Mesenchymal cell targeting by TNF as a common pathogenic principle in chronic inflammatory joint and intestinal diseases

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Tumor necrosis factor (TNF) is key to the pathogenesis of various arthritic diseases and inflammatory bowel disease (IBD). Anti–TNF therapies have proved successful in the clinical treatment of these diseases, but a mechanistic understanding of TNF function is still lacking. We have investigated early cellular mechanisms of TNF function in these diseases using an established TNF transgenic model, which develops a spondyloarthritic-like disease characterized by peripheral joint arthritis, sacroiliitis, enthesitis, and Crohn’s-like IBD. Bone marrow grafting experiments demonstrated that development of arthritis requires TNF receptor I (TNFRI) expression in the radiation-resistant compartment, which is also known to be a sufficient target of TNF in the development of Crohn’s-like IBD in the same model. Early activation of synovial fibroblasts and intestinal myofibroblasts could also be demonstrated by perturbed expression of matrix metalloproteases and their inhibitors. Notably, selective Cre/loxP–mediated TNFRI expression in mesenchymal cells resulted in a fully arthritic–spondyloarthritic and intestinal phenotype, indicating that mesenchymal cells are primary and sufficient targets of TNF in these pathologies. Our results offer a novel mechanistic perspective for TNF function in gut and joint pathologies and indicate early common cellular pathways that may also explain the often observed synovial–gut axis in human disease.
which render them capable of homing equally efficiently to the intestinal mucosa and joints (10). An experimental model in support of this hypothesis, the HLA-B27 transgenic rat, a spontaneous model for SpAs, develops colitis, gastritis, and arthritis (11). Although adaptive immune responses and, more specifically, CD8+ effector function are required for the development of Crohn’s-like IBD in the Tnf/ARE model (12), arthritis develops independently of the adaptive immune compartment (9), indicating that T cell responses may not be commonly required in the pathogenesis of these two diseases. Therefore, alternative mechanisms are likely to be responsive for TNF-mediated pathology in gut–joint diseases. Current hypotheses suggest that TNF delivers innate activation and proinflammatory signals through its action on various cell types, including myeloid/monocytic, lymphocytic, endothelial, mesenchymal, or epithelial cell types (5, 13). Using TNF-driven disease models, we demonstrate for the first time that mesenchymal cells, such as the fibroblasts/myofibroblasts of the joint and the intestine, are primary responder cells sufficient for full pathogenic TNF/TNFRI signaling in arthritis, spondylitis, and Crohn’s-like IBD. Our findings provide novel mechanistic insights into the cellular and molecular events underlying TNF function in joint–gut axis diseases and establish an early dominant role for mesenchymal cell responses in their pathogenesis.

RESULTS AND DISCUSSION

Tnf/ARE mice as a model for SpAs

Tnf/ARE mice have been described previously (9, 12) to develop chronic polyarthritis starting at weeks 5–6 and Crohn’s-like IBD starting at week 6. Prompted by the coincidence of both joint and intestinal disease in these mice, we have examined whether additional features of spondyloarthritic disease are developing in this model. Histological analysis of sacroiliac joints from Tnf/ARE mice revealed bilateral infiltration of the sacroiliac joints, a prominent feature of ankylosing spondylitis. Invasion of inflammatory tissue was detected in the subchondral bone and iliac BM of Tnf/ARE mice (Fig. 1). Initial signs of enthesitis were evident by week 4 (not depicted). No gender bias was apparent. These data establish Tnf/ARE mutant mice as a disease model resembling human SpAs. Interestingly, the Tnf/ARE model is characterized by the combined presence of arthritis–spondyloarthritides with a CD-like pathology localized primarily in the small intestine, as typically occurs in patients suffering from SpAs (2).

