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IL-18 Receptor β-Induced Changes in the Presentation of IL-18 Binding Sites Affect Ligand Binding and Signal Transduction

Chengbin Wu,1 Paul Sakorafas, Renee Miller, Donna McCarthy, Susanne Scesney, Richard Dixon, and Tariq Ghayur

Interleukin-18, initially defined as an IFN-γ-inducing factor, is a proinflammatory cytokine that belongs to the IL-1 cytokine family (1). Like IL-1β, IL-18 is expressed as an inactive pro-peptide (proIL-18) and is processed by caspase-1 to form an active mature protein (2, 3). It has been shown to have a broad range of effector functions beyond lymphocyte activation that implicate IL-18 as an important regulator of both innate and acquired immunity (4, 5). IL-18 expression is elevated at sites of chronic inflammation in human autoimmune diseases (6–8), and blockage of its activity showed efficacy in animal models of rheumatoid arthritis (7), multiple sclerosis (9), colitis (10), and liver disease (11). Hence, IL-18 is a potential drug target for the treatment of inflammatory indications.

The binding of IL-18 to its target cells is mediated by specific cell surface receptors, which are similar to the IL-1R system. The receptor of IL-18 is composed of an α-chain (IL-18Rα) (12, 13) and a β-chain (IL-18Rβ) or AcPL (14). The α-chain of the IL-18R complex alone can bind IL-18 with an affinity of 18.5 nM (12) and does not signal (14). Like the IL-1R accessory protein of the IL-1R complex, IL-18Rβ does not bind IL-18 alone, but forms a functional high affinity (0.4 nM) receptor complex with IL-18Rα that is able to signal in response to IL-18 (15). It has been proposed that IL-18Rβ does not directly interact with IL-18, and it is IL-18Rα that is solely responsible for IL-18 binding. Given the difference in affinities between IL-18Rα and IL-18Rαβ complex for IL-18 binding, it is likely that IL-18Rα and IL-18Rαβ complex may present different contact sites for IL-18. These differences may involve conformational changes, leading to different orientations as well as different numbers of contacting sites. Our current investigation, through the use of site-specific antagonists, shows that a mAb is able to efficiently block IL-18 binding to IL-18Rα, but not to the IL-18Rαβ complex. Interestingly, this Ab is still able to effectively neutralize IL-18-mediated signaling transduced through the IL-18Rαβ complex. We have mapped the epitope of this Ab and showed that the C-terminal 17-aa sequence of human IL-18 is critical for signal transduction through the heterodimeric receptor, but is not required for binding to the same receptor. Our data could provide valuable insights regarding the complex mechanism of IL-18 binding to its receptor heterodimer.

Materials and Methods

Determination of IL-18R expression in L428 and KG-1 cells

Expression of IL-18R subunits was studied using human Hodgkin’s lymphoma cell line L428 (obtained from Hayashibara Biochemical Laboratories, Okayama, Japan), human myelomonocytic cell line KG-1 (purchased from American Type Culture Collection, Manassas, VA), and KG-1 cells pretreated with 10 ng/ml recombinant human TNF-α (rhTNF-α2; Promega, Madison, WI) for 24 h. IL-18R expression was analyzed by flow cytometry using a PE-labeled anti-human IL-18Rα mAb (R&D Systems, Minneapolis, MN) according to standard procedures. IL-18Rβ expression was also assessed by flow cytometry using a mouse anti-human IL-18Rβ mAb (R&D Systems, Minneapolis, MN) for cell staining, followed by a secondary FITC-labeled rabbit anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for detection.

IL-18R binding assay

Anti-human IL-18 mAbs 125-2H and mAb318 were purchased from R&D Systems, and B-K22 was obtained from Cell Sciences (Norwood, MA). IL-18 binding protein (IL-18BP-Fc fusion protein) and rhIL-18 were purchased from R&D Systems. Human L428 or rhTNF-α-stimulated KG-1 cells were harvested, washed several times, and resuspended to 5 × 10⁶ cells/ml in RPMI. The viability of the cell cultures, as determined by vital

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2 Abbreviations used in this paper: rh, recombinant human; IC₅₀, 50% inhibitory concentration; IL-18BP, IL-18 binding protein; TNT, transcription and translation; wt, wild type.

