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Original article

NF-κB inhibition accelerates apoptosis of bovine neutrophils

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Abstract – Apoptosis is one of the major events that contribute to the regulation of the immune system. For human neutrophils, evidence has been produced that the transcription factor NF-κB is critical in influencing the ultimate outcome of a cell's fate. However, such research has not yet been performed on bovine neutrophils. This urged us to examine the possible involvement of NF-κB in apoptosis of these cells. At first, we investigated whether p65 and p50, the most important members of the NF-κB family, are expressed in isolated blood neutrophils. The presence of both members was demonstrated on the RNA and protein level. Then the effect on bovine neutrophil apoptosis of gliotoxin, a potent and specific inhibitor of NF-κB, was examined. The rate of constitutive apoptosis was found to be greatly accelerated by inhibition of NF-κB. Furthermore, gliotoxin dramatically augmented the limited pro-apoptotic effect of TNF-α, an important inflammatory mediator. The results were obtained in six cows by annexin-V-FITC staining of externalized phosphatidylserine and subsequent flow cytometric analysis. Additional measurement of caspase-3/7 activity and evaluation of morphological criteria confirmed the outcome of this experiment. Finally, NF-κB activity was assessed under these conditions. The activity of p50 was found to be minimally affected by gliotoxin, while significantly lower active p65 values were observed. Still, the highest percentage of apoptosis, which was caused by incubation with both gliotoxin and TNF-α, did not correspond to the lowest activity of p65. We conclude that NF-κB p65 promotes the survival of bovine neutrophils by delaying the initiation of apoptosis.

bovine neutrophil / NF-κB / apoptosis

1. INTRODUCTION

Neutrophils play a critical role in the first line of defense against invading micro-organisms. These cells circulate in the blood vessels until they are attracted to inflamed tissue, where the neutrophil life span is extended by inflammatory mediators in order to destroy the pathogen. Once the infection is cleared, residual neutrophils die by apoptosis ensuring their removal by phagocytes and the rapid resolution of inflammation. Neutrophil apoptosis minimizes the risk of loss of toxic cell contents to the surrounding tissues and thus clearly controls the duration and intensity of an inflammatory response [15].

Apoptotic cell death represents a complex sequential process involving different cellular events to which many factors contribute.

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Apoptosis is defined by morphological features, with nuclear condensation [37] and cell shrinking [4] being mentioned in particular. However, characterization of the molecular events makes a more valuable contribution to the understanding of this kind of programmed cell death. Many studies have reported caspases as being central regulators of apoptosis [11]. Following proteolysis of pro-caspases, specific substrates are cleaved and thereby activated or inactivated, resulting in a proper execution of the apoptotic program.

Several cellular proteins are capable of inhibiting apoptosis. Since nuclear factor κB (NF-κB) controls the expression of many survival genes [2, 14], this transcription factor is known to prevent the induction of programmed cell death. In resting cells, NF-κB family members are sequestered in the cytoplasm, bound to their inhibitors which belong to the IκB family. Following cell activation, IκB proteins become phosphorylated, ubiquitinated and degraded by the proteasome. Subsequently, NF-κB can enter the nucleus and bind to the DNA in order to promote transcription [38].

In vitro studies with human neutrophils have shown that a range of inflammatory mediators known to be present at inflamed sites inhibit the apoptosis process in these cells [6, 9, 17, 34]. It has also been reported that incubation with some of these agents leads to the activation of NF-κB [23]. Furthermore, a major induction of human neutrophil apoptosis was seen after inhibiting NF-κB [21, 29, 30, 43]. These data strongly suggest that NF-κB is a major regulator of the human neutrophil life span.

There is accumulating evidence that, also in neutrophils, the anti-apoptotic activity of NF-κB would depend on gene induction. More specifically, the expression of A1 (Bfl-1) and Mcl-1, two anti-apoptotic members of the Bcl-2 family of proteins [3, 7], seems to be up-regulated by anti-apoptotic stimuli and down-regulated when apoptosis is induced [8, 20, 26, 27]. At least the expression of A1 has been shown to be dependent upon activation of NF-κB [12, 45]. In addition, the use of gliotoxin as a specific inhibitor of NF-κB [33] leads to depletion of A1 and Mcl-1 [27], even though the expression of the latter protein is not NF-κB regulated [1]. Furthermore, the loss of the expression of an inhibitor of apoptosis (IAP) may facilitate the induction of neutrophil apoptosis [31]. The transcription of IAP is also under control of NF-κB [40, 44].

