Tle1 tumor suppressor negatively regulates inflammation in vivo and modulates NF-κB inflammatory pathway

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Tle1 (transducin-like enhancer of split 1) is a corepressor that interacts with a variety of DNA-binding transcription factors and has been implicated in many cellular functions; however, physiological studies are limited. Tle1-deficient (*Tle1*$^{\Delta/\Delta}$) mice, although grossly normal at birth, exhibit skin defects, lung hypoplasia, severe runting, poor body condition, and early mortality. *Tle1*$^{\Delta/\Delta}$ mice display a chronic inflammatory phenotype with increased expression of inflammatory cytokines and chemokines in the skin, lung, and intestine and increased circulatory IL-6 and G-CSF, along with a hematopoietic shift toward granulocyte macrophage progenitor and myeloid cells. *Tle1*$^{\Delta/\Delta}$ macrophages produce increased inflammatory cytokines in response to Toll-like receptor (TLR) agonists and lipopolysaccharides (LPS), and *Tle1*$^{\Delta/\Delta}$ mice display an enhanced inflammatory response to ear skin 12-O-tetradecanoylphorbol-13-acetate treatment. Loss of Tle1 not only results in increased phosphorylation and activation of proinflammatory NF-κB but also results in decreased Hes1 (hairy and enhancer of split-1), a negative regulator of inflammation in macrophages. Furthermore, *Tle1*$^{\Delta/\Delta}$ mice exhibit accelerated growth of B6-F10 melanoma xenografts. Our work provides the first in vivo evidence, to our knowledge, that TLE1 is a major counterregulator of inflammation with potential roles in a variety of inflammatory diseases and in cancer progression.

TLE1 | tumor suppressor | inflammation | NF-κB | HES1

Transducin-like enhancer of split 1 (TLE1) belongs to a family of corepressor proteins called transducin-like enhancer of split, or TLEs. Groucho, the TLE homolog in *Drosophila*, has crucial roles in neurogenesis, segmentation, and sex determination (1). These corepressors do not bind directly to DNA but rather interact with many different classes of transcription factors and help create a repressor complex (1, 2).

Vertebrates express five different TLEs (*TLE1–4*, *AES*), although the distinct functions of each of the TLEs has not been well determined. TLE1, the most studied among the Groucho family proteins in mammalian systems, is widely expressed in different tissues and cell types and has been implicated in neuronal differentiation (3, 4) and pancreatic beta cell development (5). TLE1 has tumor suppressor activity (6–8) as well as oncogenic functions in cancer (9, 10). TLE1 associates with many important transcription factors integral for cell proliferation and differentiation, including Runx2 to block rRNA expression (11), HES1 to suppresses MASH2 expression (12), and TCF/LEF proteins to block Wnt target gene activation (13). TLE1 also represses NF-κB activity (14, 15). Involvement in these diverse cellular functions and diseases was studied primarily in vitro or using in vivo overexpression systems. The major physiological function of TLE1, however, remains poorly understood.

A few recent studies suggest TLE1 might regulate immune function. For example, a single noncoding nucleotide polymorphism within the TLE1 locus was associated with inflammatory bowel diseases (16), and in human monocytes, increased TLE1 expression was important for zymosan-mediated inhibition of interleukin 12 (IL-12) p70 expression (17) as well as *Bacillus anthracis* toxin-induced immune suppression (18). However, the importance of TLE1 for overall immune function has not been characterized.

We previously identified *TLE1* and *TLE4* as tumor suppressor genes deleted in AML (acute myeloid leukemia) (19) whose loss cooperated with AML1-ETO (acute myeloid leukemia 1-eight twenty one) in leukemia development (6). We recently characterized *Tle4* knockout mice and showed that Tle4 has a critical role in maintaining hematopoietic stem cell (HSC) function and in bone development (20). To better understand the physiological roles of Tle1, we developed *Tle1* knockout mice. We found the loss of Tle1 leads to an excessive immune activation through a combination of constitutive activation of the NF-κB inflammatory pathway in skin, lung, and intestine as well as decreased Hes1-mediated immune suppression in macrophages. Tle1 deficiency also resulted in lung hypoplasia, decreased overall survival, and enhanced transplanted tumor growth. Our

Significance

This study provides the first physiological evidence, to our knowledge, that Tle1 (transducin-like enhancer of split 1) is a major negative regulator of inflammation. We show that the loss of Tle1 in mice leads to increased activity of the proinflammatory NF-κB pathway as well as decreased activity of Hes1 (hairy and enhancer of split-1), a negative regulator of inflammation. In addition, Tle1 loss resulted in decreased growth and survival with increased myelopoiesis and lung hypoplasia. Loss of Tle1 also sensitized mice to inflammatory stimuli and facilitated cancer progression. Our study opens the way to further investigations for a role of Tle1 in human inflammatory disease and cancer progression.

