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Macrophages Promote Progression of Spasmolytic Polypeptide-Expressing Metaplasia After Acute Loss of Parietal Cells

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BACKGROUND & AIMS: Loss of parietal cells causes the development of spasmolytic polypeptide-expressing metaplasia (SPEM) through transdifferentiation of chief cells. In the presence of inflammation, SPEM can advance into a more proliferative metaplasia with increased expression of intestine-specific transcripts. We used L635 to induce acute SPEM with inflammation in mice and investigated the roles of inflammatory cells in the development of SPEM. **METHODS:** To study the adaptive immune system, *Rag1* knockout, interferon-γ–deficient, and wild-type (control) mice received L635 for 3 days. To study the innate immune system, macrophages were depleted by intraperitoneal injection of clodronate liposomes 2 days before and throughout L635 administration. Neutrophils were depleted by intraperitoneal injection of an antibody against Ly6G 2 days before and throughout L635 administration. Pathology and immunohistochemical analyses were used to determine depletion efficiency, metaplasia, and proliferation. To characterize SPEM in each model, gastric tissues were collected and levels of *Cftr*, *Dmbt1*, and *Gpx2* mRNAs were measured. Markers of macrophage polarization were used to identify subpopulations of macrophages recruited to the gastric mucosa. **RESULTS:** Administration of L635 to *Rag1* knockout, interferon-γ–deficient, and neutrophil-depleted mice led to development of proliferative SPEM and up-regulation of intestine-specific transcripts in SPEM cells, similar to controls. However, macrophage-depleted mice given L635 showed significant reductions in numbers of SPEM cells, SPEM cell proliferation, and expression of intestine-specific transcripts, compared with control mice given L635. In mice given L635, as well as patients with intestinal metaplasia, M2 macrophages were the primary inflammatory component. **CONCLUSIONS:** Results from studies of mouse models and human metaplastic tissues indicate that M2 macrophages promote the advancement of SPEM in the presence of inflammation.

Keywords: Immune Depletion; Acute Injury; Gastric Cancer; CD68.

Gastric adenocarcinoma is the second highest cause of cancer-related death in the world.[1] Due to a lack of early clinical manifestations, gastric cancer frequently presents as late-stage disease. *Helicobacter pylori* infection is the major predisposing factor for gastric cancer, causing chronic inflammation and oxyntic atrophy in the gastric mucosa.[2] The parietal cell loss disrupts the homeostatic glandular environment and chief cells transdifferentiate into spasmolytic polypeptide expressing metaplasia (SPEM).[3,4] Increasing data suggest that intestinal metaplasia arises from SPEM in humans, supporting the hypothesis that SPEM is the critical initial preneoplastic metaplasia predisposing to gastric adenocarcinoma.[5–7]

Helicobacter felis infection in mice recapitulates the inflammatory and preneoplastic cascade of human *H pylori* infection.[3] In the murine *Helicobacter* infection model, SPEM develops after 6 to 12 months of infection. As in human infection with *H pylori*, there appears to be 2 phases in the development of metaplasia. First, the infection induces parietal cell loss or oxyntic atrophy. Oxyntic atrophy is required for the induction of metaplasia in humans.[8,9] In the presence of ongoing inflammation, metaplasia then evolves and expands. Given the long latency for SPEM development in *H felis*–infected mice, our group developed 2 acute SPEM models. The administration of either drugs DMP-777 or L635 induces SPEM by selectively ablating parietal cells.[10,11] Mice treated with DMP-777 for 14 days develop SPEM without the presence of significant inflammation, likely due to the ability of DMP-777 to also inhibit elastase activity.[10] In contrast, L635-treated mice develop an advanced proliferative SPEM with intestinal characteristics (previously designated as SPEM-IC) in just 3 days of treatment,[3,4] which is associated with both loss of parietal cells and a prominent inflammatory infiltrate. The phenotype of mice treated with L635 for 3 days is similar to that for mice infected with *H felis* for 6 months or more.[4] The L635 model appears to bypass the initial phases of *H felis* infection that leads to oxyntic atrophy by directly inducing parietal cell loss acutely. Although mice do not develop typical goblet cell intestinal metaplasia in either the L635 treatment or *Helicobacter* infection models, they do develop advanced

Abbreviations used in paper: **IFN, interferon; IFNγKO, interfon-γ knockout; IL, interleukin; KO, knockout; Rag1KO, Rag1 knockout; SPEM, spasmolytic polypeptide-expressing metaplasia; TNF, tumor necrosis factor.**

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proliferative SPEM that is characterized by the expression of specific up-regulated intestinal transcripts (*Cftr*, *Dmbt1*, and *Gpx2*) and an increase in proliferative SPEM cells.[4,12,13] Similarly, Varon et al observed an increase in intestinal characteristics in murine metaplasia associated with long-term *Helicobacter* infection.[14] Studies with DMP-777 treatment demonstrate that loss of parietal cells even without inflammation leads to development of SPEM from transdifferentiation of chief cells; however, the presence of inflammation in L635-treated mice leads to more rapid SPEM induction, as well as promotion of both increased proliferation and a more intestinalized phenotype.[4] Inflammation is a key factor in the advancement of SPEM to a more aggressive metaplastic phenotype. The precise immune cell populations responsible for the progression of metaplasia are not known.

