER EFFECTS OF IL-18**

Protective effects of IL-18 have been reported in different murine models of carcinogenesis (see Table 1). PAMP-mediated activation of the inflammasomes may be relevant during carcinogenesis in pathogen-associated cancers, such as gastric cancer. Indeed, Casp1 is activated, and IL-1β and IL-18 are processed as a consequence of Helicobacter pylori infection. Studies in Il1Rβ−/− and Il18−/− mouse strains indicate that IL-18 counteracts the proinflammatory effects of IL-1β and limits IL-1-mediated gastritis [44]. Indeed, Il1Rβ−/− mice infected with Helicobacter show a limited control of the colonization level but develop less gastritis and gastric pre-neoplastic lesions as a result of reduced IFN-γ- and Th17-mediated inflammatory responses. Oppositely, Il18−/− mice show reduced Helicobacter colonization, a modest defect in IFN-γ but strongly enhanced mucosal production of IL-17, and rapidly progressive gastric immunopathology. Therefore, activation of Casp1 by Helicobacter triggers IL-1-mediated Th17 responses that limit bacterial growth but induce gastritis. These effects are counterbalanced by IL-18 that limits Th17-mediated gastric immunopathology and prevents the onset of gastric cancer.

Another study showed that upon in vitro or in vivo exposure to Helicobacter, mouse DCs produce IL-18 and mediate the conversion of naïve CD4+ T cells in CD4+CD25+Forkhead box P3+ regulatory T cells, endowed with immune-suppressive properties. IL-18, produced by DCs, takes part in this process, which limits T cell-driven gastric immunopathology. Indeed, depletion of DCs in newborn mice infected with H. pylori limits the infection but also worsens T cell-driven gastric immunopathology [45]. Similar to mouse models, also in humans, increased levels of IL-18 expression are found in gastric mucosa during H. pylori infection, where IL-18 may contribute to Th1 responses [46].

IL-18 has a protective role also in mouse models of inflammation-driven colon carcinogenesis induced by azoxymethane and dextran sulfate [47]. Mdy88KO mice, which have defects in IL-1β and IL-18 systems, show increased colorectal tumor development [48]. Il1Rβ−/− mice show no increase in colorectal tumorigenesis, whereas Il18−/− and Il18Rβ−/− mice are highly susceptible to colitis and colorectal cancer development. These findings suggest a protective role of IL-18 but not of IL-1 in colorectal cancer development [48]. Another report shows that Casp1−/−, Asc−/−, and Nlrp3−/− mice are more susceptible to experimental colitis and colitis-related carcinogenesis. At early stages of tumorigenesis, their colon tissue shows reduced IL-18 production, increased macrophage infiltration, and COX-2 expression and higher numbers of proliferating epithelial cells in the dysplastic regions. In addition, Nlrp3−/− and Casp1−/− mice show reduced IFN-γ mRNA and protein expression and STAT1 signaling in their colon tissues during early phases of

![Figure 2. Role of IFN-γ and IL-27 in the induction of IL-18BP and other mechanisms of immune escape in cancer cells.](www.jleukbio.org)
The inhibition of angiogenesis is important for the anti-tumor effect of IL-18 and is mediated by IFN-γ-dependent induction of the angiogenic chemokines CXCL9 and CXCL10 and down-regulation of angiogenin expression [53].

The combination of IL-18 with other cytokines, such as IL-12 [54] or costimulatory molecules (e.g., CD80) [38], increases the IL-18-mediated anti-tumor effects. For example, IL18 and IL12A/B or CD80 genes have been integrated successfully in the genome of oncolytic viruses, with the aim to trigger synergistically T cell-mediated anti-tumor immune responses [55, 62]. IL-2/IL-18 fusion proteins also display enhanced anti-tumor properties relative to either cytokine alone and low toxicity in preclinical models [63].

IL-18 also demonstrated potent adjuvant activity in combination with a variety of cell-based or molecularly defined anti-cancer vaccines. For instance, a lung cancer cell vaccine, genetically modified to coexpress GM-CSF and IL-18, inhibits tumorigenesis, suggesting a role of IFN-γ and STAT1 as mediators of IL-18 anti-tumor effects. Indeed, IL-18 administration to Casp1−/− mice reduces the signs of colitis and epithelial cell dysplasia and restores pSTAT1 [49]. Altogether, these data support the concept that IL-18 has a protective role at early stages of the colorectal carcinogenesis.