TNFRI expression on radiation-resistant, tissue stroma–residing cells is necessary and sufficient for the induction of arthritis

The dominant role of TNFRI in mediating TNF pathogenic signals in modeled arthritis and IBD has been previously established (7, 9). To gain further insight into the cellular specificity of TNFRI-mediated signaling, we used BM engraftment experiments into lethally irradiated recipients, using TNFRI-deficient mice (14) as either recipients or donors, to restrict TNFRI expression in either hematopoietic or radiation-resistant tissue stromal cells. Using this system, we have previously reported the capacity of BM-derived cells from the Tnf/ARE mice to induce IBD and, more importantly, that radiation-resistant, tissue stroma–residing cells are sufficient TNF targets for the induction of IBD (12). We have now extended these previous findings by demonstrating that at 12 wk after transplantation, lethally irradiated WT mice transplanted with Tnf/ARE BM cells also develop arthritis (Table I; and Fig. S1 A, available at http://www.jem.org/cgi/content/full/jem.20070906/DC1), indicating that a source of pathogenic TNF production in this model resides within the hematopoietic compartment. Transfer of Tnf/ARE TnfRI−/− BM into WT irradiated recipients resulted in overt arthritis, similar to the Tnf/ARE-reconstituted WT mice, as indicated by histopathological analysis of joints performed 12 wk after engraftment (Table I and Fig. S1 B), suggesting that radiation-resistant, stroma–residing cells are sufficient TNF targets for the induction of arthritis. Reciprocal engraftment of Tnf/ARE BM into TnfRI−/− recipients did not result in any arthritic manifestation 12 wk after transplantation (Table I and Fig. S1 C), indicating that radiation-resistant, tissue stroma–residing cells are also required TNF targets for the induction of arthritis. Similar results were obtained using the huTNF transgenic Tg197 model of arthritis (unpublished data). Collectively, these results demonstrate that in TNF-mediated arthritis, TNFRI expression on radiation-resistant cells is a necessary and sufficient condition for the development of disease.

Early activation of mesenchymal cells in modeled arthritis and Crohn’s-like IBD

In inflamed tissues, mesenchymal cells become activated and act as both recruiters of lymphocytes, by the increased expression of adhesion molecules (15, 16), and as effectors of tissue destruction, through the production of several matrix degrading enzymes such as matrix metalloproteinases (MMPs) (17, 18). Importantly, TNFRI-mediated mesenchymal MMP secretion in human fetal small intestine explant cultures has been previously associated with intestinal injury (19). To examine whether mesenchymal cells of Tnf/ARE mice are activated before the onset of disease, we isolated synovial fibroblasts (SFs) of ankle joints and intestinal myofibroblasts (IMFs) from WT and Tnf/ARE mice before the appearance of the inflammatory infiltrate (at 4 wk of age) (9, 12). Increased proteolytic activity against gelatin caused by MMP9 expression was detected in SF and IMF cell extracts in contrast to MMP2 activity, which remained unaltered (Fig. 2 A). Additionally, semiquantitative RT-PCR revealed the up-regulation of MMP3 and MMP9 in Tnf/ARE cultures (Fig. S2,
collagen VI (ColVI) promoter cassette (Fig. S3 A, available at http://www.jem.org/cgi/content/full/jem.20070906/DC1), which is known to drive gene expression in mesenchymal cell types (20). The specificity of Cre-mediated recombination in the ColVI-Cre mice was assessed using the ROSA26 and Z/EG reporter mouse strains (21, 22). X-gal staining in various tissues of ColVI-Cre ROSA26 flx/+ mice indicated Cre-mediated recombination in joint SFs, articular chondrocytes and myocytes, skin keratinocytes and dermal fibroblasts, the muscle layer around arteries of the heart (not depicted), and underneath the epithelial layer of the gut (Fig. S3, B–E). Cre activity was not detected in hematopoietic tissues (spleen and thymus; not depicted). Efficiency of Cre-mediated recombination in ex vivo–cultured SFs measured by flow cytometric analysis using fluorodeoxyglucose staining was >80% (Fig. S3 F).

The localization of X-gal–positive cells neighboring the epithelium of ColVI-Cre ROSA26 flx/+ reporter mice indicated Cre recombinase expression in the subepithelial myofibroblast layer (Fig. S3, D and E). Indeed, in ColVI-Cre Z/EG mice (22), GFP-expressing cells stained positive for αSMA (Fig. S3, J–L) but negative for the CD31 endothelial cell marker (Fig. S3, G–I), indicating Cre-mediated recombination in subepithelial myofibroblasts.

