Functional screening of five PYPAF family members identifies PYPAF5 as a novel regulator of NF-κB and caspase-1

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Abstract PYRIN-containing Apaf-1-like proteins (PYPAFs) are a recently identified family of proteins thought to function in apoptotic and inflammatory signaling pathways. PYPAF1 and PYPAF7 proteins have been found to assemble with the PYRIN–CARD protein ASC and coordinate the activation of NF-κB and pro-caspase-1. To determine if other PYPAF family members function in pro-inflammatory signaling pathways, we screened five other PYPAF proteins (PYPAF2, PYPAF3, PYPAF4, PYPAF5 and PYPAF6) for their ability to activate NF-κB and pro-caspase-1. Co-expression of PYPAF5 with ASC results in a synergistic activation of NF-κB and the recruitment of PYPAF5 to punctate structures in the cytoplasm. The expression of PYPAF5 is highly restricted to granulocytes and T-cells, indicating a role for this protein in inflammatory signaling. In contrast, PYPAF2, PYPAF3, PYPAF4 and PYPAF6 failed to colocalize with ASC and activate NF-κB. PYPAF5 also synergistically activated caspase-1-dependent cytokine processing when co-expressed with ASC. These findings suggest that PYPAF5 functions in immune cells to coordinate the transduction of pro-inflammatory signals to the activation of NF-κB and pro-caspase-1.

Key words: PYPAF5; PYRIN-containing Apaf-1-like protein; Caspase-1; NF-κB; ASC; Inflammation

1. Introduction

Apaf-1 plays a central role in apoptosis by transducing pro-apoptotic signals to the activation of pro-caspase-9 [1,2]. During apoptosis, cytochrome *c* is released from mitochondria into the cytoplasm, where it induces the oligomerization of Apaf-1 in the presence of dATP. Oligomerization of Apaf-1 results in the formation of an apoptosome that recruits and activates pro-caspase-9. Apaf-1 has a tripartite domain structure consisting of an N-terminal caspase-recruitment domain (CARD) that mediates recruitment of pro-caspase-9 to the apoptosome, a central nucleotide-binding site (NBS) domain, and a C-terminal domain comprised of WD-40 repeats. The NBS domain mediates Apaf-1 oligomerization in the presence of dATP, whereas the WD-40 repeats function as binding sites for cytochrome *c*. Thus, Apaf-1 functions as a sensor-like molecule that signals apoptosis in response to the release of cytochrome *c* from mitochondria.

A search for Apaf-1-like molecules has resulted in the identification of numerous proteins that belong to the NBS/leucine-rich repeat (LRR) family of inflammatory regulators. CARD4 (Nod1), CARD15 (Nod2) and CARD12 (Ipaf/Clan) each contain an N-terminal CARD domain(s) that mediates assembly with a downstream signaling partner and a central NBS domain that likely coordinates nucleotide-dependent oligomerization [3–8]. In addition, each protein contains a C-terminal LRR domain that has been proposed to function as a binding site for specific upstream regulators. CARD4 and CARD15 signal the activation of NF-κB following assembly with RICK, a CARD-containing kinase that mediates inflammatory signaling for both the innate and adaptive immune systems [9,10]. Intriguingly, CARD15 variants confer susceptibility to Crohn's disease and Blau syndrome, two chronic inflammatory disorders [11–13]. In addition to coordinating the activation of NF-κB, Apaf-1-like proteins have also been found to regulate the activation of pro-inflammatory caspases. CARD12 is thought to play a critical role in inflammatory signaling by binding to and activating pro-caspase-1, a CARD-containing caspase that processes pro-interleukin-1β (IL-1β) and pro-interleukin-18 into active cytokines. The inflammatory signals that regulate the assembly and activation of these protein complexes are not presently understood.

A second group of Apaf-1-like proteins that contain N-terminal PYRIN domains instead of CARD domains has been identified recently [14–17]. We have named these proteins PYPAFs for PYRIN-containing Apaf1-like proteins [18,19]. The PYRIN domain is a novel protein–protein interaction domain that shares homology with pyrin, a protein that is associated with a rare inflammatory disorder called familial Mediterranean fever [20]. This domain is a new member of the death domain–fold superfamily that functions to mediate homotypic interactions between PYRIN-containing proteins [14,15,18,19]. PYPAF1 (cryopyrin) variants cause several autoinflammatory diseases, including Muckle–Wells syn-

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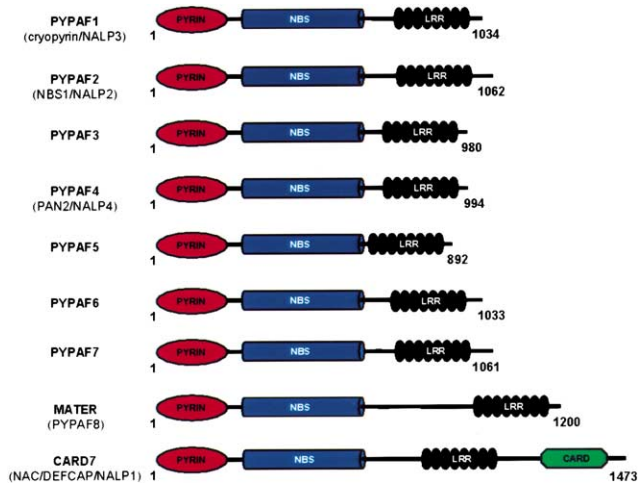

