IL-4 Induces Apoptosis in A549 Lung Adenocarcinoma Cells: Evidence for the Pivotal Role of 15-Hydroxyeicosatetraenoic Acid Binding to Activated Peroxisome Proliferator-Activated Receptor γ Transcription Factor

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*J Immunol* 2003; 170:887-894; doi: 10.4049/jimmunol.170.2.887

http://www.jimmunol.org/content/170/2/887
IL-4 Induces Apoptosis in A549 Lung Adenocarcinoma Cells: Evidence for the Pivotal Role of 15-Hydroxyeicosatetraenoic Acid Binding to Activated Peroxisome Proliferator-Activated Receptor γ Transcription Factor

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The proinflammatory cytokine IL-4 is secreted in large amounts during allergic inflammatory response in asthma and plays a pivotal role in the airway inflammation. IL-4 has been shown to up-regulate 15-lipoxygenase and produce 15(S)-hydroxyeicosatetraenoic acid (15(S)-HETE) in A549 cells via the Janus kinase/STAT6 pathway under coactivation of CREB binding protein/p300. IL-4 has also been shown to up-regulate peroxisome proliferator-activated receptor γ (PPARγ) nuclear receptors in macrophages and A549 cells. In this study we demonstrate that 15(S)-HETE binds to PPARγ nuclear receptors and induces apoptosis in A549 cells. Moreover, pretreatment of cells with nordihydroguaiaretic acid, a 15-lipoxygenase inhibitor, prevented PPARγ activation and apoptosis. The latter was accomplished by the interaction of the 15(S)-HETE/PPARγ complex with the adapter protein Fas-associating protein with death domain and caspase-8, as shown by transfection of Fas-associating protein with death domain dominant negative vector and cleavage of caspase 8 to active subunits p41/42 and p18. Whereas IL-4 and PPARγ ligands failed to induce cleavage of Bid and release of cytochrome c from mitochondria, they caused translocation of the proapoptotic protein Bax from cytoplasm to mitochondria with a concomitant decrease in the Bcl-xL level. We therefore believe that in unstimulated cells Bcl-xL and Bax form a heterodimer, in which Bcl-xL dominates and prevents the induction of apoptosis, whereas in IL-4-stimulated cells the 15(S)-HETE/PPARγ complex down-regulates Bcl-xL, and the resulting overwhelming of Bax commits the cell to apoptosis via caspase-3. However, this pathway does not rule out the direct caspase-8-mediated activation of caspase-3. In conclusion, IL-4-induced apoptosis may contribute to severe loss of alveolar structures and infiltration of eosinophils, mononuclear phagocytes, etc., into the lung tissue of chronic asthma patients.
the symptoms of asthma, highlighting their potential therapeutic application (17). Earlier, we and others have shown that IL-4 up-regulates 15-lipoxygenase in airway epithelial cells (18), monocytes (9), and A549 lung epithelial carcinoma cells (19) via the Janus kinase/STAT6 pathway (20–22). In A549 cells activation of 15-LOX by IL-4 requires the coactivation of histone acetyltransferases CREB-binding protein/p300 and led to a sizable production of 15(S)-HETE (21). IL-4 has also been shown to up-regulate the nuclear receptor and transcription factor PPARγ and transcription of the CD36 gene (23, 24). PPARγ receptors belong to the family of PPARs and were initially characterized as regulators of adipocyte differentiation and lipid metabolism (25–28). A number of lipids, including 15(S)-HETE, have been shown to be their ligands in adipocytes and various other tissues (26, 27).

Pathogenesis of asthma is also related to the balance between survival and apoptosis of inflammatory cells. A high degree of apoptosis has been reported in the epithelium of asthma patients (3, 29), which is enhanced by inhaled as well as oral corticosteroids (3). In the present study we have shown for the first time that the 15(S)-HETE produced by IL-4-activated A549 cells physically binds to PPARγ transcription factor as a ligand and leads to apoptosis of A549 cells via the death receptor and caspase-3 pathway.

