Glucocorticoid regulation of cell survival in normal and cytokine-treated gastric chief cells

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Abstract

Gastric chief cells secrete pepsinogen and are found at the base of gastric glands. Chief cells constitutively express the anti-apoptotic protein Bcl-x_L, which may enable them to live longer than other gastric cells. We reveal that the cytokines released as part of the inflammatory response during *Helicobacter pylori* infection dose-dependently disrupt chief cell monolayer integrity and viability. We also show that glucocorticoids dose-dependently increase expression of Bcl-x_L, and hypothesize that this accounts for the ability of glucocorticoids to protect chief cells from cytokine-induced cell death. In addition, we show that glucocorticoids restrict the ability of cytokines to damage chief cell monolayer integrity. Finally, we propose two mechanisms by which cytokines overwhelm the protective ability of glucocorticoids, resulting in chief cell deletion and gastric atrophy during *Helicobacter pylori* infection.

1 Introduction

1.1 Gastric anatomy and chief cells

The stomach is an organ between the esophagus and the small intestine that assists in the digestion of proteins. It is divided into three regions: the cardiac region (closest to the esophagus), the pyloric region (closest to the small intestine), and the fundic region (constituting the bulk of the stomach area) [20]. The layers of tissue that line the stomach surface are collectively called the mucosa, and include simple columnar epithelium, lamina propria, and muscularis mucosae. Within the fundic region, the surface is lined by mucous cells that secrete mucus. These cells also line the gastric pits. Pits are continuous with gastric glands, which contain a long neck segment (containing mostly parietal cells) and a short base segment (containing mostly chief cells) [10] (Figure 1). Parietal cells secrete HCl via an H^+/K^+-ATPase [20], and chief cells release pepsinogen from granules within their apical cytoplasm via exocytosis into the lumen of the gastric gland [10]. In the lumen, the secreted HCl activates pepsinogen to pepsin, which can hydrolyze peptide bonds to initiate protein digestion.

Chief cells develop from undifferentiated stem cells that move downward towards the base of the gastric gland. Chief cells live for approximately 190 days, and are then replaced. This turnover rate is significantly longer than for other gastric cells, as parietal cells live for 54 days and surface cells for 3–4 days. Thus, chief cells must possess one or more highly specific mechanisms to regulate cell survival and cell death (apoptosis). In this study, we investigate potential regulatory mechanisms for chief cell survival which involve glucocorticoids, Bcl-x expression, and tight junctions. We postulate that the rapid turnover of chief cells that occurs during *Helicobacter pylori* infection may be due to cytokines, which affect the regulation of tight junctions and overwhelm the ability of Bcl-x to protect against apoptosis.

