IL-18 Binding Protein Protects Against Contact Hypersensitivity

Thomas Plitz, Pierre Saint-Mézard, Masataka Satho, Susanne Herren, Caroline Waltzinger, Marcelo de Carvalho Bittencourt, Marie H. Kosco-Vilbois and Yolande Chvatchko

J Immunol 2003; 171:1164-1171; doi: 10.4049/jimmunol.171.3.1164
http://www.jimmunol.org/content/171/3/1164

References
This article cites 39 articles, 17 of which you can access for free at:
http://www.jimmunol.org/content/171/3/1164.full#ref-list-1

Why The JI? Submit online.
• Rapid Reviews! 30 days* from submission to initial decision
• No Triage! Every submission reviewed by practicing scientists
• Fast Publication! 4 weeks from acceptance to publication

*average

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
IL-18 Binding Protein Protects Against Contact Hypersensitivity

Thomas Plitz,* Pierre Saint-Mézard,† Masataka Satho,† Susanne Herren,2* Caroline Waltzinger,* Marcelo de Carvalho Bittencourt,3* Marie H. Kosco-Vilbois,2* and Yolande Chvatchko*" 

Allergic contact dermatitis, the clinical manifestation of contact hypersensitivity, is one of the most common disorders of the skin. It is elicited upon multiple cutaneous re-exposure of sensitized individuals to the sensitizing agent. In this study, we demonstrate that using IL-18 binding protein (IL-18BP) to neutralize IL-18 significantly reduced clinical symptoms in a murine model of contact hypersensitivity. Furthermore, IL-18BP alleviated the relapses during established disease, as indicated by significant protection during re-exposure of mice that had previously undergone a contact hypersensitivity response without treatment. Although edema was not influenced, IL-18BP reduced the number of T cells homing to sites of inflammation, resulting in diminished local production of IFN-γ. Thus, by preventing the accumulation of effector T cells to the target tissue, IL-18BP appears to be a potent protective mediator to counter skin inflammation during contact hypersensitivity. Taken together with the evidence that IL-18 is present in tissue samples of the human disease, our data reinforces IL-18BP as a candidate for this therapeutic indication. The Journal of Immunology, 2003, 171:1164–1171.

Contact hypersensitivity (CHS) is a T cell-mediated inflammation of the skin. Clinically it is often manifested as allergic contact dermatitis, one of the most frequent dermatological problems. Immunological disorders of the skin have been associated with aberrant expression of IL-18. In humans, IL-18 is up-regulated in psoriatic lesions and serum levels of IL-18 are elevated in patients with atopic dermatitis (1–3). In the mouse, cutaneous overexpression of IL-18 induces the development of atopic dermatitis-like skin inflammation (4).

IL-18 has initially been described as an IFN-γ-inducing factor belonging to the IL-1 family of cytokines (5, 6). Similar to IL-1β, IL-18 is synthesized as a biologically inactive precursor molecule, pro-IL-18, that can be converted by caspase-1 into the mature cytokine (7). IL-18 and IL-1 receptors belong to the Toll-like receptor family, and the IL-18R and IL-1R signaling pathways are analogous (8). However, despite the structural homology to IL-1α, IL-18 shares biological properties with IL-12. IL-18 strongly augments IL-12-driven development of Th1 cells and synergistically enhances the production of IFN-γ by Th1 and NK cells (9). This is made possible by the mutual up-regulation of IL-12R and IL-18R expression on Th1 cells by IL-18 and IL-12, respectively (10,11). However, IL-18 does not induce Th1 development in the absence of IL-12 (12). IL-18 directly enhances cytotoxic capacities of NK cells, NKT cells and CTL by up-regulating FasL expression as well as perforin-mediated cytotoxic activity (13–15), features that have been described as mandatory for CHS (16). Furthermore, IL-18 does not exclusively promote Th1-type immunity, as recent studies revealed that in the absence of IL-12, IL-18 can augment Th2-type immune responses by favoring the development of Th2 cells (17, 18). Thus, IL-18 should be seen as a more general enhancer of T cell-mediated immunity.