Materials and Methods

**Cell culture**

A 549 cells (lung adenocarcinoma cells) were cultivated in DMEM containing 10% FCS, 50 U/ml of penicillin, and 50 μg/ml of streptomycin. For microscopy, cells were grown on glass coverslips, treated for 72 h with 670 pM IL-4 (R&D Systems, Wiesbaden-Nordenstadt, Germany), and then assayed for apoptosis. Nordihydroguaiaretic acid (NDGA; Sigma-Aldrich, Deisenhofen, Germany) was used as a lipoxygenase inhibitor at a concentration of 10 μM. Normal human bronchial epithelial cells (BEAS-2B) were obtained from American Type Culture Collection (Weilhem, Germany) and were cultivated in a modified LHC-9 medium with all the necessary growth factors supplied by Clonetics, Palo Alto, CA.

**Assays for apoptosis**

Apoptosis was detected by staining cells with annexin V and propidium iodide and by TUNEL assay. For the annexin V assay, coverslips were washed with PBS and incubated for 15 min at room temperature with a solution of annexin V-fluos and propidium iodide (Roche, Mannheim, Germany). Cells were then washed twice with PBS and observed under a fluorescence microscope. Normal cells do not stain, while apoptotic cells are stain green (annexin V-fluos), and necrotic cells stain red (propidium iodide). A minimum of 200 cells were counted.

TUNEL assay was performed by washing paraformaldehyde-fixed cells on a coverslip once with PBS and then permeabilized using 0.5% saponin at room temperature for 30 min. After washing with TdT buffer, cells were incubated with 0.5 μM biotin dUTP and 150 U/ml of TdT (Roche) in a humidified chamber at 37°C for 30 min. After washing twice with PBS, the cells were incubated with a 1/1000 solution in PBS of streptavidin-conjugated HRP (Life Technologies, Karlsruhe, Germany) for 10 min at room temperature. Coverslips were then washed for 30 min with three washes of PBS. Color was developed with True Blue (KPL Laboratories, Wedel, Germany) peroxidase substrate, and coverslips were observed under a light microscope. Apoptotic cells were stained blue.

**Western blot analysis**

Cells were scraped in ice-cold RIPA buffer (PBS, 1% Nonidet P-40, 0.1% SDS, 0.5% sodium deoxycholate containing 1 mM PMSF, 1 mg/ml pepstatin, and 1 mg/ml leupeptin). Cell homogenates were collected by centrifugation at 12,000 rpm at 4°C. Protein concentrations were determined using Lowry’s assay (Bio-Rad, Munich, Germany). SDS-PAGE electrophoresis was performed with 50 μg of each protein on polyacrylamide gels of varying concentrations. The protein was transferred onto a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) by a semidry transfer method. After blocking with 5% skimmed milk solution in PBS and 0.05% Tween 20, the blot was incubated with the primary Ab for 1 h. A 5-min wash was followed by incubation with HRP-conjugated secondary Ab (Santa Cruz Biotechnology, Heidelberg, Germany) for 1 h. The signal was visualized using chemiluminescent substrate (Santa Cruz Biotechnology, Santa Cruz, CA). The primary Abs used were anti-Bcl-xL (ICN, Eschwege, Germany), anti-caspase-3 (Santa Cruz Biotechnology), anti-caspase-8 and anti-Bid (Cell Signaling Technologies, Beverly, MA).

**Plasmids and transfection**

For PPARγ reporter assay, well-characterized PPAR-responsive promoter region for acyl-coenzyme A oxidase (581–471) fused to the minimal globin promoter upstream of a luciferase reporter (pGL3 Basic; Promega, Mannheim, Germany), termed pPPARγ-LUC, was used. Cotransfections were performed with Rous sarcoma virus promoter-galactosidase to normalize the transfection efficiency. Transient transfections with various plasmids were performed with Polyfect transfection reagent (Qiagen, Hilden, Germany) and 1.5 μg of DNA. PPARγ dominant negative (PPAR-DN) plasmid was a gift from Prof. K. K. Chatterjee (Oxford University, Oxford, U.K.), and Fas-associating protein with death domain double negative (FADD-DN) plasmid was a gift from Dr. M. L. Schmitz (German Cancer Center, Heidelberg, Germany). Luciferase and galactosidase activities were measured according to the manufacturer’s (Promega) instructions.