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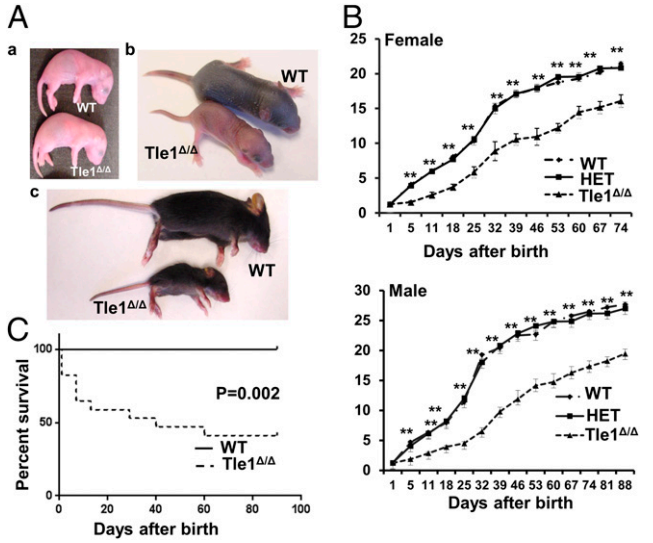

Fig. 1. Loss of Tle1 reduces growth and survivability. (*A*) *Tle1*$^{\Delta/\Delta}$ mice appear similar to WT and het at birth (*a*) but by 4 d (*b*) have delayed pigmentation and at 19 d (*c*) are runted with poor body condition. (*B*) Growth of *Tle1*$^{\Delta/\Delta}$ mice was significantly impaired relative to het and WT. A similar trend was observed in both male and female *Tle1*$^{\Delta/\Delta}$ mice; $n = 3$–6; $^{*}P < 0.05$, $^{**}P < 0.01$; all error bars indicate mean ± SEM. (*C*) Kaplan–Meier curve for *Tle1*$^{\Delta/\Delta}$ and WT mice. Fifty percent of mice died within 60 d, most between 8 and 20 d ($n = 17$–20).

results demonstrate a critical and previously unidentified role of Tle1 in suppressing in vivo inflammation.

Results

***Tle1*$^{\Delta/\Delta}$ Mice Have Growth Retardation and Reduced Fitness.** The creation of *Tle1*$^{\Delta/\Delta}$ mice (Fig. S1) is described in *SI Results*. *Tle1*$^{\Delta/\Delta}$ mice were born at normal frequency and appeared similar to wild-type (WT) and heterozygous (het) littermates at birth (Fig. 1 *A*, *a*). Delayed pigmentation was evident at 3 or 4 d of life (Fig. 1 *A*, *b*). Compared with WT and het littermates, *Tle1*$^{\Delta/\Delta}$ mice generally became progressively runted (Fig. 1 *A*–*C* and Fig. S2). By day 5, *Tle1*$^{\Delta/\Delta}$ mice on average weighed only 37% of WT and het mice. Knockout mice also exhibited significantly reduced fitness, with 50% of *Tle1*$^{\Delta/\Delta}$ mice dying before day 60, the majority between 8 and 20 d of life (Fig. 1*C*). Although among the surviving *Tle1*$^{\Delta/\Delta}$ mice there was some catch-up growth after weaning, older mice on average had 23–27% less body weight compared with age-matched het and WT mice (Fig. 1*B*). A similar effect was seen in females and males (Fig. 1*B*). In addition, *Tle1*$^{\Delta/\Delta}$ mice have shorter intestinal length, fewer hair follicles with disorganized skin epidermis basal cell layer, and pulmonary lung hypoplasia (*SI Results* and Figs. S3 and S4).

Tle1 Deficiency Skews Hematopoiesis Toward the Myeloid Lineage by Hematopoietic Cell Extrinsic Stimuli. Complete blood counts (CBCs) showed a higher neutrophil count in the *Tle1*$^{\Delta/\Delta}$ mice (Fig. 2*A*). We saw a trend toward a decrease in lymphocytes that was not significant, although as demonstrated below, we did find a significant decrease in B cells. The cellularity of the bone marrow (BM) was comparable between WT and *Tle1*$^{\Delta/\Delta}$ mice (Fig. S5*A*); however, Tle1-deficient BM showed a trend toward a higher percentage of granulocyte macrophage progenitors at 2 wk (*Tle1*$^{\Delta/\Delta}$, 0.296; Het, 0.179; $P = 0.2819$) and 4 wk (*Tle1*$^{\Delta/\Delta}$, 0.344; Het, 0.175; $P = 0.1183$) and a significant increase at 12 wk (*Tle1*$^{\Delta/\Delta}$, 0.238; Het, 0.173; $P = 0.034$) (Fig. 2*B*). By contrast, the frequencies of HSCs (Fig. S5*B*), common myeloid progenitors (CMPs) (Fig. S5*C*), megakaryocyte-erythroid progenitors (MEPs) (Fig. S5*D*), common lymphoid progenitors (CLPs) (Fig. S5*E*), or CD3e-positive T-cell populations (Fig. S5*F*) were not significantly altered. Tle1 knockout mice also displayed increased myeloid ($Mac1^{+}Gr1^{+}$) and macrophage ($Gr1^{-}Mac1^{+}F4/80^{+}$) and decreased B-cell populations (B220) in their BM, spleen, and peripheral blood (PB) (Fig. 2 *C*–*E*). BM methylcellulose assays also revealed a higher number of granulocyte macrophage and macrophage colonies (Fig. 2*F*). These data suggest that loss of Tle1 results in myeloid expansion. To determine whether this hematopoietic phenotype is cell-intrinsic or -extrinsic, one million CD45.2 BM cells from *Tle1*$^{\Delta/\Delta}$ or WT mice were transplanted into lethally irradiated CD45.1 recipient mice. *Tle1*$^{\Delta/\Delta}$-derived BM cells reconstituted all of the lineages similar to WT, indicating the

