

Leptin is an eosinophil survival factor

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Background: Leptin regulates food intake, as well as metabolic, endocrine, and immune functions. It exerts proliferative and antiapoptotic activities in a variety of cell types, including T cells. Leptin also stimulates macrophages and neutrophils, and its production is increased during inflammation.

Objective: We sought to examine the expression of leptin receptors on eosinophils and the effect of recombinant leptin on proapoptotic pathways in these cells.

Methods: The presence of leptin receptor was examined by means of RT-PCR and immunofluorescence analysis in freshly isolated blood eosinophils and tissue eosinophils. The effect of recombinant leptin on apoptotic pathways in eosinophils was studied by using flow cytometric, immunoblotting, and immunofluorescence techniques.

Results: Human eosinophils express leptin surface receptors under *in vitro* and *in vivo* conditions, and leptin delays apoptosis of mature eosinophils *in vitro*. The antiapoptotic effects of leptin were concentration dependent and blocked by an anti-leptin receptor mAb. The efficacy of leptin to block eosinophil apoptosis was similar to that of GM-CSF. Leptin delayed the cleavage of Bax, as well as the mitochondrial release of cytochrome *c* and second mitochondria-derived activator of caspase, suggesting that it blocks proapoptotic pathways proximal to mitochondria in eosinophils. Using pharmacological inhibitors, we obtained evidence that leptin initiates a signaling cascade involving phosphatidylinositol-3-OH kinase and mitogen-activated protein kinase-dependent pathways in eosinophils.

Conclusion: Leptin is a survival cytokine for human eosinophils, a finding with potential pathologic relevance in allergic and parasitic diseases. (J Allergy Clin Immunol 2005;116:1228-34.)

Key words: Apoptosis, eosinophils, inflammation, leptin, signal transduction

Eosinophils are prominent effector cells in many allergic and parasitic inflammatory responses.¹ They are terminally differentiated leukocytes and as such are short-lived.² The numbers of eosinophils normally do not exceed 1% to 3% of peripheral blood leukocytes. The physiologic form of eosinophil death is apoptosis. Delayed eosinophil apoptosis, contributing to eosinophilia,

Abbreviations used

ECP:	Eosinophil cationic protein
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
MAPK:	Mitogen-activated protein kinase
PI3K:	Phosphatidylinositol-3-OH kinase
PS:	Phosphatidylserine
Smac:	Second mitochondria-derived activator of caspase

has been demonstrated in nasal polyp explants,³ as well as in purified blood eosinophils from patients with bronchial asthma⁴ and atopic dermatitis⁵ and some patients with the hypereosinophilic syndrome.⁶ The eosinophil hematopoietins IL-3, IL-5, and GM-CSF represent important eosinophil survival factors.⁷ These survival cytokines have been described to induce the antiapoptotic Bcl-2 family member Bcl-x_L^{8,9} and to inhibit the translocation of the proapoptotic member Bax to mitochondria impeding the release of cytochrome *c* from these organelles into the cytoplasm of eosinophils.¹⁰

Leptin is a pleiotropic cytokine involved in different biologic systems. It shares structural similarities with some cytokines, including IL-6, IL-11, IL-12, and IL-15, as well as with granulocyte colony-stimulating factor, oncostatin M, ciliary neurotrophic factor, and leukemia inhibitory factor.¹¹ Leptin binds to the short and long forms of leptin receptors, which are generated by differential splicing.¹² The leptin receptors are expressed in multiple cells and tissues, including kidney, lung, adrenal gland, hematopoietic precursor cells, and bone marrow, as well as in neutrophils, monocytes, and T cells.¹³ The signal transduction pathways regulated by leptin are diverse and include those characteristic for both cytokine and growth factor receptor signaling.¹⁴

Leptin plays a key role in the regulation of body weight but also exerts other biologic functions that modulate hematopoiesis, angiogenesis, and immune responses.¹³ Interestingly, leptin production is increased in patients with atopic asthma and other inflammatory diseases, such as IgE-associated atopic eczema-dermatitis syndrome.^{15,16} Moreover, leptin mediates both proliferative and antiapoptotic activities in a variety of cell types, including T cells¹⁷ and monocytes.¹⁸ Here we demonstrate that leptin delays eosinophil apoptosis *in vitro*. Analyzing the intracellular events in these cells revealed that leptin activates both phosphatidylinositol-3-OH kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling pathways, resulting in the inhibition of the mitochondrial death pathway.

