ABSTRACT

We studied the role of endogenous interleukin (IL)-18 in hepatic metastasis by blocking this cytokine using the naturally occurring IL-18 binding protein (IL-18BP). A single i.p. dose of IL-18BP given 30 min before intrasplenic injection of murine B16 melanoma (B16M) cells reduced the number of hepatic metastatic foci by 75% and metastatic volume by 80%. Same treatment reduced the intraperitoneal retention of luciferase-transfected B16M by 50% and abolished VCAM-1 up-regulation in the hepatic microvasculature, as assessed by reverse transcription-PCR, Western blot, and immunohistochemistry. Twelve hours after IL-18BP, hepatic sinusoidal endothelium (HSE) cells were isolated, and adhesion of B16M cells to these cultured HSE cells was reduced to the level of vehicle-treated mice. IL-18BP treatment of mice with established micrometastases resulted in a 25% decrease in metastasis number and 40% decrease in metastasis volume, suggesting inhibition of endogenous growth factors. Indeed, the addition of IL-18BP to normal HSE abolished the release of melanoma cell growth factor(s) induced by B16M. IL-18 promoted the in vitro growth of B16M and human melanoma cells, which was IL-1 dependent. These data demonstrate a significant role of endogenous IL-18 in hepatic metastasis by up-regulating melanoma cell adhesion to HSE cells and tumor growth, implicating a possible antimetastatic benefit of neutralizing IL-18.

INTRODUCTION

IL-18 is a new member of the IL-1 family that plays a central role in the immune response by acting on Th1 cell differentiation, cell-mediated cytotoxicity, and inflammation (1). It has been reported that constitutive (2) or genetically manipulated (3) production of IL-18 can lead to enhanced antitumor response and improved survival. Furthermore, hypervascularization of IL-18-treated tumors has been reported (4). Thus, IL-18 has been proposed as a novel adjuvant therapy against cancer diseases (5). On the other hand, human (6) and rodent (7) cancer cells produce IL-18, and circulating concentrations increase during metastatic disease as compared with levels in early cancer patients (8). In addition, murine B16M cells can escape immune recognition by up-regulating the expression of reactive oxygen intermediate-mediated Fas ligand via IL-18-dependent mechanism (7). Furthermore, IL-18 increases the adhesion of murine B16M cells to cultured HSE via a VCAM-1-dependent mechanism (9). Thus, the role of IL-18 in cancer progression and metastasis remains controversial.

IL-18 shares with IL-1 the same family of receptors, and several signal transduction pathways seem identical to those of IL-1 (10). Thus, aside from the actions of IL-18 on immune cells, IL-18 has important proinflammatory effects (11), e.g., IL-18 can even contribute to tissue damage, as during acute liver inflammation (12). Using B16M cells, we have reported that hepatic sinusoidal cells also release IL-18 in response to melanoma cell interaction with liver microvasculature, which increases VCAM-1-dependent adhesion of cancer cells to HSE cells (9, 13). This step is critical in metastasis progression, as shown by the reduced metastatic activity occurring in B16M cell-injected ICE and IL-1β KO mice (9). This appears to be regulated by an inflammatory cascade, whereby soluble products from B16M cells trigger HSE cells to sequentially release TNF-α, IL-1β, and IL-18, the latter cytokine being responsible for the up-regulation of VCAM-1 via HO2-dependent nuclear factor-κB (13) and the adherence of B16M cells. Furthermore, IL-18 activates endothelial cell migration via αV/β3 integrin and increases angiogenesis in vivo via TNF-α-independent mechanisms (14). To further characterize the prometastatic effects of IL-18, we studied the effects of IL-18BP on B16M metastatic mechanisms in vivo and in vitro. IL-18BP was produced as described previously (15). Because B16M cell adhesion to HSE cells is mainly VCAM-1 mediated (16, 17), we also assessed VCAM-1 expression in HSE cells either in situ or ex vivo once isolated on the 12th h after the injection of tumor cells in untreated and IL-18BP-pretreated mice. Because treatment with IL-18BP once metastases were established still reduced their development, we considered that IL-18 may function as a growth factor for melanoma cells and, hence, studied the effect of IL-18BP on B16M cell proliferation in response to the supernatant from B16M CM-treated HSE cells in vitro. We also determined proliferation rate of several human melanoma cell lines in response to recombinant human IL-18.