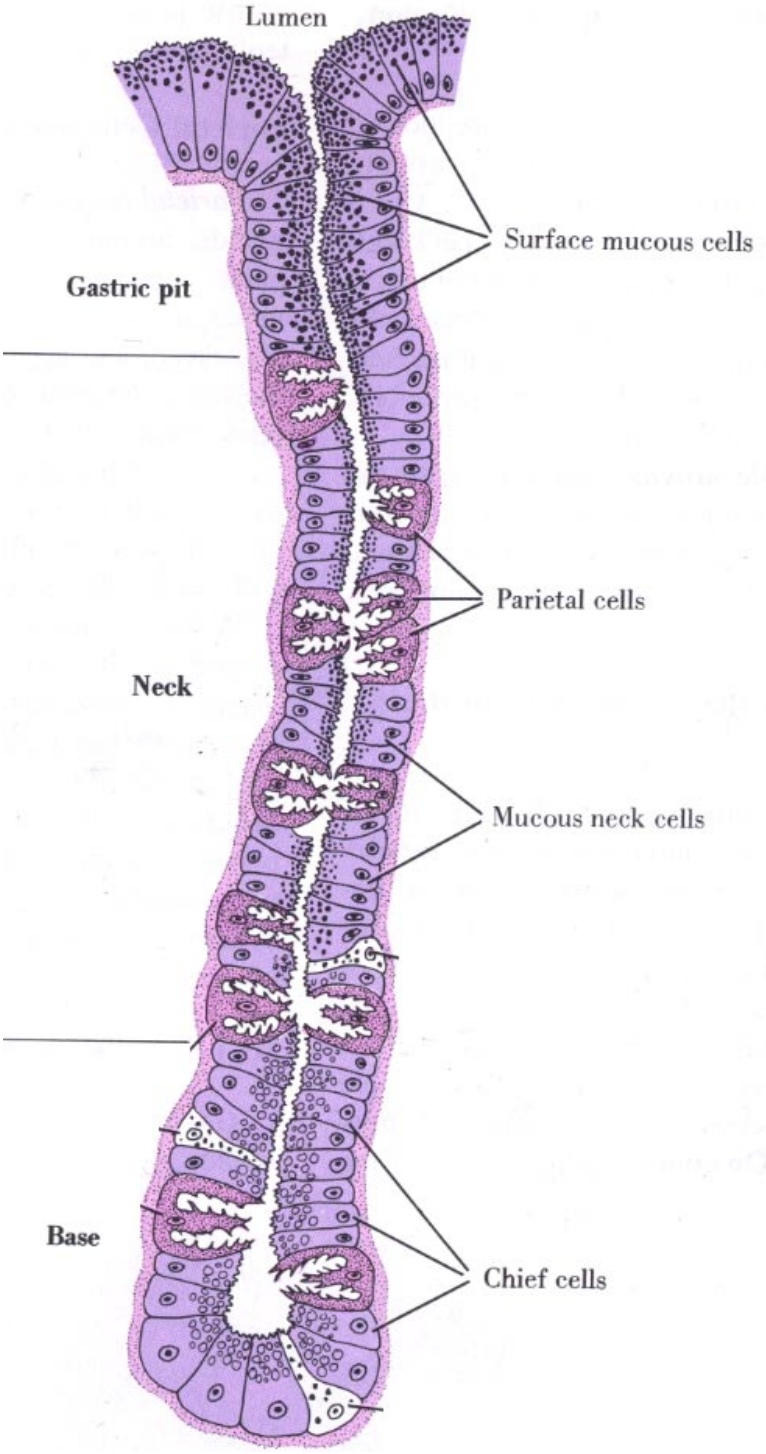

Figure 1: This diagram [20] shows the location of the different cells in the gastric mucosa. Chief cells are found at the base of gastric glands.

1.2 Apoptosis, Bcl-x, and chief cells

Apoptosis occurs when a cell is induced to commit suicide in a controlled manner [9]. Apoptosis is important in the maintenance of tissue homeostasis, which often requires the timely and controlled death of specific cells [6]. By mediating the programmed death of cancerous cells, apoptosis also serves as a defense mechanism against tumorigenesis. An apoptotic cell undergoes cytoplasmic shrinking, DNA degradation, nuclear condensation, and membrane blebbing [1], in addition to activation of cell death proteins and pathways that are cell-type specific.

Apoptosis is regulated through complex signaling pathways, which include pro-apoptotic and anti-apoptotic (pro-survival) proteins [6] from the Bcl-2 family of proteins. Proteins in the Bcl-2 family possess at least one of four conserved motifs known as Bcl-2 homology (BH) domains [1]. Anti-apoptotic proteins in this family generally contain at least BH1 and BH2, whereas many (but not all) pro-apoptotic proteins lack these domains. Different tissues express different members of the Bcl-2 protein family, but the ratio of pro-apoptotic to anti-apoptotic proteins appears to determine whether or not a cell undergoes apoptosis [1]. The mechanism through which these proteins control apoptosis is not completely understood, but appears in certain cases to involve heterodimerization between pro-apoptotic and anti-apoptotic proteins [1].

Little is known about the expression of Bcl-2 family proteins in the stomach, in general, or in chief cells. Neu *et al.* [17] found that rat gastric chief cells express the pro-survival protein, Bcl-2, and the pro-apoptotic protein, Bax, a finding that was confirmed by Hagen *et al.* (unpublished data). Recently, rat chief cells were also shown to express Bcl-x, a second survival protein that is usually expressed in long-lived cells.

The *bcl-x* gene undergoes alternative splicing, leading to two ubiquitous isoforms: Bcl-x_L and Bcl-x_S [2]. (Three other isoforms have been identified, but are not ubiquitous.) Bcl-x_L is a pro-survival protein that, like Bcl-2, contains the BH1 to BH4 domains [1]. Bcl-x_S

contains a deletion of 63 amino acid residues relative to Bcl-x_L, and contains only BH3 and BH4 domains [2]. Minn *et al.* [14] showed that Bcl-x_S is pro-apoptotic, and can antagonize Bcl-x_L. This is consistent with the observation that the deleted residues in Bcl-x_S are those with greatest similarity to Bcl-2 [2]. Pecci *et al.* [18] identified five promoters (P1 to P5) of the *bcl-x* gene in mice, and reported that tissue-specific promoter choice influences alternative splicing and, thus, the balance of Bcl-x isoforms. Promoter choice in chief cells may affect the ratio of Bcl-x_L/Bcl-x_S and regulate the balance between apoptosis and survival.

1.3 Tight junctions and chief cells

Tight junctions are structures that seal adjacent cells together [21]. They are present in epithelial tissues, including the simple columnar epithelium that lines the gastric lumen. Tight junctions are important in gastric glands because they prevent mucus, HCl, and pepsinogen from leaking out of the stomach lumen. Tight junctions also selectively prevent ions and molecules, especially secreted H^+, from entering gastric cells from the stomach lumen. The apical membrane of gastric cells, which faces the lumen, is generally impermeable to these molecules. However, without tight junctions, these molecules would be able to pass between gastric cells and then enter gastric cells via the basolateral membrane, which is more permeable. Thus, tight junctions are important in the gastric mucosa because they can protect gastric cells, including chief cells, from ions and molecules that would affect their function and survival.

Tight junctions are composed of a series of transmembrane proteins. Two cells forming a tight junction possess proteins called claudins in their plasma membranes. The claudins in the membranes of the two cells bind to each other, thus sealing the two cells together [21]. Occludin is also found in the membranes of two cells forming a tight junction, but its role in tight junction formation is less clearly defined [21]. Finally, ZO proteins anchor claudins and occludin to the cytoskeleton of the cells forming a tight junction [21].

Transepithelial electrical resistance (TER) is commonly used to verify that tight junctions are present in a monolayer of epithelial cells. A high TER is associated with a tight monolayer that has low ion permeability. Conversely, a low TER indicates a leaky monolayer with high permeability. Thus, measurement of TER is used in experiments to determine whether a treatment affects monolayer integrity and permeability [21].