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dye staining with 0.4% trypan blue stain, was 95% in the binding experiments. To examine the potency of mAbs to inhibit IL-18R binding, 100 µL of the cell suspension (5 × 10^6 cells/well) was incubated with a constant amount of [125I]-labeled rhIL-18 (2 nM) with or without serial dilutions of either human IgG1 or anti-IL-18 Abs/IL-18BP in 96-well, U-bottom, microtiter plates for ~4 h on ice in a final total volume of 0.2 mL. The level of nonspecific binding in each experiment was determined by adding a 2800-fold excess of unlabeled rhIL-18 to control wells. Thereafter, 75 µL of the mixture was transferred to 1.0-mL test tubes containing a 300-µL mixture of dibutyrylphosphate (Sigma-Aldrich, St. Louis, MO) and di-nonylphthalate (ICN Pharmaceuticals, Costa Mesa, CA) at a 2:1 volume ratio. Free [125I]-labeled rhIL-18 was removed by centrifugation for 5 min. Each test tube end containing the cell pellet was cut with a microtome scissors. The pellet contained cell-bound [125I]-labeled rhIL-18, and the aqueous phase above the phthalate oil mixture contained excess free [125I]-labeled rhIL-18. Cell pellets were collected in counting tubes, and cell-bound radioactivity was determined using a Packard Cobra gamma counter. All results were determined in duplicate and expressed as the average. Each experiment was repeated three times. The data shown are from one representative experiment. Data are presented as the percent inhibition of total [125I]-labeled rhIL-18 binding to the L428 or rhTNF-α-stimulated KG-1 cells against the concentrations of rhIL-18 antagonists. Fifty percent inhibitory concentration (IC50) values were then calculated with a nonlinear, four-parameter, sigmoidal curve-fitting method using Origin software version 6.0 (Microcal Software, Northampton, MA).

Analysis of IL-18-induced IFN-γ production by human KG-1 cells

IL-18 bioassay using KG-1 cells was performed as described previously (16). Briefly, rhIL-18 preparations (in a final concentration of 2 ng/ml) were added to KG-1 cells (3 × 10^5/ml) in RPMI medium containing 100 ng/ml hTNF-α and incubated at 37°C for 16–20 h. For the Ab neutralization assay, IL-18 samples (in a final concentration of 2 ng/ml) were preincubated with mAb (in final concentrations between 0 and 10 µg/ml) or IL-18BP (in final concentrations between 0 and 1 µg/ml) at 37°C for 1 h and then added to KG-1 cells (3 × 10^5/ml) in RPMI medium containing 100 ng/ml rhTNF-α, followed by incubation at 37°C for 16–20 h. The culture supernatants were collected, and human IFN-γ production in each sample was determined by ELISA (R&D Systems). Data are presented as the percent inhibition of total IFN-γ production in the absence of IL-18 antagonists.

Surface plasmon resonance

Real-time binding interactions between captured Ab (mouse anti-rhIL-18 Ab captured on a biosensor matrix via goat anti-mouse IgG, and IL-18 BP/Ab was captured by goat anti-human Fc) and rhIL-18 were measured by surface plasmon resonance using the BIAcore system (BIAcore, Piscataway, NJ) according to the manufacturer’s instructions and standard procedures. Briefly, IL-18 was diluted in HBS running buffer (BIAcore), and 50-µL aliquots were injected through the immobilized protein matrixes at a flow rate of 5 µl/min. The concentrations of rhIL-18 used were 62.5, 125, 187.5, 250, 375, 500, 750, 1000, 1500, and 2000 nM. To determine the dissociation constant (off-rate), association constant (on-rate), and Kd, BIAcore kinetic evaluation software (version 3.1) was used.

Generation of IL-18 mutant proteins

A series of human-mouse proIL-18 chimeric constructs were generated by overlapping PCR using human and mouse proIL-18 cDNA as templates, and the final PCR products were subcloned into pcDNA3.1 TOPO vector (Invitrogen, San Diego, CA). The plasmids, each containing a proIL-18 wild-type (wt) or mutant construct, were used as templates for in vitro transcription and translation (TNT) using the TNT Quick Coupled System (Promega) according to the manufacturer’s instructions. Briefly, 1 µg of DNA and 1 µl of methionine were added to 40 µl of reaction mixture and incubated at 30°C for 90 min. The resulting protein products were processed into the mature form of IL-18 by caspase-1 digestion at 25°C for 1 h. The wt human and mouse IL-18 processed using the above procedure was quantified by ELISA (17). To quantify the pro and mature forms of mutant IL-18 proteins, [35S]methionine was used in parallel TNT reactions. Before and after caspase-1 processing, wt and mutant samples were applied on SDS-PAGE and quantified densitometrically using a Storm 860 image analysis system (Appligene). The biological activity of each mutant protein was analyzed by stimulating TNF-α-primed KG-1 cells with each mutant protein at concentrations in the range of 0.1–50 ng/ml, and resulting IFN-γ production was measured by ELISA. The activities of wt and mutant IL-18 proteins at 2 ng/ml, which was within the linear range of the dose-response curve, are shown.

