IEX-1 Deficiency Protects against Colonic Cancer
Irina V. Ustyugova, Liang Zhi, Joel Abramowitz, Lutz Birnbaumer, and Mei X. Wu

Abstract
The immediate early response gene X-1 (IEX-1) is involved in regulation of various cellular processes including proliferation, apoptosis in part by controlling homeostasis of reactive oxygen species (ROS) at mitochondria. The present study shows reduced inflammatory responses and colorectal cancer in IEX-1 knockout (KO) mice treated with azoxymethane/dextran sulfate sodium (DSS). However, DSS induced worse colitis in RAG<sup>−/−</sup> IEX-1<sup>−/−</sup> double KO mice than in RAG and IEX-1 single KO mice, underscoring an importance of T cells in IEX-1 deficiency-induced protection against colon inflammation. Lack of IEX-1 promoted the differentiation of interleukin (IL)-17-producing T cells, concomitant with upregulation of G<sub>0</sub>/G<sub>1</sub> expression, a gene that is well-documented for its role in the control of inflammation in the colon. In accordance with this, T-helper 17 (T<sub>H17</sub>) cell differentiation was compromised in the absence of G<sub>0</sub>/G<sub>1</sub>, and deletion of G<sub>0</sub>/G<sub>1</sub> in T cells alone aggravated colon inflammation and colorectal cancer development after azoxymethane/DSS treatment. Null mutation of IEX-1 also enhanced both proliferation and apoptosis of intestinal epithelial cells (IEC) after injury. A potential impact of this altered IEC turnover on colon inflammation and cancer development is discussed. These observations provide a linkage of IEX-1 and G<sub>0</sub>/G<sub>1</sub> expression in the regulation of TH17 cell differentiation and suggest a previously unappreciated role for IEX-1 in the control of colon epithelial homeostasis.

Introduction
Prolonged intestinal inflammation or chronic inflammatory bowel disease (IBD) often results in colorectal cancer development, the second most common cancer in the developed countries, responsible for estimated 51,370 deaths in the United States in 2010 alone (refs. 1, 2; www.cancer.gov). Chronic IBD is characterized by a mucosal damage and ulceration, involving rectum and colon (3). It manifests as bloody mucoid, diarrhea, concomitant with DNA damage, epithelial dysplasia, and intestinal inflammation, followed by episodes of disease inactivity. Ulcerative colitis can be induced by oral administration of dextran sulfate sodium (DSS) to rodents, which mimics human colonic inflammation occurring in the entire colon (4).

When repetitive DSS administration was combined with a procarcinogen, azoxymethane, a nearly 100% incidence of carcinoma was induced in the colon, arguing strongly a connection between recurrent inflammation and cancer development (5, 6).

The NF-kB is known as a master inflammatory mediator and controls the expression of many genes linked to inflammation. One of its targets is the immediate early response gene X-1 (IEX-1) that is known for its involvement in the control of cell growth, apoptosis, and intracellular reactive oxygen species (ROS) homeostasis (7, 8). Its expression can be quickly induced, in response to a variety of stressors, in epithelial, vasculature, and endocrine tissues, as well as inflammatory cells (9). Intracellularly, IEX-1 is localized to the endoplasmic reticulum, nuclei, and mitochondria. In mitochondria, it targets the F1F0-ATPase inhibitor (IF1) for degradation, resulting in elevated F1F0-ATPase activity and reduced mitochondrial ROS (mROS) production (8). In contrast, its null mutation increases mROS production that appears to augment T-helper 17 (T<sub>H17</sub>) cell differentiation and protect against DSS-induced colitis (10). The protective role of interleukin (IL)-17A is consistent with its ability to fortify tight junctions between intestine epithelial cells (11) and its neutralization with anti-IL-17A antibody worsening DSS-induced colitis in mice (10, 12).

The present investigation shows that null mutation of IEX-1 prevents mice from not only colitis but also cancer development after DSS or azoxymethane/DSS treatment. The prevention was primarily attributed to altered
differentiation or function of T cells, in part by upregulation of Gαi2 expression. Accordingly, deletion of Gαi2 in T cells alone aggravated colon inflammation and tumor formation. Although IEX-1–deficient animals also displayed a significantly higher level of proliferation and apoptosis in intestinal epithelial cells (IEC) than wild-type IECs, the fast turnover of IECs might exert both beneficial and detrimental effects on colon inflammation and cancer development and it might not be as critical as T cells in IEX-1 deficiency-mediated protection against colitis or colon cancer development.

