Proprotein convertase furin is preferentially expressed in T helper 1 cells and regulates interferon gamma

Marko Pesu, Linda Muul, Yuka Kanno, and John J. O’Shea

Interleukin 12 (IL-12) is a major inducer of interferon gamma (IFN-γ) and the principal mediator of T helper 1 (Th1) differentiation. To identify IL-12–regulated genes, which might contribute to Th1 differentiation and IFN-γ regulation, we employed microarray analysis. Surprisingly, a ubiquitously expressed proprotein convertase (PC), furin, was one of the most consistently IL-12–induced genes in T cells, and among PCs was the only one regulated by this cytokine. Furin was preferentially expressed in differentiated Th1 cells in a Stat4-dependent manner. Expression of furin enhanced IFN-γ secretion, whereas inhibition of furin interfered with IFN-γ production. Thus, we conclude that IL-12 induction of furin might represent a new aspect of IFN-γ regulation and control of Th1 differentiation.

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RT-PCR, Western blot, ELISA, and ChIP

RNA was isolated and mRNAs were quantified by real-time polymerase chain reaction (PCR; ABI PRISM7700; Applied Biosystems, Foster City, CA). Western blotting was performed using antifurin (Santa Cruz Biotechnology, Santa Cruz, CA) and antiactin antibodies (Chemicon, Temecula, CA). IFN-γ and IL-4 were detected by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN) or Cytometric Bead Array (BD Biosciences, San Jose, CA). Chromatin immunoprecipitation (ChIP) was performed as described. Anti-Stat4 was from Santa Cruz Biotechnology and anti-Stat5 was from R&D Systems. The eluted DNA samples were analyzed by quantitative (q) PCR with murine furin (Fur) and oncostatin M (Osm) promoter site-specific primers using ABI PRISM7700.

Results and discussion

To obtain insights into developing human Th1 cells, we surveyed T-cell gene expression in response to a 6-hour stimulation with IL-12 using Affymetrix human genome microarrays. We identified a number of genes known to be regulated by IL-12, but also consistently observed the induction of the gene encoding a ubiquitously expressed proprotein convertase (PC), furin with IFN-γ production. Thus, we conclude that IL-12 induction of furin might represent a new aspect of IFN-γ regulation and control of Th1 differentiation.
Furin is selectively regulated by IL-12 and Stat4 and is preferentially expressed in Th1 cells. (A) Human T cells were preactivated, rested, and restimulated with cytokines (10 ng/mL, 50 U/mL for IL-2) for 6 hours. Furin and GAPDH mRNA levels were analyzed by real-time PCR. Furin expression was normalized to GAPDH, and unstimulated samples were given an arbitrary value of 1. (B) T cells were treated with IL-12 and the relative expression of different proprotein convertases was analyzed by RT-PCR as in panel A. (C-D) CD4+ T cells from wild-type, Stat4-, or Ifng-deficient mice were stimulated with indicated cytokines for 24 hours, and furin was analyzed by RT-PCR. Furin expression in unstimulated wild-type cells was assigned the value of 1. (E) Murine CD4+ T cells were stimulated with IL-2 or IL-12 for 1 hour as indicated, and Stat4 and Stat5 binding to mouse Fur and Osm promoters was analyzed by chromatin immunoprecipitation. The amount of immunoprecipitated DNA was quantified by quantitative PCR and normalized to the input value, and is expressed as fold-enrichment relative to normal rabbit serum control. Furin primers forward: GAAAGGCTGGCCAGGTTCTAC, reverse: GAGAAGA, Taqman MGB probe: TGTGCCTGGGTTGC; OSM primers forward: AATTCGAAGAAAACGGGAGGA, reverse: TTTCTGGGAGGGCTGACCTGG, Taqman MGB probe: CCATTGCGCGTGG. (F) Naive CD4+ T cells were purified using negative selection columns and activated with plate-bound anti-CD3 and anti-CD28 for 3 days in the presence of IL-2 (50 ng/mL), IL-12 (10 ng/mL), and anti–IL-4 Ab (5 mg/mL) for Th1 condition or in the presence of IL-2 (50 ng/mL), IL-4 (40 ng/mL), and anti–IL-12 Ab (5 mg/mL) for Th2 condition. On day 3, the polarizing cytokines were re-added and the cells were cultured 4 additional days without neutralizing antibodies. Furin, IFN-γ, and IL-4 mRNA expression levels, normalized to GAPDH, are shown with expression in naive cells being assigned an arbitrary value of 1. Furin and actin protein levels from Th1 and Th2 samples were analyzed by Western blot (insert). All experiments were performed at least 3 times. One representative experiment is shown, and error bars depict intraexperimental variation. *P < .001.