In view of its immune-enhancing properties, IL-18 has been investigated for anti-tumor activity in preclinical models (Table 1). Administration of rIL-18 mediated melanoma or sarcoma regression in syngenic mice through the activation of CD4+ T and/or NK cell-mediated responses [50, 51]. Several other reports indicate that administration of rIL-18 or Il18 gene transfer has anti-tumor effects in different experimental models (reviewed in ref. [61]). In some of these studies, the IL-18 anti-tumor effects required IFN-γ and involved antiangiogenic mechanisms [52, 53].

### Table 1. Anticancer effects of IL-18 in murine models

| Tumor type                                                                 | Model                                                                 | Major findings                                                                 | Ref. |
|---------------------------------------------------------------------------|----------------------------------------------------------------------|--------------------------------------------------------------------------------|------|
| Helicobacter-mediated gastritis and preneoplastic gastric lesions         | IIIR−/− and IIIS−/− mice                                            | IL-18 counteracts IL-1 driven inflammation and limits Helicobacter pathogenic effect | [44] |
| Dextran sulfate-induced colitis and colorectal tumorigenesis              | Mydd8−/−, IIIS−/−, and II18R−/− mice                                 | Protective role of IL-18 in inflammation-driven colitis and tumorigenesis       | [48] |
| Colitis-related tumorigenesis                                             | Casp1−/−, Asc−/−, and Nhp3−/− mice                                   | IL-18 administration reduces colitis-related tumorigenesis                     | [49] |
| B16 melanoma in syngeneic mice                                           | Pulmonary metastases by i.v. injection of B16 cells, 1 day before therapy | rIL-18 reduced the number of metastatic foci through an NK-mediated effect and shows synergistic activity with α-galactosyl-ceramide | [50] |
| CL8-1 melanoma and methylcholanthrene 205 sarcoma in syngeneic mice      | IL-18 administration starting before and/or after i.d. injection of tumor cells | IL-18 has potent anti-tumor effects mediated by CD4+ T cells and NK cells       | [51] |
| SCK mammary carcinoma cell line expressing IL-12 or IL-18 in syngeneic mice | Concurrently injected, distant parental SCK cells                     | IL-12 and IL-18 cooperate in inhibiting tumor angiogenesis and growth of parental cells | [52] |
| B16F10 melanoma-secreting IL-18 in syngeneic mice                        | s.c. Injection of IL-18-secreting B16F10 cells                       | Inhibition of tumor growth and microvessel formation                            | [55] |
| B16 melanoma in syngeneic mice                                           | Treatment started at the time of B16 cell s.c. injection             | Systemic IL-18 and B7-1-expressing B16 cells synergistically suppress B16 tumor and metastasis formation | [54] |
| B16F10 melanoma in syngeneic mice                                        | Injection in established s.c. tumors                                | Oncolytic viruses coexpressing IL-12 and IL-18 suppress tumor growth via T cell responses | [55] |
| LL/2 lung cancer in syngeneic mice                                       | Prophylactic vaccination before s.c. LL/2 challenge                 | LL/2 expressing GM-CSF and IL-18 suppress parent tumor growth via T cell responses | [56] |
| MC38 and MC38F3 expressing MUC1 antigen in MUC1 transgenic mice          | Prophylactic treatment or therapy, 3 days after tumor cell i.v. injection | DNA vaccination with MUC1/IL-18-expressing plasmid suppresses metastases in MUC1 transgenic mice | [57] |
| CT26 colon and C2F2 breast carcinoma in syngeneic mice                   | Therapy started 14 days after CT26 cell s.c. injection or 6 days after C2F2 i.v. injection | Systemic injection of IL-18-expressing, attenuated Salmonella inhibits tumor growth | [58] |
| RMA-S lymphoma cells or B16-RAE-1α cells in syngeneic mice               | Adoptive transfer, 3 h after radiotherapy of Day 7 tumors            | IL-12/IL-15/IL-18-activated NK cells injected in mice inhibit tumor growth       | [59] |
| Human Ramos lymphoma xenografts in SCID mice                             | Established s.c. tumors                                             | IL-18, combined with anti-CD20 mAb, enhances ADCC by NK cells and inhibits lymphoma xenograft growth | [60] |

---

*a*Selected references.
tumor growth and increases survival of mice bearing LL/2 tumors [56]. Administration of DNA plasmid vaccines encoding the human tumor antigen MUC1 and mouse IL-18 is an effective treatment for pulmonary metastases in MUC1-transgenic mice, whereas MUC1- or IL-18-encoding plasmids alone have no effect. CD8\(^+\) T cells and IFN-\(\gamma\) mediate the anti-tumor immunity in this model [57].

An attenuated strain of *Salmonella typhimurium*, engineered to express IL-18, inhibits the growth of s.c. tumors or pulmonary metastases in syngeneic mice by systemic administration without toxic effects. This treatment induces accumulation of T and NK cells and granulocytes in tumors and intratumor production of cytokines [58].