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Supported by grants from the Swiss National Science Foundation (310000-107526) and OPO-Foundation (Zurich).

Received for publication June 6, 2005; revised July 20, 2005; accepted for publication September 2, 2005.

Available online October 24, 2005.

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0091-6749/\$30.00

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doi:10.1016/j.jaci.2005.09.003

METHODS

Reagents

Recombinant human leptin was purchased from R&D Systems (distributed by Bühlmann AG, Basel, Switzerland), and human GM-CSF was from Novartis Pharma GmbH (Nürnberg, Germany). The anti-human Fas mAb (CH11) was from LabForce AG (Nunningen, Switzerland). Anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH) mAb was from Chemicon International, Inc (Temecula, Calif). Rabbit polyclonal anti-human Bax antibody and anti-cytochrome *c* mAb were from Becton Dickinson Biosciences (Basel, Switzerland). The anti-eosinophil cationic protein (ECP) mAb (EG1) and polyclonal anti-second mitochondria-derived activator of caspase (anti-Smac) antibody were purchased from Molecular Probes (distributed by Invitrogen Corp, Groningen, Netherlands) and Pharmacia & Upjohn Diagnostics AB (Uppsala, Sweden), respectively. The goat polyclonal anti-leptin receptor (sc-1834) was from Santa Cruz Biotechnology, Inc (LabForce AG). Mouse and rabbit horseradish peroxidase-conjugated secondary antibodies were obtained from Amersham Pharmacia Biotech (Dübendorf, Switzerland). The pharmacologic inhibitors SB 203580 (inhibitor of p38 kinase) and LY 294002 (inhibitor of PI3-kinase) and polymyxin B sulphate were from Calbiochem (Juro, Lucerne Switzerland). All other reagents were, unless otherwise stated, from Sigma (Buchs, Switzerland).

Cells

Peripheral blood eosinophils were purified from control individuals, as described previously.^{8,19} The resulting cell populations contained less than 2% contaminating cells. Cell purity was assessed by staining with Diff-Quik (Medion GmbH, Düringen, Switzerland) and light microscopic analysis. Written informed consent was obtained from all blood donors, and the study was approved by the ethics committee of the Canton Bern.

Cell cultures

Human eosinophils were cultured at 1×10^6 /mL in complete culture medium (RPMI-1640 containing 10% FCS) in the presence and absence of recombinant GM-CSF (50 ng/mL), anti-Fas mAb (1 μ g/mL), and leptin (0.0001–5 μ M, usually 0.5 μ M) for the indicated times. In some experiments we added 15 μ g/mL polymyxin B. Leptin-blocking experiments were performed with anti-human leptin receptor mAb (240–480 μ M). SB 203580 and LY 294002 (both at 50 μ M) were added 30 minutes before adding leptin and left in the culture.

Determination of cell death and apoptosis

Eosinophil death was assessed on the basis of uptake of 1 μ M ethidium bromide and flow cytometric analysis (FACS Calibur). DNA fragmentation and redistribution of phosphatidylserine (PS) were measured to determine whether cell death was apoptosis.^{20–22}

Immunoblotting

Freshly isolated eosinophils and cultured eosinophils (1×10^6 /mL) were washed with cold PBS supplemented with a protease inhibitor cocktail (Sigma) and lysed with RIPA buffer (50 mM Tris HCl, 150 mM NaCl, 1% Nonidet P-40, 1 mM ethyleneglycol-bis-[β -aminoethylether]-N,N,N',N'-tetraacetic acid, and 0.25% sodium deoxycholate supplemented with a protease inhibitor cocktail), with frequent vortexing on ice for 15 minutes. Equal amounts of cell lysates were mixed with running buffer, boiled, and subjected to gel electrophoresis on 12% NuPage-Gels (Novex, distributed by Invitrogen). Separated proteins were electrotransferred onto polyvinylidene difluoride membranes (Immobilion-P; Millipore, Volketswil, Switzerland). The filters were incubated with blocking solution (Tris-buffered saline/0.1% Tween-20 and 5% non-fat dry milk) at room temperature