125H binding to mutant IL-18 proteins

Binding of IL-18 mutants to 125H was analyzed by ELISA. Briefly, IL-18BP (IL-18BP-Fc chimera, 5 µg/ml; R&D Systems) was coated on 96-well plates as capturing reagent overnight at 4°C, followed by blocking with assay diluent (BD Pharmingen, San Diego, CA) at 37°C for 1 h. After washing, wt or mutant IL-18 TNT preparations (100 ng/ml) were added to the wells and incubated overnight at 4°C, followed by detection with 125H-2H (2 µg/ml) and HRP-anti-mouse IgG Fc (human IgG absorbed; Sigma-Aldrich) as the secondary Ab. As a control, another anti-hIL-18 mAb, Ab318, which was reactive with all the mutants, was also used as a detecting Ab to show proper capturing of all mutant proteins by IL-18BP. Following substrate incubation, samples were read using a microplate reader (Molecular Devices, Sunnyvale, CA).

Results

Expression of IL-18Rα and β subunits and binding of IL-18

IL-18R is expressed on a variety of cell types, including T and B cells and myeloid, mononcytoid, erythroid, and megakaryocytic cell lines. Among the many cell lines tested, L428 exhibited the strongest level of total IL-18 binding, but failed to secret IFN-γ in response to IL-18 (12, 18), and KG-1 cells produced IFN-γ in response to IL-18 stimulation (18). However the IL-18R expression patterns (i.e., whether they express only IL-18Rα or both IL-18Rα and β, and the receptor numbers and affinities) on these cells have not been assessed in detail. We have examined these two cell lines with regard to the level of IL-18Rαβ expression using flow cytometry and receptor binding assay. Previous data showed that TNF-α could significantly increase the response of KG-1 cells to IL-18 in IFN-γ production, suggesting that TNF-α might up-regulate the expression of IL-18Rα and/or IL-18Rβ (18). Therefore, we also analyzed TNF-α-primed KG-1 cells for receptor expression and IL-18 binding. The flow cytometric data showed that both L428 cells and KG-1 cells express IL-18Rα (Fig. 1). Rousing KG-1 cells were expressed much less IL-18Rα than L428 cells; however, upon TNF-α stimulation, IL-18Rα expression in KG-1 cells was significantly up-regulated (Fig. 1). Our results also showed that both L428 cells and KG-1 cells expressed low levels of IL-18Rβ, and the level of IL-18Rβ expression was significantly increased in TNF-α-primed KG-1 cells (Fig. 1). The expression of both IL-18Rα and β on L428 cells was not altered when cells were primed with TNF-α.
were treated with TNF-α, since these cells did not express either p55 or p75 of TNF receptors as measured by flow cytometry (data not shown). Based on the above observations, we concluded that L428 cells primarily expressed low affinity IL-18Rα and did not respond to IL-18, while TNF-α-primed KG-1 cells expressed high affinity IL-18Rαβ heterodimer and responded to IL-18 as measured by IFN-γ production. We therefore used these two cell lines to analyze IL-18 binding to IL-18Rα and the IL-18Rαβ complex.

Neutralizing mAb 125-2H blocks IL-18 binding to IL-18Rαβ effectively, but not to the IL-18Rαβ complex