The activity of IL-18 can be neutralized by a natural inhibitor, IL-18 binding protein (IL-18BP) (19). IL-18BP is a secreted protein that binds IL-18 with high affinity and prevents its interaction with the IL-18R. Different isoforms of IL-18BP have been demonstrated, four in human and two in the murine system, and biological activity is limited to particular isoforms (20).

Several cell types residing in the skin are capable of producing IL-18, including dermal dendritic cells, epidermal Langerhans cells, and keratinocytes (KC), the latter being the principal cell type of the epidermis (21–23). Human KC constitutively express pro-IL-18, but the production of the processed form is under debate (22, 24). However, several reports demonstrate that KC upregulate IL-18 transcription and secrete biologically active IL-18 following stimulation with proinflammatory mediators as well as contact sensitizers (25, 26). This is in keeping with the capability of KC to respond to the same stimuli with caspase−1-dependent release of mature IL-18 (27). Furthermore, KC express IL-18R suggesting an autocrine or paracrine action of IL-18 on these cells (28). The bioavailability of IL-18 in the skin may be determined by the local concentration of IL-18BP. Similar to intestinal endothelial cells that produce IL-18BP during chronic inflammation, KC of the skin express IL-18BP in response to elevated levels of IFN-γ, thus limiting the proinflammatory capacity of IL-18 (29, 30).

CHS is elicited following cutaneous re-exposure of a sensitized individual to the sensitizing agent. Sensitization is asymptomatic and affected individuals develop clinical manifestations only after re-exposure to the agent. Thus, therapeutic intervention for CHS...
must target the elicitation phase of the response. To determine the contribution of IL-18 to CHS, we treated mice with neutralizing amounts of IL-18BP during the elicitation phase of CHS to 2,4-dinitrofluorobenzene (DNFB). We provide evidence that IL-18 significantly contributes to the elicitation of CHS by augmenting the recruitment of IFN-γ-produc- ing Th cells to the inflammatory focus. Importantly, administration of IL-18BP not only reduced symptoms after the primary re-exposure to DNFB but also signifi- cantly decreased inflammation in mice that had previously under- gone CHS without treatment. Taking together the documented role of IL-18 in T cell activation and the production of IL-18 by resi- dent skin cells with our observations that IL-18BP can impair CHS even when treatment commences after the resolution of a primary flare, clearly underscores the potential therapeutic capacity of IL-18BP in inflammatory skin diseases.

Materials and Methods

Animal resources

C57BL/6 mice were obtained from IFFA Credo (Lyon, France). Animals were housed in a conventional mouse facility and were used between 8 and 14 wk of age. All animal experiments were approved by Swiss veterinary authorities.

Reagents

Recombinant human IL-18BP isofrom a was obtained from Serono manufac- turing facilities. DNFB, acetone, Evans blue, formaldehyde, BSA, PMA, ionomycin, brefeldin A, and LPS (Escherichia coli 026:B6) were pur- chased from Sigma-Aldrich (St. Louis, MO). Murine IL-18 was from MBL (Wate- rtown, MA). The culture medium generally used in this study was Iscove's DMEM supplemented with 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin (all Life Technologies, Grand Island, NY). HBSS without calcium and magnesium as well as 2.5% trypsin was obtained from Life Technologies (Carlsbad, CA). Single cell suspensions prepared from ears were preincubated with the 2.4G2 mAb (BD PharMingen) to block FcγR before the second application of DNFB to the ears at day 19. For these experiments, IL-18BP treatment started 30 min before challenge at day 5. To assess cytokine production by T cells present in the inflamed ears, cells were harvested, washed, and labeled with combinations of FITC-anti-CD8 and CyChrome anti-CD4 or FITC anti-CD3 and biotin-conjugated mAbs to mouse CD45 (30-F11), CD4 (GK1.5), CD8a (53-6.7), NK1.1 (PK136), GR-1 (RB6-8C5), CD11b (M1/70), CD11c (HL3), and IFN-γ-producing cells directly ex vivo, cells obtained from