To obtain mesenchymal cell–specific expression of TNFRI, we used previously generated mutant mice carrying a conditional gain-of-function allele for this receptor (TnfRI flxneo mice). In these mice, TNFRI expression is inhibited by the presence of a floxed neomycin cassette but is restored upon Cre-mediated neo excision (23). The specificity of recombination for the floxed neo TNFRI allele was examined in

Table I. Arthritis development following TnfΔARE BM reconstitution of lethally irradiated recipients

<table>
<thead>
<tr>
<th>Donor genotype</th>
<th>Recipient genotype</th>
<th>Arthritis development</th>
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<tbody>
<tr>
<td>WT</td>
<td>WT</td>
<td>0/20</td>
</tr>
<tr>
<td>TnfΔARE/+</td>
<td>WT</td>
<td>19/21</td>
</tr>
<tr>
<td>TnfΔAREΔ ARE</td>
<td>TnfRI flx Neo</td>
<td>0/8</td>
</tr>
<tr>
<td>TnfΔAREΔ ARE</td>
<td>TnfRI flx Neo</td>
<td>25/25</td>
</tr>
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BM isolated from 2-mo-old female B6.129 mice (donor genotype) was engrafted into 6–8-wk-old syngeneic, lethally irradiated, female mice (recipient genotype). Assessment of arthritis development was based on histopathological evaluation of joint samples at 12 wk after transfer.
ColVI-Cre TnfRIfl/flneo Tg197 mice exhibited progressive inflammatory arthritis starting at 5–6 wk of age and with 100% incidence (10 out of 10 mice), similar to the timing and course of disease seen in Tg197 mice (Fig. 3 G). Littermate control Tg197 TnfRIfl/flneo mice remained healthy even at 50 wk of age (15 out of 15 mice; Fig. 3 H), further confirming the sufficiency of TNFRI signaling in mesenchymal cells for the development of TNF-driven arthritis. Southern blot analysis of tissue DNA from ColVI-Cre Tg197 TnfRIfl/flneo mice confirmed the recombination of the TNFRIfl allele in joints, skeletal muscle, skin, gut, and heart (Fig. 3 I). These findings demonstrate that mesenchymal cells are common primary targets for TNF in the development of inflammatory polyarthritis, Crohn’s-like IBD, and anklylosing spondylitis, and that selective expression of TNFRI on these cells is sufficient to orchestrate the complete development of such SpA-related pathologies.
Collectively, the data in this study demonstrate that mesenchymal cell targeting by TNF represents a common pathogenic mechanism in chronic inflammatory joint and intestinal diseases. Deregulated MMP expression was detected as an early marker of SF and intestinal subepithelial fibroblast activation by TNF. Increased levels of MMP3 and other MMPs have been reported in CD (24) and arthritic patients (25). Sustained perturbations on MMP expression are now strongly

Figure 3. TNFRI-expressing mesenchymal cells are sufficient targets of pathogenic TNF. (A–D) Histological examination of joint (8-wk-old) and ileal (16-wk-old) sections from ColVI-Cre Tnf^{ARE/-} TnfRI^{flxneo/flxneo} (A and C) and Tnf^{ARE/-} TnfRI^{flxneo/flxneo} mice (B and D). (E and F) Histological examination of sacroiliac joint sections from 10-mo-old ColVI-Cre Tnf^{ARE/-} TnfRI^{flxneo/flxneo} (E) and Tnf^{ARE/-} TnfRI^{flxneo/flxneo} mice (F). (G and H) Histological examination of joint sections from 7-wk-old ColVI-Cre Tg197 TnfRI^{flxneo/flxneo} (G) and Tg197 TnfRI^{flxneo/flxneo} mice (H). Paraffin sections were stained with hematoxylin and eosin. Bars: (A, B, G, and H) 600 μm; (C–F) 100 μm. (I) Southern blot analysis of BamHI-digested tissue DNA from ColVI-Cre TnfRI^{flxneo/flxneo} mice for detection of the recombined loxP TnfRI allele. (J) Flow cytometric analysis or the detection of TNFRI expression in ColVI-Cre Tnf^{ARE/-} TnfRI^{flxneo/flxneo} SFs derived from the cultures of individual mice. White lines indicate that intervening lanes have been spliced out.
implicated in various processes central to inflammation and immunity, such as cytokine and chemokine production, leukocyte recruitment, and tissue repair (26). It is thus possible that, in susceptible hosts, dysfunctional responses to tissue injury, infections, or other environmental stimuli may lead to sustained TNF/TNFRI-dependent activation of mesenchymal cells. Failure to regulate these proinflammatory and tissue-repair activities may be responsible for driving the transition of acute-to-chronic tissue inflammation and fibrosis (15). SFs do play a critical role in these processes, as they have been suggested to be capable of propagating both inflammation and joint destruction in rheumatoid arthritis (27, 28). An equally important role may be played by the intestinal subepithelial myofibroblast, which is also considered important in maintaining tissue architecture, regulating inflammation, and repair in the gut (29). Intestinal inflammation has also been prominently linked to epithelial barrier dysfunction (13). It was recently demonstrated that NF-κB-deficiency sensitizes epithelial cells to TNFRI-dependent apoptosis, linking innate immunity to intestinal inflammation (30). It will also be interesting to investigate whether TNFRI-mediated signaling on IFMs may contribute to epithelial barrier dysfunction in CD.