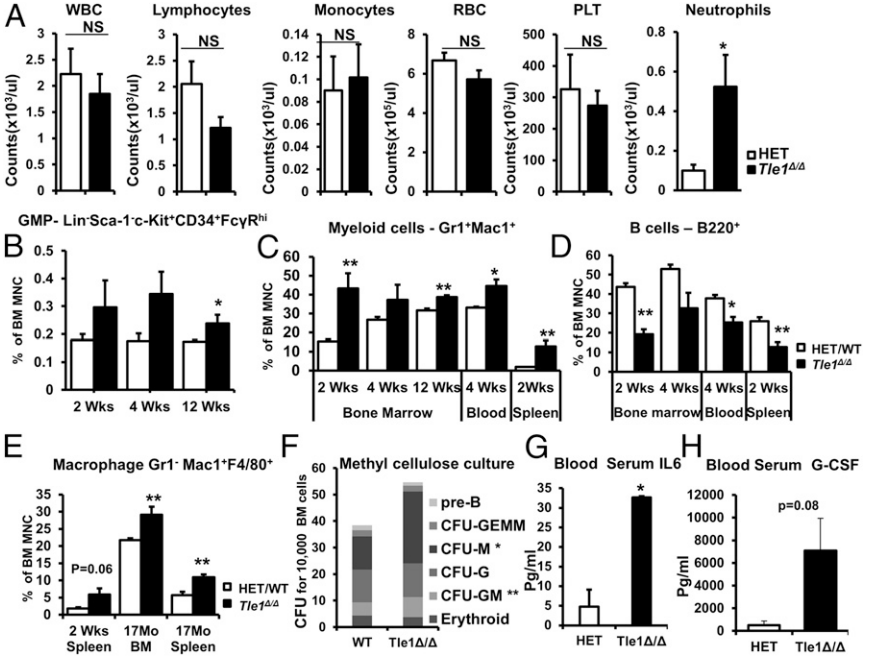

Fig. 2. Hematopoiesis in *Tle1*$^{\Delta/\Delta}$ mice is skewed toward the myeloid lineage with increased serum IL-6 and G-CSF. (*A*) PB counts for the indicated population at day 23 ($n = 5$–6). (*B*) Percentage of BM GMP-$Lin^{-}Sca\text{-}1^{-}c\text{-}Kit^{+}CD34^{+}Fc\gamma R^{hi}$ granulocyte macrophage progenitor cells. (*C*) Percentage of Gr1- and Mac1-positive myeloid cells. (*D*) Percentage of B220-positive B cells. (*E*) Percentage of $Gr1^{-}Mac1^{+}F4/80^{+}$-positive macrophage cells. (*F*) CFU-C assay for myeloid progenitors. (*G*) Serum IL-6 level. (*H*) Serum G-CSF level. $n = 3$–6 mice per genotype in *A*–*H*; $^{*}P < 0.05$, $^{**}P < 0.01$; all error bars indicate mean ± SEM.

myeloid expansion observed in the *Tle1* knockout mice was not cell-autonomous (Fig. S6 *A*–*C*).

Tle1-Deficient Mice Have Increased Expression of Inflammatory Cytokines and Chemokines in the Intestine, Skin, and Lung. Because myeloid cells and macrophages are key regulators of inflammation, we next asked whether the myeloid expansion observed in $Tle1^{\Delta/\Delta}$ mice is associated with increased inflammation. We analyzed mRNA expression of inflammatory cytokines IL-6 (interleukin-6), IL-1β, and TNF-α (tumor necrosis factor-α) and chemokines M-CSF (macrophage colony stimulating factor), GM-CSF (granulocyte macrophage-CSF), and G-CSF (granulocyte-CSF) at 2, 8, and 21 d after birth of the severely affected animals from the lung, liver, small intestine, skin, and BM. At day 2, the small intestine of $Tle1^{\Delta/\Delta}$ mice had significantly higher IL-1β, IL-6, GM-CSF, and G-CSF expression compared with WT (Fig. 3*A*). IL-1β, IL-6, and G-CSF expression was also significantly higher in the skin, and TNF-α levels were higher in the small intestine and BM of 8-d-old $Tle1^{\Delta/\Delta}$ mice (Fig. 3*B*). At day 21, IL-6, IL-1β, TNF-α, and chemokines M-CSF, GM-CSF, and G-CSF were significantly increased in the lungs, and IL-6 was also increased in the skin and small intestine (Fig. 3*C*). The analysis of serum from 21-d-old mice revealed a corresponding increase of the inflammatory cytokine IL-6 (Fig. 2*G*) and the chemokine G-CSF (Fig. 2*H*) in $Tle1^{\Delta/\Delta}$ mice compared with WT mice. We also observed increased expression of IL-1β in 1.5-y-old Tle1-deficient mice (Fig. S7). Thus, Tle1 deficiency leads to increased local and systemic production of inflammatory cytokines and chemokines.