Assay to determine vascular permeability

Two hours before challenge with DNFB on the ears, mice were injected retro-orbitally with 100 µl of 7.5 mg/ml Evans blue in saline. Mice were sacrificed 24 h later. DNFB painted and control ears collected, dried over- night at 80°C and the dry weight was determined. The ears were minced and the dye was extracted with 1 ml of formalin for 24 h at 55°C. Samples were filtered to remove tissue debris and their OD 620 was mea- sured to assess the content of the dye. Vascular leakage specific for CHS was determined as the content of Evans blue per milligram of dried tissue found in the DNFB-painted ear corrected for the background value deter- mined for the control ear of the same animal.

Enumeration of CD8+ T cells in the inflamed ears by immunohistochemistry

Ear samples were collected 24 h after challenge and embedded in tissue- freezing medium (OCT; Miles, Torrance, CA). Cryostat sections (6 µm) were prepared in acetone for 10 min and preincubated in PBS containing 5% FCS for 30 min. To label CD8+ T cells, the sections were incubated with rat anti-CD8 (KT-15 rat IgG2a; Immunotech, Marseille, France) or isotype- control Ab, followed by biotinylated rabbit anti-rat IgG (Vector Labora- tories, Burlingame, CA) and alkaline phosphatase (AP)-conjugated strepta- vidin (StreptAB complex/AP; DAKO, Glostrup, Denmark). Color development for bound AP was performed with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (DAKO). The sections were counter- stained with Fast Red.

The number of CD8+ T cells was determined in three microscopic fields per section and the counts for each section were normalized to 500 basal cells. Results are the mean of two ears per group and are representative of three experiments.

Preparation of single cell suspensions from the ear

DNFB-painted ears were harvested 24 h after challenge, pooled by treatment group (n = 5–10), rinsed with 70% ethanol, splited with the aid of forceps, and placed dermal side down on 7.5 ml of HBSS. Five milliliters of 2.5% trypsin were added to obtain a final trypsin concentration of 1%. After 15 min of incubation at 37°C, the ear halves were transferred to 10 ml of 80% FCS in HBSS on ice to stop the digestion. Cells were dislodged from the tissue by gently meshing the ear halves dermal side down through a nylon sieve (Cell Strainer, Falcon; BD Biosciences, Mountain View, CA). Sieves containing big debris were removed, cells were washed twice in cold complete medium, and adjusted to 106/ml before proceeding with further analysis.

Analysis of inflammatory infiltrates by FACS

All flow cytometric analysis for surface Ags was performed using FITC-, PE-, CyChrome-, or biotin-conjugated mAbs to mouse CD45 (30-F11), CD4 (GK1.5), CD8a (53-6.7), NK1.1 (PK136), GR-1 (RB6-8C5), CD11b (M1/70), CD11c (HL3), and γδ TCR (GL3) obtained from BD PharMingen (San Diego, CA). Single cell suspensions prepared from ears were preincubated with the 2.4G2 mAb (BD PharMingen) to block FcγR binding, and then incubated with the relevant mAbs for 30 min on ice. Subse- quently, cells were washed with 1% BSA in PBS and incubated with streptavidin-CyChrome (BD PharMingen) for 20 min. Finally, cells were washed, fixed in Cytofix buffer (BD PharMingen), and analyzed with a FACSCalibur (BD Biosciences).

Quantification of cytokine production

To assess cytokine production by T cells present in the inflamed ears, single cell suspensions were cultured at 105 cells per well in 96-well plates in the presence or absence of 10 µg of anti-CD3 mAb (clone 145-2C11; BD PharMingen). Supernatants were harvested after 20 h of infection and 2 µg/ml brefeldin A in fresh medium added. After a further 2 h of incu- bation, cells were harvested, washed, and labeled with combinations of FITC anti-CD8 and CyChrome anti-CD4 or FITC anti-γδ TCR and biotin- conjugated NK1.1 followed by streptavidin-CyChrome. Subsequently, cells were fixed and permeabilized in Cytofix/Cytoperm buffer and intra- cellular IFN-γ was labeled with PE-conjugated anti-IFN-γ mAb (XM1G12; BD PharMingen). PE-conjugated anti-IG1 (BD Pharmingen) was used as an isotype control. Cells were analyzed with a FACSCalibur.