1.4 Glucocorticoid regulation of chief cell survival

Glucocorticoids are steroid hormones implicated in the regulation of the *bcl-x* gene. Viegas *et al.* [26] reported that treatment of cells with dexamethasone (a synthetic glucocorticoid) allowed glucocorticoid receptor to bind to two hormone response element (HRE)-like sequences on the distal promoter P4 of *bcl-x*. This resulted in the expression of the anti-apoptotic Bcl-x_L isoform. Another study by Chang *et al.* [4] showed that dexamethasone suppressed apoptosis in the human gastric cancer TMK-1 cell line through modulation of *bcl-x* expression. Inducing apoptosis in the cells led to increased expression of Bcl-x_S and cell death. In contrast, treatment with dexamethasone decreased Bcl-x_S expression and increased Bcl-x_L expression to favor cell survival.

Glucocorticoids are also implicated in the regulation of tight junctions. A study by Zettl *et al.* [27] found that glucocorticoids induce mouse mammary epithelial cells to form tight junctions by modulating the expression of tight junction proteins in cells in a similar manner as described for *bcl-x*.

Because *bcl-x* is expressed at a high level in gastric chief cells and because adjacent chief cells form tight junctions, we postulate that glucocorticoids might be involved in the control of chief cell survival by regulating *bcl-x* expression and/or monolayer integrity. Tseng *et al.* [24] reported that adrenalectomized rats exhibit a dramatic decrease in the number of mature gastric chief cells, suggesting that glucocorticoids may regulate the survival of chief cells.

We hypothesize that glucocorticoids protect chief cells from cell death, in part, by increasing the expression of the anti-apoptotic Bcl-x_L isoform and by preserving monolayer integrity.

1.5 *Helicobacter pylori*, cytokines, and chief cell death

Helicobacter pylori (HP) is a gram-negative rod-shaped bacterium that infects the stomach of more than half of the global population [16]. It survives the acidic environment of the stomach by catalyzing the breakdown of urea into the basic bicarbonate and ammonia species [13]. HP colonizes the gastric mucosa and causes gastritis, followed by atrophy, metaplasia, and cancer. Atrophy, defined as the deletion of parietal and chief cells in gastric glands, is the major factor that predisposes to gastric cancer progression during HP infection [13].

Although specific pathways remain to be defined, infection with HP triggers an inflammatory response in the gastric mucosa [22]. This is associated with the release of proinflammatory cytokines from T helper-1 (T_H1) cells, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and interferon (IFN)-γ [8], [22]. These cytokines attract leukocytes to the site of infection. In addition, IL-1β and TNF-α inhibit the secretion of gastric acid by parietal cells, allowing HP to further colonize the gastric mucosa [8]. In the fundus, the increase in inflammation is associated with increased apoptosis of gastric epithelial cells, resulting in gastric atrophy. In cells of the intestinal mucosa, cytokines released during inflammatory bowel disease are known to alter tight junction structure, increasing their permeability and making them leaky [3], [5].

In our experiments, we use T_H1 cytokines (TNF-α, IL-1β, and IFN-γ) to simulate the cytokines released by inflammatory cells during HP infection. We hypothesize that this combination of cytokines causes chief cell death. Our experiments aim to confirm this hypothesis, and to shed light on the mechanisms by which cytokines kill chief cells. One possibility is that the cytokines stimulate apoptosis of chief cells by upregulating the pro-apoptotic Bcl-x_S

isoform or by downregulating the anti-apoptotic Bcl-x_L isoform. Alternately, the cytokines may disrupt the integrity of the chief cell monolayer, possibly by altering the expression of tight junction proteins. Another possibility is that the cytokines kill chief cells through some combination of these two mechanisms. For example, a change in Bcl-x protein production may result in disruption of the chief cell monolayer, ultimately causing anoikis (detachment-induced apoptosis). Because Bcl-x expression and monolayer integrity are regulated by glucocorticoids, we also investigate the ability of dexamethasone (a synthetic glucocorticoid) to protect chief cells from cell death induced by cytokines.

2 Materials and Methods

2.1 Chief cell isolation and purification

Stomachs were surgically removed under heavy anesthesia from non-fasted rats weighing between 180 and 200 g. The pyloric region was cut, and the stomach was everted to expose the epithelium. After ligating both ends of the stomach and filling with protease solution, successive fractions of cells were collected every 30 minutes from buffer solutions that covered the epithelium and were maintained at 37°C in a shaking water bath. Early fractions containing surface cells, neck cells, and parietal cells were discarded. The final fraction contained mostly chief cells and was kept for further purification.

This chief cell fraction was pelleted by centrifugation, resuspended in buffer, filtered, and then subjected to centrifugal elutriation at 40 ml/minute to eliminate the non-chief cell population. The elutriated chief cell fraction was then further purified by density gradient centrifugation. For this, the suspension of chief cells was transferred to tubes that contained a linear 40–80% density gradient of Accudenz (Accurate Chemical, Westbury, NY) and centrifuged at 500×g for 10 minutes. Because chief cells have the highest density of all gastric cells, they were collected from the bottom of the linear density gradient tubes,

washed in buffer, pelleted by centrifugation, and resuspended in a 1:1 mixture of Ham's F12 and Dulbecco's Modified Eagle medium supplemented with heat-inactivated 10% fetal bovine serum, antibiotics, insulin, and hepatocyte growth factor. Cells were counted using a hemocytometer, and 1.6×10^6 cells/ml were plated. Hydrocortisone or dexamethasone, both commercially available glucocorticoids, were also added in some studies as described below.