The identification of mesenchymal cells as a cellular subset linking joint and intestinal pathologies provides an alternative cellular basis to understand the common occurrences of these pathologies in humans, as well as the remarkable response of a significant number of patients to anti-TNF therapies (6). Mesenchymal cell replacement by stem cell or BM transplantation may offer future therapeutic approaches for the resolution of chronic inflammatory processes. The identification of TNF-responsive mesenchymal cells as dominant contributors to joint and gut inflammatory diseases suggests that attenuation of the TNFRI pathway in these cells or their precursors may hold promise for more rational and effective therapeutic interventions.

### MATERIALS AND METHODS

**Mice.** The generation of Tg197 (7), TgΔAHE (9), and mutant mice carrying a conditional gain-of-function allele for TgRI (TgRIΔinv) mice have been previously described (23). TgRIΔinv-/- (14) and Z/EGR (22) mice were purchased from the Jackson Laboratory. ROSA26Gv/+ (21) mice were provided by P. Soriano (Fred Hutchinson Cancer Research Center, Seattle, WA). The ColVI promoter cassette used for the generation of ColVI-Cre mice was provided by G. Bressan (University of Milano, Milano, Italy). All mice were bred and maintained on C57BL/6J and on mixed C57BL/6J × 129Sv/6 × CBA genetic backgrounds in the animal facilities of the BSRC “Alexander Fleming.”

**Table II. Incidence of arthritic and intestinal pathology in TnfΔARE+/ TnfRIflxneo/flxneo ColVI-Cre mice and littermates**

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<thead>
<tr>
<th>Genotype</th>
<th>Arthritic</th>
<th>Intestinal</th>
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<tbody>
<tr>
<td></td>
<td>wk 8</td>
<td>wk 16</td>
</tr>
<tr>
<td>TnfΔARE+/ TnfRIflxneo/flxneo</td>
<td>0/7</td>
<td>0/10</td>
</tr>
<tr>
<td>CoVI-Cre TnfΔARE+/ TnfRIflxneo/flxneo</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>TnfΔARE+/ TnfRIflxneo+</td>
<td>5/5</td>
<td>21/21</td>
</tr>
<tr>
<td>TnfΔARE+/ TnfRIflxneo+/</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>TnfΔARE+/ TnfRIflxneo+/</td>
<td>5/6</td>
<td>4/4</td>
</tr>
<tr>
<td>TnfΔARE+/ TnfRIflxneo+</td>
<td>83%</td>
<td>100%</td>
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**RNA isolation and RT.** RNA was isolated with TRIzol (Invitrogen). RT to complementary DNA was performed with 12 μg RNA by MMLV-RT (Promega) at a final volume of 20 μl according to manufacturer’s instructions. The volume of the reaction was increased to 50 μl and the dilutions used in reactions were always performed in accordance with the housekeeping gene content. The sequences of the primers are as follows: MMP3 sense, 5'-CCACAGACCTTT-GAGTCTGAGAGATTT-GAGTCCTGAGAGATTT-GGCAGCC-3'; TIMP1 sense, 5'-ACAAAGTCCACAGACCCGACATA-3'; TIMP1 antisense, 5'-GGACCTGATCCGTCCACAAAC-3'; MMP3 antisense, 5'-GGACCTGATCCGTCCACAAAC-3'.

**Online supplemental material.** Fig. S1 shows the histopathological manifestations of BM chimeras. Fig. S2 shows the immunohistochemical detection of MMPs in mesenchymal cells of TgΔAHE mice before disease onset.
Fig. S3 describes the generation and characterization of ColVI-Cre mice. Fig. S4 demonstrates the flow cytometric analysis of TNFRI expression in mesenchymal and nonmesenchymal cells of ColVI-Cre TgRF1×mMig mice. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20070906/DC1.

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