The supernatants were assayed for IL-2, IL-4, IL-5, TNF-α, and IFN-γ using the mouse Th1/Th2 cytokine cytometric bead array kit (BD Phar- Mingen). Levels of IL-10 and RANTES were determined by ELISA (R&D Systems). All kits were used according to the manufacturer’s instructions.

To detect IFN-γ-producing cells directly ex vivo, cells obtained from the ears were incubated at 106 cells per ml with 50 µg/ml PMA and 500 µg/ml ionomycin in complete medium for 4 h at 37°C. Cytokine secretion was determined by the addition of 2 µg/ml brefeldin A for the last 2 h of the incubation period. Ab labeling for subsequent FACS analysis was per- formed as described above.

Statistical analysis

Data are expressed as mean ± SEM. The statistical significance of the differences between the means of each experimental group was determined.
by performing one-way ANOVA followed by Bonferroni’s multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

**Results**

**Therapeutic treatment with IL-18BP protects against CHS**

To experimentally induce CHS, we sensitized mice with the hapten DNFB on their shaved backs. We elicited CHS 5 days later by painting DNFB onto the ears. Inflammation was scored as the increase in swelling of the DNFB-challenged vs the control ear painted with solvent only. To examine the contribution of IL-18 to the elicitation phase of CHS, we treated mice with IL-18BP in a therapeutic fashion, i.e., from the time of ear-challenge onwards. For all experiments, we used human IL-18BP isoform a, which neutralizes murine IL-18 with high efficiency (Fig. 1). The observed IC$_{50}$ of 96.5 pM is comparable to the value obtained for the neutralization of human IL-18 in a KG-1 cell assay (Fig. 1 and data not shown).

Administration of IL-18BP during the elicitation phase at days 5–8 significantly reduced swelling of the DNFB-challenged ears for the total duration of the response (Fig. 2A). At the peak of the inflammation at days 6 and 7, ear swelling in IL-18BP-treated C57BL/6 mice reached only an average of 52.4 ± 4.4% and 46.8 ± 4.1% of the vehicle-treated control, respectively (Fig. 2B). We did not observe any difference in the effectiveness of treatment starting the administration of IL-18BP either at day 4 or 30 min before challenge at day 5. Ear swelling in nonsensitized, but challenged, control animals remained below 6% (Fig. 2B), indicating that the observed swelling was CHS-specific for DNFB and not due to a nonspecific inflammation caused by high concentrations of the hapten. The protective effect was IL-18BP-specific, as injections of the same amount of the irrelevant protein BSA did not alter ear swelling (data not shown). To further investigate the

**FIGURE 1.** Human IL-18BPa neutralizes murine IL-18. Mouse spleen cells were stimulated with murine IL-18 and LPS in the presence of increasing amounts of human IL-18BPa or an irrelevant protein. IFN-γ in the supernatant was measured after 24 h. A representative experiment of 10 is shown.

**FIGURE 2.** Treatment with IL-18BP during elicitation protects against CHS. C57BL/6 mice were sensitized with DNFB at day 0 and challenged 5 days later on the ears. Ear swelling was measured daily and expressed as the increase in swelling of the DNFB-challenged vs the vehicle-painted control ear. The animals were treated daily with IL-18BP or the vehicle only. A, Kinetic of the CHS response with IL-18BP treatment at days 5–8 ($n = 5$ mice per group, one representative experiment is shown). B, Ear swelling observed 24 and 48 h after challenge, respectively. The swelling is expressed relative to the vehicle-treated control. Treatment was started at day 4. Data are the mean of 13 (day 6) and 7 (day 7) independent experiments. C, Treatment with IL-18BP from days 19–22 in C57BL/6 mice that had undergone a first CHS response to DNFB without treatment and then had been rechallenged at day 19 after sensitization. The swelling is expressed relative to vehicle-treated controls at day 20. The data are the mean of two independent experiments ($n = 13$ mice per group). ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$ (vehicle vs IL-18BP-treated group).
capacity of IL-18BP to interfere with elicitation of CHS, we administered IL-18BP from days 19–22 to mice that had previously undergone a CHS response to DNFB (from day 5 onwards) without treatment and were rechallenged with DNFB at day 19 after sensitization (Fig. 2C). Even after rechallenge, which results in a considerably stronger CHS response, IL-18BP treatment significantly reduced ear swelling to between 65 and 77% as compared with vehicle-treated controls (Fig. 2C). These observations imply that IL-18 is necessary to fully activate or correctly target previously primed effector T cells during the elicitation of CHS.

