We have identified a novel member of the interleukin-1 (IL-1) receptor family, which we have termed AcPL. In transient transfection assays, we were unable to demonstrate a role for AcPL in IL-1-induced activation of NFκB. Interleukin-18 (interferon-γ-inducing factor) is another member of the IL-1 family of cytokines, and it has recently been shown that IL-18 has a weak affinity for IL-1R-rp1. We examined whether AcPL might function alone or in concert with IL-1R-rp1 to mediate IL-18 signaling. We found that both IL-1R-rp1 and AcPL expression were required for induction of NFκB activity and for activation of c-Jun N-terminal kinase in response to IL-18. Furthermore, a dominant negative version of AcPL specifically inhibited IL-18 signaling. In vitro immunoprecipitation assays demonstrated that AcPL alone was unable to bind IL-18 with any appreciable affinity. We propose that although IL-1R-rp1 binds the cytokine, IL-1R-rp1 and AcPL proteins are both required for IL-18 signaling, analogous to the requirement for both IL-1R and IL-1RAcP in IL-1-mediated responses.

Interleukin-18 (interferon-γ-inducing factor/IL-18) has a wide range of immunoregulatory functions, including stimulation of interferon-γ production, induction of natural killer (NK) cell cytotoxicity, potentiation of Th1 differentiation, and inhibition of osteoclast proliferation (1–4). IL-18-deficient mice displayed an even greater perturbation of NK cell cytotoxicity, potentiation of Th1 differentiation, and inhibition of osteoclast proliferation (1–4). IL-18 has been shown to down-modulate IL-1 responses (15). Signaling by IL-1 is dependent not only on expression of IL-1 type 1 but also on expression of the IL-1RAcP (16, 17). Although IL-1RAcP does not bind IL-1 directly, it does increase the affinity of IL-1R for cytokine binding. Furthermore, the IL-1RAcP in the active receptor complex is responsible for recruitment of the IL-1R-activated kinase (IRAK) (18). Two other members of the family, IL-1R-rp1 and T1/SST2, are unable to bind IL-1 but do respond to IL-1 stimulation in reporter assays when expressed in chimeric form with IL-1R extracellular and transmembrane regions fused to the cytoplasmic domains of these receptors (19–22). IL-1R-rp2 is another orphan family member, incapable of IL-1 binding (23). Interestingly, IL-1R type I, IL-1R type II, IL-1R-rp1, and T1/SST2 all map to the same region of chromosome 2, indicating that perhaps these receptors arise from a common ancestral gene (22, 24).

A recent search of the expressed sequence tag data base revealed a sequence with homology to IL-1RAcP. Reasoning that this might be a new member of the IL-1R family, we utilized the corresponding IMAGE clone to screen a cDNA library to obtain full-length sequence. Analysis of the entire open reading frame verifies that this is a novel member of the IL-1R family, with the IL-1R hallmark domains showing high conservation. Based on its homology to IL-1RAcP we have termed this new protein AcPL (for accessory protein-like). AcPL was unable to mediate signaling by IL-1 but did play a role in signaling via the structurally related cytokine IL-18. Although AcPL did not bind IL-18 in vitro, coexpression of IL-1R-rp1 and AcPL was required for IL-18 responsiveness in terms of NFκB induction and JNK activation. Furthermore, a mutant version of AcPL lacking the cytoplasmic domain inhibited IL-18 signaling. We therefore propose that both the previously identified IL-1R-rp1 and the novel AcPL are involved in signaling by IL-18.

**EXPERIMENTAL PROCEDURES**

**Cloning and Mapping of AcPL**—Expressed sequence tag mu27d04.r1 (GenBank accession number AA203986) was obtained from the IMAGE consortium, and the insert was labeled with [32P]dCTP by random priming and used to probe an EL46.1 (mouse thymocyte) cDNA library. Hybridization was carried out at 42 °C in hybridization solution containing 50% formamide. After the full-length open reading frame was defined by an EL46.1 cDNA clone, it was verified by obtaining independent isolates from 7B9 (mouse T cell) and LDA11 (mouse bone

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‡ Thrombocytopenia was induced by treatment with an antibody directed against the FcγR. Expression of IL-1RAcP was measured by using a monoclonal antibody directed against the receptor.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF077347 and AF077346.