**Detection of PPARγ ligand**

Labeled fatty acids as ligands to PPARγ were detected by immunoprecipitating PPARγ in pretreated cells and then detecting the fatty acid attached to it by radio-TLC. A549 cells (1 × 10⁵) were incubated with 0.25 μCi of [14C]arachidonic acid (sp. act., 55 mCi/mmol; Amersham International, Freiburg/Breisgau, Germany) for 24 h. After incorporation of the radioactive arachidonic acid (AA), cells were washed and treated according to the experimental set-up (670 pM IL-4 and 10 μM NDGA). Cell lysate was prepared and immunoprecipitated with anti-PPARγ Ab in 1 ml of RIP buffer for 1 h at 4°C. The immune complexes were allowed to bind to protein A/agarose (Santa Cruz Biotechnology) for 1 h. The beads were spun down, washed three times with RIP buffer, and resuspended in 200 μl of PBS. This solution was acidified with HCl to pH 3.5, and lipids were extracted three times with ethyl acetate. After drying under a nitrogen stream the sample was reconstituted in ethyl acetate and loaded onto a silica TLC plate (Merck, Darmstadt, Germany) and developed with hexane/ether/acetic acid (50:50:0.1 v/v/v) as the solvent system. For quantification, the TLC plate was scanned on a radio-TLC scanner (Berthold Instruments, Wildbad, Germany). AA, 5-, 12-, and 15-HETEs; and various PGs were run on the side as standards.

**Preparation of mitochondria**

Cells were washed twice with PBS and trypsinized, and the cell pellet was collected. The pellet was resuspended in 5 vol of homogenization buffer (20 mM HEPES 7.5, 1.5 mM MgCl₂, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, and 250 mM sucrose) and homogenized for 5 min using a tight-fitting Dounce homogenizer (Kontes, Vineland, NJ). The effectiveness of the procedure was checked by a trypan blue exclusion test. Nuclei and cell debris were pelleted at 2,000 × g. Mitochondria were pelleted by centrifugation at 10,000 × g for 30 min at 4°C. The pellet was resuspended in RIPA buffer (see above). The supernatant was further centrifuged at 100,000 × g for 45 min at 4°C. The supernatant was used as the

**Preparation of mitochondria**

Cells were washed twice with PBS and trypsinized, and the cell pellet was collected. The pellet was resuspended in 5 vol of homogenization buffer (20 mM HEPES 7.5, 1.5 mM MgCl₂, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, and 250 mM sucrose) and homogenized for 5 min using a tight-fitting Dounce homogenizer (Kontes, Vineland, NJ). The effectiveness of the procedure was checked by a trypan blue exclusion test. Nuclei and cell debris were pelleted at 2,000 × g. Mitochondria were pelleted by centrifugation at 10,000 × g for 30 min at 4°C. The pellet was resuspended in RIPA buffer (see above). The supernatant was further centrifuged at 100,000 × g for 45 min at 4°C. The supernatant was used as the...
cytosolic extract. The protein concentration was determined in both mitochondrial and cytosolic fractions. Equal amounts of protein were used for additional experiments.

**Statistics**

Data are presented as the mean±SD. Statistical comparisons between groups were made using Student’s t test for paired observations. Significance was achieved at the p<0.05 level.