MATERIALS AND METHODS

Culture of Murine and Human Melanoma Cells. B16M cells (B16F10 subline) were used. Human melanoma cell lines (MM, SK23, HMB2, VUP, and A375) were kindly provided by Ian A. Hart (St. Thomas Hospital, London, United Kingdom). All cell lines were cultured in endotoxin-free DMEM (Life Technologies, Inc., Grand Island, NY) supplemented with 10% FCS and penicillin-streptomycin. Cultures were maintained and passed as described previously (13, 18).

Hepatic Metastasis. Syngeneic C57BL/6j mice (male, 6–8 weeks old) were obtained from IFFA CREDO (L’Arbresle, France). Hepatic metastases were produced by the intrasplenic injection into anesthetized mice (Nembutal, 50 mg/kg i.p.) of 3 × 10⁵ viable B16M cells suspended in 0.1 ml of HBSS (Life Technologies, Inc.). The volume fraction of liver occupied by metastases was determined histologically and recorded as the (number of metastases per 100 mm² of liver, based on the mean number of foci detected in fifteen 10 × 10 mm² sections/liver) were also determined using stereochemical procedures described previously (18).

B16M Cell Adhesion Assay to Primary Cultured HSE. Saline or 3 × 10⁵ B16M cells were intrasplenically injected into mice 18 h before HSE isolation. Some mice additionally received an i.p. injection of 12.5 μg/kg IL-18BP for 30 days. This article must therefore be hereby marked advertisement. This article must therefore be hereby marked charges. This article must therefore be hereby marked advertisement.

Received 9/2002; accepted 11/13/02.

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1. Supported in part by Grant 15641/2001 from the Basque Country University, Leioa, Grant EX-1999-125 from the Department of Education, University and Research from the Basque Country Government, Grant SAF99–0042 from the Plan Nacional de I+D de la Comisión Interministerial de Ciencia y Tecnología (to F. V-V.), Grant AI-15614 from the Basque Country Government, Grant SAF99-0042 from the Plan Nacional de I+D de la Comisión Interministerial de Ciencia y Tecnología (to F. V-V.), Grant AI-15614 from the Basque Country Government, Grant SAF99–0042 from the Plan Nacional de I+D de la Comisión Interministerial de Ciencia y Tecnología (to F. V-V.), Grant AI-15614 from the Basque Country Government, Grant SAF99–0042 from the Plan Nacional de I+D de la Comisión Interministerial de Ciencia y Tecnología (to F. V-V.), Grant AI-15614 from the Basque Country Government, Grant AI-15614 from the Basque Country Government, Grant AI-15614 from the Basque Country Government, Grant AI-15614 from the Basque Country Government, Grant AI-15614 from the Basque Country Government.

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3. The abbreviations used are: IL, interleukin; B16M, B16 melanoma; TNF, tumor necrosis factor; IL-18BP, interleukin-18 binding protein; IL-1Ra, interleukin-1 receptor antagonist; HSE, hepatic sinusoidal endothelium; B16M-Luc, luciferase-transfected B16M; LAL, liver-associated lymphocyte; VCAM, vascular cell adhesion molecule; KO, knockout; CM, conditioned medium; ICE, interleukin-1β-converting enzyme.

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min before B16M cells. Then, resulting HSE cells from these mice were isolated and identified as described previously (19) and cultured for only 5 h before being used in adhesion assays as described previously (13).