A key role for bovine neutrophil apoptosis in the resolution of an inflammatory response has been documented in a growing number of reports [32]. Still, in contrast to humans, research into the role of NF-κB in regulating the life span of bovine neutrophils has not yet been carried out. Therefore, we first investigated whether bovine blood neutrophils express p65 and p50, the most important members of the NF-κB family. Still, the major purpose of the present study was to examine the in vitro effect of gliotoxin, a potent and specific inhibitor of NF-κB [33], on bovine neutrophil apoptosis and on the NF-κB activity of p65 and p50.

2. MATERIALS AND METHODS

2.1. Isolation and culture of bovine blood neutrophils

In this study, 6 healthy heifers of the Holstein-Friesian breed were selected from the Ghent University dairy farm (Biocentrum Agri-Vet, Melle, Belgium). Bovine peripheral blood was collected using sterile polypropylene tubes (IMI, Montegrotto Terme, Italy) pre-filled with an equal volume of Alsever as an anticoagulant solution (3 mM citric acid monohydrate, 27 mM trisodium citrate dehydrate, 72 mM NaCl and 125 mM D-glucose, pH 6.1). After centrifugation (300 *g*, 15 min), plasma and the buffy coat were removed. Red blood cells were lysed during incubation with an ice-cold isotonic NH_4Cl solution (138 mM NH_4Cl and

21 mM Tris, pH 7.4) for 10 min. The remaining cell suspension was washed twice with phosphate buffered saline (PBS) (200 *g*, 10 min). Contaminating mononuclear cells were then removed by density gradient centrifugation (1000 *g*, 20 min) over Percoll with a specific gravity of 1.094 g/mL, prepared according to the manufacturer's protocol (Sigma-Aldrich, Bornem, Belgium). The remaining cell pellet was again washed twice with PBS and used for further applications. Over 98% of the isolated cells were granulocytes, of which less than 5% were eosinophils.

Isolated neutrophils were resuspended at a final concentration of 3×10^6/mL in RPMI 1640 with 10% fetal calf serum (both from Invitrogen, Merelbeke, Belgium). The cultures were supplemented with 0.1 μg/mL gliotoxin (Sigma-Aldrich), 10 ng/mL rhTNF-α (Alexis, Zandhoven, Belgium) or with both effectors. Incubation was performed at 37 °C in a 5% CO_2 incubator (Binder, Tuttlingen, Germany) for 6 h.

2.2. NF-κB p65 and p50 mRNA detection following polymerase chain reaction (PCR)

Total cellular RNA was extracted from 1×10^6 neutrophils after cell lysis with Trizol® reagent (Invitrogen). First, mRNA was converted into cDNA by reverse transcriptase (60 min, 42 °C) and then the cDNA was screened with specific primer pairs (Eurogentec, Seraing, Belgium). The primer sequences used were the following: for p65 GGACTTCTCAGCCCTTCT and CCATCAGTGTGTGCTTTG; for p50 CTTGCTGCTAAATGCTGCTC and CCAGGTTCTGTAGGACTGTATCTTC. For amplification, 40 cycles were applied (30 s at 94 °C, 45 s at 59 °C for p50 or 60 °C for p65 and 45 s at 72 °C) on a Mastercycler (Eppendorf, Hamburg, Germany). Finally, amplification products were loaded on a 2.5% agarose gel (Helena Biosciences, Sunderland, UK) and visualized after electrophoresis under UV-light using ethidium bromide (Sigma-Aldrich). A 100 base pair (bp) marker (Genecraft, Münster, Germany) was used for sizing the fragments.