The abbreviations used are: IL, interleukin; mIL, murine IL; JNK, c-Jun N-terminal kinase; IL-R, IL-1 receptor; IRAK, IL-1-activated kinase; PCR, polymerase chain reaction; NK, natural killer.

1 The abbreviations used are: IL, interleukin; mIL, murine IL; JNK, c-Jun N-terminal kinase; IL-R, IL-1 receptor; IRAK, IL-1-activated kinase; PCR, polymerase chain reaction; NK, natural killer.

2 It was also recently shown that IL-18 can activate IRAK, recruit TRAF6, and induce translocation of NFκB (3, 11), which are all involved in IL-1 signaling (12–14).

3 There are several members of the IL-1R family, many of which are currently orphan receptors. The type I and type II IL-1 receptors bind IL-1α, IL-1β, and IL-1ra. The type II IL-1 receptor lacks a cytoplasmic domain, thus rendering it a decoy receptor that has been shown to down-modulate IL-1 responses (15).

4 Although IL-1RAcP does not bind IL-1 directly, it does increase the affinity of IL-1R for cytokine binding. Furthermore, the IL-1RAcP in the active receptor complex is responsible for recruitment of the IL-1R-activated kinase (IRAK) (18). Two other members of the family, IL-1R-rp1 and T1/SST2, are unable to bind IL-1 but do respond to IL-1 stimulation in reporter assays when expressed in chimeric form with IL-1R extracellular and transmembrane regions fused to the cytoplasmic domains of these receptors (19–22). IL-1R-rp2 is another orphan family member, incapable of IL-1 binding (23). Interestingly, IL-1R type I, IL-1R type II, IL-1R-rp1, and T1/SST2 all map to the same region of chromosome 2, indicating that perhaps these receptors arise from a common ancestral gene (22, 24).

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**Cloning of a Novel Receptor Subunit, AcPL, Required for Interleukin-18 Signaling**

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AcP and IL-18 Signaling

mature (sternal) DNA libraries using PCR amplification. Primers corresponded to nucleotides 15 to 13 and nucleotides 1892 to 1916 (relative to the initiating ATG: +1 to +3) of mAeCL. Amplification was under standard PCR conditions for 32 cycles using a KlenTaq-Vent (16:1) mixture of polymerases. Products were cloned directly into pGEM-T Easy (Promega). A human cDNA clone, QQ1352, randomly sequenced at ImmuneX from an NK cell library had a high degree of homology to murine AcPL and was used as a probe to isolate human AcPL clones from peripheral blood lymphocyte, peripheral blood T cell, and NK cDNA libraries. The region of clone QQ1352 used as a probe was homologous to murine AcPL nucleotides 1196–1753. A full-length clone was not obtained from any of the libraries, so vector-anchored PCR was carried out in each of the libraries to obtain the 5′ end of the open reading frame. As for the murine sequence, the reported sequence represents sequence from at least three independent isolates over the entire open reading frame.

The chromosome map position of human AcPL was determined by radiation hybrid mapping using the Stanford G3 Radiation Hybrid Panel (Research Genetics). The primers used, by homology to IL-1R (24), corresponded to intron 4, 5′-CACATCATTGAGGAAATGTACCC-3′, and exon 5, 5′-CTAAAATCATCTTGACACAACAGGC-3′. Amplification was carried out under standard PCR conditions for 40 cycles.

Northern Analysis—A human multiple tissue blot was purchased from CLONTECH Laboratories, Inc. and contained 2 μg of mRNA from normal human spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocyte. This was hybridized overnight with a 32P-labeled antisense hAcPL riboprobe in hybridization buffer containing 50% formamide at 63 °C and then washed at 68 °C in 0.1× SSC/0.1% SDS. After exposure, the blot was rehybridized with a random-prime labeled probe against β-actin for standardization.