**Enzyme Immunoassay.** Recombinant murine IL-18 concentration in serum from hepatic blood was detected using competitive enzyme immunoassays (R&D Systems, Inc., Minneapolis, MN), according to the manufacturer’s instructions.

**Studies on VCAM-1 Expression.** The quantification from transcripts of the gene encoding for VCAM-1 and β-actin as the endogenous RNA control and Western blotting on VCAM-1 and β-tubulin proteins were made as described previously (13).

**Quantitative Assay on Hepatic Retention of Circulating Melanoma Cells.** B16M cells were permanently transfected by lipofection as described previously (20), using plasmid pRec/cytomegalovirus-luciferase, a construct containing the Photinus pyralis luciferase gene coding sequence under transcriptional control of the cytomegalovirus promoter, and the neomycin resistance gene encoding resistance to the G418 antibiotic (Life Technologies, Inc., Gaithersburg, MD). A group of 300,000 viable B16M-Luc cells was intrasplenically injected into C57BL/6J mice (n = 30). Part of the mice (n = 15) received an i.p. injection of 25 μg/kg IL-18BP 30 min before B16M-Luc cells. All mice were killed by cervical dislocation 18 h later, and livers were processed as described previously (20) to measure luciferase activity by chemiluminescence using the standard luciferase assay kit (Promega Co., Madison, WI), as reported (13).

**Melanoma Cell Proliferation Assay.** Conditioned media from confluent HSE cells were obtained from 24-well plates containing ~8 × 10^6 cells/well after an incubation period of 24 h. In some experiments, HSE cells were cultured in the presence of B16M-CM obtained as described above. Then, HSE supernatants were removed, centrifuged at 1000 g for 10 min, and subjected to 0.22-μm filtration. Different conditioned media were added to 3 × 10^4 B16M cells and seeded into each well of a 96-well microtiter plate, in the presence or not of 1 μg/ml IL-18BP. Control B16M cells were cultured in the presence of basal medium (DMEM) used in generating the HSE-conditioned media. After 48-h incubation, B16M cell proliferation was measured using sulforhodamine 101 protein assay, as described previously (21). The same proliferation assay was used to determine the in vitro growth rate of several human melanoma cell lines (MMJ, SK23, HMB2, VUP, and A375 cells) and B16M cells, given 0.1 ng/ml recombinant human or murine IL-18 (PeproTech, Inc., Rocky Hill, NJ), respectively. In some experiments, B16M cells were incubated with 100 ng/ml IL-1Rα (kindly provided by Amgen Biologicals) 30 min before IL-18 addition.

**Immunohistochemistry and Quantitative Histology.** Indirect immunofluorescence analysis of VCAM-1 (CD106) expression in liver was performed on 10-μm-thick frozen sections obtained from mouse livers on the 12th h after intrasplenic injection of saline or B16M cells. Staining was performed overnight, as described previously (13). The intensity of the VCAM-1 expression on scanned digitzed liver tissue images was densitometrically analyzed using the NIH image analysis program for Macintosh.

**Statistical Analysis.** The data were expressed as means ± SE. Statistical analysis was performed by SPPS statistical software for Microsoft Windows, release 6.0 (Professional Statistic, Chicago, IL). Homogeneity of variance was tested by using the Levene test. If the variances were homogenous, data were analyzed by using unpaired Student’s t test (program Statview 512 for Macintosh; Abacus Concepts, Inc.). The criterion for significance was P ≤ 0.05 for all comparisons.