Fig. 1. Domain structure of PYPAF family members. Shown are PYPAF1, PYPAF2, PYPAF3, PYPAF4, PYPAF5, PYPAF6, PYPAF7, MATER (PYPAF8) and CARD7. PYRIN domains (red shading), NBS domains (blue shading) and LRR domains (black shading) are indicated.

drome, familial cold urticaria, and chronic infantile neurological cutaneous and articular syndrome [21–23]. We recently identified PYPAF1 and PYPAF7 as PYPAF family members that assemble together with ASC and signal the activation of NF-κB and pro-caspase-1 [18,19]. The recruitment and activation of pro-caspase-1 by these PYPAF proteins is mediated by the C-terminal CARD domain of ASC [19,24]. To determine if other PYPAF proteins regulate inflammatory signaling pathways, we screened five other family members for their

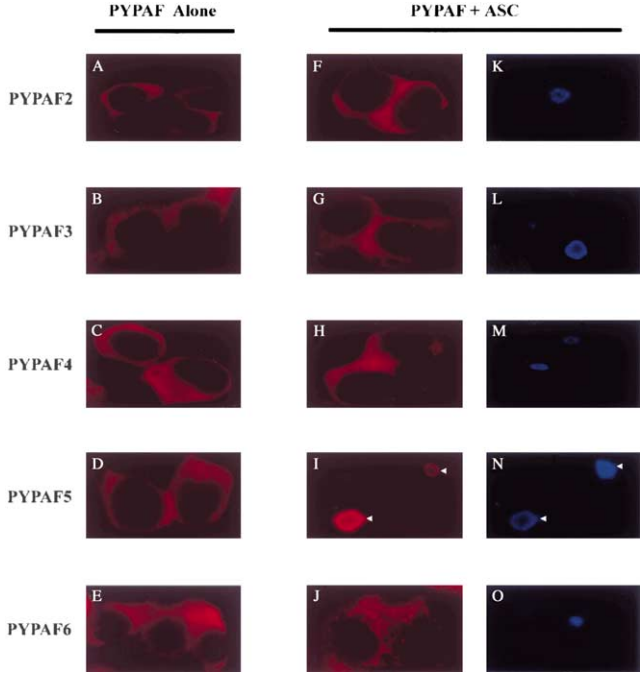

Fig. 2. PYPAF5 is recruited to ASC punctate structures. HA-tagged ASC (blue staining) and FLAG-tagged PYPAF proteins (red staining) were expressed in 293T cells. A–E show localization of PYPAF proteins when expressed alone. F–J and K–O show the immunostaining patterns observed when each protein was co-expressed with ASC. Note the co-localization of PYPAF5 (I and N) to the ASC punctate structures (arrows).

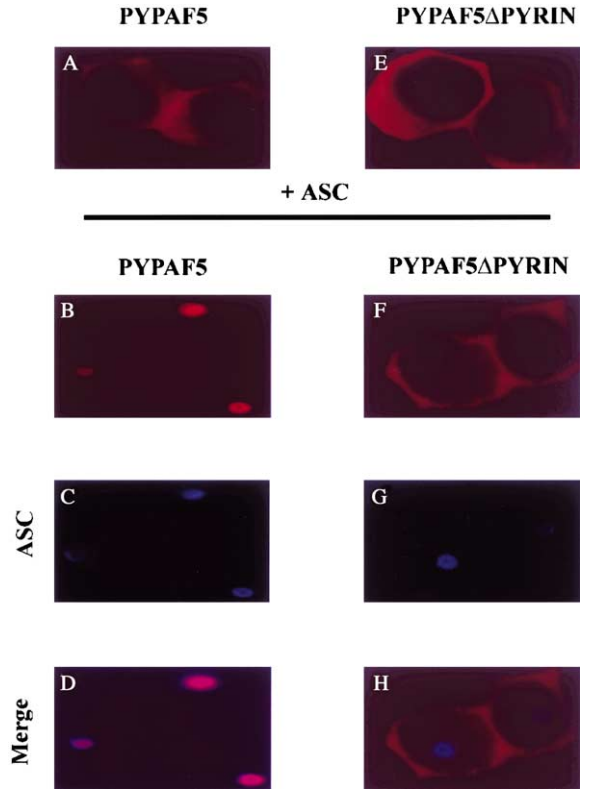

Fig. 3. The PYRIN domain of PYPAF5 is needed for recruitment to ASC punctate structures. HA-tagged ASC (blue staining) and FLAG-tagged PYPAF5 proteins (red staining) were expressed in 293T cells. A and E show PYPAF5 and PYPAF5ΔPYRIN, respectively, when expressed alone. B–D and F–H show the immunostaining patterns observed when each protein was co-expressed with ASC. Note the co-localization of PYPAF5 (B–D) and the lack of co-localization of PYPAF5ΔPYRIN (F–H) to the ASC punctate structures (arrows).

ability to activate NF-κB and pro-caspase-1. We report here that ASC recruits PYPAF5 to distinct cytoplasmic loci. In addition, PYPAF5 shows restricted expression to immune cells and synergistically activates NF-κB and caspase-1 when co-expressed with ASC. These findings identify PYPAF5 as a cell-type-specific upstream regulator of pro-inflammatory signaling pathways.