Materials and Methods

Animals

IEX-1 knockout (IEX-1 KO) and wild-type control mice on the mixed 129Sv/C57BL/6 background (F1) were generated by gene targeting (13). Gαi2–floxed (Gαi2fl/fl) mice in which loxP sites were introduced by homologous recombination before exon 2 and after exon 4 of the Gna12 gene were generated as detailed in Supporting Materials. To specifically delete Gαi2 in T cells, Gαi2fl/fl–CD4-Cre mice were made by backcrossing Gαi2–floxed mice on the mixed 129Sv/C57BL/6 background (F1) with CD4-Cre transgenic mice (C57BL/6NTacTgN/CD4-Cre, line # 4196) for 3 generations (F3) and then used to generate CD4-Cre, Gαi2fl/fl, and Gαi2fl/fl–CD4-Cre littermates for the study. CD4-Cre mouse breeding pairs were distributed by Taconic under NIAID contract N01-AO-02740 (14). IEX-1–/–, Rag1–/– double KO mice on C57BL/6 background were generated by cross-breeding IEX-1–deficient C57BL/6 mice with Rag1–deficient C57BL/6 animals (Jackson laboratory). All mice were housed in conventional cages at the animal facilities of Massachusetts General Hospital in compliance with institutional guidelines. The Institutional Animal Care and Use Committee for Massachusetts General Hospital approved all animal experiments.

Colitis and tumor induction

Acute colitis was induced in the mice by DSS treatment (15), which is a commonly used experimental model of IBD (16). Briefly, mice at 8 to 10 weeks of age were given 3.5% DSS (molecular weight, 36,000–50,000; MP Bio-medicals) in drinking water for 9 days, followed by 5 days of regular drinking water. The mice were weighed daily, and the value was expressed as a percentage of a body weight decrease relatively to the initial body weight on day 0. Colon cancer was induced by a combination of azoxymethane with DSS following a published protocol (17). The mice were first injected intraperitoneally (i.p.) with 12.5 mg/kg azoxymethane (Sigma), followed by 3 cycles of DSS ingestion. The first cycle was administrated 5 days after azoxymethane injection and consisted of 5 days of 2.5% DSS ingestion followed by 16 days of regular water. The second cycle was identical to the first one. The third cycle consisted of 4 days of 2% DSS followed by 10 days of regular water. The animals were sacrificed on the final day of the protocol, and colons were removed for analysis.

Morphologic and histologic analysis

The treated or untreated control animals were sacrificed at indicated times. Their colons were removed, opened longitudinally, rinsed free of feces with PBS, pinned to the surface of a board at both ends to maintain a linear shape, and fixed in 10% formalin neutral buffer solution. On the following day, the colons were photographed at high resolution and tumors were counted in each individual colon. The colon samples were then embedded in paraffin, cut at 5 μm, and stained with hematoxylin and eosin (H&E).

Bromodeoxyuridine staining

Mice were i.p. injected with 1 mL (1 mg/mL) bromodeoxyuridine (BrdUrd) and sacrificed 2 hours later. Colons were removed, fixed, and sectioned as earlier. BrdUrd staining was carried out using a Zymed BrdU labeling kit (Zymed) as per the manufacturer’s instructions. BrdUrd-positive cells were counted in a total of 75 crypts in each group, and an average number of positive cells per crypt was presented.

Apoptosis in IECs

To measure apoptosis in IECs, IEX-1 KO and wild-type littermates at 8 to 10 weeks of age were given 3.5% DSS in drinking water for 3 days. The mice were sacrificed and their distal colons were dissected, flushed to remove feces with cold Ca2+– and Mg2+–free Hank’s buffer containing 100 U/mL penicillin, 100 μg/mL streptomycin sulfate, and 25 μg/mL gentamicin. The colons were opened longitudinally and cut laterally into small pieces. The colon pieces were then incubated at 4°C for 1 hour in 50-mL tube containing cold Hank’s buffer supplemented with 0.02% EDTA and 10 mmol/L dithiothreitol (DTT) and then vortexed for 2 minutes to dislodge IECs. The vortex procedure was repeated once after collecting the cells released from the colons. The resultant cells were pooled and layered over a 5%, 15%, 40% discontinuous Percoll gradient in Hank’s buffer followed by centrifugation for 30 minutes at 560 × g at 4°C. Epithelial cells were recovered at the interface between 5% and 15% Percoll, washed, and collected for intracellular staining with epithelial-specific antibody against cytokeratin (pan-Cytokeratin, C-11; Santa Cruz Biotechnology) and an In Situ Cell Death Detection Kit (terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling, TUNEL) according to the manufacturer’s instruction (Roche Applied Science). The stained cells were evaluated on FACSARia and the flow data were analyzed in FlowJo software (Tree Star Inc). Apoptotic index (%) was expressed as the percentage of apoptotic cells (TUNEL+) relative to the number of cytokeratin-positive cells examined.