**IL-18BP does not reduce vascular leakage during CHS**

At least two processes that are not necessarily mutually exclusive could contribute to the swelling observed during CHS. These include leakage of liquid from the vasculature into the surrounding tissue causing edema as well as the extravasation of inflammatory cells from the blood vessels to the site of tissue damage. To monitor edema caused by the CHS reaction, we sensitized mice at day 0 and injected Evans blue i.v. 2 h before challenge with DNFB. Mice were sacrificed 24 h later and the dye was extracted from the ear tissue. Vascular leakage in the DNFB-challenged ear was assessed as the amount of Evans blue per milligram of dried ear tissue corrected for the background value determined for the solvent only painted ear of the same animal. Vascular leakage is expressed relative to nonsensitized control animals (n = 10 mice per group). The data are the mean of two independent experiments. ***, p < 0.001 vs nonsensitized control.

**IL-18BP treatment reduces the number of αβ T cells infiltrating the DNFB-challenged ear**

To examine the inflammatory infiltrate at the site of challenge, we performed FACS analysis of single cell suspensions prepared from DNFB-challenged ears. We elicited CHS and treated the animals with IL-18BP or vehicle from day 4 onwards. We sacrificed the mice at the peak of the response, collected the challenged ears, pooled them by group, and prepared single cell suspensions. Subsequently, we determined the proportion of CD45⁺ cells contained in the preparations by FACS (Fig. 4A) as a first measure of the number of leukocytes that had infiltrated the ears. As expected, samples obtained from the inflamed ears of vehicle-treated mice contained on average a higher number of CD45⁺ cells (4.6 ± 0.4%) as compared with the preparations from ears of nonsensitized but challenged control mice (2.8 ± 0.1%, Fig. 4B). The CD45⁺ cells contained in the control samples were mainly γδ T cells and CD11c⁺ dendritic cells of the skin (data not shown). The proportion of these cells was not substantially changed by the DNFB painting (see Fig. 6D and data not shown). The average percentage of CD45⁺ cells following IL-18BP treatment was...
3.8 ± 0.2% (Fig. 4B). Subtracting the baseline of cells contributed by CD45^+ γδ T cells and dendritic cells enumerated in the naïve situation of the nonsensitized controls, an overall reduction to 56% in inflammatory cells was observed as compared with the vehicle-treated group.

Further characterization of the CD45^+ population in the ear preparation revealed that the inflammatory infiltrate 24 h after challenge was composed of CD8^+ and CD4^+ T cells (Fig. 4A), neutrophils (GR-1^+), monocytes/macrophages (CD11c^−, CD11b^hi), and NK cells (NK1.1^−) (data not shown). These cell types were barely detectable in naïve or nonsensitized but challenged control ears. IL-18BP decreased the number of infiltrating CD4^+ and CD8^+ T cells (Fig. 4A) to an average of 47.6 ± 10.2% and 68.5 ± 10.9% of the vehicle-treated control, respectively (Fig. 4C). However, the reduction in ear swelling caused by IL-18BP did not strictly correlate with decreased infiltration of neutrophils, monocytes/macrophages, and NK cells.

