Immune-enhancing effects of endogenous glucocorticoids on gastric macrophages contribute to the development of gastric inflammation and metaplasia

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Short title: Immune-enhancing effect of corticoids on gastric macrophages

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Infection of the gastric mucosa with *Helicobacter pylori* is considered the major risk factor for gastric cancer, which remains a significant cause of cancer-related mortality worldwide. Gastric macrophages contribute to the development of gastric cancer by promoting chronic gastric inflammation and oxidative stress, consistent with a typical M1 macrophage phenotype (1). Gastric macrophages also contribute to gastric tumor progression following polarization to an M2 macrophage phenotype that can that enhance angiogenesis and epithelial cell proliferation (1). However, the factors that control macrophage polarization and function in the gastric environment, particularly in the context of *H. pylori* infection are still not fully understood.

Glucocorticoids are a group of steroid hormones produced by the adrenal gland that are widely recognized for their anti-inflammatory potential (2). Glucocorticoids are recognized by the glucocorticoid receptor (GR), which acts as a transcription factor and transcriptional modulator, or through non-genomic mechanisms, leading to the regulation of numerous cellular signaling networks. Glucocorticoids block inflammation by interfering with pro-inflammatory signaling pathways downstream of pattern recognition receptors (e.g., NF-κB) and T cell receptors (e.g., AP-1), by inhibiting synthesis of eicosanoids, cytokines, and chemokines, and by blocking leukocyte recruitment. Because of these broad anti-inflammatory actions, synthetic corticoids are one of the most widely used groups of anti-inflammatory drugs in both human and veterinary medicine, with applications in allergic, autoimmune, and inflammatory diseases and in transplantation (2). Therefore, glucocorticoids also were expected to prevent gastritis. Indeed, a previous study by Dr. Busada's team showed that loss of endogenous glucocorticoids upon adrenalectomy, which removes all endogenous corticoids, led to spontaneous gastric inflammation followed by metaplasia in female mice, supporting an anti-inflammatory role for glucocorticoids in gastritis (3). Moreover, in adrenalectomized mice, depletion of monocytes and macrophages prevented spontaneous inflammation and metaplasia, indicating that gastric macrophages are important drivers of gastric inflammation (4).

In a new report from the Busada lab recently published in the *American Journal of Physiology*, Khadka et al. (5) analyzed the impact of macrophage-specific deletion of the glucocorticoid receptor (GR) on the inflammatory response to *Helicobacter* infection using a *LysM*^{cre} *Nr3c1*$^{flox/flox}$ mouse model (termed MyGRKO mice). These mice produce endogenous glucocorticoids at physiological levels, but their myeloid cells, which include monocytes, macrophages, and granulocytes, are unable to respond to the glucocorticoids through their cognate GR. Therefore, it was hypothesized that the MyGRKO mice would have an increased

inflammatory response to *H. pylori* infection. Indeed, increased expression of IL-1β and IL-6 was seen in peritoneal macrophages from MyGRKO mice stimulated with high dose LPS. However, reduced inflammatory responses were detected upon *H. pylori* exposure in several parallel lines of investigation. ATACSeq analysis of thyoglycolate-elicited peritoneal macrophages showed that co-administration of *H. pylori* led to significant chromatin reorganization with a strong upregulation of anti-inflammatory TGF-β signaling pathways in the MyGRKO mice compared to wild type controls. Likewise, RNASeq revealed a decreased induction of gene sets associated with cytokine signaling in MyGRKO compared to control mice. In a murine model of gastric *H. pylori* infection with the pre-mouse Sydney Strain 1 (PMSS1), recruitment of macrophages and T cells to the gastric mucosa was impaired in MyGRKO mice. Intriguingly, MyGRKO mice also showed a reduced development of precancerous lesions, i.e., gastric atrophy and metaplasia, in response to *H. pylori* infection, as evidenced by reduced parietal cell loss and reduced expression of metaplasia markers. A similar reduction in precancerous lesions was found in MyGRKO mice infected with the model pathogen *Helicobacter felis,* which induces severe inflammation, atrophy, and metaplasia in wild type mice (6). These results indicate that disruption of glucocorticoid signaling impairs macrophage activation in response to *Helicobacter* in the stomach, suggesting that the endogenous glucocorticoids enhanced macrophage activation. Surprisingly, no impact of GR deletion on gastric *Helicobacter* colonization levels was found, indicating that glucocorticoids regulate only select functions of gastric macrophages.

Although the majority of studies have focused on anti-inflammatory effects of glucocorticoids, the notion that glucocorticoids may have stimulatory effects in specific situations is not entirely novel. In a 2017 review paper, Cain and Cidlowski (2) stated that, "*there is evidence that glucocorticoids promote immune responsiveness under certain conditions, which presents an often overlooked challenge to the clinical dogma of glucocorticoids acting solely as immunosuppressive agents*". The effects of glucocorticoids are known to be highly pleotropic, regulating up to 20% of all human genes (7). Therefore, context-dependent effects can be expected.