T17 polarization and flow cytometric analysis

Single-cell suspensions were prepared from spleens and treated with a mixture of rat anti-mouse antibodies (Ab) against CD19, CD32, and CD8 followed by depletion of Ab-bound cells with BioMag goat anti-rat IgG (Polyscience) as per manufacturer’s instructions. The freshly isolated
CD4+ T cells were plated in 6-well plate at 0.6 × 10^{6}/well in 2 mL of complete medium and stimulated with 0.5 μg/mL anti-CD3 and 1 μg/mL anti-CD28 in the presence of 1 ng/mL TGF-β and 50 ng/mL IL-6 (PeproTech). After 4 days of incubation, the cells were restimulated with 50 μg/mL phorbol 12-myristate 13-acetate (PMA) and 750 μg/mL ionomycin for 4 hours in the presence of 1 μg/mL Golgi Plug (BD Biosciences). The stimulated cells were stained first with PE-anti-CD4 Ab and then permeabilized in a permeabilizing buffer followed by intracellular staining with anti-IL-17A-Alexa Fluor 700 Ab (TC11-18H10.1; BioLegend). The stained cells were evaluated on FACSaria, and the flow data were analyzed in FlowJo software as above.

Real-time reverse transcriptase PCR of inflammatory cytokines and Goi2

Wild-type and IEX-1-KO mice at 8 to 10 weeks of age were given with drinking water with or without 3.5% DSS for 36 hours. The mice were sacrificed and colons were dissected, rinsed thoroughly with ice cold Hank’s buffer, and opened longitudinally to expose the intestinal mucosa. The mucosal layers were harvested by gentle scraping with a glass slide. Total RNA was extracted from the resultant scraping samples using a TRIzol reagent (Invitrogen) as per the manufacturer’s instructions followed by treatment with DNase-I (bovine pancreas; Sigma) for 1 hour at room temperature and reverse transcription with phenol/chloroform/isoamyl alcohol mixture to remove DNase-I. The purified RNA was reverse-transcribed and amplified by real-time PCR using a SYBR Green PCR kit (Applied Biosystems) on an Mx4000TM Multiplex Quantitative PCR System (Stratagene). The primers used were: forward, CCGTTCACACTCAGATCATCTTCT and reverse, GCTAC-GACGTGGA-CTTTCT and reverse, GCTAC-GACGTGGGCTA-CTTCT and reverse, GCTAC-GACGTGGGCTA-CTTCT for TNFα; forward, 5'-CAGGATGC-3' and reverse, 5'-TGAAGTGTGTTTC-TGAAGTGTGTTTC for IL-1β; forward, TAGTCCTTCCTACCC-CATTTCC and reverse, TGGGTCTTATGGCACTCTTTC for IL-6; forward, TTAACCACTCGATCGCAACCA and reverse, GCCATTGCTCAATGGTACCCTG for CCL2; forward, AGTGAATCGGCCGTTCAATGC, and reverse, GGCAAACTTTTGTACCGC for MIP-2; and forward, GGCTGTATTCCCCTACTGC and reverse, CCAG-TTGCTACACATGCTCATG for β-actin. For quantifying Gαt2 mRNA in T cells, T cells isolated from indicated mice were subject to RNA extraction, reverse transcription, and real-time PCR as described earlier using Gαt2 specific primers: forward, 5'-CAATGACTGACGCGTACTAC-3' and reverse, 5'-TGAAGTGTGTTTCCGATGTCG-3'. No PCR products were generated from non-reverse-transcribed samples run in parallel. Fold induction of mRNA was determined from the threshold cycle (Ct) values normalized for β-actin expression and then normalized to the value derived from controls.

Statistical analysis

The statistical analysis was based on the calculation of arithmetic mean and standard deviation (SD). The difference between 2 means was compared by 2-tailed unpaired Student t test assuming equal variances. One-way ANOVA was used for multiple group comparisons. A P value of less than 0.05 was considered statistically significant.