Tle1 Modulates NF-κB–Mediated Inflammation. Because Tle1 overexpression has been shown to block NF-κB activation (14, 15), we further studied the effect of TLE1 on NF-κB nuclear translocation and phosphorylation (21). Overexpression of TLE1 inhibited both basal as well as LPS-induced NF-κB activation in THP-1 human monocyte cells, as evidenced by a decrease in nuclear NF-κB translocation using ImageStream analysis. The mean difference between nuclear and cytoplasmic (mean nuc–cyto diff) NF-κB was –24 ± 0.8 in unstimulated cells with empty vector, whereas it was –28 ± 0.9 in TLE1-expressing cells. After LPS stimulation, the mean nuc–cyto diff in cells with empty vector increased to 13 ± 1.2, whereas it is –7 ± 0.8 in the TLE1-transduced cells, indicating a greater proportion of NF-κB remains in the cytoplasm (Fig. 4*A*). Overexpression of Tle1 decreased LPS-mediated NF-κB phosphorylation in the Raw264.7 mouse macrophage cell line (Fig. 4*B*, *Bottom*). We found increased expression of phosphorylated NF-κB (serine 536) in the skin, lung, and intestine of 10-d-old $Tle1^{\Delta/\Delta}$ mice compared with WT mice (Fig. 4*B*, *Top*) and in LPS-treated $Tle1^{\Delta/\Delta}$ BMDMs compared with WT BMDMs (Fig. 4*B*, *Middle*). We also found an increase in many NF-κB pathway genes involved in inflammation and antiapoptosis in the lungs of 21-d-old $Tle1^{\Delta/\Delta}$ mice (Fig. 4*C*). Most significantly, angiotensinogen (Agt) was expressed sixfold higher in $Tle1^{\Delta/\Delta}$ mice compared with WT mice. We found overexpression of the neutrophil chemoattractants CXCL1 and CXCL3 as well as the inflammatory cytokines IL-1β and myeloid growth factor CSF2. We also observed increased expression of inflammatory genes *C-Rel*, *Snap25*, and *Tnfrsf1b*. These data are consistent with Tle1 modulation, NF-κB nuclear translocation, phosphorylation, and increased expression of NF-κB inflammatory target genes, which in turn promotes inflammation in $Tle1^{\Delta/\Delta}$ mice. Next, we asked whether suppression of NF-κB activation using a bortezomib proteasome inhibitor can ameliorate inflammation in $Tle1^{\Delta/\Delta}$ mice and decrease the expansion of myeloid cells. Repeated injection of bortezomib twice a week for 5 wk significantly reduced the percentage of $Gr1^{+}mac1^{+}$ myeloid cells in the spleen of $Tle1^{\Delta/\Delta}$ mice (2.54) compared with PBS vehicle (7.38)-treated mice (Fig. 4*D*). These results demonstrate that Tle1 modulates NF-κB activation and expression of inflammatory target genes and provide evidence that at least part of this inflammatory phenotype in $Tle1^{\Delta/\Delta}$ mice can be ameliorated by NF-κB inhibition in vivo.

Tle1 Loss Enhances Inflammatory Response to TPA and Accelerated B6-F10 Tumor Growth. To confirm that Tle1 regulates inflammation, we examined models involving inflammation. First, we analyzed the sensitivity of $Tle1^{\Delta/\Delta}$ and WT mouse ears to 12-O-tetradecanoylphorbol-13-acetate (TPA), an inflammation-inducing compound. Repeated treatment with TPA produced only mild swelling and minimal redness in the ears of WT mice. In contrast, an enhanced inflammatory response was seen in $Tle1^{\Delta/\Delta}$ mice with severe redness, scaling (Fig. S8*A*), and swelling (Fig. 5*A*). The histology of untreated ears of $Tle1^{\Delta/\Delta}$ mice was similar to WT. The ears of TPA-treated $Tle1^{\Delta/\Delta}$ mice had a thicker epidermal layer, with increased hematopoietic cell infiltration (Fig. 5*B*). Analysis of expression of inflammatory cytokine and chemokine mRNA levels using a PCR array revealed that TPA-treated $Tle1^{\Delta/\Delta}$ ears produced higher inflammatory cytokines and chemokines compared with treated WT ears (Fig. 5*C*). Thus, the loss of Tle1 enhanced an inflammatory response to TPA through the increased production of inflammatory cytokines and chemokines and attraction of inflammatory cells.