**Results**

**IL-4 causes apoptosis in A549 cells**

Cells treated with IL-4 and various inhibitors underwent morphological changes and were examined for apoptosis. The TUNEL assay showed that IL-4 (670 pM) induces apoptosis in A549 cells (Fig. 1A). Annexin V staining showed similar results (Fig. 1B). Apoptotic cells stained green, and necrotic cells stained red. This was further confirmed with a cell death detection ELISA, in which cytoplasmic DNA-histone complexes were detected (Fig. 2A). Moreover, 15(S)-HETE (30 μM) and 5 μM 15-deoxy-Δ12,14 PGJ2 (15-PGJ2), a PPARγ ligand, also caused apoptosis when preincubated for 72 h. Upon preincubation of cells with 10 μM NDGA, a 15-LOX inhibitor, IL-4-induced apoptosis was almost completely abolished (Fig. 2A). To determine whether this observation can be extrapolated to other cell lines, human bronchial epithelial cells (BEAS-2B) were treated in a similar manner and assayed for apoptosis by the cell death detection ELISA. Indeed, the findings (Fig. 2B) confirmed that IL-4 and other effectors induced apoptosis in BEAS-2B cells through a similar pathway as in A549 cells. Consequently, additional experiments were confined to A549 cells.

The activation of caspase-3, a downstream caspase involved in the effector phase of apoptosis, was also studied. IL-4 caused cleavage of the 34-kDa procaspase-3 to the active 20- and 10-kDa forms. The increased activity of caspase-3 using DEVD-pNA as a substrate supported these findings (Fig. 3). Upon incubation with a peptide inhibitor of caspase-3, Z-VAD-FMK (100 μM), 1 h before IL-4 treatment, the onset of apoptosis was abrogated, as measured by a cell death detection ELISA (Fig. 2A). This inhibitor also prevented apoptosis induced by 15(S)-HETE and 15-PGJ2 (not shown). The presence of an anti-IL-4 Ab in the cell culture medium inhibited the induction of apoptosis, thus adding support to a role for IL-4 in A549 apoptosis (not shown). To clarify the role of NDGA in the inhibition of IL-4-induced apoptosis, 15(S)-HETE (30 μM) was added to cells treated with NDGA (10 μM) and IL-4 (670 pM). 15(S)-HETE overcame the suppression of caspase-3 activity caused by NDGA (Fig. 3, last column), thus supporting the prominent role of 15-lipoxygenase in the apoptosis of A549 cells. NDGA alone did not cause any increase in caspase-3 activity.

**IL-4-induced apoptosis is mediated by PPARγ**

A549 cells were transiently transfected with PPARγ-DN vector and treated with 670 pM IL-4. IL-4-induced apoptosis was completely abolished, indicating the prominent role of PPARγ in the induction of apoptosis (Figs. 2A and 3). In analogy, upon treatment of PPARγ-DN-transfected cells with 15-PGJ2 no signs of apoptosis were observed (not shown).

**15(S)-HETE is a ligand for PPARγ in IL-4-stimulated A549 cells**

In an earlier report we demonstrated that IL-4 treatment of A549 cells resulted in the up-regulation of 15-lipoxygenase, which, in

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**FIGURE 1.** IL-4 induces apoptosis in A549 cells. A, A549 cells were treated with 670 pM IL-4 for 72 h, fixed with paraformaldehyde, and permeabilized with 0.5% saponin. After washing, cells were subjected to TUNEL assay (see Materials and Methods). Apoptotic cells are stained blue. Untreated A549 cells (left panel) and A549 cells with IL-4 (right panel) are shown. Both photographs were taken at ×400 magnification and are representative of three separate experiments. B, Apoptosis hallmarks are detected by annexin V-FITC. A549 cells were treated with 670 pM IL-4 for 72 h and incubated for 15 min with annexin V and propidium iodide. Apoptotic cells are stained green, necrotic cells are stained red, and normal cells are unstained (×1000 magnification). The photograph is representative of three separate experiments.
turn, augmented the production of 15(S)-HETE (22). It was therefore of interest to determine whether 15(S)-HETE could serve as a ligand for PPAR\textsubscript{\gamma/H9253}\textsuperscript{9253} in this cell system. A549 cells were labeled with $[^{14}\text{C}]$AA and treated with 670 pM IL-4 for 72 h. Total protein extracts prepared from the cells were subjected to immunoprecipitation with PPAR\textsubscript{\gamma/H9253}\textsuperscript{9253}-Ab and protein A/agarose. The lipids were extracted from the immune complex and analyzed by TLC. In IL-4-treated cells a solitary radioactive lipid peak was observed. It was identified as 15(S)-HETE by radio-TLC by cochromatography of standard 15(S)-HETE (Fig. 4). Untreated cells or cells treated with NDGA (10 \mu M) before IL-4 challenge failed to show any radioactive ligand for PPAR\textsubscript{\gamma/H9253}\textsuperscript{9253}. Moreover, IL-4 increased PPAR\textsubscript{\gamma/H9253}\textsuperscript{9253}-dependent promoter activity (Fig. 5), which could be inhibited by 10 \mu M NDGA. Inasmuch as 15(S)-HETE was also observed to