Results

IEX-1 deficiency prevents from colon tumor formation

The toxic reagent, DSS directly damages mucosal epithelium and causes an acute intestinal inflammation (15). When giving drinking water containing 3.5% DSS over a 9-day period of time, wild-type animals suffered from a body weight loss up to 7.4% ± 1.5% by day 8, concurrent with colonic bleeding in some mice. The animals gained a body weight gradually and reached a pre-DSS treatment level by day 4 after switching to regular drinking water (Fig. 1A). In contrast, IEX-1−/− animals did not experience a significant body weight loss (1.26% ± 0.4%), with almost no fluctuations over a 9-day course of DSS treatment (Fig. 1A). Similar protection against colitis induced by 5% DSS was also showed in the absence of IEX-1 (10). Consistent with reduced inflammation in the absence of IEX-1, inflammatory cytokine TNFα, and chemokine MCP1 (CCL2), but not IL-1β, IL-6, or MIP-2 (CCL3), were produced in the colon mucosa at a significantly lower level in IEX-1 KO mice than in wild-type control mice treated with 3.5% DSS for 36 hours (Fig. 1B). To determine whether lack of IEX-1 also protected mice from colon cancer, the mice were i.p. injected with a single dose of azoxymethane followed by 3 cycles of DSS administration as previously described (18). Azoxymethane is a procarcinogen structurally similar to cycasin, a naturally-occurring compound. It causes the formation of O2-methylguanine adducts in DNA, leading to G→A transition after replication and tumor formation in the distal colon of rodents. Repeated administration of DSS after azoxymethane treatment greatly enhances the incidence of azoxymethane-induced tumors (17). The treatment gave rise to 8 ± 2.5 tumors per colon in wild-type animals, but only 2 ± 1 in IEX-1−/− mice, in agreement with reduced colitis in the mice (Fig. 1D, P < 0.01). The majority of the tumors in wild-type animals were localized at the distal colon, whereas tumors in IEX-1−/− animals were located primarily in the distal colon (Fig. 1C). Microscopic analysis of H&E-stained sections from wild-type animals showed the presence of colonic adenomas with a high degree of dysplasia, a loss of entire crypts and surface epithelium, and massive infiltration of leukocytes into mucosa and submucosal edema (Fig. 1E). In contrast, IEX-1−/− mice displayed much less damage to the epithelium and lacked significant morphologic changes in crypt architecture, confirming a protective role of IEX-1 deficiency in colon inflammation and tumor development.

Aggravated colitis in Rag1−/− IEX-1−/− mice

We previously showed that adoptive transfer of IEX-1 KO CD4+ CD45RBhigh cells caused delayed and less severe colitis in Rag1−/− mice than in adoptive transfer of wild-type...
CD4⁺CD45RB<sup>high</sup> cells (10). While this observation corroborated diminished pathogenicity of CD4<sup>+</sup> T cells lacking IEX-1, it could not rule out that intestine epithelium or other inflammatory, non-T cells were also involved in the disease resistance in IEX-1 KO mice. We thus generated Rag<sup>−/−</sup> IEX-1<sup>−/−</sup> double KO mice to address whether diminished inflammation in IEX-1 KO mice could be ascribed to altered function of T cells alone or both T cells and non-T cells. As shown in Fig. 2, Rag<sup>−/−</sup> and IEX-1<sup>−/−</sup> single KO animals both experienced much less severe colitis than Rag<sup>−/−</sup> IEX-1<sup>−/−</sup> double KO mice or wild-type mice upon DSS exposure. The less severe colitis in Rag<sup>−/−</sup> mice or IEX-1<sup>−/−</sup> mice than wild-type mice probably resulted in part from either a lack of pathogenic T cells in Rag<sup>−/−</sup> mice or increased differentiation of IL-17-producing cells in IEX-1<sup>−/−</sup> mice as previously showed (10, 19). However, worse colitis in Rag<sup>−/−</sup> IEX-1<sup>−/−</sup> double KO mice than single KO mice stresses a T cell–independent mechanism involved, reinforcing a predominant role of T cells in the attenuated colitis and cancer development in IEX-1 KO mice. Despite worse colitis in Rag<sup>−/−</sup> IEX-1<sup>−/−</sup> double KO mice compared with single KO mice, the disease in Rag<sup>−/−</sup> IEX-1<sup>−/−</sup> double KO mice was till significantly less severe than that of wild-type mice. Rag<sup>−/−</sup> IEX-1<sup>−/−</sup> double KO mice suffered from an 8% decrease in their body weight, compared with an 18% of a body weight loss in wild-type animals (Fig. 2), which had to be sacrificed in accordance with institutional guidelines, resulting in a premature termination of the study. Notably, these groups of mice were on C57BL/6 genetic background and C57BL/6 mice were more susceptible to DSS-induced colitis than the mice on...
mixed 129Sv/C57BL/6 background as previously described (20).