Plasmids and Cell Culture—For expression, full-length murine and human AcPL were generated by PCR and cloned into pDC302, a variant of pDC302 (25). The human AcPLFc expression vector joins the extracellular portion of the receptor (amino acids 1–356) to the CH2 and CH3 domains of human IgG1 and was generated as described previously (26). Expression vectors encoding IL-1R-rp1 and IL-1R-rp1-Fc have been described previously (22). mAcPL-CytO contains Thr17 to Leu39 of mAeCL, with an immunoglobulin κ light chain signal peptide substituted for the native signal peptide, followed by a modified FLAG (27) epitope tag (METDTLLLWLIVVLPVGSGDYKDEGTGTS). mIL-1R-rp1Accto contains Met1 to Glu39 with the native signal peptide and no epitope tag. The NFκB-Luciferase plasmid has been described previously (28) and contains three NFκB sites positioned in a minimal e-Fos promoter driving expression of the luciferase gene. IL8p-Luc contains the previously described hIL8 promoter (29) subcloned into pGL2-Basic (Promega). The human IL-18 sequence was cloned into pDC206 (30) for transient overexpression.

Cos7 (monkey kidney) cells were maintained in Dulbecco’s modified Eagle’s medium/5% fetal bovine serum and S49.1 (murine T) cells were maintained in RPMI 1640/5% fetal bovine serum supplemented with 1 mM nonessential amino acids, and 55 μM 2-mercaptoethanol.

Reporter and JNK Assays—To assess NFκB activation, Cos7 cells were transiently transfected by the DEAE-dextran method as described (31), using 150 ng of each receptor and 700 ng of the reporter plasmid (unless otherwise noted) or 5 × 105 cells. Two days post-transfection, cells were stimulated with 10 ng/ml IL-1 or 40 ng/ml IL-18 (PeproTech, Inc.) for 4 h. Cells were lysed and luciferase activity assessed using Reporter Lysis Buffer and Luciferase Assay Reagent (Promega Corp.). Inc.) for 4 h. Cells were lysed and luciferase activity assessed using Reporter Lysis Buffer and Luciferase Assay Reagent (Promega Corp.). S49.1 cells (1 × 106) were electroporated (320 V, 960 microfarad) with 20 μg of reporter DNA and 5 μg of each receptor-encoding DNA. After 2 days cells were stimulated and luciferase levels were measured as described above.

Activation of JNK activity was assessed as described previously (32). Briefly, 2 days post-transfection Cos7 cells were stimulated with IL-18 for 15 min, lysed, and immunoprecipitated with a combination of two anti-JNK antibodies (C-17 and FL, Santa Cruz Biotechnology, Inc.). This immunocomplex was assayed for activity by addition of glutathione-activated /32P-labeled S-glutathione-activated myelin basic protein (Upstate Biotechnology, Inc.) and γ-32P]ATP in kinase buffer. The reaction was allowed to proceed for 30 min at room temperature, after which Laemmli Loading buffer was added to stop the reaction, and products were electrophoresed on a 4–20% acrylamide gel, stained, dried, and analyzed on a STORM PhosphorImager.

Immunoprecipitation of /32P/35S/IL-18—Cos7 cells (4.5 × 105) were transiently transfected with an expression plasmid for human IL-18 (1 μg) by the DEAE-dextran method. Two days after transfection, cells were starved for 1 h then labeled in /35S]Cys-/35S]Met containing medium for 4 h. The supernatants were removed, subjected to centrifugation, and adjusted to 0.4 μM NaCl/1.0% Triton (X-100) in the presence of protease inhibitors. Fc receptor protein (1 μg) was added to the supernatants with 30 μl of (50%) protein G-Sepharose (Boehringer Mannheim). Precipitations were carried out overnight at 4 °C, washed extensively in buffer (0.4 μM NaCl, 0.05% SDS, 1.0% Nonidet P-40), and then separated by electrophoresis in a 4–20% Tris-Glycine gel (Novex). The gel was fixed, incubated in Amplify (Amersham Pharmacia Biotech), dried, and exposed to X-Omat AR film (Kodak).