As CD8^+ effector T cells have been shown critical for the development of CHS, we performed immunohistochemistry to analyze their number and localization in the inflamed tissue. In ears of vehicle-treated animals, CD8^+ T cells were predominantly found in the dermis and at the dermal-epidermal junction (Fig. 5A). The same distribution of CD8^+ T cells was observed in ears obtained from IL-18BP-treated mice (Fig. 5B). However, the number of CD8^+ T cells detected in the tissue sections of IL-18BP-treated mice was markedly reduced as compared with the vehicle-treated controls (Fig. 5D), corroborating the data obtained by FACS analysis (Fig. 6, A and B). Enumeration of CD8^+ T cells was not significantly altered in the different groups analyzed (Fig. 6D) and these cells did not produce IFN-γ (Fig. 6D). Intracellular labeling of IFN-γ^+ cells directly ex vivo further corroborated these data. Here again, the majority of IFN-γ^+ cells were CD8^+ T cells with significant numbers of CD4^+ T cells also positive for intracellular IFN-γ (Table I). IL-18BP treatment reduced the number of both CD8^+ as well as CD4^+ IFN-γ^+ producing T cells (Table I). Interestingly, in the inflamed ear, IFN-γ was mainly produced by CD8^+ and to a lesser extent CD4^+ T cells, but not by NK1.1^− cells or γδ T cells as suggested earlier (32). Taken together, this data demonstrates that the reduction of IFN-γ production in the DNFB-challenged ears observed after IL-18BP treatment is due to an impaired recruitment of αβ T cells.

**FIGURE 5.** IL-18BP decreases the number of infiltrating CD8^+ T cells. Immunohistochemistry of ear sections obtained from vehicle-treated (A), IL-18BP-treated (B), or nonsensitized control mice (C) 24 h after challenge. CD8^+ T cells are labeled in dark blue (red arrows point to examples). Green arrows indicate examples of black melanocytes. D, Enumeration of infiltrating CD8^+ T cells. The number of CD8^+ T cells in the skin (dermis and epidermis) was determined in three microscopic fields per section and the counts for each section were normalized to 500 basal cells. Results are the mean of two ears per group and are representative of three experiments. ***, p < 0.001 vs vehicle-treated control.

**Discussion**

Allergic contact dermatitis, the clinical manifestation of contact hypersensitivity, is one of the most common disorders of the skin with considerable social and economic impact. As it is often impossible to avoid sensitization, therapies ameliorating the inflam-
mation caused by re-exposure to the sensitizing agent are needed. In this study, we demonstrate that treatment of mice with IL-18BP during elicitation of experimentally induced CHS to DNFB significantly reduced inflammation. Furthermore, IL-18BP was also protective against DNFB rechallenge of mice that had previously undergone a CHS response without treatment. This is of interest as multiple exposures to the sensitizing agent are frequently encountered in patients with allergic contact dermatitis. Thus, this work establishes a role for IL-18 in the elicitation phase of CHS. Although the edema associated with CHS was not influenced, the IL-18BP treatment reduced the number of αβ T cells infiltrating the site of challenge, resulting in diminished local production of IFN-γ.

We demonstrate that in localized inflammation like CHS, IL-18 predominantly contributes to homing of primed αβ T cells to the site of inflammation rather than their capacity to produce IFN-γ.

IL-18 can increase the permeability of microvessels. Intrathoracic instillation of rIL-18 resulted in enhanced vascular leakage in a model of immune complex-mediated alveolitis (33). In the same model, blockade of endogenous IL-18 by IL-18BP or neutralizing anti-IL-18 Ab reduced pulmonary vascular permeability (33). Furthermore, IL-18 can synergize with IL-12 in the production of vascular permeability factor (34). Therefore, we tested whether IL-18BP alleviated CHS by reducing the edema caused by elevated levels of IL-18. Our study shows that IL-18BP does not
Table I. IFN-γ-producing cells in the inflammatory infiltrate

<table>
<thead>
<tr>
<th>Marker</th>
<th>Vehicle (0.18)</th>
<th>IL-18BP</th>
<th>Nonsensitized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total T cells</td>
<td>100%</td>
<td>77.8%</td>
<td>0% (ND)</td>
</tr>
<tr>
<td>CD4+</td>
<td>100% (0.05)</td>
<td>58.2%</td>
<td>0% (ND)</td>
</tr>
<tr>
<td>CD8+</td>
<td>100% (0.13)</td>
<td>84.6%</td>
<td>0% (ND)</td>
</tr>
</tbody>
</table>

* The values in bold face designate the proportion of IFN-γ-producing cells positive for the indicated marker relative to the vehicle-treated group. The proportion of positive cells relative to the total number of cells obtained from the ears is given in parentheses. Data from one representative experiment is shown. Here, the IL-18BP treatment reduced the swelling to 58% of the vehicle control. ND, not detectable.