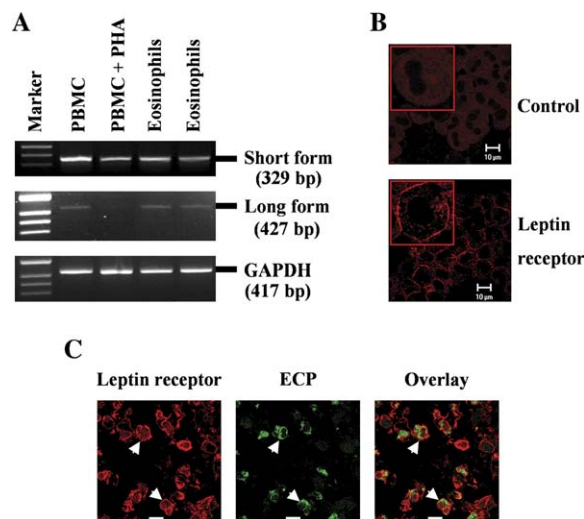


FIG 1. Eosinophils express leptin receptors. **A**, RT-PCR. Eosinophils express both the short and long form of the leptin receptor. **B**, Immunofluorescence. Eosinophils express leptin surface receptors (ring-like staining pattern). The bars represent 10 μ m. *Upper left corner*, Magnification of one cell. **C**, Immunofluorescence. Leptin surface receptors were readily detected on eosinophils (white arrows), which infiltrated into the nasal mucosa (polyp tissues). The bars represent 10 μ m.

for 1 hour. The primary anti-Bax antibody (1:1000) was incubated in blocking solution overnight at 4°C. For loading controls, stripped filters were incubated with anti-GAPDH mAb (1:3000). Filters were washed in Tris-buffered saline/0.1% Tween-20 for 30 minutes and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (1:3000) at room temperature for 1 hour. After another washing step, filters were developed by using an ECL technique (ECL-Kit, Amersham), according to the manufacturer's instructions.

RT-PCR

Total RNA was isolated from eosinophils by using TRIzol solution (Invitrogen, Basel, Switzerland), according to the manufacturer's instructions. First-strand synthesis was performed with total RNA, oligo (dT) 15 primer (Promega, distributed by Catalys AG, Wallisellen, Switzerland), and Superscript Reverse Transcriptase (Invitrogen). Primers for long isoform of human leptin receptor (5'-GAA GAT GTT CCG AAC CCC AAG AAT TG-3' and 5'-CTA GAG AAG CAC TTG GTG ACT GAA C-3'),²³ short isoform of human leptin receptor (5'-CCA TTG AGA AGT ACC AGT TCA GTC TTT ACC-3' and 5'-GGG AAG TTG GCA CAT TGG GTT CA-3'),²³ and GAPDH (5'-CCC CTT CAT TGA CCT CAA CTA C-3' and 5'-GAG TCC TTC CAC GAT ACC AAA G-3')²⁴ amplifications were synthesized (MWG-Biotech AG, Ebersberg, Germany). The cycling parameters for leptin receptor cDNA amplification were as follows: 95°C for 5 minutes; 40 cycles of 95°C for 30 seconds, 60°C for 60 seconds, and 68°C for 60 seconds; and 72°C for 10 minutes. Leptin receptor short isoform (329 bp), leptin receptor long isoform (427 bp), and GAPDH (417 bp) PCR products were separated on 1% agarose gels and visualized by means of ethidium bromide staining.

Confocal laser scanning microscopy

Cytospins were prepared from freshly purified eosinophils and eosinophils that were cultured in the presence and absence of leptin, GM-CSF, and anti-Fas mAb for 13 hours. Cells were fixed and permeabilized as previously described.^{21,25} Immunofluorescent stainings were performed at room temperature for 1 hour by using