**RESULTS**

**IL-18 Level Increases in the Hepatic Venous Blood during Hepatic Colonization of Intrasplenically Injected B16M Cells.** IL-18 increased (P < 0.01) in hepatic venous blood over basal level from the 12th h after the injection of B16M cells until day 3 (Fig. 1). This elevation coincided with sinusoidal inflammation associated with B16M cell implantation (18). Serum levels of IL-18 increased again (P < 0.01) on day 7 after the injection of the B16M cells, which was concomitant with the growth of hepatic micrometastases with a diameter <1 mm. At day 12, the elevated serum levels of IL-18 appeared to correlate with the presence of hepatic metastatic growth. In vitro, levels of IL-18 in the 24-h supernatants of B16M cells were below detection; however, steady-state levels of IL-18 mRNA were present in these cells (data not shown) but similar to those reported previously (9). On the other hand, the supernatant from cultured B16M cells induced IL-18 secretion from HSE cells in vitro (11, 16), suggesting that elevated levels of IL-18 in hepatic blood likely are from tumor-activated liver sinusoidal cells rather than from the B16M cells themselves.

**Pretreatment of Mice with IL-18BP Reduces B16M Hepatic Metastasis Density and Metastatic Volume.** Mice were i.p. injected with 25 μg/kg IL-18BP and 30 min later, received an intrasplenic injection of B16M cells (preimplantation treatment). Control mice were pretreated with saline (vehicle). As shown in Fig. 2A–D, there was a dramatic reduction in the number of metastatic foci in the liver (metastatic density), as well as the percentage of liver tissue occupied by the cancer tissue (metastatic volume). In studies published previously using the same model, pretreatment of mice with IL-1Rα also resulted in comparable reductions in B16M hepatic metastases, but a dose of 10 mg/kg IL-1Rα was required (18). Pretreatment of mice with neutralizing antibodies to mouse IL-18 2 h before the intrasplenic injection of B16M cells resulted in the same reduction in the metastasis density and volume that we observed in mice pretreated with IL-18BP (data not shown).

We extended these studies by comparing preimplantation with IL-18BP to postimplantation treatment. For the postimplantation schedule, mice received 12.5 μg/kg IL-18BP i.p., as single daily dose, from days 7 to 11 after the initial injection of B16M cells. As shown in Fig. 2A–D, this postimplantation treatment schedule resulted in a reduction in the metastatic volume (40% inhibition, P < 0.05). This observation suggests a role of IL-18 in the mechanism of metastatic growth in the liver. However, the metastatic density (number of metastatic foci) decreased only by 25% (Fig. 2, A and B), suggesting that there was an IL-18-dependent prometastatic effect in this model.
that cannot be explained solely on the reduction in microvascular implantation of B16M cells in the days after the intrasplenic injection. Again, there was a similar reduction in the metastatic volume in mice receiving neutralizing antibodies to mouse IL-18 on days 5, 7, and 9 after the injection of the B16M cells (data not shown). These findings suggest that during the postimplantation phases, IL-18 was acting as a growth factor via a direct proliferative effect on tumor cells or by increasing the blood supply to the growing tumors.

The combination of a single preimplantation treatment (25 μg/kg) plus 5 days of postimplantation treatment with IL-18BP (12.5 μg/kg) resulted in a near total eradication in both metastatic density and volume (Fig. 2, A and B). These results are also visually represented in Fig. 2E.

Effect of Pretreatment with IL-18BP on in Vivo Intrasinusoidal Retention of B16M Cells and Adherence to HSE ex Vivo. Mice pretreated with either IL-18BP or saline received an intrasplenic injection of B16M-Luc cells, and after 12 h, the intrahepatic retention of tumor cells was evaluated by measurement of chemiluminiscence of luciferase activity in liver homogenates. For these studies, a dose of 12.5 μg/kg was used. As shown in Fig. 3A, IL-18BP significantly (P < 0.01) reduced by 50% the intrahepatic retention of B16M-Luc cells, as compared with mice given saline injection. This finding suggests that IL-18 blockade during intrahepatic infiltration of B16M cells inhibited their intrasinusoidal retention. This dose also resulted in ~50% reduction in hepatic
metastasis density with respect to saline-pretreated control mice (data not shown).