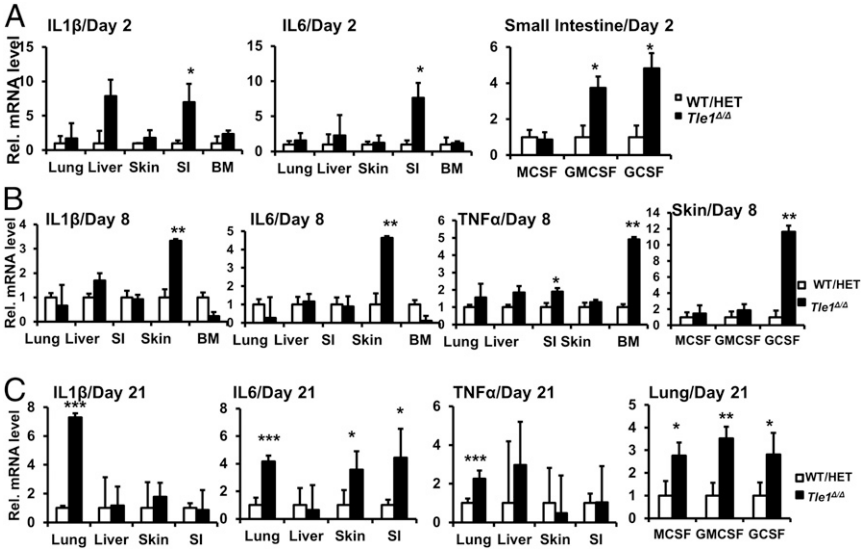

Fig. 3. Deficiency in Tle1 results in systemic inflammation with increased inflammatory cytokines and chemokine expression. Expression of IL-1β, IL-6, TNF-α, M-CSF, GM-CSF, and G-CSF was analyzed from liver, lung, skin, small intestine, and BM in WT (open bar) and $Tle1^{\Delta/\Delta}$ mice (closed bar). All error bars are expressed as mean + SEM ($n = 3$); $^{*}P < 0.05$, $^{**}P < 0.01$. (*A*) Two-day-old mice. (*B*) Eight-day-old mice. (*C*) Mice 21 d old.

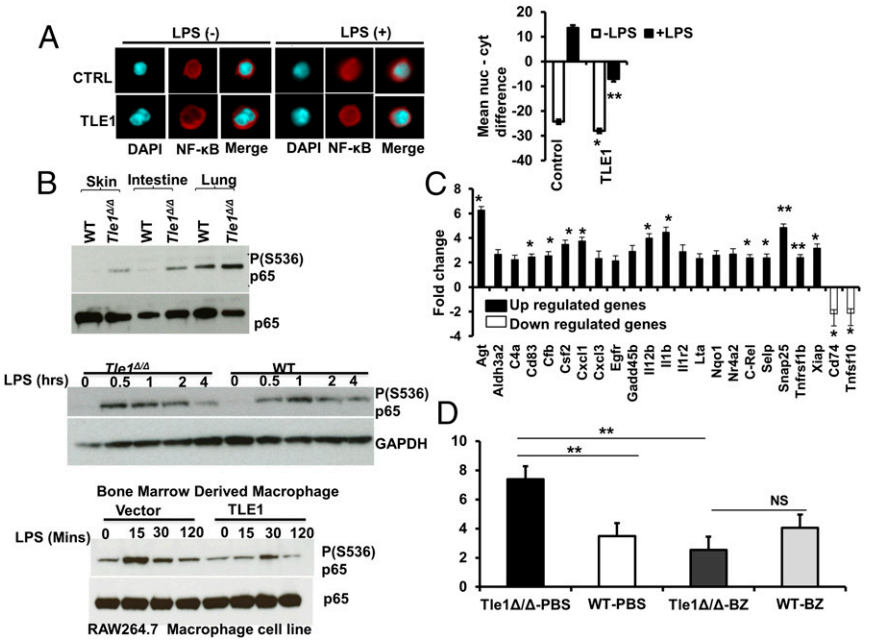

Fig. 4. TLE1 modulates NF-κB–mediated inflammation. (*A*) Inhibition of nuclear translocation of NF-κB. Empty vector or TLE1-transduced THP-1 cells were exposed to LPS for 1 h. The level of NF-κB protein in the nucleus and cytoplasm was visualized (*Left*) and measured by ImageStream before or after LPS exposure. The mean nuc–cyto diff is shown (*Right*). A negative value indicates a relatively greater cytoplasmic concentration. (*B*) Western blots showing the level of phosphorylated NF-κB (S536) in 10-d-old $Tle1^{\Delta/\Delta}$ and WT mice (*Top*) and in $Tle1^{\Delta/\Delta}$ and WT BMDMs after exposure to 100 ng/mL LPS for the indicated time (*Middle*) and RAW264.7 macrophage cell line transfected with vector or TLE1 after exposure to 10 ng/mL LPS for the indicated time (*Bottom*). Data are representative of three independent experiments. (*C*) Expression of NF-κB target genes in the lungs of $Tle1^{\Delta/\Delta}$ 21-d-old mice compared with WT mice measured using PCR array ($n = 3$). (*D*) Percentage of $Gr1^{+}Mac1^{+}$ myeloid cells in the $Tle1^{\Delta/\Delta}$ and WT mice after 5 wk of intraperitoneal injection of PBS or bortezomib 0.75 mg/kg ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$.