2.3. NF-κB p65 and p50 protein detection following western blotting (WB)

Total cell extracts were prepared by resuspending 5×10^6 neutrophils in 500 μL Laemmli buffer (62.5 mM Tris HCl, pH 6.8; 50 mM dithiotreitol, 10% glycerol, 2% sodium dodecyl sulfate and 0.1% bromophenol blue) supplemented with a cocktail of protease inhibitors (Calbiochem, La Jolla, USA). The samples were sonicated and boiled for 5 min at 95 °C and 20 μL were loaded on a 10% denaturing polyacrylamide gel followed by blotting on a nitrocellulose membrane (both from Bio-Rad Life Science, Nazareth, Belgium). After blocking in Tris-buffered saline with 1% Tween 20 (TBS-T) and 5% milk powder for 1 h, overnight incubation with primary anti-p65 (sc-372) or anti-p50 (sc-8414) antibodies (Santa Cruz Biotechnology, Heidelberg, Germany) was carried out. The membranes were then washed and a secondary antibody conjugated to horseradish peroxidase (HRP) was applied for 1 h. Finally, proteins were detected with a ChemiDoc™ System (Bio-Rad Life Science) using Supersignal® West Dura (Perbio Science, Erembodegem, Belgium). A molecular weight ladder with bands from 10 to 200 kDa (Westburg, Leusden, The Netherlands) was used for sizing the proteins.

2.4. Phosphatidylserine translocation assay

Flow cytometric detection of exposed phosphatidylserine was used to quantify the percentage of apoptotic neutrophils. After centrifugation (200 *g*, 10 min) of 1×10^6 neutrophils, the cell pellet was resuspended in 100 μL incubation buffer (10 mM Hepes, 140 mM NaCl and 5 mM $CaCl_2$, pH 7.4) containing 2 μL annexin-V-Fluos labeling reagent (Roche Diagnostics, Vilvoorde, Belgium) and 2 μL propidium iodide (PI)

(50 μg/mL; Sigma-Aldrich). Following 10 min incubation in the dark, 400 μL PBS was added and the samples were immediately analyzed using a FACScan flow cytometer (Becton Dickinson Biosciences, Erembodegem, Belgium). Cells positive for annexin-V-FITC and negative for PI were defined as apoptotic.

2.5. Morphological cell death assessment

After cytocentrifugation (55 *g*, 5 min) of 2×10^5 neutrophils, slides were air dried, fixed with pure methanol and stained with Hemacolor® (Merck, Darmstadt, Germany) according to Pappenheim. Evaluation of apoptotic morphology was based on cell shrinking and chromatin condensation characteristics.

2.6. Caspase-3/7 activation assay

For the assessment of the caspase-3 and -7 activity, the Caspase-Glo™ Assay (Promega, Leiden, The Netherlands) was used according to the manufacturer's protocol. Briefly, 2×10^6 neutrophils were lysed on ice during an incubation of 30 min with 100 μL PBS supplemented with 1% saponin (Sigma-Aldrich) and a cocktail of protease inhibitors (Calbiochem). After centrifugation (8000 *g*, 10 min), the supernatant was frozen at –80 °C until further analysis. In a 96-well plate, 5 μL of freshly thawn protein lysate were diluted with lysis buffer to a final volume of 100 μL. Subsequently, the same volume of Caspase-Glo™ 3/7 reagent was added. After an incubation of 1 h at room temperature, chemiluminescence was measured with a Microlumat LB 96 P luminometer (Berthold Technologies, Vilvoorde, Belgium) and expressed in relative light units per second (RLU/s).

2.7. NF-κB activation assay

For the detection and quantification of the activity of NF-κB p65 and p50, the TransAM™ Transcription Factor Assay Kits (Active Motif, Rixensart, Belgium) were used according to the manufacturer's protocol. In brief, 2×10^6 neutrophils were lysed on ice during an incubation of 30 min with 100 μL PBS supplemented with 1% saponin (Sigma-Aldrich) and a cocktail of protease inhibitors (Calbiochem). After centrifugation (8000 *g*, 10 min), the supernatant was frozen at –80 °C until further analysis. For each sample, 5 μL of freshly thawn protein lysate were incubated for 1 h in wells of a 96-well plate containing immobilized NF-κB consensus oligonucleotide. After extensive washing, the NF-κB complex bound to the oligonucleotide was identified using the supplied anti-p65 or anti-p50 antibody. Addition of a secondary antibody conjugated to HRP allowed a chemiluminescent readout on a Microlumat LB 96 P luminometer (Berthold Technologies) with an expression in RLU/s.