IL-18 may be an important factor for NK-based cell therapies. Mouse NK cells, preactivated in vitro with a combination of IL-12, IL-15, and IL-18, persist with sustained effector function in vivo when transferred into syngeneic mice. These cytokine-stimulated NK cells display "memory-like" features, as they produce more IFN-\(\gamma\) than naïve NK cells when restimulated in vitro with cytokines or antibodies engaging NKRs [64]. A subsequent study shows that the adoptive transfer of IL-12/15/18-activated NK cells, combined with irradiation, inhibits the growth of established mouse tumors through mechanisms requiring host CD4\(^+\) T cells [59].

The finding that human IL-12/15/18-pretreated NK cells also display memory-like features and secrete more IFN-\(\gamma\) in response to cytokines or to K562 cells in vitro suggests possible applications in human cancer therapy [65]. Human IL-12/15/18-pretreated NK cells display increased IL-2R\(\alpha\) (CD25) expression, which allows the formation of high-affinity trimeric IL-2Rs. Therefore, IL-12/15/18-pretreated NK cells respond to picomolar concentrations of IL-2 with increased proliferation, survival, and cytotoxicity. This finding provides a rationale for IL-12/15/18 NK-adoptive cell-therapy strategies combined with low-dose IL-2 [66]. Another report proposes the use of IL-18-containing combinatorial cytokine adjuvants to induce the intranodal helper NK functions, which activate DCs to recruit and activate T cells [67].

A recent study examined the effects of IL-18 on NK cell function mediated through FcγRs. IL-18 augments IFN-\(\gamma\) production and ADCC of NK cells mediated by anti-CD20 antibody (Rituximab) against Burkitt lymphoma cells [60]. Moreover, IL-18 and Rituximab cooperate in mediating regression of human lymphoma xenografts in immune-deficient mice. Altogether, these studies indicate that IL-18 activates NK or T cell-mediated anti-tumor immune responses in different preclinical tumor models.

It has been proposed that endogenous IL-18 may have anti-tumor properties in some human cancers. For example, the normal colon and ovarian epithelia are capable to process and release mature IL-18, which is involved in local immunity to pathogens. However, during neoplastic transformation, colon or ovarian cancer cells may develop mechanisms that limit the potential Th1/CTL-mediated anti-tumor immunity triggered by IL-18. Indeed, colon and ovarian cancer cells are unable to process IL-18 and express only pro-IL-18 as a result of defective Casp1 expression or activation [68–70]. Moreover, pro-IL-18, present in some ovarian cancer cells, is partially resistant to in vitro digestion with Casp1 as a result of the presence of a Casp1-resistant splice variant of pro-IL-18 [71]. Immune-reactive IL-18 is present at very high levels in ovarian cancer ascites and is also elevated in the patient sera. However, biochemical analyses of ascites show the predominance of the 25-kDa pro-IL-18, whereas the 18-kDa mature form is undetectable. In agreement with this finding, IFN-\(\gamma\) is not increased in patients’ sera and is undetectable in most ascites [72]. Altogether, these data suggest that the loss of the capacity to process IL-18 during neoplastic transformation may represent a potential mechanism of escape from Th1/IFN-\(\gamma\)-mediated responses, at least in the colon and ovary.

Another potential mechanism limiting IL-18 activity in some cancers is related to the increased expression of its natural inhibitor IL-18BP. In prostatic cancer, urinary and serum IL-18BP levels are increased, and serum IL-18BP levels correlate with the Gleason score. IFN-\(\gamma\) up-regulates IL-18BP production by prostate cancer cells in vitro, and costimulation with other cytokines, such as TNF-\(\alpha\) or IFN-\(\alpha\), increases IL-18BP production further [73]. Furthermore, in ovarian cancer, IL-18BP is increased in sera and even more in the ascites. This is likely related to local production, as tumor-associated myeloid cells and cancer cells show IL-18BP expression in vivo by immunohistochemistry [74]. Several findings suggest that different factors present in the microenvironment mediate IL-18BP expression in the tumor cells. Indeed, ovarian cancer cell lines do not express IL-18BP in vitro, unless treated with IFN-\(\gamma\) or IL-27, and IL-27 is a heterodimer cytokine—member of the IL-12 family—that mediates pSTAT1 in ovarian cancer cells. A potential role of IL-27 in vivo is suggested by the expression of the 2 IL-27 chains (IL-27A and EBV-induced 3) in ovarian cancer-associated leukocytes [74]. Prostate or ovarian cancer-derived IL-18BP is functional and inhibits IL-18 biologic activity in vitro. Altogether, these findings suggest that IL-18BP, produced in the microenvironment of ovarian and prostate tumors, may limit the activity of therapeutic IL-18 and represent a potential mechanism of tumor escape from Th1 responses by inhibiting endogenous IL-18, eventually present (Fig. 2). IL-18BP may then contribute to other known mechanisms of immune regulation at the tumor site, such as the induction of PD-L1 or of the immune-suppressive enzyme IDO in tumor cells, which can be driven by IFN-\(\gamma\) [75], or alternatively, by IL-27 in ovarian cancer [unpublished results].