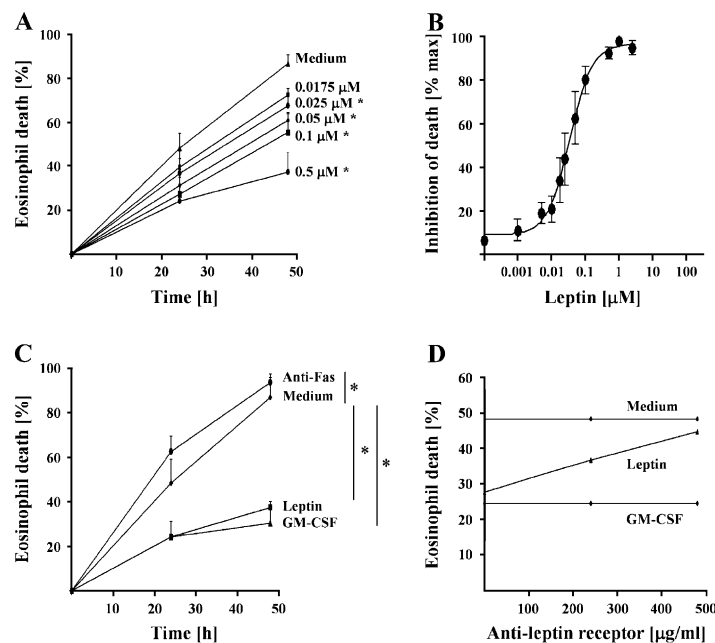


FIG 2. Leptin delays eosinophil apoptosis *in vitro*. **A**, Dose-dependent inhibition of eosinophil death by leptin ($n = 5$). **B**, Concentration-effect curve of leptin in 24-hour cultures ($n = 5$). **C**, Leptin ($0.5 \mu\text{M}$, $n = 4$) and GM-CSF (50 ng/mL , $n = 6$) similarly maintained eosinophil survival. **D**, Anti-leptin receptor antibody dose-dependently abolished the antideath effect of leptin ($0.1 \mu\text{M}$) but not that of GM-CSF (50 ng/mL). This panel is representative of 3 independent experiments (24-hour cultures). * $P < .05$.

the following primary antibodies diluted in PBS plus 3% BSA plus 2% normal goat serum: anti-cytochrome *c* mAb (1:100), polyclonal anti-leptin receptor antibody (1:10), and polyclonal anti-Smac antibody (1:1500). Incubation with appropriate tetramethyl rhodamine isothiocyanate (TRITC)- and FITC-conjugated secondary antibodies was performed in the dark at room temperature for 1 hour. The anti-fading agent Slowfade (Molecular Probes, Eugene, Ore) was added, and the cells were covered with cover slips. To quantify the amount of cells with diffuse staining pattern (cytochrome *c* and Smac experiments), 100 cells were counted in 3 different regions, and the mean number of cells demonstrating diffuse staining was calculated.

Immunofluorescent staining was also performed on 5- μm -thick paraformaldehyde-fixed paraffin-embedded tissue sections from nasal polyps, as previously described.²⁶ Immunostaining was performed at 4°C overnight with goat polyclonal anti-leptin receptor (1:10), together with anti-ECP mAb (1:50) in blocking buffer. Incubation with secondary antibodies and mounting was performed as described earlier. All slides were analyzed by means of confocal laser scanning microscopy (LSM 510; Carl Zeiss, Heidelberg, Germany) equipped with Ar and HeNe lasers.

Statistical analysis

Statistical analysis was performed with the Mann-Whitney *U* test. If no data from individual experiments are provided, the figures show mean levels $\pm$ SD. A *P* value of less than .05 was considered statistically significant.

RESULTS

Leptin receptor expression by eosinophils

We measured the expression of leptin receptor at mRNA and protein levels in freshly isolated normal blood

eosinophils. In agreement with previously published work in neutrophils,²⁷ we observed that freshly isolated eosinophils expressed mRNA for the short form of the leptin receptor. In contrast, the long form was rather difficult to detect, perhaps because of lower expression (Fig 1, A). Freshly purified PBMCs and PHA-activated PBMCs (5-hour stimulation) served as controls. Fresh PBMCs expressed both short and long leptin receptors, but PHA activation appeared to decrease the levels of the long form (Fig 1, A). To determine whether the expression of mRNA correlates with protein expression, we performed immunofluorescence analysis on freshly purified blood eosinophils. Eosinophils demonstrated a ring-like staining pattern, suggesting leptin receptor surface expression (Fig 1, B). To demonstrate leptin receptor expression on eosinophils under *in vivo* conditions, we analyzed nasal polyp eosinophils using a double-immunofluorescence technique. Infiltrating eosinophils were identified with an anti-ECP mAb. Eosinophils expressed leptin receptors, demonstrated by a ring-like staining pattern, which is consistent with its expression on the cell surface (Fig 1, C).