Increased proliferation and apoptosis in IECs in IEX-1 KO animals

We next investigated whether lack of IEX-1 altered intestinal homeostasis. Normally, the intestinal epithelium of adult mammals undergoes continuous self-renewal in which stem cells at the bottom of the crypts give rise to progenitor cells that proliferate and differentiate as they move up the crypt surface (21). This process can be tracked by intraperitoneal injection of BrdUrd 2 hours before colon harvest, which labels proliferating cells. In untreated (day 0) wild-type and IEX-1 KO animals, BrdUrd-labeled cells were located primarily within the bottom third of crypts in the colon (Fig. 3A). On average, 7 ± 3 and 5 ± 2 cells were BrdUrd positive in the control and IEX-1 KO animals, respectively, which was without statistical significance. By day 5 of DSS treatment, labeled cells were seen extending into the midregions and throughout the colonic crypts in IEX-1 KO animals, whereas wild-type epithelial cells did not undergo a similar expansion. Counting of BrdUrd-positive cells showed a higher number of proliferating cells per crypt in IEX-1 KO mice than in wild-type animals (Fig. 3B, \( P < 0.01 \)). Strikingly, the increased IEC proliferation was concurrent with increasing apoptosis in the cells (Fig. 3C). The altered proliferation and apoptosis of IECs may exert both detrimental and beneficial effects on colon inflammation and cancer development.

A high level of Gai2 in IEX-1 KO cells is concurrent with increased T\(_{17}\) differentiation

A worse colitis in Rag\(^{-/-}\)IEX-1\(^{-/-}\) double KO mice compared with IEX-1 KO mice confirmed an importance of T cells in IEX-1 deficiency-induced protection against colon inflammation. Our laboratory previously showed that lack of IEX-1 enhanced Gai2 expression in the vasculature (13). Interestingly, an absence of this gene in mice produced spontaneous IBD, with a T\(_{12}\)-skewed autoimmunity in the colon (22). In light of reciprocal regulation of T\(_{17}\) and T\(_{11}\) subsets, Gai2 might be upregulated in IEX-1–deficient T cells, favoring T\(_{11}\) over T\(_{17}\) cell differentiation. To determine this, Gai2 expression was analyzed in T cells isolated from wild-type and IEX-1–deficient mice by reverse transcriptase RT-PCR (RT-PCR) (Fig. 4A) or Western blotting (Fig. 4B). We found an approximate 60% increase in Gai2 expression in IEX-1 KO T cells compared with wild-type counterparts (\( P < 0.01 \)). Moreover, CD4\(^{+}\) T cells isolated from the spleen of Gai2\(^{-/-}\) mice developed significantly fewer T\(_{17}\) cells than wild-type CD4\(^{+}\) T cells under T\(_{17}\)-polarizing conditions (Fig. 4C and D). Interestingly, an opposite trend was observed with IEX-1–deficient CD4\(^{+}\) T cells that produced a 2-fold or 6-fold higher number of T\(_{17}\) cells than wild type or Gai2\(^{-/-}\) counterparts, respectively, under a similar condition (Fig. 4C and D). The observation suggests an indispensable role for Gai2 in the regulation of T\(_{17}\) cell differentiation.