We next pretreated mice with 12.5 μg/kg IL-18BP, injected B16M cells into the spleen, and after 12 h, isolated HSE cells. HSE cells were cultured for 4 h, and the percentage of B16M cells adhering was evaluated (Fig. 3B). In HSE cells from mice not injected with tumor cells (control cells), the percentage of adhering B16M cells was 20%. In contrast, the percentage of cells adhering to HSE cells from mice injected intrasplenically with B16M cells but pretreated saline increased to 30%. However, B16M cells adhering to HSE derived from mice pretreated with IL-18BP fell to 20%, the level of adherence of control mice not injected with tumor cells. These results suggest that intrasplenic injection of B16M cells increases the basal adhesive quality of HSE for B16M cells (from 20 to 30%), which lasts for 7–8 h after the isolation of HSE from the animal. Furthermore, these results demonstrate that this up-regulation is IL-18 dependent in vivo. The mechanism was tumor specific because, as reported previously, intrasplenic injection of autologous primary cultured epidermal keratinocytes did not significantly increase B16M cell adhesion to HSE (13).

Using the cells from the studies depicted in Fig. 3A–B, the effect of IL-18BP preimplantation treatment on VCAM-1 gene and protein expression was assessed in lysates from these HSE cells. As shown in Fig. 3C, the ratio of VCAM-1/β-actin mRNA increased in HSE cells isolated from B16M cell-injected mice to 0.36, compared with cells isolated from mice not injected with tumor cells (0.27).
However, when mice were pretreated with IL-18BP before the injection of B16M cells and HSE isolated after 12 h were analyzed, the increased VCAM-1 steady-state mRNA was completely abrogated (0.18). There was also a reduction observed with IL-18BP-treated mice not injected with tumor cells (0.27 compared with 0.22). Both observations suggest that VCAM-1 mRNA levels are IL-18 dependent.

After isolation, the total synthesis of VCAM-1 was also assessed in lysates of these same cells by Western blotting. Additionally shown in Fig. 3C is the ratio of VCAM-1-β-tubulin synthesis. In mice injected with IL-18BP before the saline (no tumor cells), there was a marked reduction in VCAM-1 synthesis (from 2.48 units to 1.24). The injection of B16M cells into the spleen resulted in a significant increase of VCAM-1 synthesis compared with the level in HSE cells from saline-injected mouse livers; however, when these mice were pretreated with IL-18BP, there was a near complete reduction in the synthesis of VCAM-1 (from 3.18 units to 1.23).

Immunohistochemical staining and densitometrical quantification of VCAM-1 expression on these hepatic tissue samples from above experimental design are shown in Fig. 3D. These studies revealed a marked increase of VCAM-1 expression level in the hepatic sinusoidal wall 12 h after the injection of B16M cells (175,321 units), as compared with saline-injected control (16,844 units). However, tumor-induced VCAM-1 expression was nearly completely abrogated in mice pretreated with IL-18BP before B16M cell injection (31,259 compared with 175,321). Altogether, these results are consistent with the importance of VCAM-1 expression in mediating the binding of B16M cells to IL-18-activated HSE, facilitating the inflammatory component of the implantation step of hepatic colonization by B16M cells (9, 13). Moreover, gene expression and synthesis of VCAM-1 appears to be IL-18 dependent.