2. Materials and methods

2.1. Expression plasmids

Plasmids expressing full-length PYPAF2 (accession number AF464764), PYPAF3 (accession number AF464765), PYPAF4 (accession number AF479747), PYPAF5 (accession number AF479748) and PYPAF6 (accession number AY095145) with a C-terminal FLAG epitope were constructed using either pCI (Promega) or pCMV-4 (PYPAF4, Stratagene). The plasmid expressing a PYPAF5 truncation mutant lacking the PYRIN domain (PYPAF5ΔPYRIN, residues 81–892) with a C-terminal FLAG epitope was also constructed using pCI (Promega). Plasmids expressing mouse pro-IL-1β, pro-caspase-1, dominant negative IKKγ and inactive pro-caspase-1 (C285) were described previously [6,18].

2.2. PYPAF5 expression analysis

Total RNA from cells in culture was extracted (Qiagen) and expression profiles were determined by real time quantitative PCR analysis (Taqman™). In brief, an oligonucleotide probe (5′-ACGGAGCGGGCGTTCCTCTCCT-3′) was designed to anneal to PYPAF5 between two PCR primers (forward: 5′-CCGTGTCCGAG-

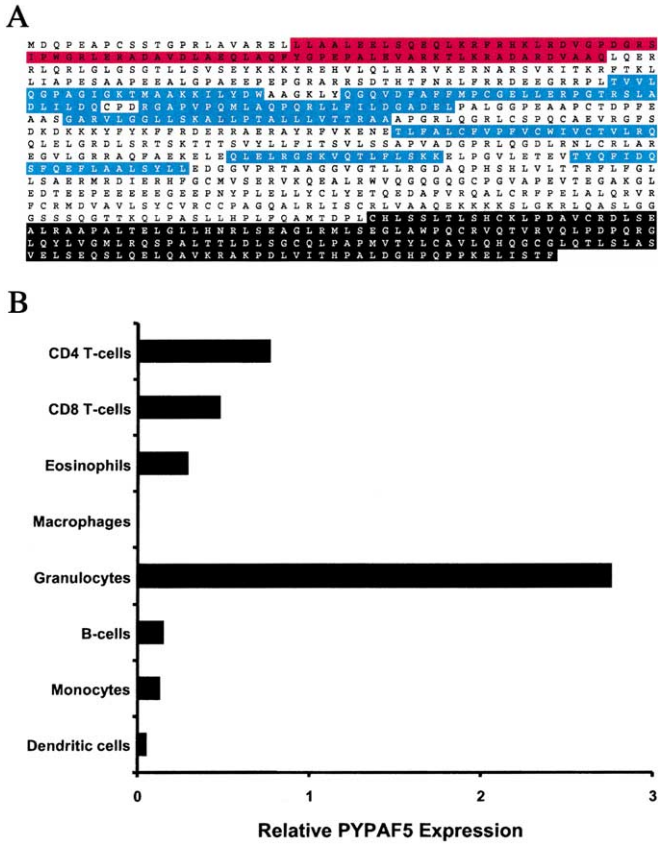

Fig. 4. Sequence and expression of PYPAF5. A: Amino acid sequence of PYPAF5. N-terminal PYRIN domain (residues 22–96, red shading), NBS domain (residues 194–513) with seven consensus motifs that are found in the NACHT subfamily of NTPases (blue shading), and C-terminal domain (residues 729–892) comprised of at least four LRRs. B: PYPAF5 expression in various immune cells by real time quantitative PCR. Level of PYPAF5 expression in various cell types was normalized to the expression of β2 microglobulin.

TACAAGAAGAAG-3′ and reverse: 5′-CGCGATGAGCAGCTTG-GT-3′). The probe was then fluorescently labeled with FAM (reporter dye) on the 5′ end and TAMRA (quencher dye) on the 3′ end. Probe and PCR primers designed for β2 microglobulin were described previously [18]. PCR reactions were performed on cDNAs from various cell types using primers and probes for both PYPAF5 and β2 microglobulin genes. Fluorescent emissions generated during PCR reaction was measured by Sequence Detector 7700 (Perkin Elmer Life Sciences). The expression level of PYPAF5 was normalized to the expression of β2 microglobulin for each sample.