Based on the efficacy of IL-18 in preclinical studies of cancer immunotherapy, clinical trials of IL-18 have been conducted (Table 2). Phase I clinical studies of IL-18 in advanced solid tumors and lymphomas showed limited toxicity and evidence of immune modulatory activity and identified a bioactive dose range [76, 77]. IL-18 administration had biologic effects on the immune system, as indicated by the increase in plasma concentrations of the proinflammatory cytokines IFN-\(\gamma\) and GM-CSF, soluble Fasl, and IL-18BP. In addition, IL-18 induced a transient lymphopenia, reaching a maximum at 2 h from infusion. The reduction in lymphocyte counts was more evident for NK cells than for CD8\(^+\) and CD4\(^+\) T cells, and the remaining NK and T cells showed increased expression of Fasl and the activation marker CD69. In addition, these studies showed some clinical activity and disease stabilization in a fraction of patients.

A Phase II study of IL-18 was performed in untreated Stage IV melanoma [78]. Patients were randomized to different groups receiving different dose levels of i.v. IL-18 for 5 days,
repeated every 28 days. Among 63 evaluable patients, a partial response and 4 disease stabilizations, lasting for 6 months or more, were observed. It was concluded that rIL-18 was well tolerated but had limited activity in metastatic melanoma as a single agent.

The in vitro and in vivo synergy of IL-18 and anti-CD20 mAb in a preclinical study [60] led to the design of a Phase I study of IL-18 and Rituximab in patients with B cell lymphoma. IL-18 administration was followed by an increase in plasma proinflammatory cytokines, CXC chemokines (CXCL9 and CXCL10), and the CC chemokine, CCL2. The IL-18-induced lymphopenia, recorded also in other studies [77] is possibly a result of lymphocyte activation and their subsequent extravasation into tissues. Indeed, an increased tumor infiltration of CD69<sup>+</sup>-activated lymphocytes was recorded in a patient with mantle cell lymphoma [79]. Objective tumor responses were seen in 5 patients out of 18 treated, including 2 complete and 3 partial responses. It was concluded that further studies of human rIL-18 and anti-CD20 mAb in B cell malignancies are warranted [79].

A Phase I dose-escalation study of i.v. IL-18, in combination with pegylated liposomal doxorubicin (Doxil) in recurrent epithelial ovarian cancer, has been concluded recently (NCT00659178). rIL-18, in combination with Doxil, is safe and mediates biologic responses. One out of 10 evaluable patients showed a partial response and approximately one-third had stable disease. The authors concluded that this combination therapy is feasible and deserves further evaluation in a Phase II trial [80].

Collectively, clinical trials show that IL-18 has low toxicity in humans but limited therapeutic effects as a single agent. Nonetheless, IL-18 may be incorporated as an immune-enhancing molecule in combinational therapies with other agents (e.g., mAb, cytotoxic drugs, or vaccines).

In this respect, a Phase I study of IL-18, in combination with the anti-CD20 antibody ofatumumab, after autologous peripheral blood stem-cell transplantation for lymphoma, is ongoing (NCT01768338). Moreover, a Phase I study of cyclophosphamide/fludarabine lymphodepletion, followed by adoptive transfer of vaccine-primed peripheral blood autologous T cells, is ongoing (NCT02277392). Treatment, will be conducted in patients with recurrent ovarian, fallopian-tube, or primary peritoneal cancer, who were vaccinated previously with a whole-tumor vaccine (NCT02277392).

**TUMOR-PROMOTING ACTIVITIES OF IL-18**

Although the preclinical studies and some clinical trials suggest that IL-18 has anti-tumor activities, other studies indicate that IL-18 has a dual role in tumors, as it may exert proinvasive,
proangiogenic, and immune-regulatory activities in different tumor models (Table 3) [42, 43].