B16M-derived Factor(s) Stimulate(s) B16M Cell Proliferation via HSE-derived IL-18: Mediating Role of IL-1. Postimplantation treatment with IL-18BP was effective in reducing hepatic metastasis volume (Fig. 2), suggesting that IL-18 is a direct growth factor for B16M. Alternatively, IL-18 stimulates local production of B16M cell growth factors. Therefore, freshly isolated HSE cells were stimulated with supernatants from B16M cell cultures. For these experiments, the B16M cells were cultured without exogenous stimulation, and therefore, the biological activity of B16M supernatants reflects constitutive production, as well as the release of putative growth factors. Diluted B16M supernatants were added to primary cultured HSE obtained from healthy mice. After 24 h, the HSE-CM was harvested and added back to new B16M cells. CM from B16M-CM-treated HSE cells significantly (P < 0.05) stimulated proliferation of B16M cells compared with CM obtained from unstimulated HSE cultures (Fig. 4A). The ability of tumor-activated HSE cells to increase B16M proliferation was completely neutralized by IL-18BP. IL-18BP added directly to B16M cells did not affect basal proliferation. We also observed using flow cytometry with antimurine IL-18 receptor α chain antibody that only 4% B16M cells constitutively expressed IL-18 receptors on their cell surface (data not shown). The interpretation of these results is that B16M cells constitutively release a factor or factors which stimulate HSE to release a growth factor(s) for B16M cells; the release of the putative B16M cell growth factor(s) is IL-18 dependent. To support this mechanism, the addition of murine IL-18 also significantly (P < 0.01) increased B16M cell proliferation by 2-fold (Fig. 4B). However, importantly, the addition of IL-1Ra completely abrogated the growth-promoting effect of IL-18 on B16M cell proliferation. These findings suggest that the paracrine effects of IL-1 contribute to the growth of B16M.

IL-18 Is a Growth Factor for Human Melanoma Cell Lines. We next studied the effect of recombinant human IL-18 on the proliferation of established human melanoma cell lines. Each of the human melanoma cell lines responded to the addition of 100 pg/ml exogenous IL-18 with increased proliferation (Fig. 4C). Concentrations of IL-18 (from 10 pg/ml to 100 ng/ml) did inhibit proliferation of these cells. Each cell line, regardless of the basal level of proliferation, exhibited an increase of 1.5 to 2-fold in growth after 48 h. However, in the presence of 100 ng/ml IL-1Ra completely abrogated the IL-18-dependent HMB2 cell proliferation (data not shown). These findings indicate that paracrine effects of IL-1 also contribute to the effect of IL-18 on human melanoma cell growth.

**DISCUSSION**

Previously, we reported that IL-18 is a proinflammatory cytokine that regulates IL-1β-dependent metastatic spread of B16M cells in the liver via VCAM-1 expression on HSE cells (9). Important for this
model was the discovery that IL-18 has a pivotal position in the hierarchy of cytokines released to up-regulate VCAM-1 expression in HSE cells on activation by melanoma-derived factors in vitro. This study on the adhesion and growth of metastatic B16M cells in the liver during in vivo IL-18 blockade demonstrates that neutralization of IL-18BP significantly reduces B16M metastasis formation in the liver. Two antitumoral mechanisms were identified using neutralization of IL-18. First, IL-18BP efficiently down-regulated VCAM-1 expression in tumor-activated HSE cells, which reduced IL-18-dependent adhesion of B16M cells to HSE. As a consequence, in vivo retention of B16M cells in the liver microvasculature and metastasis implantation efficiency were decreased. The second antitumor mechanism of IL-18 neutralization was the finding that postimplantation treatment with IL-18BP reduced hepatic metastasis volume. In this regard, IL-18BP neutralized the growth-promoting effect of tumor-activated HSE cells. Not unexpectedly, recombinant IL-18 stimulated the proliferation rate of B16M cells. IL-18 also increased the proliferation of several human melanoma cell lines.

It has already been reported that systemic levels of IL-18 are significantly increased in patients with metastatic disease as compared with levels in patients at early stages of their malignant disease (8). In our mouse model, IL-18 serum levels increased in a biphasic fashion: (a) during intravascular cancer cell circulation and retention in the liver; and (b) during macrometastatic development. This may have mechanistic implications for metastasis progression because the first peak of IL-18 may promote VCAM-1-dependent adhesion of B16M cells to HSE cells. VCAM-1 is critical for efficient cancer cell implantation into the liver. This process appears to depend on IL-18-inducible up-regulation of both VCAM-1 and very late antigen-4 expression on HSE cells (present study) and B16M cells, respectively. Thereafter, the second peak of IL-18 in the serum may reflect the promotion of tumor cell proliferation, contributing to metastasis growth. Thus, IL-18 may up-regulate both metastatic cell implantation and development in the liver at the same time, explaining why IL-18BP administration exhibits these antimitastatic effects when given as combined treatment schedule (before cancer cell injection and later along metastasis development).