2.3. Immunostaining assays

293T cells were transfected in poly-D-lysine-coated glass chamber slides (BioCoat, Becton-Dickinson Labware) with plasmids expressing HA-tagged ASC and FLAG-tagged PYPAFs using SuperFect transfection reagent (Qiagen). Cells were fixed 24 h after transfection in 4% paraformaldehyde, permeabilized and blocked in a buffer containing 0.3% Triton X-100, and incubated with the following primary and secondary antibodies: rabbit anti-HA polyclonal Y-11 (Santa Cruz Biotechnology), mouse anti-FLAG monoclonal antibody M2 (Sigma), Alexa-350 goat anti-rabbit IgG (Molecular Probes), and Alexa-598 goat anti-mouse IgG (Molecular Probes).

2.4. Reporter gene assays

The NF-κB assay was described previously [25]. 293T cells were transfected with pNF-κB luciferase reporter (Stratagene), pRL-TK Renilla reporter (Promega), and indicated expression plasmids. Cells were harvested and firefly and Renilla luciferase activities were determined using the Dual-Luciferase reporter assay system (Promega).

2.5. IL-1β secretion assays

COS-7L cells (Gibco) were co-transfected in 12-well (22-mm) plates using LipofectAMINE 2000 transfection reagent (Invitrogen) with plasmids encoding mouse pro-IL-1β and indicated expression plasmids (total DNA = 1.04 μg). Supernatants were collected 24 h after transfection and subjected to ELISA for mouse IL-1β according to the manufacturer's protocol (R&D Systems). The caspase-1 inhibitor z-WEHD-FMK was added to cells following transfection at a final concentration of 100 μM (R&D Systems).

3. Results and discussion

3.1. PYPAF family members are putative regulators of NF-κB and caspase-1

The PYPAF family of signaling proteins is comprised of at least eight members (Fig. 1). In addition, six other genes that encode novel PYPAFs are also found in the HTG database of genomic sequences (J. Bertin, unpublished data). Each family member contains an N-terminal PYRIN domain, a central NBS domain, and a C-terminal domain comprised of multiple LRRs. In addition to these domains, CARD7 also contains a C-terminal CARD domain and is therefore a member of both the CARD and PYRIN protein families [14] (Fig. 1). We recently identified PYPAF1 and PYPAF7 as upstream activators of NF-κB signaling and pro-caspase-1 [18,19]. The PYRIN–CARD protein ASC plays a central role in PYPAF1 and PYPAF7 signaling by recruiting pro-caspase-1 to the PYPAF/ASC signaling complex. In this study, we screened five other PYPAF family members (PYPAF2, PYPAF3, PYPAF4, PYPAF5 and PYPAF6) for their ability to activate downstream inflammatory signaling pathways.