In macrophages, the effects of corticosteroids appear to be dose-dependent, where high dose corticoids suppress, but low dose corticoids enhance inflammatory gene expression (8). Endogenous corticosteroid levels as in the study by Khadka (5) are likely to be low in situations where the experimental animals are not subjected to specific stressors. Also, the timing of the inflammatory challenge and the corticosteroid application appears to affect outcomes of corticosteroid stimulation. In a study on liver cells, corticosteroids had proinflammatory effects if they were administered before LPS challenge (9), consistent with an increased immune responsiveness in high stress situations. In Khadka's study, baseline levels of endogenous corticosteroids would have been present prior to the *H. pylori* challenge. The strength and location of the inflammatory insults also likely affect the outcome of GR signaling. In contrast to Khadka's study, two previous studies with MyGRKO mice supported a predominantly anti-inflammatory role for endogenous glucocorticoids in macrophages (10, 11). However, these studies involved models of sepsis induced by systemic LPS delivery. In a study by Kleiman et al., GR-mediated suppression of IL-1β signaling in macrophages protected mice from sepsis induced by LPS (11). Likewise, Bhattacharyya et al. showed that LPS application caused significantly increased mortality in MyGRKO compared to wild type mice, due to increased stimulation of proinflammatory signaling through p38 MAPK, JNK, ERK1/2, and Akt (10). A transcriptomics analysis of human macrophages exposed to glucocorticoids found that glucocorticoid treatment strengthened innate immune signatures associated with chemokine

signaling, but downregulated expression of MHC-II and co-stimulatory molecules that are involved in the activation of adaptive T cell responses (12). The decreased T cell recruitment to the *H. pylori*-infected gastric mucosa in MyGRKO mice described by Khadka et al. is consistent with the stimulatory effects of GR signaling on chemokine production and immune cell recruitment, but also indicates that the differential effects of GR signaling in the immune system do not simply split along the innate vs. adaptive immunity lines. Physiologically, increased systemic glucocorticoid levels signal stress, and the glucocorticoid-induced increase in chemokine, cytokine, and pattern recognition receptor expression may prime the immune system for a more rapid response upon infection in situations where the organism is vulnerable (2).

One other important finding was the reduction of gastric atrophy and metaplasia in the MyGRKO mice with both the *H. pylori* and the *H. felis* infection models, which suggests that endogenous corticosteroids can enhance cancer-inducing activities of macrophages. Gastric cancer and pre-cancerous lesions are driven by a combination of inflammatory mediators, particularly mutagenic reactive oxygen species, and the *H. pylori* virulence factor CagA (13). CagA is a bacterial oncoprotein that triggers activation of multiple carcinogenic signaling pathways after intracellular delivery via a type IV secretion system. Given that *H. felis* lacks CagA and that the PMSS1 strain of *H. pylori* commonly loses the ability to deliver CagA through mutations to the CagY gene in mice (14), it is likely that proinflammatory mediators including reactive oxygen species from macrophages and neutrophils were the major drivers of the pro-carcinogenic lesions in the MyGRKO model. A mostly pro-inflammatory pathway of gastric carcinogenesis may have amplified the impact of functional changes in the inflammation-promoting myeloid cells in this model.

Some open questions remain that may be the subject of future studies. While the peritoneal *H. pylori* challenge model was useful for obtaining sufficient cell numbers for sequencing studies, peritoneal macrophages differ from gastric macrophages in their development and function. Therefore, it would be of interest to confirm key findings such as the activation of anti-inflammatory TGF-β pathways with gastric macrophages from *Helicobacter*-infected mice. Also, it would be interesting to study the MyGRKO mice in a true gastric cancer model rather than in models of premalignant lesions. In contrast to the pro-inflammatory M1 macrophages that drive early gastric inflammation, M2 macrophages and anti-inflammatory tumor-associated macrophages predominate the gastric tumor environment (1). In this situation, lack of endogenous glucocorticoids could prevent M2 macrophage polarization, enabling more effective anti-tumor immune responses, which would be consistent with the common paradigm that glucocorticoids block inflammation. However, if lack of GR signaling in macrophages prevents the development of precancerous lesions, the MyGRKO mice may never develop cancer.

In summary, the recent publication on the role of macrophage regulation by glucocorticoids in *H. pylori* infection by Khadka et al. (5) contributes to a growing body of work that is shifting the paradigm that glucocorticoid responses are exclusively anti-inflammatory and anti-proliferative and points to a more nuanced and context-dependent role for glucocorticoid signaling in gastric pathogenesis. The findings from this study also suggest that macrophage GR signaling may have immunostimulatory effects beyond an elevated baseline cellular “preparedness” induced by stress that leads to a greater response to danger signals, since macrophage GR signaling was shown to impact both acute *H. pylori* and chronic *H. felis* infection as well as longtime sequelae of chronic tissue inflammation, i.e. gastric metaplasia.

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