Leptin delays eosinophil apoptosis

Because leptin delivers antiapoptotic signals in T cells¹⁷ and monocytes,¹⁸ we investigated whether leptin delays apoptosis of eosinophils that spontaneously occurs after culturing these cells.⁷ Leptin delayed spontaneous eosinophil death in a dose- and time-dependent manner (Fig 2, A). Maximal inhibition of eosinophil death was reached with $0.5 \mu\text{M}$; higher concentrations had no further effect

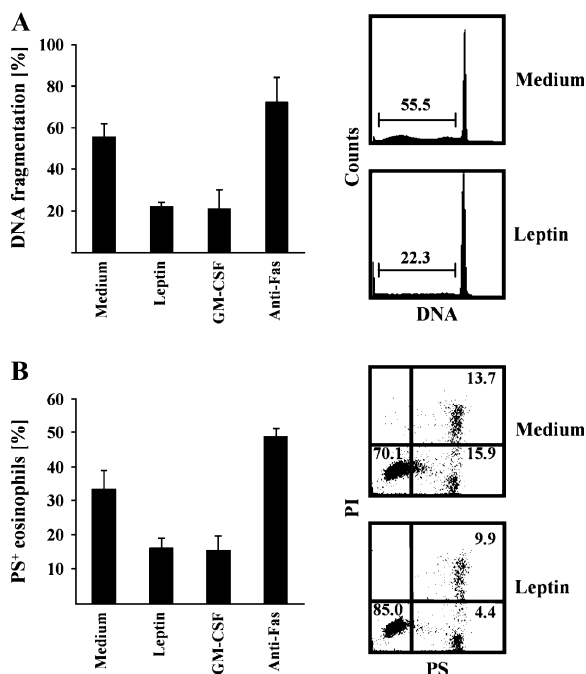


FIG 3. Leptin delays eosinophil apoptosis *in vitro*. **A**, Leptin reduced the formation of hypodiploid DNA in eosinophils (19-hour cultures, $n = 3$). **Right**, Representative examples of flow cytometric analysis. **B**, Leptin reduced PS redistribution in eosinophil membranes (15-hour cultures, $n = 3$). **Right**, Representative examples of flow cytometric analysis. The indicated numbers in these panels are relative cell numbers in percentages. * $P < .05$.

(Fig 2, B). The median effective concentration of leptin used for these experiments was approximately 0.025 μ M (400 ng/mL). Optimal concentrations of leptin and GM-CSF had similar antideath effects on eosinophils (Fig 2, C). Anti-Fas stimulation induced eosinophil death in this system, as expected.^{20,21} An anti-leptin receptor mAb was used to demonstrate specificity of leptin actions. This mAb dose-dependently inhibited the survival effect of leptin but not of GM-CSF (Fig 2, D). The anti-leptin receptor mAb had no effect on eosinophil viability when used in the absence of cytokine stimulation. Moreover, a control mAb had no effect, and leptin effects were not blocked when optimal concentrations of polymyxin B were added, excluding any potential nonspecific effects through LPS (data not shown).

We next investigated whether the antideath effect mediated by leptin was due to inhibition of apoptosis. Leptin reduced DNA fragmentation, a characteristic feature of apoptotic eosinophils,¹⁰ with the same efficacy as GM-CSF (Fig 3, A). In contrast, anti-Fas stimulation accelerated eosinophil apoptosis in this *in vitro* system. We also analyzed redistribution of PS, another hallmark of apoptotic cells. Again, leptin and GM-CSF significantly blocked apoptosis, whereas anti-Fas stimulation resulted in increased PS redistribution (Fig 3, B). In addition, we investigated whether the antiapoptotic effect of leptin was the result of GM-CSF gene activation in eosinophils by using a neutralizing anti-GM-CSF mAb. Although