Several possibilities exist regarding IL-18 derivation and its up-regulation during hepatic metastasis progression in this model. Because the numbers of cancer cells were very low at early stages of the metastatic process, first elevation of IL-18 may depend on host cells activated by tumor-derived factor(s). This is supported by our previous finding on HSE cell production of IL-18 in response to B16M-CM in vitro, via TNFα-dependent IL-1β (13), and is in agreement to the reported rapid induction of liver proinflammatory cytokines in response to intrasplenic injection of different murine cancer cell variants expressing very late antigen-4 integrin; (b) by increasing melanoma cell growth at metastatic sites via IL-1-dependent mechanism, consistent with the role of IL-1 in melanoma metastasis (18); and (c) by inducing other factors which support melanoma growth in the liver, such as vascular endothelial growth factor. However, each of these proposed mechanisms is inhibited by exogenous IL-18BP, demonstrating that endogenous IL-18 significantly contributes to metastasis in this model. The near complete prevention of implantation and growth of metastasis obtained in mice administering the combination of pre and postimplantation treatment confirms that a majority of hepatic B16M metastases are IL-18 dependent and explains our previous findings in ICE-KO mice (9). Thus, we were able to recreate the phenotype of the ICE-KO mouse using combination of pre and postimplantation with IL-18BP. Finally, we believe that IL-18BP is primarily neutralizing IL-18 and not any agonist member of the IL-1 family. In fact, the unusually high affinity of IL-18BP for mature IL-1Ra neutralized IL-18-dependent B16M cell proliferation suggests that IL-1 is one candidate factor.

In summary, we demonstrated that the production of IL-18 in the liver microenvironment regulates hepatic development of B16M metastases through three well-defined actions: (a) by increasing VCAM-1-dependent B16M cell adhesion to HSE, which is a compulsory step in the initial hepatic implantation of circulating melanoma cell variants expressing very late antigen-4 integrin; (b) by increasing melanoma cell growth at metastatic sites via IL-1-dependent mechanism, consistent with the role of IL-1 in melanoma metastasis (18); and (c) by inducing other factors which support melanoma growth in the liver, such as vascular endothelial growth factor. However, each of these proposed mechanisms is inhibited by exogenous IL-18BP, demonstrating that endogenous IL-18 significantly contributes to metastasis in this model. The near complete prevention of implantation and growth of metastasis obtained in mice administering the combination of pre and postimplantation treatment confirms that a majority of hepatic B16M metastases are IL-18 dependent and explains our previous findings in ICE-KO mice (9). Thus, we were able to recreate the phenotype of the ICE-KO mouse using combination of pre and postimplantation with IL-18BP. Finally, we believe that IL-18BP is primarily neutralizing IL-18 and not any agonist member of the IL-1 family. In fact, the unusually high affinity of IL-18BP for mature IL-18 (400 pM) makes any biological effect of other cytokines the result of neutralizing IL-18 (15, 27).

The main clinical implication of this study is that IL-18BP may be a potent metastasis preventive biological for those melanoma patients at high risk of systemic dissemination and hepatic colonization. Because IL-18BP administration has no toxicity and fewer side effects, an additional clinical implication of these results is that IL-18BP doses can potentially be increased to match the growth rate of a given melanoma.

4 Unpublished observations.

5 Unpublished observations.
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Interleukin-18 Binding Protein Reduces B16 Melanoma Hepatic Metastasis by Neutralizing Adhesiveness and Growth Factors of Sinusoidal Endothelium

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