RESULTS

Cloning of AcP—A search of the expressed sequence tag data base revealed that IMAGE clone 640615 (GenBank™ Accession number AA203986) had homology to IL-1RαCp. We used this IMAGE clone to screen an EL46.1 cDNA library to isolate the full-length open reading frame. Subsequently, full-length sequence was also isolated from two other murine T cell libraries by PCR using gene-specific primers. We have therefore verified that the same sequence was obtained from three different library sources. The murine sequence was highly homologous to a human NK cell clone, QQ1352, randomly sequenced at ImmuneX. Clone QQ1352 was therefore used to probe peripheral blood T cell, peripheral blood lymphocyte, and NK cell libraries, and the full-length human sequence was obtained from all three libraries. The predicted amino acid sequence of both the murine and human forms of AcP is presented in Fig. 1. Murine and human forms of AcP share 65% identity. Overall, AcP shows 25% identity to IL-1R type I, 27% identity to IL-1RαCp, and 26% identity to IL-1R-rp1. A BLAST (33) search of the data base does not reveal any other proteins with significant homology to AcP besides the noted IL-1R family members.

Similar to other members of the IL-1R family, hAcP is predicted to contain a signal peptide (14 amino acids), a 342-amino acid extracellular segment, a single transmembrane region, and a 222 amino acid cytoplasmic domain. The extracellular region classifies this receptor as a member of the immunoglobulin superfamily. In comparison with type I IL-1R, Ig domain 1 is poorly conserved, yet domains 2 and 3 are highly conserved in AcP. There are three potential glycosylation sites that are conserved in both the murine and human sequence (as well as one in each species that is not conserved). The cytoplasmic domain of hAcP is 32% identical to that of hIL-1R.

By Northern blot analysis, human AcP was expressed strongly in peripheral blood leukocytes and spleen and to a lesser extent in colon. Weak expression was detected in prostate and small intestine mRNA (which may not be visible on reproduction). The predominant mRNA product was approximately 3.8 kilobases, with minor bands at approximately 2.6 and 8.0 kilobases (Fig. 2). Expression was also detected in lung mRNA by Northern analysis (data not shown). No expression was detected in thymus, prostate, testis, ovary, or small intestine (Fig. 2) nor in heart, brain, kidney, or muscle mRNA (data not shown). In agreement with these results we were unable to detect expression of AcP in human fetal brain, murine heart, or murine brain libraries by PCR.

Radiation hybrid analysis using the Stanford G3 panel placed human AcP on chromosome 2, most closely linked to AFM3161tg5, with a logarithm of odds score of 12.72 (data not shown). This is the same region of chromosome 2 to which IL-1R type I, IL-1R type II, IL-1R-rp1, and T1/ST2 have been mapped (Stanford Human Genome Center bin 57) (22, 24).