2.8. Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). For the percentage of apoptosis, the caspase activity and the NF-κB activity, pairwise comparisons between the control group and the three other treatment groups were made after 6 h of incubation using a mixed model with cow as random effect and treatment as fixed effect. The Bonferroni's multiple comparisons adjustment technique was used with a global confidence level of 95%. The correlation study was conducted according to Pearson. The SAS System software (SAS Institute GmbH, Heidelberg, Germany) was used for all analyses.

3. RESULTS

3.1. Constitutive expression of NF-κB p65 and p50 in bovine neutrophils

In freshly isolated bovine neutrophils, the presence of p65 and p50 as important members of the NF-κB family was investigated on the RNA as well as on the protein

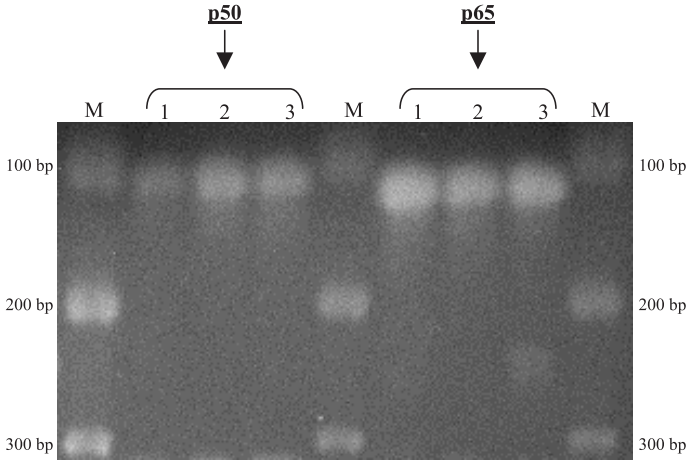

Figure 1. PCR analysis of neutrophils of cows. mRNA of p65 and p50 are seen as bands of 111 and 104 bp, respectively. M represents the 100 bp DNA marker.

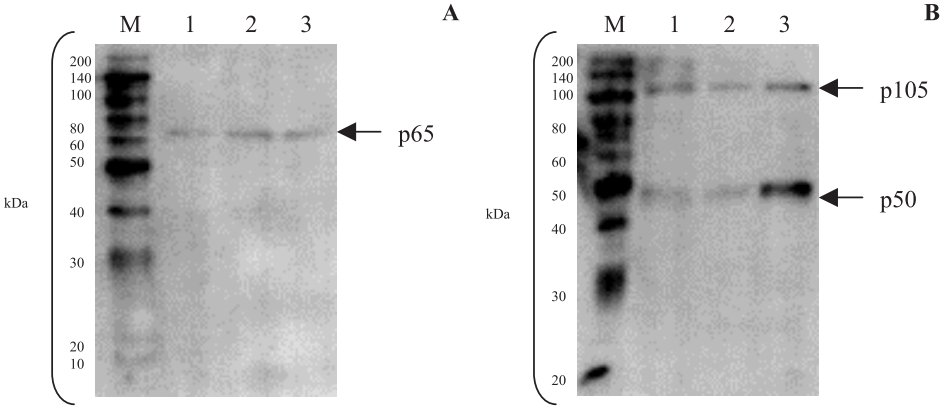

Figure 2. Western blotting analysis of total cell extracts of 3 cows: (A) shows p65 protein, (B) shows p50 and p105 protein. M represents the molecular weight marker with bands from 10 to 200 kDa.

level. Using specific primer pairs in PCR, bands of 111 bp and 104 bp were detected, corresponding to mRNA from p65 and p50 respectively (Fig. 1). After western blotting analysis, both p65 and p50 could also be detected as protein bands at their corresponding molecular weight (Fig. 2). When using the anti-p50 antibody, an additional protein band was visualized since this antibody also recognizes the p50 precursor p105. Taken together, these experiments demonstrate the presence of p65 and p50 mRNA and protein in bovine neutrophils.