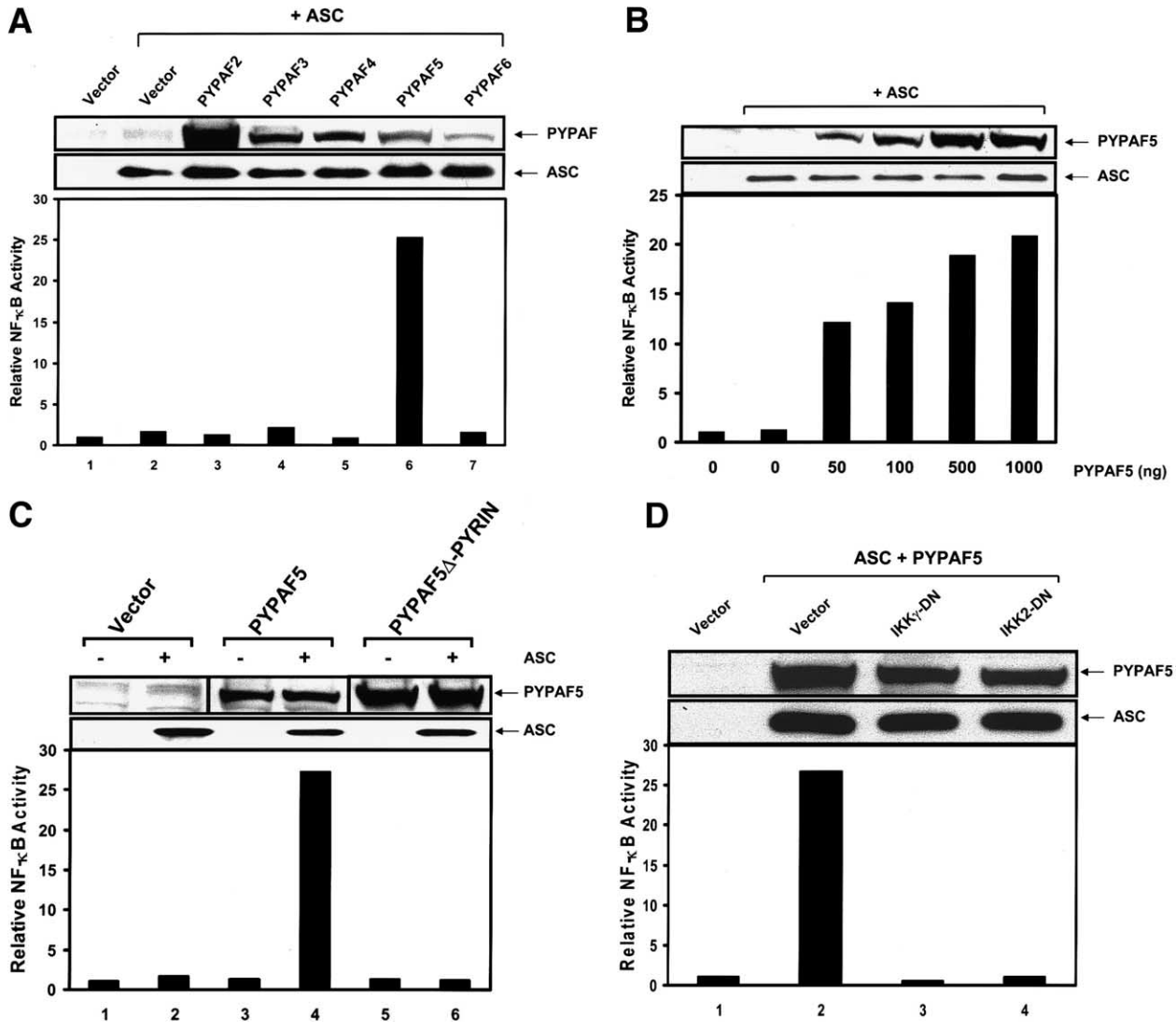

Fig. 5. PYPAF5 augments ASC-induced NF-κB activity. A: PYPAF5 synergizes with ASC to induce NF-κB activation. Plasmids expressing PYPAFs (1000 ng) and ASC (32 ng) were transfected into 293T cells, and relative luciferase activities were determined to measure the induction of NF-κB activity. B: PYPAF5 activates NF-κB in a concentration-dependent manner. C: The N-terminal PYRIN domain of PYPAF5 is required for synergy. 293T cells were transfected with plasmids expressing PYPAF5, and PYPAF5ΔPYRIN (1000 ng) with or without ASC (32 ng). The amount of DNAs in each transfection was kept constant by addition of empty vectors. Relative luciferase activities were then determined as a measure of NF-κB activity (lower panel). Immunoblot analysis shows expression of PYPAF5 and ASC (upper panels). D: PYPAF5 and ASC activate NF-κB through the IKK complex. 293T cells were transfected with plasmids expressing 32 ng of ASC and 500 ng of PYPAF5 with either 500 ng of empty vector or dominant negative mutants of IKKγ (IKKγ-DN) or IKK2 (IKK2-DN). Relative luciferase activities were then measured. Immunoblot analysis shows expression of PYPAF5 and ASC (upper panels).

3.2. Recruitment of PYPAF5 to ASC punctate structures

We first performed cellular co-localization studies to determine whether the PYPAF proteins interact with ASC. FLAG-tagged PYPAF2, PYPAF3, PYPAF4, PYPAF5 or PYPAF6 were co-expressed with HA-tagged ASC in cells and detected using a mixture of anti-HA and anti-FLAG antibodies. When expressed alone, the PYPAFs showed a broad cytoplasmic distribution that excluded the nucleus (Fig. 2A–E). As observed previously, ASC localized to cytoplasmic punctate structures when expressed alone ([26], and data not shown). However, PYPAF5 was found to co-localize with ASC when co-expressed (Fig. 2I and N). PYPAF2, PYPAF3, PYPAF4 and PYPAF6 failed to be recruited to the ASC punctate structures (Fig. 2, compare F and K, G and L, H and M, J and O). We also examined the ability of a PYPAF5 mutant lacking the N-terminal PYRIN domain (PYPAF5ΔPYRIN) to be recruited to the ASC punctate structures. When expressed alone, PYPAF5ΔPYRIN showed a broad cytoplasmic distribution similar to that of PYPAF5 (Fig. 3A and E). However, PYPAF5ΔPYRIN failed to associate with the ASC punctate structures, demonstrating that the PYRIN domain of PYPAF5 is necessary for recruitment (Fig. 3, compare B–D to F–H). We have been unable to detect an interaction between PYPAF5 and ASC by performing co-immunoprecipitation experiments when both proteins are overexpressed in cells. Our inability to detect an interaction is likely due to the transient nature of the interaction and/or the relative insolubility of PYPAF5/ASC complexes that form in the cell when these proteins are co-expressed. Nonetheless, the co-localization of PYPAF5 to the ASC punctate structures is required for the