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** A, IL-4-induced apoptosis is mimicked by 15(S)-HETE and 15-PGJ\textsubscript{2}, but is inhibited by inhibitors of 15-LOX and caspase-3 as well as by transfection of PPAR\textsubscript{\gamma/DN} plasmid into A549 cells. A549 cells were treated with IL-4 (670 pM) and exposed to 5 \mu M 15-PGJ\textsubscript{2} and 30 \mu M 15(S)-HETE for 72 h. The assay was performed with a cell death detection ELISA kit. Values represent the mean \pm SD absorbance at 405 nm. Apoptosis was also induced by incubation of cells with 5 \mu M 15-PGJ\textsubscript{2} and 30 \mu M 15(S)-HETE. For blocking of 15(S)-HETE-induced apoptosis, cells were transiently transfected with PPAR\textsubscript{\gamma/DN} plasmid or were preincubated with 10 \mu M NDGA 1 h before challenge with agonists. For inhibition of caspase-3, A549 cells were preincubated with 100 \mu M Z-VAD-FMK, a caspase-3 inhibitor, for 1 h before adding IL-4. All experiments were performed in triplicate and represent the mean \pm SD. B, IL-4 induces apoptosis in normal human bronchial epithelial cells. BEAS-2B cells were exposed to IL-4, 15(S)-HETE, and 15-PGJ\textsubscript{2} as described above for experiments with A549 cells. Apoptosis was measured using a cell death detection ELISA. The mean \pm SD absorbance values at 405 nm are reported. * Significance compared with the untreated cells ($p < 0.05$).
increase PPARγ-dependent promoter activity, an interaction between 15(S)-HETE and PPARγ receptors may be implicated. An analogous increase in PPARγ promoter activity was seen when cells were treated with 5 μM 15-PGJ₂.

**PPARγ up-regulates cleavage of caspase-8**

Caspase-8 is one of the key upstream factors involved in the up-regulation of caspase-3 activity. Caspase-8 exists as an inactive 54-kDa molecule, which is autocleaved into active p41/42 and p18 molecules. A549 cells treated with IL-4 showed significantly higher levels of the cleaved products compared with untreated cells or cells treated with 10 μM NDGA before IL-4 induction (Fig. 6A). Similar up-regulation of caspase-8 cleavage was observed with 5 μM PGJ₂ and 30 μM 15-HETE. Caspase-8 cleavage was totally abolished in A549 cells transiently transfected with PPARγ-DN vector (Fig. 6B).

**IL-4-induced apoptosis involves the death domain receptor pathway**

Death domain receptors are a family of cell receptors that regulate the survival of the cell in response to various factors, such as Fas ligand, TNF-α, and TRAIL. Upon activation, these receptors use specific adapter proteins to activate the caspase-8 pathway. FADD is such a vital adapter protein, which physically binds to caspase-8 and is involved in the regulation of Fas- and TNF-α-mediated apoptosis (30). To verify the involvement of death domain receptors in IL-4- and PPARγ-induced apoptosis in A549 cells, we transfected A549 cells with a FADD-DN vector. This mutant lacks the death effector domain, thus blocking the transmission of signal. As shown in Fig. 6B, IL-4-induced cleavage of caspase-8 was completely blocked in cells transfected with FADD-DN vector. Moreover, IL-4-induced cleavage of caspase-8, as measured by ELISA, was completely abolished in cells transfected with FADD-DN (not shown). These findings strongly suggest the involvement of death domain receptors in IL-4- and PPARγ-induced apoptosis in A549 cells.