2.2 Chief cell culture and treatment

Transwell plates and 6-cm circular dishes, both coated with type 1 collagen, were used to culture cells. Cells grown for 96 hours to achieve a confluent monolayer; cell culture medium was replaced on a regular basis. Three experiments were done with cultured chief cells.

2.2.1 Glucocorticoids and chief cell survival

To assess the effect of glucocorticoids on cell viability, barrier properties, and Bcl-x protein production in the absence of cytokine challenge, chief cell cultures were given the following glucocorticoid treatments:

a) no glucocorticoid; b) 10^{-9} M dexamethasone; c) 10^{-6} M dexamethasone; d) 1 μg/ml hydrocortisone.

2.2.2 Chief cell survival in the presence of $\mathbf{T_H1}$ cytokines

Using an *in vitro* assay to assess the effect of inflammation during HP infection on cell viability, barrier properties, and Bcl-x protein production, chief cell cultures were treated with or without a cytokine mix (cytomix) containing 10 ng/ml each of TNF-α, IL-1β, and IFN-γ. A dose dependence was tested by varying the percentage of the cytomix used to treat cell cultures:

a) no cytomix; b) 10% cytomix; c) 30% cytomix; d)100% cytomix.

2.2.3 Chief cell survival with cytokines in the presence of glucocorticoids

To investigate the ability of glucocorticoids to protect against chief cell death induced by the T_H1 cytomix, chief cell cultures were treated in a dose-dependent manner both with the cytomix and with dexamethasone as described below:

a) 10^{-9} M dexamethasone and 10% cytomix; b) 10^{-9} M dexamethasone and 30% cytomix; c) 10^{-9} M dexamethasone and 100% cytomix; d) 10^{-6} M dexamethasone and 10% cytomix; e) 10^{-6} M dexamethasone and 30% cytomix; f) 10^{-6} M dexamethasone and 100% cytomix.

To investigate the effects of dexamethasone and cytokine treatments on the monolayer integrity and barrier properties of chief cells, the transepithelial electrical resistance (TER) was measured in the Transwell plates at 0, 6, 12, 24, 30, 36, and 48 hours following each treatment. After 48 hours of treatment, the cell cultures in the Transwell plates were used for the cell viability assay.

2.3 Cell viability assay

The cell viability assay was used to determine the relative numbers of living, viable cells following the glucocorticoid and cytokine treatments. For each group of treated cells, the cell culture medium in Transwell plates was aspirated. The cells were washed with phosphate-buffered saline and fixed with ice-cold 100% methanol at 4°C for 15 minutes. The cells were stained with 0.1% crystal violet solution, incubated at room temperature for 5 minutes, washed with warm tap water.

After drying overnight, Transwells were then treated with 0.5% sodium dodecyl sulfate (SDS) solution and agitated for 30 minutes at room temperature to facilitate cell lysis and elaboration of the stain into solution. Lysates were transferred to wells on a 96-well plate, diluted with SDS, and the absorbances at 590 nm and 650 nm were determined by spectroscopy. Stain intensity, and thus cell viability, was determined by subtracting the

absorbance readings.

2.4 SDS-PAGE and Western blotting

Using the 6-cm dishes of cultured chief cells, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were used to determine the relative expression of Bcl-x following glucocorticoid and cytokine treatments, as described in Section 2.2. Chief cells were washed and treated with Radio-Immunoprecipitation Assay (RIPA) Buffer containing proteolytic inhibitors to enable cell lysis and protein solubilization while preventing degradation of individual polypeptides. The cells were scraped, sonicated to disrupt cell integrity, and cleared of cell debris by centrifugation. The supernatant was collected, and overall protein concentration of each sample was determined using the bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL).

Protein samples were separated by SDS-PAGE. Equal amounts of each protein sample (10 μg) were mixed with gel buffer containing 2-mercaptoethanol which reduces (breaks) disulfide bonds. The samples were loaded onto a polyacrylamide gel (12.5% base and 4.0% stack) and allowed to run for 35 minutes with a current of 200 A.

The resulting gels were electroblotted onto nitrocellulose membranes for 60 minutes at 15 V, and the presence of protein was confirmed by Ponceau staining. Each blot was blocked overnight at 4°C in a solution containing 5% nonfat dry milk to block non-specific binding sites. Blots were incubated for 2 hours at room temperature with the primary antibody in 1% nonfat milk solution. Blots were then washed and incubated for 1 hour at room temperature with the secondary antibody in 1% nonfat milk solution. The primary antibody used was rabbit anti-Bcl-$x_{S/L}$ L-19 (1:500) (Santa Cruz Biotechnology, Santa Cruz, CA). The secondary antibody used was goat anti-rabbit horseradish peroxidase (HRP)-conjugated antibody (1:2000). Blots were developed using the Enhanced Chemiluminescence (ECL) Kit (Amersham, Arlington Heights, IL), and the bands visualized by autoradiography using Blue

Bio Film (Denville Scientific, South Plainfield, NJ).