3.2. Effect of gliotoxin on phosphatidylserine exposure in bovine neutrophils

Phosphatidylserine exposure in bovine neutrophils was measured directly after isolation and at different time points (2, 4 and

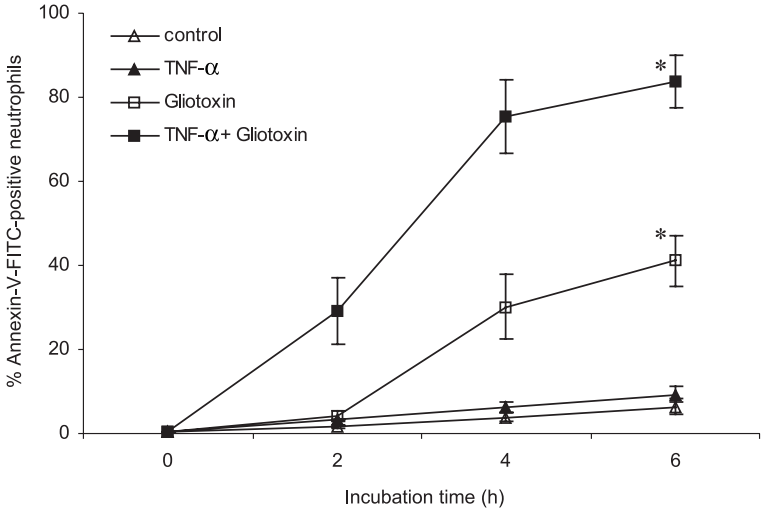

Figure 3. Effect of gliotoxin (0.1 μg/mL) and/or TNF-α (10 ng/mL) on cell surface exposure of phosphatidylserine in bovine neutrophils after isolation and after 2, 4 and 6 h of culture. Data are means ± SEM of 6 cows. Statistically significant differences compared to the control at 6 h are indicated with an asterisk (global confidence level of 95% with Bonferroni's multiple comparisons adjustment technique).

6 h) following incubation with 0.1 μg/mL gliotoxin and/or 10 ng/mL TNF-α (Fig. 3). A time-dependent increase of the percentage of annexin-V-FITC-positive/PI-negative neutrophils was observed in all samples. The constitutive rate of apoptosis was greatly increased when gliotoxin was added. After 6 h of incubation, a significant difference in the percentage of apoptotic cells between the control (on average 6.34%, $n = 6$) and gliotoxin (41.05%) was seen ($P < 0.0001$). A remarkably higher percentage of apoptosis was observed in neutrophils incubated with a combination of gliotoxin and TNF-α (83.73%; $P < 0.0001$). TNF-α alone did not cause a significant increase in neutrophil apoptosis as compared to the control (9.25%). Annexin-V-FITC-positive/PI-positive values were less than 2% in all samples.

3.3. Effect of gliotoxin on bovine neutrophil morphology

Cytospin slides of neutrophils incubated for 6 h in control medium were compared the to slides of cells cultured in medium supplemented with gliotoxin in combination with TNF-α for the same period of time. Changes from normal cell morphology to apoptotic morphology were clearly seen (Fig. 4). Whereas non-apoptotic neutrophils were characterized by a polysegmented nucleus, apoptotic cells had a shrunken appearance with condensed nuclei. These qualitative results were in accordance with the flow cytometric data of phosphatidylserine exposure.

3.4. Effect of gliotoxin on caspase-3/7 activity in bovine neutrophils

In all samples of freshly isolated neutrophils, caspase activity was minimal (Fig. 5). This result was in accordance with the flow cytometric data of phosphatidylserine exposure which showed that an average of more than 98% of the cells were viable at that time point. After 6 h of incubation, caspase activity increased and the cells incubated in the control medium showed a 10-fold higher