Absence of Gai2 in T cells alone aggravates colon inflammation and cancer development

Gai2\(^{-/-}\) mice developed spontaneous colitis, characterized by a T\(_{11}\)-predominant autoimmunity in the colon (22), but it was not clear whether a defect in T cells alone could cause the disease, despite altered function and differentiation of the cells (22, 23, 24). We generated Gai2\(^{-/-}\)/CD4-Cre mice to delete Gai2 specifically in T cells to address this issue. Unlike Gai2\(^{-/-}\) mice, Gai2\(^{-/-}\)/CD4-Cre mice were healthy and did not show any sign of a gross defect. However, when the mice were subject to DSS-induced colitis, Gai2\(^{-/-}\)/CD4-Cre mice lost more weight.
IEX-1 in the suppression of TH17 cell differentiation and in present and previous studies highlight an importance of showing that the mice are also protected against azoxymethane/DSS treatment (10). This study extends the induced colitis as re"ed that IEX-1 KO mice were resistant to DSS-treated mice (14.9 ± 3.7) than in control animals (8 ± 1.8 in Gαi2flx/flx mice or 7.8 ± 1.5 in CD4-Cre mice) after treatment with azoxymethane/DSS (Fig. 4F, *P < 0.01). The tumors were predominantly distributed to distal colon, concurrent with signs of rectal prolapse in 80% of animals, corroborating the pathogenicity of Gαi2-deficient T cells.

**Discussion**

Inflammation is one of attributing factors to carcinogenesis, as has been shown in many studies connecting these 2 pathologic conditions (1, 2). Our previous investigation identified that IEX-1 KO mice were resistant to DSS-induced colitis as reflected by unaltered colon length, body weight, or myeloperoxidase activity of the mice before and after DSS treatment (10). This study extends the finding showing that the mice are also protected against azoxymethane/DSS-induced tumor development. Hence, our present and previous studies highlight an importance of IEX-1 in the suppression of T_{H17} cell differentiation and in the development of inflammation-associated cancer in the colon. Null mutation of IEX-1 increases differentiation of IL-17-producing T cells that play a primary role in protection against colon inflammation and cancer development in IEX-1 KO mice.

Lack of IEX-1 upregulated Gαi2 expression in T cells in association with T_{H17} differentiation. Gαi2 KO mice developed a T_{H17}-skewed autoimmunity in the colon, characterized by increasing production of IFN-γ, TNFα, IL-1β, IL-6, and IL-12p40 (22). Moreover, CD4^{+} T cells purified from the mice exhibited an intrinsic propensity to differentiate into T_{H17} cells in vitro culture (24), whereas CD4^{+} T cells lacking Gαi2 had compromised T_{H17} differentiation (Fig. 4C and D). Mice lacking Gαi2 in T cells alone developed severe colitis and a higher number of tumors than the control mice after exposing to DSS or azoxymethane/DSS. These studies stress a critical role for Gαi2 in a balance between T_{H17} and T_{H17} cell differentiation.

Lack of IEX-1 has been shown to elevate mROS production that may augment Gαi2 expression as occurs in IEX-1-deficient vascular smooth muscle cells (13). The promoter of Gαi2 gene encoding Gαi2 contains transcriptional regulatory motifs for the redox-sensitive NF-KB and nuclear factor erythroid 2–related factor transcription factors (25).
IEX-1 deficiency-mediated production of mROS may upregulate Gαt2 expression via these redox-sensitive transcription factors, which is currently under investigation. Apart from transcriptional regulation, Nishida and colleagues showed that hydrogen peroxide specifically increased Gαt protein activity but not the activity of Gαs in association with the plasma membrane (26). A high level of Gαt2 activity in IEX-1-deficient T cells may increase T H17 cell differentiation and reciprocally decrease T H1 cell differentiation, rendering IEX-1 KO mice refractory to DSS-induced colitis and azoxymethane/DSS-induced color cancer.

A high level of IL-17A in IEX-1 KO T cells but low in Gαt2−/− T cells correlates with a protective role of this cytokine in intestine inflammation, as has been showed in several studies (10, 12, 27). Moreover, elevated levels of IL-17A as well as T H17 cells were found in various tumors (28, 29, 30, 31, 32). Overexpression of IL-17A in cell lines and their subsequent implantation into mice resulted in tumor formation, suggesting its carcinogenic potential (33). However, expression of IL-17A in murine hematopoietic immunogenic tumors and grafting them into syngeneic immunocompetent mice resulted in tumor inhibition via a T cell–dependent mechanism (34). Supporting data were also reported by Muranski and colleagues showing that T H17 cells could protect against advanced B16 melanoma (35). Finally, T H17 cells in a lung melanoma model were able to promote cytotoxic T cell activation causing tumor reduction (36). These observations argue for complex inflammation and cytotoxic T cells by TH17 or differentiation of T cells. The study provides novel insight into a role of Gαt2 in regulating a balance between TH1 and TH17 cell differentiation secondarily to IEX-1 expression.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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