As a second model, we used an orthotopic melanoma cancer model. Because cancer growth is regulated by immune and inflammatory components (22), we asked whether absence of Tle1 affects tumor progression. We compared B6-F10 melanoma growth in $Tle1^{\Delta/\Delta}$ mice and WT mice using an orthotopic transplant model. Indeed, as shown in Fig. 5*D* and Fig. S8*B*, loss of Tle1 accelerated primary tumor growth.

Altogether our data support that the loss of Tle1 enhances the inflammatory response to inflammatory stimuli and accelerates tumor growth.

Tle1-Deficient Hematopoietic Cells and Macrophages Produce Enhanced Inflammatory Response to Inflammatory Stimuli. Although we did not observe increased myelopoiesis when $Tle1^{\Delta/\Delta}$ BM was transplanted into WT mice, we performed further experiments to determine if there are intrinsic defects in the regulation of inflammatory pathways in $Tle1^{\Delta/\Delta}$ hematopoietic cells. For these experiments, we examined the response of BM-transplanted mice, as well as isolated macrophages, to LPS and Toll-like receptor (TLR) agonist inflammatory stimuli.

Lethally irradiated WT mice were transplanted with BM from WT or $Tle1^{\Delta/\Delta}$ mice. After 16 wk, injection of LPS resulted in increased septic shock-induced death in $Tle1^{\Delta/\Delta}$ BM-transplanted mice (Fig. 6*A*). Given the critical role of macrophages as mediators of inflammation, we also isolated thioglycolate-induced peritoneal macrophages from $Tle1^{\Delta/\Delta}$ mice. These macrophages were stimulated with TLR agonists, and inflammatory cytokine expression was quantified. We found significantly increased expression of cytokines in response to stimulation for $Tle1^{\Delta/\Delta}$ macrophages compared with WT (Fig. 6*B*). Specifically, the expression of IL-6 and TNF-α in response to PAM3, the expression of IL-6 in response to Flagellin, and the expression of TNF-α in response to heat-killed *Listeria* Monocytogenes were greater in $Tle1^{\Delta/\Delta}$ macrophages. We confirmed this increased macrophage responsiveness using bone marrow-derived macrophages (BMDMs). Stimulating BMDMs with LPS resulted in increased expression of inflammatory cytokines IL-6 and IL-1β (Fig. 6*C*). As shown in Fig. 6 *D* and *E*, the expression of Hes1 is decreased in LPS-treated and untreated $Tle1^{\Delta/\Delta}$ BMDMs compared with WT. These data show that the loss of Tle1 with resultant decreased expression is associated with increased expression of inflammatory cytokines. Significantly higher Hes1mRNA in Tle1-deficient macrophage compared with WT BMDM (Fig. S9) indicates that decreased Hes1 protein expression is not due to decreased mRNA.

Discussion

Inflammation is exquisitely balanced by immune activators and suppressors. *Tle1* knockout mice exhibited both increased chronic inflammation as well as heightened susceptibility to inflammatory stimuli, as evidenced by enhanced cytokine expression by stimulated macrophages and enhanced inflammatory response of $Tle1^{\Delta/\Delta}$ mice

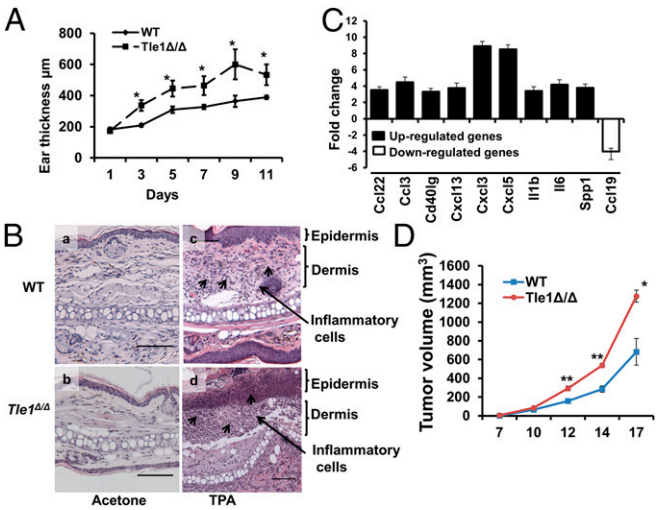

Fig. 5. Tle1-deficient mice exhibit an enhanced inflammatory response to TPA treatment and accelerated B6-F10 tumor growth. (*A*) Ear thickness in the $Tle1^{\Delta/\Delta}$ and WT mice after repeated application of TPA on the indicated day for 11 d. $n = 6$; $^{*}P < 0.05$, $^{**}P < 0.01$; error bars indicate mean ± SEM. (*B*) Ears treated with TPA for 11 d and stained with hematoxylin and eosin had marked thickening of the dermis and epidermal layers with dense immune cell infiltrates in $Tle1^{\Delta/\Delta}$ mice compared with WT mice. (Scale bar, 100 μm.) (*C*) Expression of various cytokine and chemokine mRNAs in the $Tle1^{\Delta/\Delta}$ mice ear compared with WT mice ear after application of TPA for 11 d ($n = 3$–4). (*D*) B16-F10 cells were injected intradermally, and the tumor volume (mm3) was measured ($n = 4$–6). $^{*}P < 0.05$, $^{**}P < 0.01$; error bars indicate mean ± SEM.

ears to TPA treatment. Our data indicate that loss of Tle1 results in excessive activation of NF-κB–mediated inflammatory pathways.