3 Results

3.1 Glucocorticoids upregulate Bcl-x_L expression in cultured chief cells

The synthetic glucocorticoid dexamethasone increased the expression of the anti-apoptotic Bcl-x_L isoform in chief cells (Figure 2). As the gel shows in lane 1, cultured chief cells express Bcl-x_L constitutively after withdrawal of glucocorticoid treatment for 48 hours. Thus, the fetal bovine serum used to culture the chief cells maintains the constitutive expression of Bcl-x_L for at least 48 hours. However, the addition of dexamethasone to chief cell cultures significantly increased the expression of Bcl-x_L in a dose-dependent manner, as shown in lanes 2 and 3. Bcl-x_L expression in chief cells increased significantly with 10^{-9} M dexamethasone (lane 2), and increased even further with 10^{-6} M dexamethasone (lane 3). Chief cells did not constitutively express the pro-apoptotic Bcl-x_S isoform, and glucocorticoids did not stimulate Bcl-x_S expression (Figure 2).

3.2 Chief cell viability and TER do not depend on glucocorticoid concentration in the absence of cellular stress

Dexamethasone treatment, which upregulated Bcl-x_L expression (Figure 2), did not affect chief cell viability over 48 hours (Figure 3). Hydrocortisone (1 μg/ml) was used as a control, because it is originally added to cultured chief cells to allow them to reach confluence and maturity prior to the differential 48-hour glucocorticoid treatments. As Figure 3 shows, treatment with dexamethasone (known to be about 40 times as potent as hydrocortisone) did not increase cell viability relative to treatment with hydrocortisone. Thus, in the absence

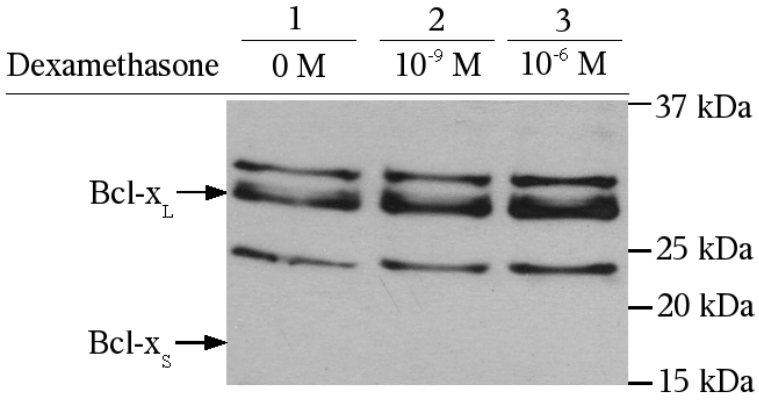

Figure 2: This Western blot shows that Bcl-x_L expression increases as glucocorticoid concentration increases. The thick band is Bcl-x_L, while the two other thin bands represent contamination. Bcl-x_S is not expressed in any lane.

of cellular stress, glucocorticoids with high potency and present in high concentrations were not required to keep chief cells alive.

In the absence of cellular stress, glucocorticoids did not affect TER to a large extent (Figure 4). From 24 to 48 hours following treatment, no changes in chief cell TER greater than 500 $\Omega \cdot cm^2$ were found for any glucocorticoid treatment (either hydrocortisone or dexamethasone). These results indicate that, in the absence of cellular stress, integrity of the chief cell monolayer was preserved for at least 48 hours regardless of glucocorticoid potency and concentration.

3.3 T_H1 cytokines reduce chief cell TER and viability

The cytomix was shown to decrease both chief cell TER and chief cell viability in a dose-dependent manner (Figures 5 and 6). When TER was measured in control chief cells that were not treated with the cytomix, TER remained stable over 48 hours. Chief cells treated with the cytomix maintained a relatively stable TER for 24 hours following treatment. However, after 48 hours, there was a dose-dependent decrease in TER. Thus, after 48 hours,

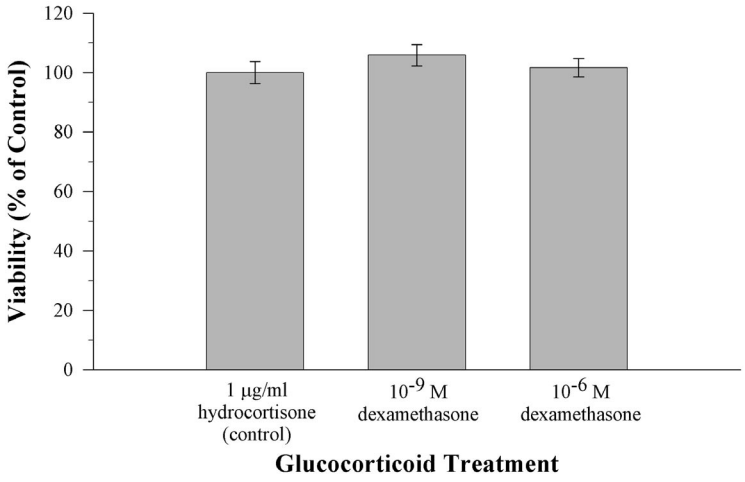

Figure 3: This graph shows chief cell viability in response to glucocorticoid treatments. Relative to hydrocortisone, dexamethasone (which is 40 times as potent) did not affect chief cell viability. Error bars in this and all subsequent graphs show standard error.