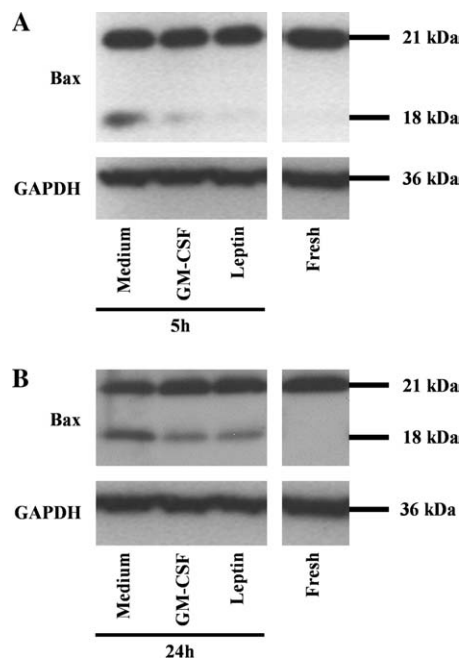


FIG 4. Leptin delays cleavage of Bax in cultured eosinophils. Delay of cleavage was also seen in GM-CSF-treated eosinophils. The filters were reprobated with an anti-GAPDH mAb to ensure equal loading of the gels. Results of 5-hour (**A**) and 24-hour (**B**) cultures are shown. The immunoblots are representative of 3 independent experiments.

this mAb neutralized the antiapoptotic effect of GM-CSF, it did not block leptin-mediated effects (data not shown). In conclusion, leptin exerts direct antiapoptotic activities on eosinophils *in vitro*.

Leptin inhibits Bax cleavage and the mitochondrial release of cytochrome c and Smac

Bax has been shown to translocate to mitochondria during eosinophil apoptosis¹⁰ and to be cleaved by calpain 1 during neutrophil apoptosis.²¹ As in neutrophils, Bax was cleaved during the process of spontaneous apoptosis in eosinophils, suggesting that calpain 1 might also play an important role in regulation of eosinophil apoptosis. In short-term eosinophil cultures (5 hours) Bax cleavage was prevented in the presence of leptin or GM-CSF (Fig 4, A). In longer eosinophil cultures (24 hours) Bax cleavage was partially blocked, suggesting that these 2 cytokines delay but do not completely block proapoptotic protease activation in eosinophils (Fig 4, B).

Mitochondria have been implicated in the regulation of eosinophil apoptosis.¹⁰ The observed cleavage of Bax, which we show here for the first time in eosinophils, supports the concept that mitochondria are involved in this process. We therefore analyzed the mitochondrial release of 2 proapoptotic factors in cultured eosinophils in the presence and absence of leptin and GM-CSF, respectively, by means of fluorescence immunostaining and

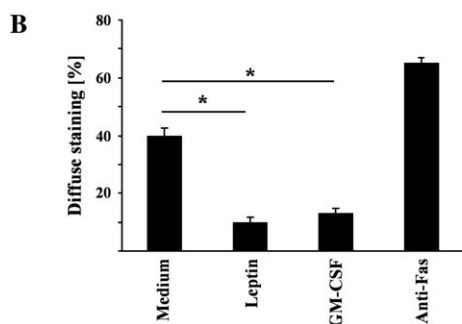
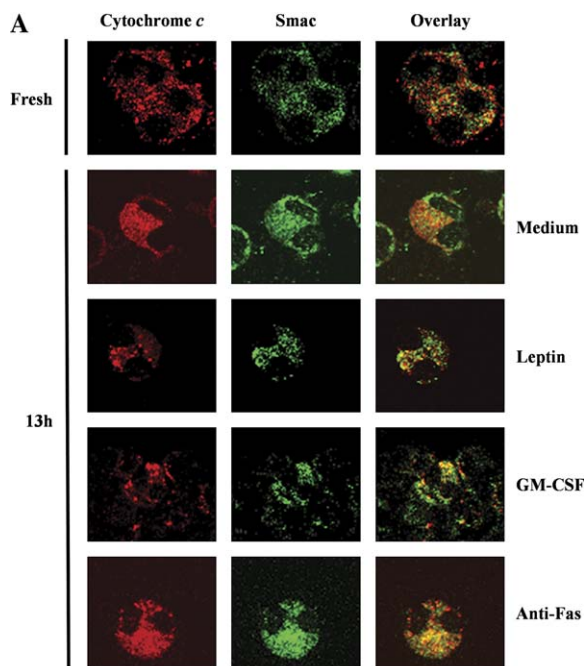


FIG 5. Leptin prevents the mitochondrial release of cytochrome *c* and Smac. **A**, Cytochrome *c* and Smac colocalized with each other and with Cox1 (data not shown), indicating that they are mitochondrial proteins in freshly isolated eosinophils. Leptin preserved the punctate pattern in cultured eosinophils, whereas we observed diffuse staining in the absence of cytokine support or after anti-Fas mAb treatment. Results are representative of 3 independent experiments. **B**, Quantitative and statistical analysis of the experiments shown in Fig 5, A. * $P < .05$ ($n = 3$).