In spite of its protective effect in initial stages of *H. pylori* infection, IL-18 may support tumor progression in advanced gastric cancer. Indeed, it stimulates the production of the proangiogenic factor, thrombospondin-1, in IL-18R-expressing gastric cancer cells through the activation of the c-Jun N-terminal kinase [81]. In another study, VEGF stimulates IL-18 production and processing in gastric cancer cells, and IL-18, in turn, promotes cell migration through tensin down-regulation and actin polymerization. Therefore, IL-18 may be part of a loop that amplifies gastric cancer cell migration, angiogenesis, and progression [82]. In addition, VEGF induces ADAM-33 expression, which up-regulates IL-18 secretion, resulting in increased gastric cancer cell migration and proliferation [83]. Besides its activity on tumor invasiveness and angiogenesis, IL-18 induced expression of a granzyme B inhibitor, protease inhibitor 9, in gastric cancer cells and decreased their susceptibility to lymphocyte-mediated cytotoxicity [84].

A recent report showed that IL-18, produced by stromal cells, is a growth factor for T-ALL cells [85]. MEK inhibitors enhance the growth of human T-ALL cells cocultured with stromal cells through the transcriptional up-regulation of *IL18* in stromal cells. In addition, rIL-18 promotes T-ALL growth in vitro through activation of the NF-κB pathway, whereas silencing of IL-18R in T-ALL cells inhibits their proliferation in vitro and in vivo.

The finding that high serum levels of IL-18 in some patients with autoimmune diseases correlate with impaired NK cell survival [98] suggested immune-regulatory activities of IL-18 on NK cells, other than the "helper NK" cell priming. This hypothesis was addressed in syngeneic tumor models. Intriguingly, whereas daily administration of IL-18 had anti-tumor effects in a syngeneic melanoma model, similar to previous reports [50], a twice/wk schedule of IL-18 showed protumor

### TABLE 3. Protumor activities of IL-18 in different models

| Tumor type                                           | Model                                         | Major findings                                                   | Ref. * |
|------------------------------------------------------|-----------------------------------------------|-----------------------------------------------------------------|--------|
| Human gastric cancer                                 | Gastric cancer cell lines in vitro           | IL-18 mediates production of the proangiogenic factor thrombospondin-1. |        |
| Human gastric cancer cells                           | SNU-601 cell line in vitro                   | VEGF-induced IL-18 promotes cell migration and proliferation through ADAM-33 induction. | [82, 83] |
| Human gastric cancer cells                           | Gastric cancer cell lines in vitro           | IL-18 induces resistance to lymphocyte-mediated cytotoxicity via granzyme B inhibitor. | [84]   |
| Human T-ALL.                                         | In vitro assays on primary T-ALL cells and T-ALL xenografts | High levels of IL-18 produced by MEK inhibitor-treated stromal cells support T-ALL cell growth. IL-18 blockade or IL-18R silencing delays xenograft growth. | [85]   |
| B16F10 melanoma and CT26 colon cancer                | IL-18, twice/wk to syngeneic mice injected i.v. with tumor cells | IL-18 enhances the development of B16F10 or CT26 lung metastases through the induction of PD-1 on NK cells. B16F10 metastases were reduced in IL-18R<sup>−/−</sup> or MyD88<sup>−/−</sup> mice. | [86]   |
| B16F10 melanoma and CT26 colon cancer                | Syngeneic mice injected i.v. with tumor cells | Silencing of IL-18 in tumor cells or IL-18BP administration restores NK-mediated immune surveillance and inhibits metastasis formation. |        |
| B16F10 melanoma                                      | Administration of IL-18, twice/wk to syngeneic mice | IL-18 induces the differentiation of MDSC from bone marrow cells in vitro. IL-18 administration increased MDSC in s.c. tumors. | [88]   |
| HPV16 E7 transgenic mice                             | HPV-associated epidermal hyperplasia         | IL-18 mediates IFN-γ production in the immune-suppressive environment of hyperplastic skin. | [89]   |
| B16 melanoma in syngeneic mice                       | Liver metastases and in vitro assays         | IL-18 mediates the development of liver metastases of B16M in vivo, promotes VCAM1 expression on endothelial cells, and favors tumor cell adhesiveness. | [90, 91] |
| B16 melanoma                                         | Liver metastases in syngeneic mice           | IL-18BP, resveratrol, or thymoquinone inhibits liver metastases. | [92, 93] |
| Human and mouse melanoma cell lines                 | In vitro assays and in vivo liver B16 metastases in syngeneic mice | IL-18 mediates VLA-4 integrin activation and cell adhesion through the sequential induction of proinflammatory factors. | [94]   |
| Human melanoma cell line xenografts                 | Study of human melanomas and their xenografts in nude mice | IL-18R/VEGF/VLA-4-expressing melanoma cells produce more metastases than melanoma cells without this phenotype. | [95]   |
| Different human cancers                              | Meta-analysis of *IL18* gene polymorphism     | −607 C > A or −137 G > C polymorphisms of the *IL18* promoter associate with increased risk of nasopharyngeal and esophageal carcinoma in the Asian population. | [96, 97] |