TLE1 has previously been shown to negatively regulate NF-κB using several in vitro systems (14–16, 23), although this has not been previously examined using animal models. NF-κB is fundamental in controlling many cellular processes, including inflammation and immune response, cell proliferation, apoptosis, and development (24–26). Our results indicate that loss of Tle1 results in increased NF-κB phosphorylation with increased expression of inflammatory cytokines and chemokines in the skin, lung, and intestine and increased expression of NF-κB inflammatory target genes in the lung. This is associated with increased committed myeloid progenitors and myeloid cells and decreased B cells in the BM along with increased circulating neutrophils, IL-6, and G-CSF in Tle1-deficient mice. It is similar to the effects seen with dysregulation of inflammatory pathways and infections (27) and the increase in G-CSF and granulopoiesis seen with constitutive activation of NF-κB in IκBa-deficient mice (28). In addition, in vitro overexpression of Tle1 in monocyte/macrophage cell lines blocks LPS-induced NF-κB nuclear translocation and phosphorylation. We also found bortezomib-mediated suppression of NF-κB activation in *Tle1*$^{\Delta/\Delta}$ mice decreased myeloid cell expansion. This finding provides evidence for the physiological role of Tle1 in negatively regulating the NF-κB inflammatory pathway and in regulating the immune response. Given that the TLEs are capable of interacting with other signaling pathways, we cannot exclude the possibility that pathways other than NF-κB influence the severe inflammatory phenotype in *Tle1*$^{\Delta/\Delta}$ mice. However, we did not find evidence of activation of other pathways such as Wnt, TGF-β, and IFN gamma in lung and TPA-treated ears using NF-κB and inflammatory/cytokine expression arrays. In addition, we did not see increased β-catenin protein, a marker of Wnt activation (Fig. S10).

Although WT recipients of *Tle1*$^{\Delta/\Delta}$ BM did not exhibit increased myelopoiesis or decreased survival or body weight as seen in Tle1-null mice, they do demonstrate increased susceptibility to LPS-induced septic shock. Isolated *Tle1*$^{\Delta/\Delta}$ macrophages from *Tle1*$^{\Delta/\Delta}$ mice expressed higher inflammatory cytokines in response to LPS and TLR agonists. Our data indicate that along with dysregulation of inflammatory pathways intrinsic to *Tle1*$^{\Delta/\Delta}$ hematopoietic cells, the inflammatory signal from nonhematopoietic cells contributes to the severe inflammatory phenotype of *Tle1* knockout mice. Indeed, *Tle1* is known to be expressed in endothelial cells and epithelial cells (29), which play a major role in the innate immune response (30, 31).

TLR-mediated activation of the Notch pathway and its target genes *Hes1* and *Hey1* negatively regulates TLR-mediated inflammatory cytokine production in macrophages (32, 33), providing an important counterregulatory feedback to prevent excessive inflammation. Notch activation also up-regulates TLEs (1, 34, 35). Tle1 forms a very stable complex with Hes1 protein, and it is generally through the association of TLEs that HES1 gains its repressive function (1, 34, 35). TLE–Hes1 interaction has been shown to be important for immune suppression in human monocytes (18) and has been suggested to play a critical role in limiting inflammation (18, 33, 36), although in vivo evidence for this was lacking. Decreased Hes1 protein in the Tle1-deficient macrophage suggests defective immune suppression may be contributing to the increased inflammatory response to the TLR agonist and LPS in these cells. The lower Hes1 protein levels in Tle1-deficient macrophages compared with WT BMDMs, despite higher Hes1 mRNA levels (Fig. S8), suggest a decrease in Hes1 protein stability. Although we did not examine the mechanism of this observed decrease in Hes1 protein levels, Hes1 protein is known to be stabilized by phosphorylation (37) or targeted for degradation by interaction with Hes6 (38). Our results suggest a combination of increased NF-κB activation with decreased Hes1-mediated immune suppression in *Tle1*$^{\Delta/\Delta}$ macrophages might be responsible for enhanced sensitivity of *Tle1*$^{\Delta/\Delta}$ BM to LPS-induced inflammation.

Along with inflammation, we observed a number of apparent developmental abnormalities in severely affected *Tle1*$^{\Delta/\Delta}$ mice, including delayed skin pigmentation, fewer hair follicles with disorganization of the epidermis, poor lung septation, and shortening of the intestine and dysmotility, in addition to growth retardation and early mortality. Although these effects may reflect an important role of Tle1 in the development of these organs, the inflammatory state caused by constitutive activation of NF-κB and expression of inflammatory cytokines and chemokines could be responsible for many of these findings. This concept is supported by prior work from several groups that found inflammation in mice can lead to delayed pigmentation (39), alveolar hypoplasia, and poor postnatal growth and decreased survival (28, 40).