microscopic analysis. Both cytochrome *c* and Smac were present in mitochondria of freshly purified eosinophils, as indicated by their colocalization in a punctate pattern (Fig 5, A). Culturing of eosinophils for 13 hours demonstrated evidence for cytochrome *c* and Smac release (= diffuse pattern) in a subgroup of cells (average of approximately 40%). Both leptin and GM-CSF preserved the punctate pattern in the majority of the cells, whereas anti-Fas treatment revealed a diffuse pattern of both cytochrome *c* and Smac in almost all cells. No difference was observed when cytochrome *c* and Smac stainings were compared. A statistical analysis of these experiments is shown in Fig 5, B. Leptin and GM-CSF had the same

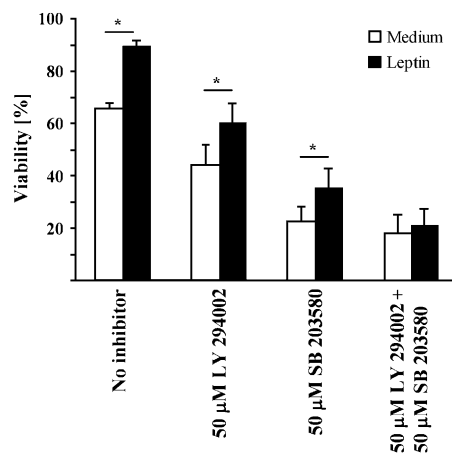


FIG 6. Pharmacologic inhibition of p38 MAPK and PI3K blocks antideath effects of leptin. Eosinophils were cultured in the presence (filled columns) and absence (open columns) of leptin (0.5 μ M) for 24 hours ($n = 3$). Only combined treatment with SB 203580 and LY 294002 completely abolished the effect of leptin in this system. The same results were observed when eosinophils were cultured for 48 hours (data not shown). * $P < .05$.

efficacy to block the transition into a diffuse cytosolic staining pattern associated with eosinophil apoptosis.

The antiapoptotic effect of leptin is mediated through PI3K and MAPK pathways

To test whether activation of PI3K and/or MAPK pathways are involved in leptin-mediated antiapoptosis, eosinophils were preincubated (30 minutes) with different concentrations of defined kinase inhibitors. Both SB 203580, a selective inhibitor of p38 MAPK, and LY 294002, an inhibitor of PI3K, accelerated spontaneous eosinophil death. Moreover, the antideath effect of leptin appeared to be partially blocked by each inhibitor, but leptin was still able to significantly increase eosinophil viability under these conditions. However, when SB 203580 and LY 294002 were used concurrently, leptin-induced survival was completely abrogated (Fig 6). In these experiments we also used PD 98059, an inhibitor of p42/44 MAPKs, which had no effect in this system (data not shown). These data suggest that PI3K and p38 MAPK pathways are involved in transducing leptin-mediated antiapoptotic signals into eosinophils.

DISCUSSION

Leptin has been implicated in the regulation of the immune system. Earlier studies suggested that leptin stimulates T_H1 immunity.²⁸ Moreover, leptin stimulates cells of the innate immune system, such as neutrophils, which have previously been shown to express leptin receptors.²⁷ However, whether these receptors are functional is a matter of debate. Here we investigated, for the first time, apoptotic pathways in eosinophils after stimulation with leptin. The following new findings are reported: (1) leptin receptors are expressed on eosinophils under *in vitro* and

in vivo conditions; (2) leptin directly activates eosinophils and delays spontaneous apoptosis of these cells; (3) leptin appears to exert its antiapoptotic function by inhibiting proapoptotic events proximal to mitochondria; and (4) the antiapoptotic function of leptin is mediated through PI3K and p38 MAPK signaling pathways.

Our study adds additional information on the potential role of leptin in regulating immune responses, which are often associated with increased levels of leptin. Besides atopic asthma,¹⁵ leptin has also been implicated in the pathogenesis of chronic inflammatory skin diseases. For instance, it was found that leptin levels were significantly increased in children with IgE-associated atopic eczema-dermatitis syndrome.¹⁶ Eosinophils play an important pathogenic role in allergic inflammatory diseases. Therefore the questions of whether leptin activates eosinophils and how the functional response or responses of such an interaction might appear seem to be of great interest. Because leptin delayed spontaneous eosinophil apoptosis, it is possible that leptin contributes to eosinophil accumulation at inflammatory sites, and perhaps it might then also stimulate the release of proinflammatory mediators from these cells.