AcP Is Not Involved in IL-1 Signaling—Because the extracellular domain of AcP was homologous to IL-1R, we first tested its ability to induce signaling in response to IL-1 stimulation. Cos7 cells were transiently transfected with full-length
murine AcPL and a luciferase reporter harboring three NFκB sites. Cells were stimulated with 10 ng/ml IL-1α and assayed for luciferase activity. As shown in Fig. 3A, cells transfected with empty vector or IL-1RAcP exhibited a reporter induction in response to IL-1α stimulation, due to endogenous monkey IL-1R expression. Overexpression of IL-1R, however, resulted in a marked enhancement of luciferase induction over background. In contrast, transfection of AcPL did not augment the background response, suggesting that it is incapable of mediating IL-1 signaling. Similar results were obtained with IL-1β stimulation and with an IL-8 promoter-containing reporter. It is possible that AcPL could function in IL-1 signaling in a role similar to IL-1RαcP, by complementing the IL-1R for IL-1 responsiveness. Due to endogenous IL-1RαcP expression in Cos7 cells, such a function for AcPL would not be uncovered in the above transfection assays. We have characterized a T cell line, S49.1, which is nonresponsive to IL-1 when electroporated with an NFκB-driven reporter but is responsive to IL-1 when IL-1R and IL-1RαcP are coexpressed. Using this system, we investigated whether AcPL could replace IL-1R or IL-1RαcP to produce an NFκB response to IL-1. We found that replacement of either IL-1R or IL-1RαcP with AcPL abolished responsiveness (Fig. 3B). Taken together with the results in Cos7 cells, we conclude that AcPL does not mediate IL-1 signaling.

AcPL Is Required for IL-18 Signaling—IL-18 has been classified as a member of the IL-1 family of cytokines based on amino acid sequence and structural homology. It has recently been shown that overexpression of hIL-1R-rp1 in Cos1 cells resulted in increased IL-18 binding and conferred an NFκB induction in response to IL-18 (9). In our hands, however, Cos1 or Cos7 cells overexpressing IL-1R-rp1 are nonresponsive to IL-18, suggesting the requirement for another receptor chain, which is expressed in the cells used by Torigoe et al. (9), but not expressed in our Cos cells. Therefore, we tested whether AcPL could function alone or as a second subunit in IL-18 responses by performing transient transfections in Cos7 cells. As shown in Fig. 4A, NFκB activation by mIL-18 required the expression of both mAcPL and mIL-1R-rp1 in these cells. We observed no NFκB induction in control, mIL-1R-rp1, or mAcPL-transfected cells stimulated with IL-18. In contrast, coexpression of mIL-1R-rp1 and mAcPL resulted in elevated levels of both basal and cytokine-stimulated NFκB activity. A control reporter lacking the NFκB sites was not activated in these assays (data not shown). Elevation of basal NFκB activity has also been reported upon IL-1R and IL-1RαcP overexpression and probably reflects the formation of receptor heterodimers in the absence of ligand due to the high density of surface expression (18). This is supported by the fact that basal reporter activity is not elevated in Cos7 cells transfected with lower amounts (10 ng each as opposed to 150 ng each) of IL-1RαcP and AcPL (data not shown).
We have also shown that both mIL-1R-rp1 and mAcPL receptor chains were required when S49.1 cells were electroporated, and reporter gene activity was measured in response to mIL-18 (data not shown). In these experiments, transfection efficiency was much less than in Cos7 cells, and we therefore did not observe an elevation in basal NFκB activity when both receptors were coexpressed.

In contrast to murine AcPL, overexpression of human AcPL alone was able to mediate a significant NFκB induction following hIL-18 stimulation in Cos7 cells (Fig. 4B). Using primers designed against human IL-1R-rp1, we were able to verify expression of IL-1R-rp1 in our Cos7 cells (data not shown). It is therefore likely that the endogenous monkey IL-1R-rp1 is capable of cooperating with hAcPL but not with mAcPL to confer IL-18 responsiveness. Alternatively, it could be that monkey IL-1R-rp1 can bind human IL-18 but not murine IL-18.

If IL-1R-rp1 and AcPL are both absolutely required for IL-18 signaling, as suggested by the above experiments, then one would expect that mutants of either receptor in which the entire cytoplasmic domain were deleted should exhibit a dominant negative effect. To test this, we transfected Cos7 cells with limiting amounts of receptor (10 ng each) and cotransfected mutant versions of either mIL-1R-rp1 or mAcPL (100 and 1000 ng). As shown in Fig. 4C, both mIL-1R-rp1Δcyto and mAcPLΔcyto were capable of inhibiting IL-18 signaling. In contrast, overexpression of these mutant receptors had no effect on IL-1 signaling (data not shown).