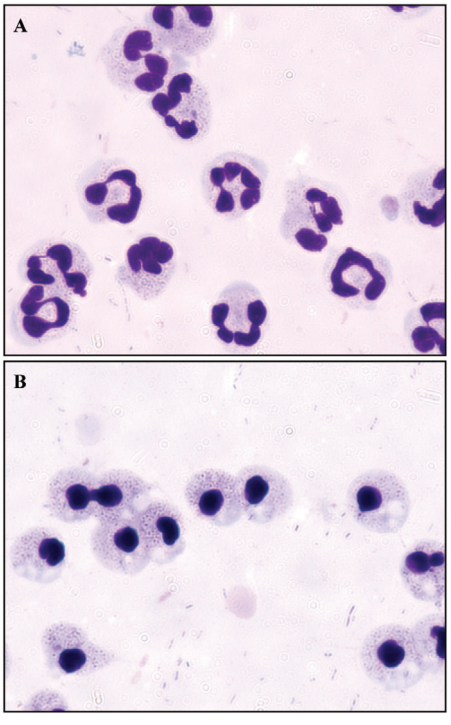

Figure 4. Cytocentrifuge preparations of neutrophils from one representative cow after 6 h of culture (magnification: 1000 ×): (A) shows neutrophils incubated in control medium, (B) shows neutrophils incubated in medium supplemented with gliotoxin (0.1 μg/mL) and TNF-α (10 ng/mL).

activity as compared to freshly isolated neutrophils. The highest values of caspase activity were obtained when the cells were incubated with gliotoxin in combination with TNF-α ($P < 0.0001$). Treatment with gliotoxin alone also significantly induced caspase activity ($P = 0.0001$). Treatment with TNF-α resulted in minimal caspase activity with values being comparable to the control at the same time point. In general, these data again fit well with the phosphatidylserine observations. Indeed, a striking correlation ($r = 0.9326$) was obtained between both apoptosis parameters.

3.5. Effect of gliotoxin on NF-κB p65 and p50 activity

NF-κB p65 and p50 activities were measured in all cell extracts of freshly isolated neutrophils and of neutrophils incubated for 6 h. The highest p65 activity was seen in freshly isolated neutrophils (Fig. 6). This activity decreased after 6 h of incubation, with the lowest values obtained in extracts of cells incubated in the presence of gliotoxin ($P = 0.0002$). Treatment with TNF-α alone did not result in a significantly lower p65 activity in comparison with the control at 6 h. The addition of TNF-α to gliotoxin abrogated the inhibition of the p65 activity by gliotoxin alone. This latter effect was not in accordance with the results obtained for the apoptosis parameters. No significant changes in p50 activity were found after 6 h of incubation in the different culture conditions. Values were even systematically comparable to those of the blank (data not shown).

4. DISCUSSION

In neutrophils the major inducible NF-κB protein complex has been shown to consist of p65 and p50 [23]. Both family members contain at least one transcription activation domain which makes them responsible for gene induction [25]. Since these observations were obtained in the human species, the need exists to demonstrate the presence of both proteins in their bovine counterpart. In this study, we report that p65 and p50 are constitutively present in bovine blood neutrophils on the mRNA level as well as on the protein level (Figs. 1 and 2). Subsequently, we set out to examine their function in these cells.

Since gliotoxin is a potent and specific inhibitor of NF-κB [33], it was used as a pharmacological tool to investigate the involvement of the transcription factor in the regulation of bovine neutrophil apoptosis. Exposure of phosphatidylserine on the outer leaflet of the plasma membrane is a

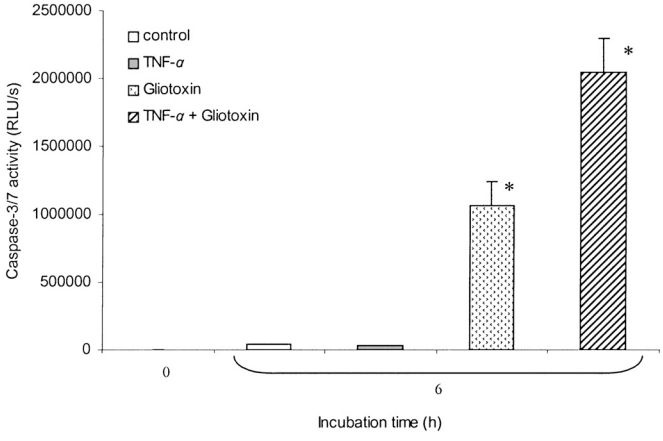

Figure 5. Caspase-3/7 activity of bovine neutrophils after isolation and after 6 h of culture with gliotoxin (0.1 μg/mL) and/or TNF-α (10 ng/mL). Data are means ± SEM of 6 cows. Statistically significant differences compared to the control at 6 h are indicated with an asterisk (global confidence level of 95% with Bonferroni's multiple comparisons adjustment technique).