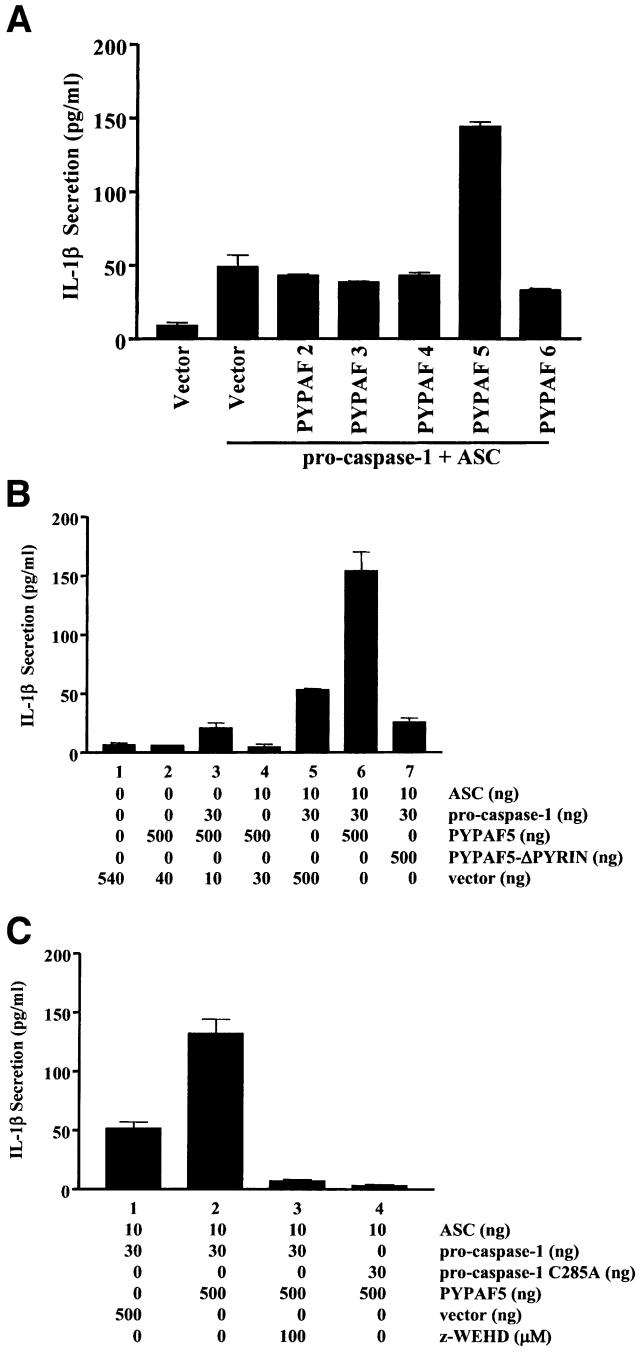

Fig. 6. PYPAF5 and ASC activate pro-caspase-1. COS-7L cells were co-transfected with plasmids encoding mouse pro-IL-1β in combination with various amounts of indicated plasmids encoding pro-caspase-1, ASC and PYPAFs. After 24 h, supernatants were collected and subjected to ELISA for IL-1β. A: Activation of pro-caspase-1 by PYPAF5. B: Activation of pro-caspase-1 by PYPAF5 is dependent on its N-terminal PYRIN domain. C: PYPAF5 and ASC induction of IL-1β secretion is dependent on active caspase-1.

synergistic activation of NF-κB and caspase-1-dependent cytokine processing (see below).

3.3. PYPAF5 is expressed in immune cells

PYPAF5 has a tripartite structure consisting of an N-terminal PYRIN domain (residues 22–96), a central NBS domain (residues 194–513) and a C-terminal domain (residues 729–892) comprised of at least four LRR motifs (Fig. 4A). The NBS domain belongs to the NACHT subfamily of NTPases and contains seven signature motifs, including the P-loop (residues 194–219, Fig. 4A) [27]. The PYRIN domain of PYPAF5 shares significant sequence similarity with the PYRIN domains of other PYRIN-containing proteins (data not shown). To determine if PYPAF5 is expressed in immune cells, peripheral blood leukocytes were fractionated into distinct cell populations. Quantitative real time PCR analysis showed PYPAF5 to be predominantly expressed in granulocytes with some expression in T-cells (Fig. 4B). Northern blot analysis using a multiple tissue expression array (CLONTECH) showed little or no expression in other tissues and cell lines (data not shown), suggesting a restricted expression of PYPAF5 to immune cells.

3.4. Activation of NF-κB by PYPAF5

We next examined whether PYPAF5 participates in NF-κB signaling pathways using a luciferase reporter plasmid. Expression of each PYPAF alone in cells failed to activate NF-κB at all protein levels examined (data not shown). In contrast, NF-κB was activated when high levels of ASC were expressed in cells (data not shown; [18,19]). However, when ASC was expressed at low protein levels that did not activate NF-κB, co-expression with PYPAF5 resulted in a potent activation of NF-κB (Fig. 5A, lane 6). Co-expression of ASC with PYPAF2, PYPAF3, PYPAF4 or PYPAF6 failed to activate NF-κB (Fig. 5A, lanes 3–5, 7). PYPAF5 activated ASC-dependent NF-κB signaling in a concentration-dependent manner (Fig. 5B). The N-terminal PYRIN domain of PYPAF5 was essential for NF-κB signaling, since deletion of this domain (PYPAF5ΔPYRIN) eliminated the synergistic induction of NF-κB activity (Fig. 5C, compare lanes 4 and 6). Immunoblot analysis revealed that ASC protein levels were not increased when co-expressed with PYPAF5, demonstrating that the activation of NF-κB was not due to increased levels of ASC (Fig. 5C, upper panels). NF-κB signaling occurred through the IKK complex because dominant-negative versions of IKK-γ and IKK-2 blocked the ability of PYPAF5 to synergistically activate NF-κB (Fig. 5D, lanes 3 and 4). Taken together, these data demonstrate that PYPAF5 is an activator of NF-κB signaling when co-expressed with ASC.