**Bid cleavage is not induced by caspase-8**

The activated caspase-8 can stimulate apoptosis either via direct cleavage and activation of caspase-3 or by the mitochondrial route involving the cleavage of the C-terminal part of Bid, which then...
leads to release of cytochrome c. IL-4 and 15-PGJ2 induction failed to induce cleavage of Bid, as analyzed by Western blotting (Fig. 7). This suggests that caspase-8 may directly activate caspase-3 upon IL-4 treatment. However, determination of cytochrome c in the cytoplasmic fraction after challenging cells with IL-4 surprisingly revealed almost no release of cytochrome c. This implicates a cytochrome c-independent functional interplay between the pro- and anti-apoptotic members of the Bcl-2 family in IL-4-induced apoptosis in A549 cells.

Discussion

PPARs are ligand-activated transcription factors belonging to the nuclear receptor family; they function as regulators of fatty acid oxidation and glucose utilization (26, 27). In addition to their critical role in homeostasis, PPARs also affect cell proliferation, differentiation, and apoptosis. PPARγ is predominantly expressed in adipose tissue, where it plays a pivotal role in adipocyte differentiation and lipid metabolism (25, 26). Upon activation by ligand binding, PPARγ heterodimerizes with retinoid X receptor protein and binds to PPAR response elements present on the promoters of various genes, thus regulating their function (30). Most of these genes are involved in lipid metabolism (26). The ligands for PPARγ are mainly synthetic anti-diabetic thiazolidinediones and 15-PGJ2 (28, 31), but also include a large number of polyunsaturated fatty acids, among them arachidonic acid metabolites such as 15(S)-HETE (24). PPARγ transcription factors have also been found in other tissues and blood cells, such as lung, macrophages, and lymphocytes (24, 26). Upon stimulation of macrophages with IL-4, up-regulation of PPARγ-mRNA and protein synthesis was observed (24).

PPARγ receptors have been shown to regulate inflammation (32, 33) and apoptosis in lung epithelial cells (34) and macrophages (35). In the present study we have shown for the first time that 15(S)-HETE is bound as a ligand to PPARγ transcription factors (Fig. 4) and is an effector of apoptosis (Figs. 2 and 3). Moreover, treatment of cells with NDGA, a 15- lipoxygenase inhibitor, prevented PPARγ activation and apoptosis. This inhibition, however, could be almost completely suppressed by the addition of 15(S)-HETE, thus ruling out the possibility that the inhibition may be due to alteration of the redox state of non-heme iron in the 15-LOX enzyme. Identical results were also obtained with the PPARγ ligand 15-PGJ2. Apoptosis was induced by a similar mechanism in IL-4-treated human monocytes (not shown). To further substantiate the crucial role of 15(S)-HETE in apoptosis via the PPARγ transcription factor, we used PPARγ-DN, in which two amino acids (L468A and E471A) have been mutated, thus impairing transcriptional activation and cofactor recruitment (36). Transfection of PPARγ-DN in A549 cells strongly inhibited IL-4-induced and 15-PGJ2-induced apoptosis (Figs. 2 and 3), supporting the prominent roles of 15(S)-HETE and 15 PGJ2 as effectors of apoptosis via PPARγ pathway. Experiments with normal bronchial epithelial cells (BEAS-2B) confirmed the observations in A549 cells and thus underlined the importance of these observations in human allergic inflammatory reactions.