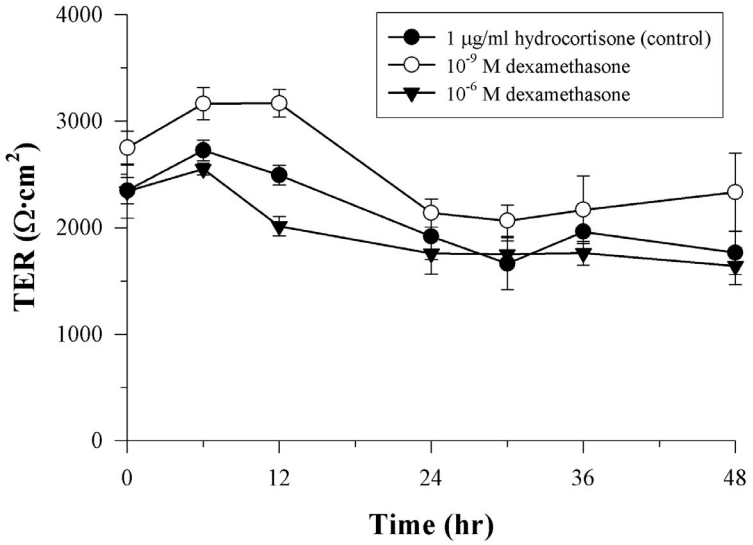

Figure 4: This graph shows the effect of different glucocorticoid treatments on TER. After an initial increase in TER in all groups, dexamethasone at 10^{-9} M or 10^{-6} M did not affect TER.

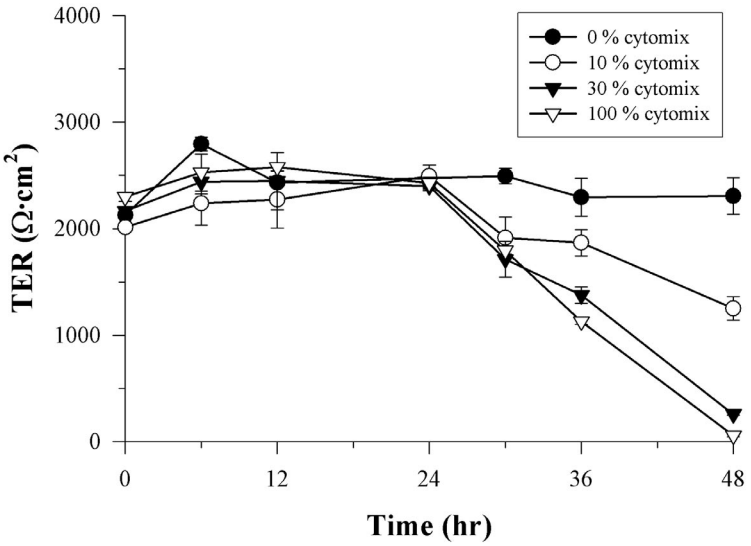

Figure 5: This graph shows the effect of increasing cytomix concentrations on chief cell TER. The cytomix decreased TER in a dose-dependent manner by 48 hours after addition to chief cell cultures.

our data suggests that cytokines disrupted the barrier properties of the chief cell cultures.

To determine whether the reduction in TER correlates with changes in viability, we measured chief cell viability in response to the cytomix and showed that the cytomix decreased chief cell viability in a dose-dependent manner (Figure 6). After treatment for 48 hours, the number of viable chief cells was reduced by 20% with 10% cytomix, 40% with 30% cytomix, and 50% with 100% cytomix. Although there is correlation between TER and survival data, it was of interest that TER was affected far more than viability after 48 hours of treatment (compare Figures 5 and 6). For instance, TER was reduced nearly to 0 with the 100% cytomix, but viability was only reduced by 50%.

3.4 Glucocorticoids protect chief cells from the effects of cytokines

When chief cells were treated with both dexamethasone and T_H1 cytokines, dexamethasone was able to protect chief cells from the effects of the cytokines on both TER and viability

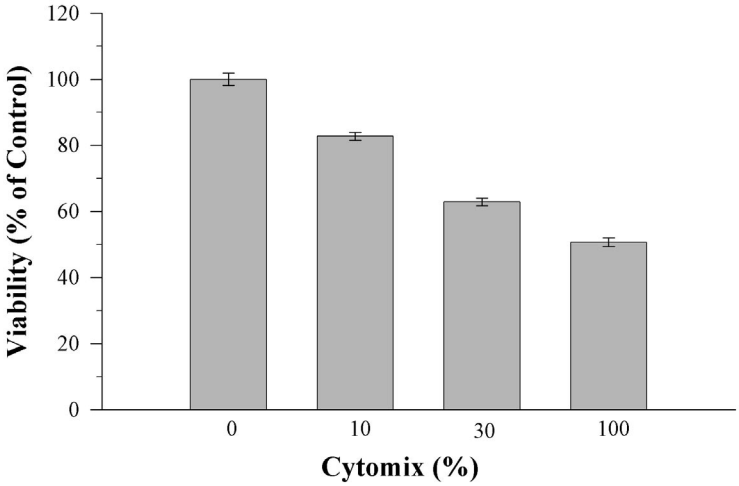

Figure 6: This graph shows the effect of increasing cytomix concentrations on chief cell viability. The cytomix decreased viability in a dose-dependent manner.

(Figures 7 and 8). Shown in Figure 7, both 10^{-9} M (A) and 10^{-6} M (B) dexamethasone were able to prevent reduction in TER over 48 hours in chief cells treated with all dilutions of the cytomix. Only in chief cells treated with 100% cytomix was dexamethasone unable to completely prevent the decrease in TER (Figure 7). These results indicate that glucocorticoids can effectively prevent a cytokine-induced decrease in TER.