The accelerated B6-F10 melanoma xenograft growth in Tle1-null mice could be due to increased inflammation. Chronic inflammation has been associated with cancer (41). *Tle1*$^{\Delta/\Delta}$ mice had elevated

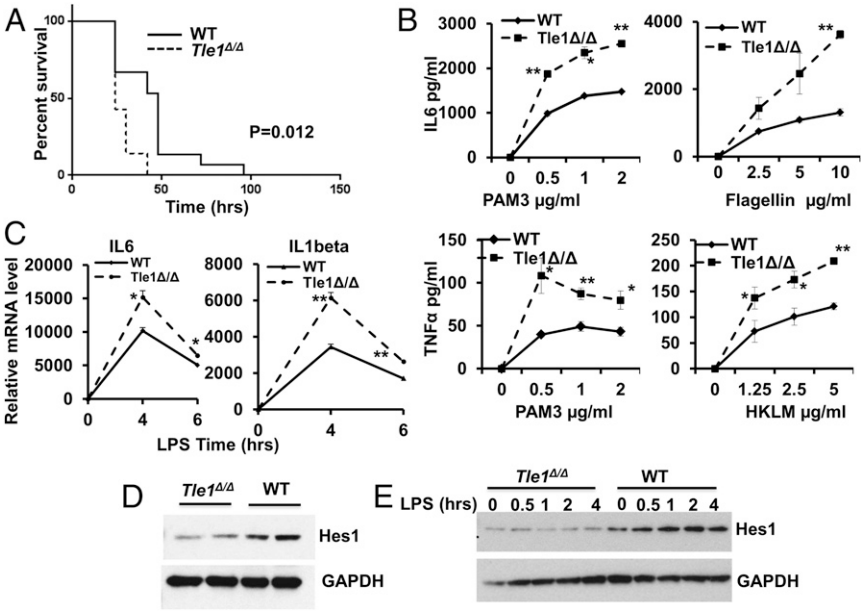

Fig. 6. Tle1 deficiency in the hematopoietic cells increases inflammatory response to LPS and TLR agonist. (*A*) Kaplan–Meier survival plots of syngenic lethally irradiated WT mice transplanted with Tle1$^{\Delta/\Delta}$ or WT BM for 16 wk and challenged with LPS (n = 10–13). (*B*) Thioglycolate-induced peritoneal macrophages were cultured and stimulated with increasing concentrations of purified TLR agonist for 24 h. Supernatants were assayed for expression of TNF-α and IL-6 using ELISA. Data (mean ± SEM) from four replicates from two independent experiments are presented after normalizing for cell numbers in each condition. (*C*) BMDMs were generated by culturing the BM cells with mouse M-CSF for 7 d. BMDMs from either WT or *Tle1*$^{\Delta/\Delta}$ mice were stimulated with LPS for the indicated time, and the expression of IL-1β and IL-6 mRNA was quantified using quantitative RT-PCR on triplicate samples from three independent experiments; $P < 0.05$, $^{**}P < 0.01$; error bars indicate mean ± SEM. (*D*) Western blot analysis of Hes1 expression in the BMDMs. Data are representative of two independent experiments. (*E*) Level of Hes1 protein in the *Tle1*$^{\Delta/\Delta}$ and WT BMDMs after exposure to 100 nm LPS for the indicated time. Data are representative of two independent experiments.

serum IL-6 and G-CSF with higher circulating myeloid cells. It has been shown that neutrophils exposed to a combination of G-CSF and IL-6 converted BM neutrophils from a tumor-suppressive to a tumor-promoting phenotype (42). NF-κB–mediated increases in myeloid-derived suppressor cells were shown to have a major role in tumor angiogenesis and immune modulation (43). This could underlie the advantage for the tumor cell growth we see in $Tle1^{\Delta/\Delta}$ mice.

The inflammatory phenotype in these $Tle1^{\Delta/\Delta}$ mice may have relevance to a variety of human inflammatory states. Increased NF-κB activation and its target genes Agt and IL-1β are observed in lungs with pulmonary fibrosis (44, 45). A genome-wide association study previously reported that an SNP variation in TLE1 was associated with colitis, and a case was made that this might be due to TLE1 interacting with NOD2 and inhibiting NF-κB activation (16). In support of a possible regulatory role of TLE1 in inflammatory bowel disease, we observed an increase in IL-1β, IL-6, and TNF-α in the intestine of $Tle1^{\Delta/\Delta}$ mice.

In summary, our work provides evidence that Tle1 negatively regulates the NF-κB inflammatory pathway and together with Hes1 provides an important role in suppressing excessive inflammation. Through the characterization of *Tle1* knockout mice, we provide the first in vivo evidence, to our knowledge, that TLE1 is a major counterregulator of inflammation and could play a role in a variety of inflammatory diseases and potentially cancer growth.

Materials and Methods

Materials and procedures for all experiments are described in *SI Materials and Methods*. Included are the generation of $Tle1^{\Delta/\Delta}$ mice, flow cytometry analysis, PCR array analysis, bortezomib treatment, LPS-induced septic shock, TPA treatment, generation and culturing of macrophage, and statistical analysis. Also provided are additional results and figures. This research was approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee.

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