The exact downstream process of PPARγ activation is still unclear. Involvement of the death domain receptor in IL-4-induced apoptosis has been observed in our experiments. Death domain receptors constitute a family of cell surface receptors including CD95 (Fas/Apo-1), TNF-α receptor, DR3, DR4, and DR5 (37). Ligands initiate the signaling cascade via receptor oligomerization and, through a set of special adapter proteins, activation of the caspase cascade. Numerous adapter proteins have been identified for these death receptors, including FADD, TNFR1-associated death domain, receptor interacting protein, and death-associated protein (38–40). The FADD protein, which is common to all receptors and directly interacts with caspase-8, consists of a death effector domain and a death domain (41). The death domain interacts with caspase-8, which is the next step in the cascade, while the death effector domain is required in conjunction with other adapter proteins for interaction with the receptors. The application of FADD-DN vector (42) lacking the death effector domain abrogated the apoptotic signal induced by IL-4 or PPARγ (Fig. 6B). The involvement of death domain receptors in IL-4-induced apoptosis can be observed by the cleavage of caspase-8 to active subunits p41/42 and p18 (Fig. 6A) This cleavage and activation are inhibited by NDGA and PPARγ-DN vector, demonstrating the vital importance of 15-LOX and PPARγ in the activation of apoptosis signal. Activated caspase-8 has been proposed to stimulate apoptosis through two parallel pathways (43, 44). In type I cells, caspase-8 directly cleaves and activates caspase-3. Type II cells use the mitochondrial pathway through the cleavage of Bid and subsequent release of cytochrome c to amplify the apoptotic signal. Caspase-3, an effector caspase, further cleaves PARP and other cellular proteins to cause apoptosis. In our hands IL-4-treated A549 cells do not exhibit Bid activation (Fig. 7), thus suggesting the involvement of the type I pathway. It was previously shown that PPARγ promotes TRAIL-induced apoptosis (45). TRAIL uses various types of death receptors, such as DR3, DR4, and DR5, to trigger apoptosis (46, 47). However, it is intriguing to note that IL-4-induced apoptosis in A549 is mediated simultaneously through two different pathways, i.e., through direct activation of caspase-3 and through mitochondrial pathway involving Bax. The
activation of Bax and its subsequent translocation to the mitochondria along with the decrease in Bcl-xL can account for the cytochrome c release (48). Inasmuch as cytochrome c release is absent (Fig. 9), the dominating role for apoptosis is apparently played by Bax, a proapoptotic Bcl-2-binding protein. Thus, the scenario can be explained in the following way. In type I cell death, binding of 15(S)-HETE to PPARγ transcription factor leads to generation of active caspase-8 through activation of FADD protein within seconds (Fig. 6), which subsequently activates downstream executor caspase-3. In type II cell death, propagation and amplification of the apoptotic signal require mitochondrial factors and are therefore delayed. Due to the absence of cytochrome c, we believe that a molecular link between caspase-3 activation and Bcl-xL is mediated by Bax. Bax may bind to Bcl-xL forming a heterodimer Bcl-xL/Bax, in which Bcl-xL dominates and prevents the induction of apoptosis (49). In unstimulated A549 cells this pathway is blocked by Bcl-xL but, in IL-4-stimulated cells the binding of 15(S)-HETE to PPARγ transcription factor down-regulates Bcl-xL (Fig. 8), and the resulting overexpression of Bax commits the cell to apoptosis via caspase-3. Bax has also been shown to be involved in a number of apoptotic pathways, especially the DNA damage-induced apoptosis involving p53 (50), without participation of death receptors. Anti-diabetic thiazolidinediones, potent PPARγ agonists, have been observed to induce apoptosis in vascular smooth muscle cells through p53 and growth arrest and DANN damage 45 pathways, although it is not clear whether PPARγ itself is the effector (51). In non-small cell lung cancer cells, it has been reported that PPARγ increases the release of 15-hydroxyeicosatetraenoic acid (15-HETE) and prostaglandin E2 (PGE2) by cultured human bronchial epithelial cells. [4. Chavis, C., I. Vachier, J. Bousquet, P. Godard, and P. Chanez. 1998. Generation of eicosanoids from 15(13,14)-dihydroxyeicosatrienoic acid is the major arachidonic acid metabolite in human bronchi: association with airway epithelium. Arch. Biochem. Biophys. 282:254.]