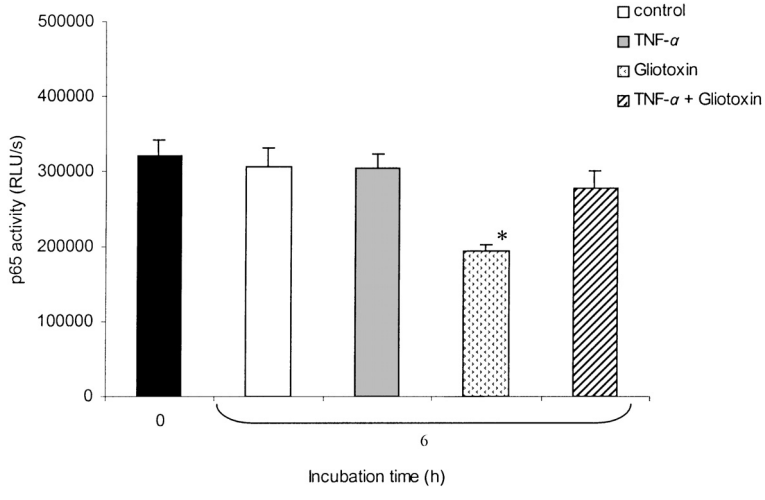

Figure 6. NF-κB activity of p65 in bovine neutrophils after isolation and after 6 h of culture with gliotoxin (0.1 μg/mL) and/or TNF-α (10 ng/mL). Data are means ± SEM of 6 cows. Statistically significant differences compared to the control at 6 h are indicated with an asterisk (global confidence level of 95% with Bonferroni's multiple comparisons adjustment technique).

feature characteristic of apoptotic cells that can be flow cytometrically detected with annexin-V-FITC which binds the phospholipid in a calcium-dependent way [42]. Our data obtained with this assay show that at least 98% of freshly isolated neutrophils were viable and that incubation gave rise to spontaneous apoptosis. Gliotoxin significantly increased this constitutive rate of apoptosis over a time course of 6 h (Fig. 3). Moreover, gliotoxin greatly enhanced the limited pro-apoptotic effect of TNF-α. These

observations were consistent with the results of similar experiments in human neutrophils [21, 36, 43]. In addition to annexin-V-FITC, PI was used as a measure of necrosis in our flow cytometric analysis. Less than 2% of neutrophils were PI-positive. Therefore we can conclude that treatment induced a purely apoptotic form of cell death.

As an additional apoptosis parameter, caspase activity was measured in all samples made after 6 h of incubation. Because inhibition of caspase-3 and -7 blocks human neutrophil apoptosis [10, 18, 19], the combined activity of both these effector caspases was assessed. The results obtained were in agreement with our flow cytometric phosphatidylserine exposure data (Fig. 5): gliotoxin increased caspase activity compared to the control at 6 h and together with TNF-α maximal activity was achieved. This demonstrates that apoptosis induced in the different culture conditions is dependent on caspase activation. The morphological study also confirmed that gliotoxin acts synergistically with TNF-α to stimulate bovine neutrophil apoptosis (Fig. 4). Taken together, our investigation of multiple complementary apoptosis parameters yielded indisputable results.