Another downstream signaling event in the IL-1 pathway is induction of JNK activity. We have observed that AE7 (murine T) cells stimulated with IL-18 show an induction of JNK activity. We therefore examined whether AcPL alone or in combination with IL-1R-rp1 was capable of mediating the induction of JNK activity by IL-18. Cos7 cells were transiently transfected with empty vector, IL-1R-rp1 and mAcPL and 50 ng of 3XNFκB-Luciferase. Versions of mIL-1R-rp1 or mAcPL lacking the cytoplasmic domain were cotransfected at 100 ng or 1 μg. In all cases the total amount of DNA remained constant by addition of empty vector (pDC304). Cells were stimulated and assayed as described above. All data represent the relative light units (RLU) per sample. Experiments were performed at least three times, with one representative experiment being presented. Open bars, medium; bars with shaded grid, IL-18.

**Fig. 3.** AcPL does not mediate NFκB signaling in response to IL-1α stimulation. A. Cos7 cells were transiently transfected with 150 ng each of the indicated receptor-encoding plasmids and 700 ng of 3XNFκB-Luciferase reporter plasmid. 2 days post transfection cells were stimulated with Dulbecco’s modified Eagle’s medium/F12/no serum (open bars) or 10 ng/ml IL-1α (bars with shaded hatching) diluted in the same medium. 4 h following stimulation luciferase activity was determined. B, S49.1 cells were electroporated with 5 μg each of the indicated receptor plasmids and 20 μg of 3XNFκB-Luciferase. Cells were stimulated and assayed as in A. Both sets of data represent the relative light units (RLU) per sample. Experiments were performed at least three times, with one representative experiment being presented.

**Fig. 4.** IL-1R-rp1 and AcPL are both required for NFκB signaling in response to IL-18. A, Cos7 cells were transiently transfected with 150 ng each of the indicated receptor-encoding plasmids and 700 ng of 3XNFκB-Luciferase reporter plasmid. 2 days post transfection cells were stimulated with Dulbecco’s modified Eagle’s medium/F12/no serum (open bars) or 40 ng/ml mIL-18 (bars with shaded hatching) diluted in the same medium. 4 h following stimulation luciferase activity was determined. B, Cos7 cells were transfected and assayed as in A, except human versions of the receptors and hIL-18 were utilized. C, Cos7 cells were transiently transfected with 10 ng each of mIL-1R-rp1 and mAcPL and 50 ng of 3XNFκB-Luciferase. Versions of mIL-1R-rp1 or mAcPL lacking the cytoplasmic domain were cotransfected at 100 ng or 1 μg. In all cases the total amount of DNA remained constant by addition of empty vector (pDC304). Cells were stimulated and assayed as described above. All data represent the relative light units (RLU) per sample. Experiments were performed at least three times, with one representative experiment being presented. Open bars, medium; bars with shaded grid, IL-18.
regarding activation of NFκB, JNK activity was only induced by IL-18 in Cos7 cells when IL-1R-Rp1 and AcPL were coexpressed (Fig. 5).

AcPL Is Unable to Bind IL-18 in Vitro—It has already been reported that overexpression of IL-1R-rp1 in Cos1 cells resulted in elevated levels of IL-18 binding (9) and that IL-1R-rp1 was able to bind IL-18 in vitro. We have investigated the ability of AcPL to bind IL-18 by immunoprecipitation of mammalian-expressed IL-18 with AcPL-Fc protein. An Fc fusion protein encoding the extracellular region of hAcPL was used to precipitate 35S-labeled IL-18 expressed in Cos7 cell supernatants. As controls, the radiolabeled IL-18 was also precipitated with Fc fusion proteins encoding the extracellular domains of IL-1R and IL-1R-rp1. As shown in Fig. 6, only IL-1R-rp1 was able to bind IL-18 in this assay. There was no detectable binding of AcPL to IL-18. Supernatants from all samples were examined for levels of hIL-18 expression prior to precipitation, and all supernatants expressed similar levels of the cytokine (data not shown).