The following 4 distinct inflammatory cell populations are most frequently associated with *Helicobacter* infection in the stomach: B cells, interferon (IFN)-γ–secreting T cells, neutrophils, and macrophages.[15] Through the manipulation of specific immune cells, previous studies have shown that T cells contribute to parietal cell loss and development of metaplasia in *Helicobacter* infection.[16] However, chronic inflammation associated with *Helicobacter* infection is predominately made up of neutrophils and macrophages. These phagocytic cells migrate into the mucosa to engulf debris and propagate the inflammatory response.[17] Similarly, during acute induction of SPEM with L635, there is a significant influx of T cells, B cells, neutrophils, and macrophages that migrate into the mucosa.[3] Still, little is known about which immune cells promote the advancement of SPEM.

In the present studies, we have sought to assess the influence of specific immune cell populations on the advancement of SPEM after the induction of parietal cell loss. To address the specific immune components, we evaluated the presence and characteristics of L635-induced SPEM in various mouse models of depleted immune cells. Rag1 knockout mice (Rag1KO) deficient in T cells and B cells, IFN-γ knockout mice (IFNγKO), neutrophil-depleted mice (Ly6G antibody-treated), and macrophage-depleted mice (clodronate-treated) were each administered L635 to induce acute parietal cell loss and SPEM. Our findings indicated that M2 macrophages are the critical immune cell driver of the induction of metaplasia after loss of parietal cells.

Methods

Treatment of Animals

L635 treatment. Each experimental group consisted of 3 male mice. L635 (synthesized by the Chemical Synthesis Core of the Vanderbilt Institute of Chemical Biology), dissolved in deionized DNA and RNA-free water, was administered by oral gavage (350 mg/kg) once a day for 3 consecutive days. Neutrophils were depleted through intraperitoneal injection of anti-Ly6G antibody (100 μg, Leaf; BioLegend, San Diego, CA) 2 days before and throughout the 3-day L635 administration. Control mice received intraperitoneal injections of a nonspecific isotype-matched IgG antibody. Macrophages were depleted by intraperitoneal injection of clodronate-containing liposomes (10 mg/kg; Encapsula NanoSciences, Brentwood, TN) 2 days before and throughout the 3 days of L635 administration. Control mice received liposomes alone (10 mg/kg). Mice were sacrificed on the third day of L635 administration.

DMP-777 treatment. Three male mice were used for each experimental group. DMP-777 (a gift from DuPont-Merck Co.) dissolved in 1% methylcellulose was administered by oral gavage (350 mg/kg) once a day for 8 consecutive days. Macrophages were depleted using 4 intraperitoneal injections of clodronate-containing liposomes (10 mg/kg) every other day of DMP-777 treatment. Control mice received liposomes (10 mg/kg) with or without DMP-777-treatment. Mice were sacrificed the ninth day.

For detailed methods, see the Supplementary Material.

Results

Rag1 and Interferon-γ Knockout Mice Develop Acute Proliferative Spasmolytic Polypeptide-Expressing Metaplasia

To determine the role of the adaptive immune system in the development of proliferative SPEM, wild-type, Rag1KO, and IFNγKO mice were administered L635 for 3 days and stomach cell lineages were analyzed. Histologic examination revealed parietal cell loss and significant inflammatory infiltration in the fundus of the stomach in all L635-treated mice (Supplementary Figure 1). Upon L635 treatment, neutrophils increased by 6-fold in wild-type mice, with no significant difference observed in L635-treated Rag1KO or IFNγKO mice (Supplementary Figure 2*A* and *C*). F4/80-positive cells increased 3- to 5-fold in wild-type, Rag1KO, and IFNγKO L635-treated mice compared with untreated mice (Supplementary Figure 2*B* and *C*). L635-treated Rag1KO and IFNγKO mice did not have a significant change in F4/80-positive cells compared with wild-type L635-treated mice (Supplementary Figure 2*C*). L635-treated Rag1KO and IFNγKO mice developed SPEM (as defined by intrinsic factor and GSII-lectin co-positive cells),[18] similar to L635-treated wild-type mice (Figure 1*A* and *C*). In addition, L635-treated Rag1KO and IFNγKO mice showed a 40- to 50-fold increase in SPEM cell proliferation similar to wild-type L635-treated mice (as defined by Ki67, gastric intrinsic factor, and GSII-lectin triple-positive cells) (Figure 1*B* and *C*). To determine whether Rag1KO or IFNγKO mice develop advanced proliferative SPEM, the expression of SPEM-associated intestinal transcripts was analyzed by quantitative real-time polymerase chain reaction (Figure 1*D*). *Cftr*, *Dmbt1*, and *Gpx2* expression was significantly up-regulated (55-fold, 30-fold, and 7-fold, respectively) in L635-treated Rag1KO mice (Figure 1*D*). L635-treated IFNγKO mice showed similar changes in *Cftr*, *Dmbt1*, and *Gpx2* expression comparable with changes observed in L635-treated wild type (Figure 1*D*). These results all indicate that the presence of T cells and B cells or IFNγ is not necessary for the development of advanced proliferative SPEM after acute parietal cell loss.