Various studies with human neutrophils have shown that NF-κB plays a crucial protective role against apoptosis [21, 29, 30, 43]. In the next part of the current study, p65 and p50 activities were therefore examined to evaluate whether this also applies to bovine neutrophils. We used a novel ELISA-based assay (TransAM™ Transcription Factor Assay Kit), which is known to correlate well with the traditional electrophoretic mobility shift assay (EMSA) [35]. From this assay, it was demonstrated that the activity of p65 was the highest in freshly isolated bovine neutrophils and decreased after 6 h of incubation (Fig. 6). The values of p65 activity were the lowest in neutrophils cultured with gliotoxin. Treatment with TNF-α also resulted in a lower p65 activity, even though this effect was again non-significant. These results were in agreement with our apoptosis data and supported the working hypothesis that NF-κB promotes survival in bovine neutrophils. In contrast, following incubation with gliotoxin and TNF-α, the p65 activity increased, an observation which was not in accordance with our apoptosis data. As for the p50 activity, no changes were found between the different culture conditions after 6 h of incubation. It was pointed out that p50 values were comparable to those of the blank. Interpretation of these data must be performed with care since the anti-human p50 antibody might not have recognized the active p50 protein of bovine origin in our samples. Indeed, this option can not be excluded since no cross-reactivity has been reported so far for the bovine species.

Gliotoxin belongs to the continuously growing group of selective NF-κB inhibitors. Although widely used, the precise mechanism by which this fungal metabolite inhibits NF-κB is still largely unknown. Some reports suggest the inhibition of the proteasome-mediated IκB degradation and the blockage of the NF-κB DNA binding activity [16, 33]. In our study, administration of gliotoxin induced apoptosis in bovine neutrophils and decreased their basal p65 activity. In human neutrophils, inhibition of the basal NF-κB activity by gliotoxin also seems to be responsible for the induction of apoptosis [43]. Because of the agreement between our observations and those in human neutrophils, the same conclusion can be drawn, namely that NF-κB promotes neutrophil survival. To the best of our knowledge, the identification of the family members composing the basal NF-κB complex in human neutrophils based on supershift experiments has remained unsuccessful [23, 24]. According to our results obtained with the ELISA-based assay, at least p65 is part of the bovine basal NF-κB complex.

TNF-α is one of the most important orchestrators of inflammation and therefore a logical choice for the study of mediators in the context of neutrophil apoptosis. When evaluating the role of this cytokine on

the neutrophil life span one should be aware that this cytokine can exert either an anti- or a pro-apoptotic effect, depending upon the concentration used and the time of exposure [28, 41]. Interestingly, the anti-apoptotic effect of TNF-α is partly ascribed to the stimulation of a survival pathway involving activation of NF-κB [22]. In the current study, TNF-α was found to slightly promote bovine neutrophil apoptosis, albeit non-significantly. Nevertheless, the dose and incubation time chosen have been previously reported to be pro-apoptotic. Furthermore, TNF-α also seemed to slightly decrease p65 activity in our study. However, in comparison with gliotoxin alone, the increase in p65 activity following incubation with gliotoxin and TNF-α seems contradictory to the maximal percentage of apoptosis observed under these conditions. Indeed, in human neutrophils, the synergistic effect of gliotoxin and TNF-α on apoptosis was correlated with a major reduction of NF-κB activity [43]. The authors even suggest that gliotoxin enhances TNF-α-induced apoptosis by inhibiting the production of a survival factor possibly mediated by NF-κB. Remarkably, in this report only the expression of an inducible form of NF-κB was down-regulated. This inducible NF-κB complex arose following TNF-α administration, but as in our study TNF-α alone did not induce p65 activity, it appears that the combined effect of gliotoxin and TNF-α is not visible at the level of NF-κB activity.

In conclusion, our data suggest that NF-κB p65 is active and promotes survival in bovine neutrophils. Still, it should be kept in mind that neutrophils in circulation will only survive for a few hours. Indeed, blood neutrophils are programmed to die spontaneously. We believe that a decrease in p65 activity probably represents an essential trigger for the onset of constitutive apoptosis. In contrast, tissue neutrophils can temporarily lose their susceptibility to apoptosis under inflammatory conditions. In human neutrophils, this loss seems to be associated with the appearance of an inducible NF-κB complex [13]. Importantly, NF-κB activity was shown to be increased in environments of inflammation [5, 39]. Since we have now observed and partly characterized the basal activity of NF-κB in bovine neutrophils, our future prospect is to investigate the existence of inducible NF-κB complexes in these innate defense cells.

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