Using three different neutralizing mAbs and IL-18BP in a receptor binding assay, we examined the interactions of IL-18 with IL-18Rα and with the IL-18Rαβ complex. Such information will allow identification of specific regions of IL-18 that might be involved in the process during which a low affinity binding to IL-18Rα is transformed into a high affinity binding to IL-18Rαβ complex. Our data showed that all three neutralizing Abs and IL-18BP were able to specifically block IL-18 binding to L428 cells (Fig. 2A). IL-18BP was the most potent inhibitor, followed by mAbs 125-2H, Mab318, and B-K22 (Fig. 2A and Table I). In TNF-α-primed KG-1 cells, the potencies of IL-18BP, Mab318, and B-K22 in blocking IL-18 binding were similar to those seen in L428 cells (Fig. 2B and Table I). However, the inhibitory potency of 125-2H in TNF-α-primed KG-1 cells was decreased dramatically (IC₅₀ = >1000 nM) compared with that in L428 cells (IC₅₀ = 1.78 nM; Table I). Interestingly, 125-2H was still very potent in neutralizing IL-18 biological activity in TNF-α-primed KG-1 cells (Fig. 3 and Table I). The neutralizing potencies of the Abs seen in the KG-1 bioassay correlated reasonably well with their affinities for IL-18 as determined by BIAcore analysis (Table I). 125-2H displayed highest affinity among all Abs tested, roughly 10- and 100-fold higher than Mab318 and B-K22, respectively. IL-18BP also exhibited high affinity to IL-18. Our data indicate that 125-2H could effectively block IL-18 binding to the α subunit of the receptor (on L428 cells), but not to the IL-18Rαβ complex (on TNF-α-primed KG-1 cells). Nevertheless, 125-2H could inhibit IL-18 biological activity in the same TNF-α-primed KG-1 cells with high potency. Collectively, these data suggest that, at least at the region of 125-2H epitope, IL-18 interacts with IL-18Rα and IL-18Rαβ complex quite differently. The 125-2H epitope region would likely be involved in the process during which a low affinity binding to IL-18Rα is transformed into a high affinity binding to IL-18Rαβ complex.

Table I. Characterizations of IL-18R binding using mAb and IL-18BP

<table>
<thead>
<tr>
<th>Ab Affinity</th>
<th>IC₅₀ (nM)</th>
<th>RBA (L428)</th>
<th>RBA (KG-1)</th>
<th>NA (KG-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kᵦₜ (M⁻¹s⁻¹)</td>
<td>Kₑᵦ (s⁻¹)</td>
<td>Kᵦ (M)</td>
<td>10⁻³</td>
</tr>
<tr>
<td>B-K22</td>
<td>5.56 x 10⁴</td>
<td>2.00 x 10⁻⁴</td>
<td>3.59 x 10⁻⁶</td>
<td>214</td>
</tr>
<tr>
<td>Mab318</td>
<td>1.11 x 10⁴</td>
<td>7.98 x 10⁻⁵</td>
<td>7.22 x 10⁻⁹</td>
<td>8.80</td>
</tr>
<tr>
<td>125-2H</td>
<td>1.09 x 10⁻⁴</td>
<td>5.32 x 10⁻⁵</td>
<td>5.33 x 10⁻¹⁰</td>
<td>1.78</td>
</tr>
<tr>
<td>IL-18BP</td>
<td>6.76 x 10⁴</td>
<td>1.88 x 10⁻⁵</td>
<td>2.82 x 10⁻¹⁰</td>
<td>0.34</td>
</tr>
</tbody>
</table>

* Real-time binding interactions between captured Ab (mouse anti rhIL-18 Ab captured on a biosensor matrix via goat anti-mouse IgG) and rhIL-18 were measured by surface plasmon resonance using the BIAcore system as described in Materials and Methods, and the dissociation constant (Kᵦₜ), association constant (Kₑᵦ), and Kᵦ values were calculated using BIAcore kinetic evaluation software. IC₅₀ values from the receptor binding assay (RBA) on L428 and KG-1 cells, and from the neutralization assay (NA) on KG-1 cells were derived from Figs. 2 and 3, respectively, using the Microcal Origin software package.

* Average of three experiments.
of IL-18 might be critically involved in the interaction with IL-18Rα/β complex, leading to signal transduction, but is not required for binding of IL-18 to the receptor complex. To define this particular region, we defined the binding epitope of 125-2H.

Generating IL-18 chimera mutants for mapping the 125-2H epitope

Previous attempts to map the 125-2H epitope using overlapping peptides were unsuccessful (C. Wu, unpublished results), suggesting that 125-2H may be against a conformational and nonlinear epitope. The intact three-dimensional structure of IL-18 appears to be required for 125-2H recognition. We have developed a functional approach to delineate the binding epitope of 125-2H using human-mouse IL-18 chimera mutants as shown in Fig. 4. Since 125-2H does not recognize mouse IL-18 (data not show), and human and mouse IL-18 share sequence and structural homologies, we replaced various regions of human IL-18 with the corresponding mouse IL-18 sequence so that the proportion of human sequence was gradually increased and the mouse sequence decreased, allowing a region specific for 125-2H binding in human IL-18 to be identified. We first determined whether the chimera IL-18 mutants HaMb1–4 were biologically active. This was done by stimulating KG-1 cells with wt or mutant IL-18 proteins (0–50 ng/ml) in the presence of TNF-α, and the secreted IFN-γ was quantified by ELISA. A dose-response curve was generated to assess the biological potency of each mutant. Only results of wt and mutant IL-18 at a 2 ng/ml concentration are shown (Fig. 5). Mouse IL-18 displayed only minimal activity on human KG-1 cells in inducing IFN-γ secretion by KG-1 cells, although their potencies were different (Fig. 5). Strikingly HaMb1 exhibited a 7-fold higher potency than wt rhIL-18, whereas HaMb2, -3, and -4 showed activities similar to that of rhIL-18 (Fig. 5).