VEGF, Vascular endothelial growth factor. *Only selected references are reported.*
activity [86]. These data suggest that the administration schedule of IL-18 may be crucial for biologic effects on tumors. IL-18 up-regulates PD-1 expression by activated, mature NK cells in lymphoid organs and reduces NK cell antimetastatic activity in a PD-1-dependent mode [86]. Further studies indicated that IL-18 converts a subset of Kit⁺ NK cells into Kit⁺ NK cells, which overexpress PD-L1 and mediate immune-ablative functions, in mouse models. Indeed, the silencing of IL-18 in tumors or its blockade by IL-18BP restores NK cell-dependent immune surveillance [87].

A recent report indicates that IL-18 induces the differentiation of MDSC expressing iNOS and arginase-1 from murine bone marrow precursors. These cells efficiently suppress T cell responses in vitro. Treatment of mice with IL-18, twice/wk, increased the accumulation of MDSC in s.c. B16 melanoma tumors [88].

Although IL-18-induced IFN-γ plays a role in the defense against infections and cancer, IFN-γ may also have immune-suppressive effects in some models of disease, as in a mouse model of HPV-associated epidermal hyperplasia, driven by transgenic expression of the HPV16 E7 oncprotein. Production of IFN-γ requires IL-18 but not IL-12 in this model. These findings indicate that IL-18 contributes to the generation of an immunosuppressive environment during viral oncogene-driven epidermal hyperplasia [89].

Moreover, IL-18 showed proangiogenic and prometastatic activities in mouse models of melanoma hepatic metastases. IL-18 mediates VCAM1 expression in endothelial cells, favoring the adhesion of melanoma cells in vitro [90]. In the B16 melanoma model, the administration of IL-18BP, which blocks endogenous IL-18, inhibits the development of hepatic metastases through inhibition of VCAM1 expression on hepatic sinusoid endothelium [91]. More recent data indicate that resveratrol [92] or the NLRP3 inflammasome inhibitor thymoquinone [93] suppresses metastases of murine melanoma cells through inhibition of IL-18-mediated VCAM1 expression and/or IL-18 secretion.

A subset of human melanomas expressing IL-18R shows enhanced prometastatic activity in nude mice relative to IL-18R-negative melanomas. The increased metastatic potential is related to a cascade of IL-18-mediated inflammatory factors leading to expression of VLA-4 integrin [94]. VLA-4, a ligand for VCAM1, mediates adhesion of circulating tumor cells to the vessel endothelium, the initial step in tissue transmigration and metastasis formation. The study of human metastatic melanomas, with or without IL-18-dependent gene-expression signatures, indicates the involvement of an IL-18-induced inflammatory phenotype in some metastasizing melanomas [95].

Altogether, these studies suggested that IL-18BP or inflammasome/Casp1 inhibitors may be regarded as a therapeutic option in cancers where IL-18 acts as a tumor-promoting agent. Indeed, IL-18BP-Fc treatment was effective in inhibiting the lung metastasis progression by blocking endogenous, tumor-released IL-18 [99]. In addition, IL-18BP may represent a useful tool for the detection of IL-18-expressing tumors. Indeed, (64)Cu-DOTA-IL-18BP-Fc shows specific accumulation by positron emission tomography in a syngeneic lung metastasis model [99].

Different studies reported the association of polymorphisms in the IL18 gene promoter (−607 C > A and −137 G > C) and the development of different human cancers, suggesting a possible functional involvement of IL-18 in human carcinogenesis. Indeed, these polymorphisms alter binding sites of specific transcription factors and result in altered IL18 gene transcription [100]. Although these studies produced controversial results, in different cancers and populations, a recent meta-analysis suggested that the −607 C > A polymorphism is associated with increased overall cancer risk, particularly in nasopharyngeal carcinoma and esophageal cancer in the Asian population [96]. Another meta-analysis indicated that the −137 G > C polymorphism is associated with increased risk of nasopharyngeal carcinoma in the Asian population but not in Caucasians [97]. On the other hand, no association between the −607 C > A polymorphisms and the risk of prostate, colorectal, breast, cervical, or other cancers was found. The divergent role of IL18 gene polymorphisms may account for differences in carcinogenic mechanisms in various cancers.