Similar to results with TER, dexamethasone also protected chief cells from cytokine-induced death (Figure 8). However, neither 10^{-9} M (A) nor 10^{-6} M (B) dexamethasone were able to exert complete protection against the cytomix. Even with dexamethasone present, the 100% cytomix decreased the number of viable chief cells by about 30% (relative to chief cells not treated with cytokines). However, this is less than the 50% decrease in chief cell viability caused by the 100% cytomix in the absence of dexamethasone.

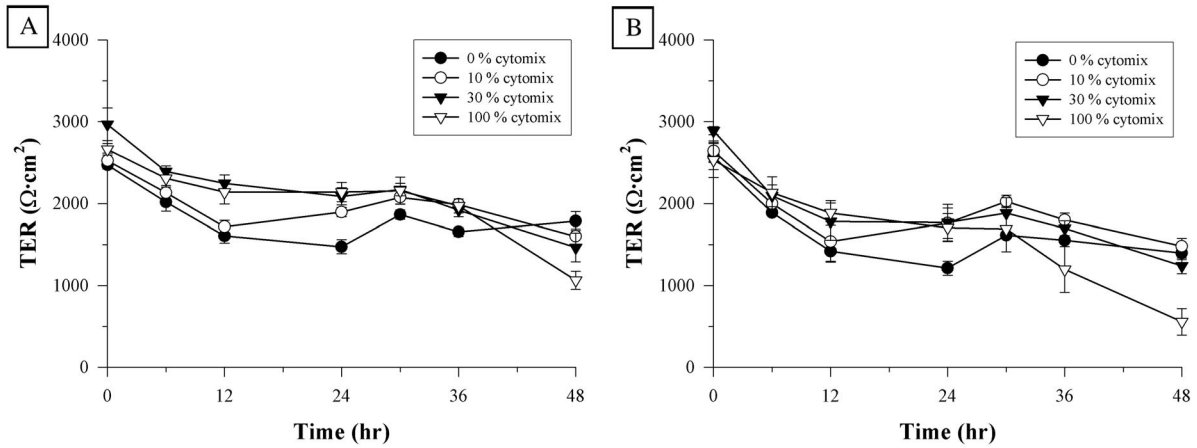

Figure 7: These graphs reveal the ability of 10^{-9} M (A) and 10^{-6} M (B) dexamethasone to protect chief cells from cytokine-induced decrease in TER.

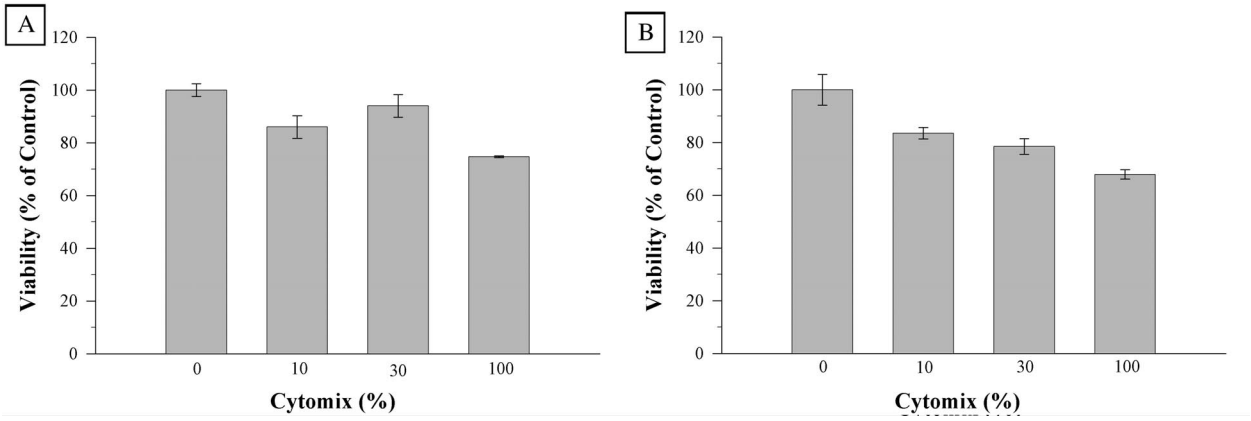

Figure 8: These graphs reveal the ability of 10^{-9} M (A) and 10^{-6} M (B) dexamethasone to preserve cell viability even in the presence of cytokines.

4 Discussion

4.1 Glucocorticoids regulate Bcl-x_L expression in chief cells

Our results showed that dexamethasone, a synthetic glucocorticoid, increased Bcl-x_L expression in chief cells in a dose-dependent manner. We propose that glucocorticoids bind to the intracellular glucocorticoid receptor (GR) in chief cells, which acts as a transcription factor by binding to hormone response element (HRE)-like sequences on one of the five promoters of the *bcl-x* gene. Because Viegas *et al.* [26] identified two HRE-like sequences on the extended P4 promoter, we hypothesize that chief cells use the P4 promoter to induce expression of Bcl-x_L.

That chief cells respond to glucocorticoids suggests the presence of a GR receptor. However, Kanemasa *et al.* [11] showed that GR receptors are found only in parietal cells in the gastric mucosa. This result is confusing in light of our present data and data showing that adrenalectomy, or reduction of glucocorticoids, significantly impacts chief cell development [24]. Further studies using RU 38486, a GR receptor antagonist, would be required to prove that the response we see to endogenous dexamethasone is specific and acting at GR receptor to increase Bcl-x_L expression in chief cells.