3.5. Activation of pro-caspase-1 by PYPAF5

We also examined whether PYPAF2, PYPAF3, PYPAF4, PYPAF5 and PYPAF6 play a role in caspase activation and cytokine processing. Active caspase-1 cleaves pro-IL-1β resulting in the release of IL-1β from cells. To measure the activation of pro-caspase-1, plasmids expressing pro-caspase-1 and mouse pro-IL-1β were transfected into COS-7L cells with plasmids encoding ASC and specific PYPAF family members. In this assay, the amount of murine IL-1β released into the culture medium 1 day after transfection correlates with the amount of intracellular caspase-1 activity [28]. When ASC was expressed at protein levels that resulted in low levels of caspase activity, co-expression with PYPAF5 resulted in a synergistic activation of pro-caspase-1 and a corresponding increase in IL-1β production (Fig. 6A). Co-expression of ASC with PYPAF2, PYPAF3, PYPAF4 or PYPAF6 failed to activate pro-caspase-1. The N-terminal PYRIN domain of PYPAF5 was essential for pro-caspase-1 activation, since deletion of this domain (PYPAF5ΔPYRIN) eliminated the synergistic production of IL-1β (Fig. 6B, lane 7). Immunoblot

analysis revealed that PYPAF5ΔPYRIN was expressed at levels similar to that of PYPAF5, indicating that loss of function was not due to reduced protein levels (data not shown). PYPAF5 and ASC were unable to synergistically induce cytokine expression when co-expressed with a caspase-1 active site mutant (C285A) (Fig. 6C, lane 4). Furthermore, addition of a caspase-1 inhibitor (z-WEHD) blocked the ability of PYPAF5 and ASC to induce the secretion of IL-1β (Fig. 6C, lane 3). Taken together, these data demonstrate that PYPAF5 and ASC synergistically activate pro-caspase-1.

3.6. Conclusions

In conclusion, we have identified PYPAF5 as a novel activator of pro-inflammatory signaling pathways. Our finding that PYPAF5 is recruited to ASC punctate structures through its N-terminal PYRIN domain suggests that these two proteins assemble together into a complex that mediates signal transduction. The restricted expression of PYPAF5 to peripheral blood leukocytes indicates a role for this PYPAF family member in inflammatory signaling. Our data further suggest that PYPAF5 and ASC participate in the activation of both NF-κB and pro-caspase-1. PYPAF5 may function in a manner analogous to APAF-1 and activate ASC and pro-caspase-1 through an induced proximity mechanism [29]. Both pro-IL-1β and pro-IL-18 are processed by caspase-1, suggesting that PYPAF5 may play an important role in cytokine production and immune regulation [30]. PYPAF1 and PYPAF7 were also recently found to activate pro-caspase-1 when co-expressed with ASC, suggesting an analogous role for these proteins in the production of IL-1 and IL-18 [18,19]. In this study, PYPAF2, PYPAF3, PYPAF4 and PYPAF6 failed to co-localize with ASC and activate NF-κB and pro-caspase-1, suggesting that these PYPAF family members may regulate other signal transduction pathways. Alternatively, these PYPAFs could be folded in such a manner that renders them unable to associate with ASC. The findings presented here indicate the PYPAF5 belongs to a subgroup of PYPAF family members that function to regulate NF-κB and cytokine production.