High levels of IL-18 were found in tumor tissues or in the systemic circulation of human cancers, including esophageal [101], gastrointestinal [102], breast [103], ovarian [72, 104], pancreatic [105], hepatocellular [106], lung [107], and renal cancer [108]; diffuse large B cell lymphoma [109]; and multiple myeloma [110]. In some instances, high levels of IL-18 were associated with advanced tumor stages and/or with a poor prognosis, suggesting that IL-18 promotes tumor progression. However, some IL-18 ELISAs show substantial cross-reactivity with the pro-IL-18 [72], leaving unresolved the question of whether the high levels of IL-18 found in tumors always correspond to an increase of the mature IL-18 form. Therefore, in most tumors, the impact of IL-18 processing and the role of endogenous bioactive IL-18 are still an open issue. For example, serum IL-18BPs and IL-18 levels were increased in pancreatic cancer patients, and calculated “free” IL-18 levels were correlated with disease severity and poor survival. Chemotherapy further increased free IL-18 levels, without affecting IL-18BPs. The authors concluded that caution in the use of IL-18 therapy should be used in pancreatic cancer in light of a potential role of IL-18 in tumor progression or angiogenesis [105]. Nonetheless, the possibility that pro-IL-18 may interfere with mature IL-18 detection and explain the apparent paradox of high, free IL-18 levels cannot be excluded, as pancreatic cancer cells secrete pro-IL-18 [111]. However, upon treatment with 5-fluorouracil, Casp1 is activated, leading to secretion of mature IL-18 by pancreatic cancer cells. This finding supports the concept that in cancer, the inflammasomes may be activated through DAMPs, produced as a consequence of spontaneous or therapy-related tumor cell death, and lead to IL-18 processing.

**CONCLUDING REMARKS**

In conclusion, the current literature uncovers a complex and sometimes divergent role of the IL-18/IL-18R/IL-18BP system in different neoplastic conditions. Anti-tumor effects of endogenous or exogenous IL-18 were reported in early stages of colon and gastric carcinogenesis and in several preclinical models of cancer immunotherapy, respectively. On the other hand, pro-cancer effects of IL-18 were described in advanced gastric cancer, in a subset of melanomas, and in T-ALL. Along this line, high levels of IL-18 were found in different cancers, and IL18 gene
polymorphisms were associated with some cancers. However, IL-18, released by ovarian and colon cancer cells, is the unprocessed, inactive form, which can cross-react with mature IL-18 in ELISA. These findings suggest that more studies on the biologic role of cancer-related IL-18 are warranted.

Altogether, the context-dependent effects of IL-18 in cancer pose the question of whether IL-18, or rather its antagonist IL-18BP, should be used for cancer therapy. Most likely, as for other biologic medicinal products, the choice should depend on the specific characteristics of a given tumor. In some tumor types, where tumor-promoting IL-18 effects prevail, IL-18BP could be helpful, whereas IL-18 therapy should be regarded with caution. This is the case of a subset of melanomas, gastric cancers, and T-ALL, which expresses IL-18R and may progress in response to IL-18. In other tumors, such as ovarian and prostate cancer, high endogenous IL-18BP levels may limit the activity of therapeutic IL-18, particularly at the tumor site. Therefore, the biologic impact of the IL-18/IL-18R/IL-18BP system in specific cancers should be considered in the design of clinical studies.

AUTHORSHIP
M.F., G.C., and S.F. wrote the manuscript together.

ACKNOWLEDGMENTS
This work was supported by a grant from Fondazione Compagnia di San Paolo, and a grant from Associazione Italiana per la Ricerca sul Cancro (AIRC; IG 13518). Grazia Carbotti is recipient of a fellowship from San Paolo, and a grant from Associazione Italiana per la Ricerca sul Cancro. This work was supported by a grant from Fondazione Compagnia di San Paolo.

DISCLOSURES
The authors have no conflict of interest to declare.

REFERENCES
1. Garlanda, C., Dinarello, C. A., Mantovani, A. (2013) The interleukin-1 family: back to the future. Immunology 139, 1003–1018.

2. Okamura, H., Tsutsu, H., Komatsu, T., Yutsudo, M., Hakura, A., Komatsu, T., Nakashima, K., Akita, K., Nambu, M., Tanabe, F., Konishi, K., Fukuda, S., Kurimoto, M. (1995) Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature 378, 88–91.

3. Puren, A. J., Fantuzzi, G., Dinarello, C. A. (1999) Gene expression, synthesis, and secretion of interleukin 18 and interleukin 1beta are differentially regulated in human blood mononuclear cells and mouse spleen cells. Proc. Natl. Acad. Sci. USA 96, 2256–2261.

4. Živogzel, L., Kepp, O., Galluzzi, L., Kroemer, G. (2012) Inflammomodules in carcinogenesis and anticaner immune responses. Nat. Immunol. 13, 343–351.