Consequently, it was interesting to investigate the effect of IL-18BP on cellular infiltration during CHS. The elicitation phase of CHS is dependent on effector as well as regulatory T cells which can be discriminated by their cytokine production profile (31). IFN-γ-producing CD8+ effector T cells mediate CHS to DNFb while the resolution of the inflammation was reported to depend on IL-4- and IL-10-secreting CD4+ T cells (31, 36). Given the fact that T cell priming is not modified by the therapeutic IL-18BP treatment starting at the time of CHS elicitation and that therefore equal amounts of primed effector T cells are available in IL-18BP as well as vehicle-treated animals, the reduced inflammation of the target tissue may be explained by the presence of elevated numbers of regulatory (IL-4- and/or IL-10-producing) T cells or by inefficient recruitment of the CD8+ effector cells. As shown in this study by FACs as well as immunohistochemistry, therapeutic IL-18BP treatment impaired the accumulation of primed CD8+ and CD4+ T cells in the site of hapten challenge. Analysis of cytokine production by the T cells present in the inflamed tissue revealed decreased local IFN-γ production while IL-4 and IL-10 were not detectable. Intracellular labeling showed that IL-18BP treatment reduced the number of IFN-γ+ effector T cells infiltrating the site of challenge, whereas IFN-γ production at the cellular level remained unchanged (Fig. 6C). In addition, IL-18BP diminished the levels of RANTES, serving as a marker for activated T cells present in the lesion. Therefore, the protection by IL-18BP is due to lower amounts of IFN-γ+ effector rather than an enhanced influx of regulatory T cells. A possible explanation for the reduced recruitment of αβ T cells to the target tissue could be an altered expression of adhesion molecules due to the functional inactivation of IL-18 during CHS. As reported earlier, IL-18 enhances the expression of VCAM-1, ICAM-1, and E-selectin on endothelial cells (37). The up-regulation of VCAM-1 on hepatic sinusoidal endothelium by IL-18 has been implicated in hepatic melanoma metastasis (38). In combination with IL-12, IL-18 induced β1 integrin and CD44-dependent adhesion of T cells to extracellular matrix (39).

In vitro experiments to stimulate T cells with combinations of IL-18 and IL-12 clearly demonstrate that these two cytokines synergize in IFN-γ production on the cellular level. However, these experiments do not consider issues of cellular homing and the possible spatially distinct availability of these cytokines in vivo. These questions may not be of importance in systemic inflammation such as LPS-induced toxic shock, because here sufficiently high levels of IL-18 and IL-12 may be present in the circulation to result in synergistic and systemic generation of IFN-γ. However, in localized inflammation such as CHS, our data suggests that IL-18 predominantly contributes to T cell recruitment. The direct effect of IL-18 on altering IFN-γ production in T cells present in the inflamed tissue seems of lesser importance, as IL-18BP did not change the amounts of IFN-γ detected by intracellular labeling of those cells.

Given its anti-inflammatory potency demonstrated in this study, without entailing the problem of general immune suppression as it is encountered with certain therapeutic approaches to deplete T cells, IL-18BP should be considered a promising candidate in the search for improved therapeutic strategies against inflammatory skin diseases.

Acknowledgments

We are indebted to P. Gruber and colleagues from Serouj manufacturing facilities for supplying rIL-18BP. We thank Drs. J.-F. Nicolas and Y. Sagot, and S. Alouani for helpful discussions. We gratefully acknowledge the technical assistance of R. Lia and S. Woltersperger in housing the mice.

References