125-2H binding requires C-terminal 17 aa of IL-18 in correct conformation

Since our first four mutants were biologically functional, we were able to analyze their interactions with 125-2H Ab in a functional assay. Increasing concentrations of 125-2H were preincubated with KG-1 cells, followed by stimulation with varying concentrations of IL-18 chimera mutants and quantification of secreted IFN-γ by ELISA. The figure shows the activities of mutants at a 2 ng/ml concentration.
with *wt* or mutant IL-18 preparations and added to KG-1 cells to induce IFN-γ production. In this neutralization assay none of the four mutants (constructs shown in Fig. 4A) was neutralized by 125-2H, while the activity of *wt* rhIL-18 was inhibited by 125-2H in a dose-dependent manner (Fig. 6A). The data suggested that the last 17 aa residues of human IL-18 were required for 125-2H recognition, since this region represented the only difference between *wt* rhIL-18 and mutant HaMb4. Based on this hypothesis, additional mutants were generated, as shown in Fig. 4B, where the last 17 residues of HaMb1 and HaMb3 were replaced by the human counterpart to generate HaMb1hc and HaMb3hc, respectively, or the last 17 residues of human IL-18 were added to HaMb1 at its C terminus to generate HaMb6. This was to determine whether this 17-aa sequence, when placed within the context of the IL-18 molecule or outside the molecule as an attachment, would render the chimera molecules recognizable to 125-2H. All three mutants were biologically active (Fig. 5). Strikingly, mutant HaMb6 displayed 10-fold higher potency than *wt* hIL-18 in the KG-1 assay (Fig. 5).

The results of the neutralization assay also showed that while 125-2H could effectively inhibit the activity of both HaMb1hc and HaMb3hc in a dose-dependent manner, it did not block the function of HaMb6 (Fig. 6B). An ELISA-based binding assay also showed that HAMb6 did not bind 125-2H, but both HaMb1hc and HaMb3hc did (Fig. 7). These results collectively demonstrate that the C-terminal 17-aa sequence of IL-18 is indispensable for 125-2H binding, and that this sequence requires a correct conformation within the tertiary structure of IL-18 for 125-2H recognition.

**Discussion**

The IL-1 cytokine family members, including IL-1α, IL-1β, IL-1R antagonist, IL-18, and several IL-1 homologues, share similar structural folds. Likewise, their receptor systems are also similar, comprising of a ligand-binding subunit (α-chain) and a signaling subunit (β-chain). Since β-chain alone does not measurably bind ligand, it is widely accepted that the α-chain is solely responsible for ligand binding. Extensive studies have been performed to understand the molecular interactions between IL-1 and IL-1Rα, and crystal structures of the complexes of IL-1RAcP/IL-1β and IL-1Ra/IL-1R antagonist have been reported (19, 20). However, the role of the receptor β-chain in the process of ligand-receptor complex interactions of IL-1 family cytokines has not been extensively evaluated.

IL-18Rα alone can bind IL-18, but does not signal, and coexpression of IL-18Rβ results in functional reconstitution of the IL-18R complex (21), exhibiting a significantly increased binding affinity for IL-18 (12, 14, 22). In TCR-triggered T cells, IL-12 can generate functional IL-18R complex through induction of IL-18Rβ expression as well as up-regulation of the IL-18Rα in a STAT4-dependent manner (23). Overexpression of IL-18Rβ is observed in lymph node cells of autoimmune MRL(lpr/lpr) mice, whereas IL-18Rα expression is normal. As a result, these cells are hyper-reactive to IL-18 stimulation in terms of both IFN-γ production and cell proliferation (24). These reports collectively show that IL-18Rβ is a crucial regulatory element in the cellular response to IL-18 and is critical for thorough understanding of the interactions between IL-18 and the functional IL-18Rαβ heterodimer.