Furthermore, the above experiment has been repeated with unpurified supernatants from cells transfected with hIL-1R-rp1-Fc and hAcPL-Fc, with the same result, eliminating the possibility that purification of hAcPL-Fc ablates its cytokine binding activity.

**DISCUSSION**

We have cloned a novel member of the IL-1R family which we show is capable of mediating cell signaling in response to IL-18. AcPL is part of the IL-18 receptor complex and is not surprising given that [1] IL-18 is structurally related to IL-1 and would be expected to recognize a receptor with a structure related to IL-1R and [2] IL-18 has already been shown to bind another member of this receptor family, IL-1R-rp1 (9). Further, given that the only other characterized member of this family requires two distinct receptor chains for signaling, it would be expected that two receptor chains would be required for IL-18 signaling. In support of this, we have observed that Cos7 or Cos1 cells transfected with IL-1R-rp1 alone were not responsive to IL-18 in terms of NFκB induction. These results suggested that another protein was required to form an active IL-18 receptor complex. The data presented in this paper suggest that the other subunit required for IL-18 responsiveness is AcPL.

We have shown that AcPL is expressed in lung, spleen, and peripheral blood lymphocytes, as well as peripheral blood T cell and NK libraries. Expression was not detected in heart, brain, kidney, or muscle mRNA. This expression pattern closely mirrors that of IL-1R-rp1 (22), although some tissues such as heart and testis express detectable IL-1R-rp1 but not AcPL. The pattern of AcPL expression also correlates well with the cell types that have been reported to be responsive to IL-18. Previous studies have shown that IL-18 induces interferon-γ production from T cells and NK cells. Costimulatory effects of IL-18 have also been reported in peripheral blood mononuclear cells and B cells (34).

Interestingly, we have also observed that IL-1R-rp1 and AcPL are capable of inducing IL-8 promoter activity in response to IL-18 (data not shown). The IL-8 promoter is directly activated by IL-1 (35), but it has not been shown to be activated directly by IL-18 to date. It was recently demonstrated that IL-8 was induced in IL-18 stimulated CD8+CD4+ and NK cells, but this induction was dependent on the induction of TNFα (36). These data suggest that in addition to activation of an artificial, NFκB-containing reporter, AcPL and IL-1R-rp1 are able to mediate IL-18 signaling to a more complex promoter.

Because AcPL alone was unable to bind IL-18, we propose that it is analogous to the IL-1RαP, which is required for IL-1 signaling but does not bind IL-1 itself. By extension of this analogy, it will be of interest to determine whether cells expressing IL-1R-rp1 and AcPL display a higher affinity toward IL-18 than do cells expressing IL-1R-rp1 alone. We have attempted to address this issue by binding recombinant IL-18, iodinated on either tyrosine or lysine residues, to Cos7 cells overexpressing IL-1R-rp1, AcPL, or both receptors together. We have been unable to demonstrate reproducible specific binding of 125I-IL-18 in these assays, suggesting that perhaps the iodination has interfered with cytokine binding. We are currently investigating alternative approaches to address this issue.

The downstream mediators of IL-18 signaling seem to be highly related to those involved in IL-1 signaling. Both our laboratory and others have recently shown that stimulation of T cells (AE7 or EL4) with IL-18 results in the recruitment of IRAK1 and TRAF6 to IL-1R-rp1 (11). In IL-1 signaling, it is IL-1RαP that is responsible for recruitment of IRAK1 to the active receptor complex, whereas IL-1R recruits IRAK2 (10, 18). It will be interesting to determine whether AcPL is able to associate with these downstream signaling molecules. Previously reported data showing that IL-1R-rp1 associates with
IRAK do not rule out the possibility that IL-1R-Rp1 is part of a larger IL-18 binding complex, rather than being directly involved in binding these proteins.

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