References

[1] Zou, H., Henzel, W.J., Liu, X., Lutschg, A. and Wang, X. (1997) Cell 90, 405–413.
[2] Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S.M., Ahmad, M., Alnemri, E.S. and Wang, X. (1997) Cell 91, 479–489.
[3] Bertin, J., Nir, W.J., Fischer, C.M., Tayber, O.V., Errada, P.R., Grant, J.R., Keilty, J.J., Gosselin, M.L., Robison, K.E., Wong, G.H., Glucksmann, M.A. and DiStefano, P.S. (1999) J. Biol. Chem. 274, 12955–12958.
[4] Inohara, N, Koseki, T., del Peso, L., Hu, Y., Yee, C., Chen, S., Carrio, R., Merino, J., Liu, D., Ni, J. and Nunez, G. (1999) J. Biol. Chem. 274, 14560–14567.
[5] Ogura, Y., Inohara, N., Benito, A., Chen, F.F., Yamaoka, S. and Nunez, G. (2001) J. Biol. Chem. 276, 4812–4818.
[6] Geddes, B.J., Wang, L., Huang, W.-J., Lavellee, M., Manji, G.A., Brown, M., Jurman, M., Cao, J., Morgenstern, J., Merriam, S., Glucksmann, M.A., DiStefano, P.S. and Bertin, J. (2001) Biochem. Biophys. Res. Commun. 284, 77–82.
[7] Poyet, J.L., Srinivasula, S.M., Tnani, M., Razmara, M., Fernandes-Alnemri, T. and Alnemri, E.S. (2001) J. Biol. Chem. 276, 28309–28313.
[8] Damiano, J.S., Stehlik, C., Pio, F., Godzik, A. and Reed, J.C. (2001) Genomics 75, 77–83.
[9] Kobayashi, K., Inohara, N., Hernandez, L.D., Galan, J.E., Nunez, G., Janeway, C.A., Medzhitov, R. and Flavell, R.A. (2002) Nature 416, 194–199.
[10] Chin, A.I., Dempsey, P.W., Bruhn, K., Miller, J.F., Xu, Y. and Cheng, G. (2002) Nature 416, 190–194.
[11] Hugot, J.P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J.P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C.A., Gassull, M., Binder, V., Finkel, Y., Cortot, A., Modigliani, R., Laurent-Puig, P., Gower-Rousseau, C., Macry, J., Colombel, J.F., Sahbatou, M. and Thomas, G. (2001) Nature 411, 537–539.
[12] Ogura, Y., Bonen, D.K., Inohara, N., Nicolae, D.L., Chen, F.F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R.H., Achkar, J.P., Brant, S.R., Bayless, T.M., Kirschner, B.S., Hanauer, S.B., Nunez, G. and Cho, J.H. (2001) Nature 411, 603–606.
[13] Miceli-Richard, C., Lesage, S., Rybojad, M., Prieur, A.M., Manouvrier-Hanu, S., Hafner, R., Chamaillard, M., Zouali, H., Thomas, G. and Hugot, J.P. (2001) Nat. Genet. 29, 19–20.
[14] Bertin, J. and DiStefano, P.S. (2000) Cell Death Differ. 7, 1273–1274.
[15] Martinon, F., Hofmann, K. and Tschopp, J. (2001) Curr. Biol. 11, 118–120.
[16] Staub, E., Dahl, E. and Rosenthal, A. (2000) Trends Biochem. Sci. 26, 83–85.
[17] Fairbrother, W.J., Gordon, N.C., Humke, E.W., O'Rourke, K.M., Starovasnik, M.A., Jian-Ping, Y. and Dixit, V.M. (2001) Protein Sci. 10, 1911–1918.
[18] Manji, G., Wang, L., Geddes, B., Brown, M., Merriam, S., Al-Garawi, A., Mak, S., Lora, J.M., Briskin, M., Jurman, M., Cao, J., DiStefano, P.S. and Bertin, J. (2002) J. Biol. Chem. 277, 11570–11575.
[19] Wang, L., Manji, G.A., Grenier, J.M., Al-Garawi, A., Merriam, S., Lora, J.M., Geddes, B.J., Briskin, M., DiStefano, P.S. and Bertin, J. (2002) J. Biol. Chem. 277, 29874–29880.
[20] The International FMF Consortium (1997) Cell 90, 797–807.
[21] Hoffman, H.M., Mueller, J.L., Broide, D.H., Wanderer, A.A. and Kolodner, R.D. (2001) Nat. Genet. 29, 301–305.
[22] Feldmann, J., Prieur, A-M., Quartier, P., Berquin, P., Certain, S., Cortis, E., Teillac-Hamel, D., Fischer, A. and de Saint Basile, G. (2002) Am. J. Hum. Genet. 71, 198–203.
[23] Dode, C., Le Du, N., Cuisset, L., Letourneur, F., Berthelot, J.M., Vaudour, G., Meyrier, A., Watts, R.A., Scott, D.G., Nicholls, A., Granel, B., Frances, C., Garcier, F., Edery, P., Boulinguez, S., Domergues, J.P., Delpech, M. and Grateau, G. (2002) Am. J. Hum. Genet. 70, 1498–1506.
[24] Srinivasula, S.M., Poyet, J.-L., Razmara, M., Datta, P., Zhang, Z.-J. and Alnemri, E.S. (2002) J. Biol. Chem. 277, 21119–21122.
[25] Wang, L., Guo, Y., Huang, W.J., Ke, X., Poyet, J.L., Manji, G.A., Merriam, S., Glucksmann, M.A., DiStefano, P.S., Alnemri, E.S. and Bertin, J. (2001) J. Biol. Chem. 276, 21405–21409.
[26] Masumoto, J., Taniguchi, S., Ayukawa, K., Sarvotham, H., Kishino, T., Niikawa, N., Hidaka, E., Katsuyama, T., Higuchi, T. and Sagara, J. (1999) J. Biol. Chem. 274, 33835–33838.
[27] Koonin, E.V. and Aravind, L. (2000) Trends Biochem. Sci. 25, 223–224.
[28] Lee, S.H., Stehlik, C. and Reed, J.C. (2001) J. Biol. Chem. 276, 34495–34500.
[29] Salvesen, G.S. and Dixit, V.M. (1999) Proc. Natl. Acad. Sci. USA 96, 10964–10967.
[30] Fantuzzi, G. and Dinarello, C.A. (1999) J. Clin. Immunol. 19, 1–11.