Figure 1. Furin is selectively regulated by IL-12 and Stat4 and is preferentially expressed in Th1 cells. (A) Human T cells were preactivated, rested, and restimulated with cytokines (10 ng/mL, 50 U/mL for IL-2) for 6 hours. Furin and GAPDH mRNA levels were analyzed by real-time PCR. Furin expression was normalized to GAPDH, and unstimulated samples were given an arbitrary value of 1. (B) T cells were treated with IL-12 and the relative expression of different proprotein convertases was analyzed by RT-PCR as in panel A. (C-D) CD4+ T cells from wild-type, Stat4-, or Ifng-deficient mice were stimulated with indicated cytokines for 24 hours, and furin was analyzed by RT-PCR. Furin expression in unstimulated wild-type cells was assigned the value of 1. (E) Murine CD4+ T cells were stimulated with IL-2 or IL-12 for 1 hour as indicated, and Stat4 and Stat5 binding to mouse Fur and Osm promoters was analyzed by chromatin immunoprecipitation. The amount of immunoprecipitated DNA was quantified by quantitative PCR and normalized to the input value, and is expressed as fold-enrichment relative to normal rabbit serum control. Furin primers forward: GAAAGGCTGGCCAGGTTCTAC, reverse: GAGAAGA, Taqman MGB probe: TGTGCCTGGGTTGC; OSM primers forward: AATTCGAAGAAAACGGGAGGA, reverse: TTTCTGGGAGGGCTGACCTGG, Taqman MGB probe: CCATTGCGCGTGG. (F) Naive CD4+ T cells were purified using negative selection columns and activated with plate-bound anti-CD3 and anti-CD28 for 3 days in the presence of IL-2 (50 ng/mL), IL-12 (10 ng/mL), and anti–IL-4 Ab (5 mg/mL) for Th1 condition or in the presence of IL-2 (50 ng/mL), IL-4 (40 ng/mL), and anti–IL-12 Ab (5 mg/mL) for Th2 condition. On day 3, the polarizing cytokines were re-added and the cells were cultured 4 additional days without neutralizing antibodies. Furin, IFN-γ, and IL-4 mRNA expression levels, normalized to GAPDH, are shown with expression in naive cells being assigned an arbitrary value of 1. Furin and actin protein levels from Th1 and Th2 samples were analyzed by Western blot (insert). All experiments were performed at least 3 times. One representative experiment is shown, and error bars depict intraexperimental variation. *P < .001.
Figure 2. Furin regulates IFN-γ production. (A) Furin-deficient LoVo cells were transfected with 0.5 μg human IFN-γ cDNA, vector alone, and 1.5 μg furin cDNA. IFN-γ protein was measured by ELISA (top panel) and IFN-γ mRNA expression was normalized to GAPDH (bottom panel), with pcDNA3-transfected cells being given an arbitrary value of 1. (B) Human T cells were rested overnight and stimulated with IL-12 with α1-AT or α1-AT as indicated for 16 hours. Shown are IFN-γ protein (top panel) and relative IFN-γ mRNA expression (bottom panel). Untransfected or α1-AT-treated cells were given an arbitrary value of 1. (C) Human Th1 cells were transfected for 1 week and restimulated with anti-CD3 and anti-CD28 for 24 hours in the presence of α1-AT or α1-AT as indicated. IFN-γ secretion is shown in the upper panel. Furin and actin protein levels from the same samples were determined by Western blot (bottom panel). An approximately 90-kDa doublet representing intact furin is identified by the arrow. (D) Human Th1 and Th2 (1 × 10^6) were prepared as in Figure 1 and were transfected with siRNAfurin (1 μg, sense: GGACUGUGGAGCCGAAUUU purchased from Dharmacon, Lafayette, CO) or nonsilencing siRNAs (siRNAnts, nontargeting siRNA no. 1 from Dharmacon) using T-cell nucleofector kit (Amaxa; approximately 45% transfection efficiency, detected by green fluorescent protein [GFP]). At 48 hours after transfection, cells were restimulated for 24 hours with anti-CD3 and anti-CD28. IFN-γ and furin mRNA levels were normalized to GAPDH and Th1 cells transfected with siRNAs were assigned an arbitrary value of 100. IFN-γ and IL-4 proteins were detected from supernatants of Th1 and Th2 cells, respectively, by Cytometric Bead Array. Western blot (top right panel) shows the effect of furin RNAi on furin and actin protein levels in human CD3+ cells. All experiments were performed at least three times. One representative experiment is shown, and error bars depict interexperimental variation.

Furin expression uncovers a potential new layer in cytokine secretion, although furin could have many targets in Th1 cells. We considered the possibility that furin might directly cleave IFN-γ and thus promote its maturation/production. A potential protease target sequence was noted in IFN-γ protein (151KKRR155), but furin enhanced the secretion of the mutant version of IFN-γ (151KAKA155), suggesting an alternate mode of regulation. Given its widespread functions, it is perhaps not surprising that deficiency of furin results in embryonic lethality. While this lethality is a major limiting factor in understanding the role of furin in T cells in vivo, hopefully this can be overcome using tissue-specific deletion of furin. Additionally, because of its diverse functions, it seems unlikely that interfering with furin function per se would be useful therapeutically. However, elucidating the mechanisms involved in IFN-γ production and secretion might provide new opportunities for therapeutic intervention.

References

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