Previously published work demonstrated that neutrophils express the short form but not the long form of leptin receptors.²⁷ Similarly, eosinophils appear to express the short form, although we obtained evidence in this study that they might also express the long form. Both forms of leptin receptors have identical extracellular and transmembrane domains but differ in their intracellular domains.¹² Although initial studies suggested that the short form is unable to deliver signals into the cells,¹² it is now clear that this is not the case. In spite of lacking the signal transducer and activator of transcription 3 docking site, the short leptin receptor is still able to bind and activate Jak2, which subsequently activates the MAPK pathway.²⁹ The data reported in this article suggest that in addition to the MAPK pathway, PI3K is also activated in leptin-stimulated eosinophils. Both MAPK^{30,31} and PI3K^{32,33} activation have previously been shown to mediate eosinophil antiapoptosis.

The leptin-initiated signaling cascades somehow block spontaneous eosinophil apoptosis before the mitochondria release their proapoptotic factors. This is indicated by the observation that leptin delays cleavage of Bax. Cleaved Bax has previously been shown to be highly efficient in proapoptotic mitochondrial triggering.³⁴ The delay of Bax cleavage by leptin was associated with inhibition of the release of cytochrome *c* and Smac into the cytosol. Cytochrome *c* forms a complex with Apaf-1 and caspase 9 that, in the presence of deoxyadenosine triphosphate, leads to the activation of caspase 3.³⁵ Smac is another proapoptotic factor released from mitochondria that inactivates members of the inhibitors of apoptosis protein family.^{36,37}

The efficacy of leptin to inhibit eosinophil apoptosis was similar to GM-CSF. However, the potency appeared to be approximately 40-fold decreased (10 ng/mL GM-CSF is usually sufficient to obtain optimal antiapoptotic effects in eosinophils). The low sensitivity of eosinophils

toward leptin might be due to the fact that they predominantly express the short form of the leptin receptor, which has been described as being less efficient in transducing leptin signals into cells compared with the long form.²⁹ The question remains whether the high concentrations of leptin that are required for the antiapoptotic action on eosinophils *in vitro* occur under *in vivo* conditions. Leptin serum levels up to 400 ng/mL (corresponding to our median effective concentration of leptin in eosinophils) were reported in children with chronic renal failure.³⁸ In addition, in obese subjects treated with leptin, serum levels of more than 700 ng/mL were measured.³⁹ Even higher leptin serum levels might have been reached (>0.1 μ M on the basis of our own calculations) when mice were treated with 2 μ g/g body weight leptin to improve wound healing.⁴⁰ These levels are in the range in which significant antiapoptotic effects on eosinophils are observed under *in vitro* conditions. Moreover, it should be noted that recombinant leptin, which has been used in this study, is known to have a lower potency than native leptin, perhaps because of differences in glycosylation.⁴¹ In addition, it is possible that higher leptin concentrations are present in inflamed tissues that contain leptin-producing adipocytes. Therefore leptin might indeed represent an eosinophil survival factor at sites of inflammation but probably not in the circulation.

In addition to eosinophils, leptin has been shown to exhibit antiapoptotic activity in T cells,¹⁷ monocytes,¹⁸ and neuroblastoma cells.⁴² In contrast, leptin has been reported to induce apoptosis in human bone marrow stromal cells.⁴³ The reason for these contrasting responses in different cell types is unclear, but differences in the expression patterns of leptin receptors and associated signaling molecules might play a role. In spite of these uncertainties, it seems that leptin, like other hormones, regulates apoptosis, and that cells of the immune system are targets of leptin. Further work is required to understand how the control of apoptosis by leptin influences innate immunity, adaptive immunity, or both.

We thank I. Schmid and E. Kozłowski (both of the Department of Pharmacology, University of Bern) for excellent technical assistance. We also thank Drs D. Simon (Department of Dermatology, University of Bern) and M. Neef (Department of Clinical Pharmacology, University of Bern) for providing blood samples.

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