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References

4. Chavas, C., I. Vachier, J. Boussuges, P. Godard, and P. Chanez. 1998. Generation of eicosanoids from 15(S)-hydroxyeicosatetraenoic acid in blood monocytes from 15-HETE-induced apoptosis involves p53 (50), without participation of death receptors. Anti-diabetic thiazolidinediones, potent PPARγ agonists, have been observed to induce apoptosis in vascular smooth muscle cells through p53 and growth arrest and DANN damage 45 pathways, although it is not clear whether PPARγ itself is the effector (51). In non-small cell lung cancer cells, it has been shown that trolgitazone induced the DNA damage-inducible growth arrest and DANN damage 153 gene. Bax and p53 form an important pathway in DNA damage-induced apoptosis (52). Caspase-3 has also been observed to activate the mitochondrial apoptotic pathway by the cleavage of anti-apoptotic Bcl-xL and Bcl-2 to pro-apoptotic components (53, 54). Thus, caspase-3 activated by other pathways can activate the mitochondrial route and provide a positive feedback loop for caspase-3 production, leading to apoptosis. In asthma, an up-regulation of IL-4 secretion in blood and higher levels of 15-HETE in lung and bronchial tissue have been found. We hypothesize therefore that IL-4-induced apoptosis is one of the major causes of hypertrophy of the bronchial smooth muscle, denuded surface epithelium, thickened basement membrane, and infiltration of eosinophils, lymphocytes, and mononuclear phagocytes as well as the apoptotic lesions observed in the lung tissue of asthma patients. It has been reported that PPARγ ligands induce apoptosis in lung cancer cells, and this may be beneficial for the therapy of such cancers (30). In contrast, in chronic inflammatory diseases such as chronic obstructive pulmonary disease, the loss of alveolar structures in the lung tissue due to apoptosis may worsen lung function (29).

13. Rapoport, S. M., and T. Schewe. 1986. The maturational breakdown of mitochondrial Bcl-xL is mediated by Bax. Bax may bind to Bcl-xL forming a heterodimer Bcl-xL/Bax, in which Bcl-xL dominates and prevents the induction of apoptosis (49). In unstimulated A549 cells this pathway is blocked by Bcl-xL but, in IL-4-stimulated cells the binding of 15(S)-HETE to PPARγ transcription factor down-regulates Bcl-xL (Fig. 8), and the resulting overexpression of Bax commits the cell to apoptosis via caspase-3. Bax has also been shown to be involved in a number of apoptotic pathways, especially the DNA damage-induced apoptosis involving p53 (50), without participation of death receptors. Anti-diabetic thiazolidinediones, potent PPARγ agonists, have been observed to induce apoptosis in vascular smooth muscle cells through p53 and growth arrest and DANN damage 45 pathways, although it is not clear whether PPARγ itself is the effector (51). In non-small cell lung cancer cells, it has been shown that trolgitazone induced the DNA damage-inducible growth arrest and DANN damage 153 gene. Bax and p53 form an important pathway in DNA damage-induced apoptosis (52). Caspase-3 has also been observed to activate the mitochondrial apoptotic pathway by the cleavage of anti-apoptotic Bcl-xL and Bcl-2 to pro-apoptotic components (53, 54). Thus, caspase-3 activated by other pathways can activate the mitochondrial route and provide a positive feedback loop for caspase-3 production, leading to apoptosis. In asthma, an up-regulation of IL-4 secretion in blood and higher levels of 15-HETE in lung and bronchial tissue have been found. We hypothesize therefore that IL-4-induced apoptosis is one of the major causes of hypertrophy of the bronchial smooth muscle, denuded surface epithelium, thickened basement membrane, and infiltration of eosinophils, lymphocytes, and mononuclear phagocytes as well as the apoptotic lesions observed in the lung tissue of asthma patients. It has been reported that PPARγ ligands induce apoptosis in lung cancer cells, and this may be beneficial for the therapy of such cancers (30). In contrast, in chronic inflammatory diseases such as chronic obstructive pulmonary disease, the loss of alveolar structures in the lung tissue due to apoptosis may worsen lung function (29).