In the present study we have demonstrated that the binding of IL-18 to IL-18Rα and to the IL-18Rαβ complex appear to be different through the use of site-specific IL-18 inhibitors, including three mAbs and IL-18BP. All four inhibitors could block the binding of IL-18 to IL-18Rα on L428 cells, but only three (two Abs and IL-18BP) blocked the binding of IL-18 to IL-18Rαβ complex on TNF-α-primed KG-1 cells. The high affinity Ab, clone 125-2H,
was not able to efficiently block IL-18 binding to IL-18Rαβ complex on TNF-α-primed KG-1 cells. This could not be due to IL-18 binding to an unknown receptor on KG-1 cells, since an anti-IL-18Rα Ab could completely block IL-18 binding to KG-1 cells in both the presence and the absence of 125-2H (data not shown). Interestingly, on the same TNF-α-primed KG-1 cells, 125-2H was a very potent neutralization agent inhibiting IL-18-mediated IFN-γ production. These observations have prompted us to propose the following. 1) The C-terminal 17-aa sequence of IL-18 might be critically involved in interactions with monomeric IL-18Rα, and therefore 125-2H can effectively block IL-18 binding to IL-18Rαβ complex, conformational changes take place in IL-18Rα that may lead to changes in the interactions with IL-18. Specifically the C-terminal region of IL-18 is probably now displaced from the IL-18Rα binding site and therefore renders 125-2H ineffective in blocking the binding of IL-18 to IL-18Rα in the heterodimeric format. 3) It is intriguing that 125-2H can inhibit signaling, but not binding, of IL-18 to IL-18Rαβ. We speculate that 125-2H may block a critical region in IL-18 that is necessary for signal transduction and could be a region that interacts with the receptor β-chain. 4) Our data also suggested that dimerization of receptors α and β occurs before (or independent of) ligand binding, since 125-2H could block IL-18 binding to IL-18Rαβ, but not to the IL-18Rαβ complex. 

Our results from 125-2H epitope mapping indicate that the C-terminal 17-aa sequence of IL-18 is required for 125-2H recognition. We have mapped this epitope through a functional approach by analyzing chimera human-mouse IL-18 mutant proteins in the IL-18 wt background.

FIGURE 8. Diagram of IL-18 binding model on L428 and KG-1 cells. The left half of the diagram represents 125-2H inhibition of IL-18 binding to L428 cells expressing only IL-18Rαβ. The white circle within the IL-18 molecule represents binding epitope of 125-2H. Since 125-2H binding epitope was directly involved in receptor binding, the interaction of IL-18 with the receptor was abolished in the presence of 125-2H. In primed KG-1 cells, shown on the right half of the picture, the presence of IL-18Rβ may induce a conformational change in IL-18Rαβ, resulting in displacement of 125-2H epitope out of the binding pocket. Therefore, the interaction of IL-18 with the receptor complex was not inhibited by 125-2H. However, 125-2H epitope might be involved in interacting with IL-18Rβ, which could be critical for signal transduction. As a result, 125-2H can inhibit IL-18-induced IFN-γ production by KG-1 cells.

These data elucidated that the C-terminal 17-aa residues of rHIL-18 were of central importance in 125-2H recognition. In addition, this 17-aa sequence required a correct conformation in the context of IL-18 tertiary structure for 125-2H recognition, demonstrating that the binding epitope of 125-2H was probably a conformational epitope. In the published IL-1β/receptor structure (19), the C-terminal sequence is positioned in the interface between IL-1 and IL-1Rα, in agreement with the idea that this part of the molecule was involved in direct contact with receptor α-chain. In Fig. 8, a possible mechanism by which 125-2H and IL-18 interact in the two cellular systems is shown. Because of the IL-18Rβ-induced changes in IL-18Rα, the epitope of 125-2H is no longer directly involved in receptor binding; instead, it may be critical for IL-18Rβ-mediated signaling.

The C-terminal region of IL-1β has been shown to be involved in receptor interaction through studies using mAbs and peptides (25–27). The crystal structure of IL-1β/IL-1R has indicated that the C-terminal region of IL-1β fits deeply into the binding pocket between Ig-like domain 2 and domain 3 of the α-chain receptor (19). Due to the unique position of the C terminus, residues at this region contribute to both receptor binding sites A and B of IL-1β (19). Given similar overall structural fold shared among IL-1 family members and based on our results from IL-18, we predict that the involvement of the C-terminal region in receptor interactions is also common throughout the family. We speculate that the receptor signaling subunit-induced binding site changes in the ligand-binding subunit of the receptor may also be shared among IL-1 family receptors.

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