4.2 Chief cells do not require glucocorticoids to survive in absence of cellular stress

Our results showed that, in the presence of serum, addition of glucocorticoid is not required to maintain chief cell survival or TER for 48 hours in culture. We show that chief cells constitutively express Bcl-x_L and, even when glucocorticoids are added to increase chief cell production of Bcl-x_L, chief cell survival is not altered. Thus, constitutively-expressed Bcl-x_L is sufficient to maintain chief cell survival for 48 hours in the absence of cellular

stress. Consistent with our results, the findings of Boise *et al.* [2] show that increased Bcl-x_L expression is important only in the face of cellular stress.

4.3 Cytokines kill chief cells: possible mechanisms

Our data clearly show that T_H1 cytokines decrease chief cell TER and viability in a dose-dependent manner. In this respect, chief cells are unique in comparison to other cells of the gastrointestinal tract. For example, Clayburgh *et al.* [5] found that T_H1 cytokines affected tight junctions between intestinal cells, resulting in barrier defects, but did not reduce the viability of epithelial cells.

One possible mechanism for cytokine-induced chief cell death involves the upregulation of Bcl-x_S by the NF-κB protein family. NF-κB proteins are known to translocate to the nucleus in response to cytokine-induced stress [23]. NF-κB proteins function as transcription factors, and regulate the *bcl-x* gene through an NF-κB binding sequence on the *bcl-x* promoter [12]. While some studies [25] have shown that NF-κB increases production of Bcl-x_L, others [7] have implicated NF-κB in the upregulation of Bcl-x_S. Thus, we propose that cytokines cause NF-κB proteins to localize to the nucleus of chief cells, where they increase production of Bcl-x_S which kills the cells. This mechanism requires further investigation: it remains to be shown that cytokines result in localization of NF-κB proteins to the nucleus and in increased expression of Bcl-x_S.

4.4 Glucocorticoids protect chief cells from cellular stress

In the absence of cellular stress, glucocorticoids had little effect on chief cell barrier properties or viability. However, in the presence of cytokine-induced cellular stress, glucocorticoids preserved the chief cell barrier and kept chief cells alive.

As previously discussed, glucocorticoids increase Bcl-x_L expression in chief cells in a dose-

dependent manner. While this increase in Bcl-x_L was not required for chief cell survival in the absence of cellular stress, we propose that the increase in Bcl-x_L is required for chief cell survival in the presence of cellular stress (such as T_H1 cytokines). Thus, the glucocorticoid-regulated increase in Bcl-x_L expression may explain the ability of glucocorticoids to protect chief cells from cytokine-induced death.

Furthermore, glucocorticoids are known to upregulate tight junction proteins such as occludin. While this upregulation of occludin is not necessary to preserve chief cell tight junctions in the absence of cellular stress, it may be necessary to protect chief cell tight junctions and barrier properties from the damaging effects of cytokines.

Of course, Bcl-x_L and occludin are not the only proteins regulated by glucocorticoids. It is possible that glucocorticoids also upregulate other protective proteins which help protect chief cells from the stress of cytokines.

4.5 Cytokines overwhelm glucocorticoids in *H. pylori* infection

HP infection triggers an inflammatory response leading to the release of proinflammatory T_H1 cytokines. Nonetheless, glucocorticoids are constitutively secreted by the adrenal glands. Based on our results, we might expect these glucocorticoids to protect chief cells from cytokine-induced cell death. However, this is clearly not the case, as it is known that chief cells are deleted during HP infection. We propose two possible mechanisms by which cytokines may overwhelm the ability of glucocorticoids to protect chief cells during HP infection.

The first mechanism is simple: the cytokines released during HP infection may be present in a concentration that is too high for the protective effects of glucocorticoids to be significant. This mechanism is consistent with our results, which show that neither 10^{-9} M dexamethasone nor 10^{-6} M dexamethasone were able to completely protect chief cells when exposed to the 100% cytokine mix. Thus, glucocorticoids cannot completely protect against

high concentrations of cytokines. As a result, chief cells are deleted in large numbers during HP infection.

As a second potential mechanism, HP infection may interfere with a step in the pathway by which glucocorticoids protect chief cells from tight junction damage and cell death. A study by Peltier *et al.* [19] found that transforming growth factor (TGF)-β1, via Smad 2/3 and AP1 transcription factor activation, increases the expression of intracellular glucocorticoid receptor in inflammatory cells. Interestingly, Monteleone *et al.* [15] recently showed that HP, via IFN-γ, induced Smad 7 and significantly reduced Smad 3 activity. As a result, chief cell expression of glucocorticoid receptor would decrease, and this would decrease chief cell responsiveness to glucocorticoids. Thus, glucocorticoids would not be able to increase production of the anti-apoptotic Bcl-x_L protein. Nonetheless, the pro-apoptotic Bcl-x_S protein might still be produced via the glucocorticoid-independent pathway involving cytokines and NF-κB. In the absence of glucocorticoid receptor, glucocorticoids would be unable to protect chief cells from tight junction damage and cell death.

While these two mechanisms are very different and highly speculative, they both explain how constitutively expressed glucocorticoids may be unable to protect chief cells from cytokines during HP infection.

5 Conclusion

In this paper, we demonstrated that cytokines kill chief cells. We also showed that glucocorticoids can protect chief cells from cytokine-induced death. We identified glucocorticoid-induced upregulation of Bcl-x_L expression and preservation of monolayer integrity as two possible protective mechanisms. Finally, we proposed two mechanisms to explain how the ability of glucocorticoids to protect chief cells from cytokines may be overwhelmed during HP infection.

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