5. Jacobs, S. R., Damania, B. (2012) NLRs, in Immunity and inflammasome cytokines in rheumatic diseases. Arthritis Res. Ther. 15, 226–247.

6. Fabbi et al. Role of IL-18 and IL-18BP in cancer
melanoma cells by inhibition of NLRP3 inflammasome.

Toxcol. Appl. Pharmacol. 270, 70–76.

Valcarcel, M., Carascal, T., Credne, O., Vidal-Vanaclocha, F. (2014) IL-18 regulates melanoma VLA-4 integrin activation through a hierarchized sequence of inflammatory factors. J. Invest. Dermatol. 134, 470–480.

Credne, O., Sahinato, M., Valcarcel, M., Carascal, T., Rierstra, P., Lopez-Guerrero, J. A., Nagore, E., Mandruzatto, S., Wang, E., Marincola, F. M., Vidal-Vanaclocha, F. (2013) Increased level of human interleukin-18 using two different schedules of administration in patients with advanced cancer. Clin. Cancer Res. 19, 4265–4273.

Robertson, M. J., Kirkwood, J. M., Logan, T. F., Koch, K. M., Kathman, S., Pandite, L. N., Oei, C., Kirby, L. C., Jewell, R. C., Bell, W. N., Thurmond, L. M., Weisenbach, J., Roberts, S., Dar, M. M. (2006) Clinical and biological effects of recombinant human interleukin-18 administered by intravenous injection to patients with advanced cancer. Clin. Cancer Res. 12, 4265–4273.

Robertson, M. J., Kline, J., Struempfer, H., Koch, K. M., Bauman, J. W., Gardner, O. S., Murray, S. C., Gernascheski, F., Weisenbach, J., Jonak, Z., Tosο, J. F. (2013) A dose-escalation study of recombinant human interleukin-18 in combination with rituximab in patients with non-Hodgkin lymphoma. J. Immunother. 36, 331–341.

Majima, T., Yoshida, R., Koga, H., Takeuchi, O., Hayashi, H., Sugawara, H., Kuranaga, N., Takayama, E., Kinoshita, M., Hiraide, H., Seki, S., Mochizuki, H. (2006) Exploitation of interleukin-18 by gastric cancers for their growth and evasion of host immunity. Int. J. Cancer 118, 388–395.

Uroz, B., Poglio, S., Gerby, B., Wu, C. L., Gross, J., Calvo, A. (2002) Clinical importance of serum interleukin-12 and interleukin-18 in lung cancer. Cancer Immunol. Immunother. 51, 251–259.

Gossmann, C., Frazer, I. H., Mattarrollo, S. R., Blumenthal, A. (2014) IL-18 enhances immunomodulatory responses by promoting differentiation into monocytic myeloid-derived suppressor cells. J. Immunol. 193, 5453–5460.

Vidal-Vanaclocha, F., Fantuzzi, G., Mendoza, L., Fuentes, F., Sancho, C., Manrique, J. M., Kirkwood, J. M. (2009) A phase 2, randomized study of SB-485232, rhIL-18, in patients with previously untreated metastatic melanoma. Clin. Cancer Res. 15, 3462–3469.

Tarhini, A., Milward, M., Mainwaring, P., Kefferd, R., Logan, T., Partick, A., Kathman, S. C., Laubscher, W. A., Dar, M. M., Kirkwood, J. M. (2009) Immunosuppressive responses by promoting differentiation into monocytic myeloid-derived suppressor cells. J. Immunol. 182, 3548–3555.

Terme, M., Ullrich, E., Aymeric, L., Meinhardt, K., Coudert, J. D., Kim, J., Kim, C., Kim, T. S., Bang, S. I., Yang, Y., Park, H., Cho, D. (2006) IL-18 enhances thrombospondin-1 production in human gastric cancer cell lines. Oncogene 25, 1468–1476.

Kim, K. E., Song, H., Hahn, C., Yoon, D., Kim, C. W., Bang, S. I., Hur, D. Y., Park, H., Cho, D. H. (2007) Interleukin-18 is a critical factor for vascular endothelial growth factor-enhanced migration in human gastric cancer cells. Oncology 68, 275–279.

Kim, K. E., Song, H., Hahn, C., Yoon, D., Park, S., Lee, H. R., Hur, D. Y., Kim, T., Kim, C. H., Bang, S. I., Bang, J. W., Park, H., Cho, D. H. (2009) Expression of ADAM33 is a novel regulatory mechanism in IL-18-secreted process in gastric cancer. J. Immunol. 182, 6347–6355.

Fabbi et al. Role of IL-18 and IL-18BP in cancer.

KEY WORDS: immune regulation · cancer immunotherapy · Th1 response