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BASIC AND TRANSLATIONAL—ALIMENTARY TRACT

Stem Cells for Murine Interstitial Cells of Cajal Suppress Cellular Immunity and Colitis Via Prostaglandin E_2 Secretion

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See Covering the Cover synopsis on page 866.

BACKGROUND & AIMS: After allogeneic transplantation, murine stem cells (SCs) for interstitial cells of Cajal (ICCs), electrical pacemaker, and neuromodulator cells of the gut, were incorporated into gastric ICC networks, indicating in vivo immunosuppression. Immunosuppression is characteristic of bone marrow− and other non-gut–derived mesenchymal stem cells (MSCs), which are emerging as potential therapeutic agents against autoimmune diseases, including inflammatory bowel disease. Therefore, we investigated whether gut-derived ICC-SCs could also mitigate experimental colitis and studied the mechanisms of ICC-SC–mediated immunosuppression in relation to MSC-induced pathways. **METHODS:** Isolated ICC-SCs were studied by transcriptome profiling, cytokine assays, flow cytometry, mixed lymphocyte reaction, and T-cell proliferation assay. Mice with acute and chronic colitis induced by dextran sulfate sodium and T-cell transfer, respectively, were administered ICC-SCs intraperitoneally and evaluated for disease activity by clinical and pathological assessment and for ICC-SC homing by live imaging. **RESULTS:** Unlike strain-matched dermal fibroblasts, intraperitoneally administered ICC-SCs preferentially homed to the colon and reduced the severity of both acute and chronic colitis assessed by clinical and blind pathological scoring. ICC-SCs profoundly suppressed T-cell proliferation in vitro. Similar to MSCs, ICC-SCs strongly expressed cyclooxygenase 1/2 and basally secreted prostaglandin E_2. Indomethacin, a cyclooxygenase inhibitor, countered the ICC-SC–mediated suppression of T-cell proliferation. In contrast, we found no role for regulatory T-cell−, programmed death receptor−, and transforming growth factor-β−mediated mechanisms reported in MSCs; and transcriptome profiling did not support a relationship between ICC-SCs and MSCs. **CONCLUSIONS:** Murine ICC-SCs belong to a class different from MSCs and potently mitigate experimental colitis via prostaglandin E_2−mediated immunosuppression.

Keywords: Immunosuppression; IBD; ICC.

The inflammatory bowel diseases (IBDs) Crohn's disease and ulcerative colitis are chronic idiopathic disorders affecting >1 million people in the United States.[1] IBD results from inappropriate inflammatory responses to intestinal microbes in a genetically susceptible host.[2] Recent studies have shown that autoimmune inflammation can be mitigated by systemically administered bone marrow−, adipose tissue−, or umbilical cord blood–derived mesenchymal stromal/stem cells[3–5] (MSCs; defined broadly as ubiquitous progenitors of all nonepithelial, non-hematopietic cells derived from the mesoderm[6,7]). There are several ongoing phase 2 and 3 clinical trials that are using MSCs for treatment of IBD (clinicaltrials.gov). Although the immunoregulatory functions of MSCs remain incompletely understood,[6] available evidence indicates important roles for prostaglandin E_2 (PGE_2)−, programmed cell death 1 (PD-1)−, regulatory T cell (Treg)−, and transforming growth factor-β (TGF-β)−mediated pathways.[3–5,8–11]

Interstitial cells of Cajal (ICCs) are mesodermally derived cells of the gastrointestinal *tunica muscularis* that generate electrical pacemaker activity and mediate neuromuscular control.[12] Populations of ICCs, identified by their characteristic expression of Kit receptor tyrosine kinase and anoctamin 1 (Ano1), a calcium-activated chloride channel, are depleted in several gastrointestinal neuromuscular disorders, significantly contributing to their pathogenesis.[13,14] In the murine gastric *tunica muscularis*, we also identified a small population of $Kit^{low/-}Ano1^{+}Cd44^{+}Cd34^{+}$ cells, which are clonogenic and capable of extensive self-renewal and differentiation into ICCs both in vitro and in vivo, fulfilling the criteria for bona fide stem cells (SCs).[15,16] When injected intraperitoneally into adult, major histocompatibility complex–mismatched, nonobese diabetic mice, an established model of ICC injury,[14] ICC-SCs (line 2×SCS70) homed to the stomach, differentiated, and engrafted into ICC networks,[16]

Abbreviations used in this paper: **Ano1, anoctamin1; CI, confidence interval; COX-1/2, cyclooxygenase 1/2; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; ICC, interstitial cell of Cajal; IL, interleukin; MLR, mixed lymphocyte reaction; MSC, mesenchymal stem cell; PBS, phosphate-buffered saline; PD-1, programmed cell death 1 (Pdcd1); PD-L1, programmed cell death 1 ligand 1 (B7-H1, Cd274 antigen); PGE_2, prostaglandin E_2; Ptger1, prostaglandin E receptor 1 (subtype EP1); Ptger, prostaglandin E receptor; Ptges, prostaglandin E synthase; Ptgs1/2, prostaglandin-endoperoxide synthase 1/2; RNA-seq, RNA deep sequencing; TGF-β, transforming growth factor−β; TNF-α, tumor necrosis factor−α; TPA, T-cell proliferation assay; Treg, regulatory T cell; SC, stem cell.**

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indicating immunosuppressive capability. Therefore, we hypothesized that, similarly to systemic MSCs to which they may be related, gut-derived ICC-SCs have immunoregulatory potential and could mitigate experimental colitis. In addition, we also studied the mechanisms of ICC-SC–mediated immunosuppression in relation to MSC-induced pathways.

Methods

Standard methods (RNA sequencing [RNA-seq], gene expression microarrays, cytokine assays, enzyme immunoassays, mixed lymphocyte reaction [MLR], and T-cell proliferation assay by carboxyfluorescein diacetate succinimidyl ester dye dilution) and additional details are described in the Supplementary Material. Isolation and maintenance of the ICC-SC lines 2×SCS2F10 and 2×SCS70 were described previously.[16] Only cells with diploid DNA content[16] were used.

Ethics Statements

Animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Protocols were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic (A36211, A60011).

Dextran Sulfate Sodium Model of Acute Colitis

C57Bl/6J mice were given 5% dextran sulfate sodium (DSS) (36,000–50,000 Da; MP Biomedicals, Illkirch, France) in the drinking water for 7 days, followed by water only for 1 day before euthanasia by CO_2 inhalation for tissue removal on day 8. 2×SCS2F10 ICC-SCs derived from C57Bl/6J mice or strain-matched dermal fibroblasts (see Supplementary Material) (5×10^6 cells/250 μL phosphate-buffered saline (PBS)/mouse; human equivalent dose:[17] 16×10^6/kg) or PBS vehicle were injected intraperitoneally 10 hours before starting DSS and on day 4. Mice were weighed daily. Disease severity was assessed by colon length, Disease Activity Index, and histology on the day of euthanasia, as described in the Supplementary Material.

$Cd4^+Cd45RB^{high}$ T-Cell Transfer Model of Chronic Colitis

We performed adoptive transfer of 300,000 $Cd4^+Cd45RB^{high}$ (naïve) T cells from healthy C57BL/6J mice by intraperitoneal injection into syngeneic mice lacking T and B cells ($RAG^{-/-}$ recipient mice; background: C57BL/6J) to induce T-cell–dependent chronic colitis as described previously.[18] 2×SCS2F10 ICC-SCs (5×10^6 cells/250 μL PBS/mouse) or PBS vehicle were injected intraperitoneally on day 7 and day 21. Disease severity assessment was performed at time of euthanasia 5–6 weeks after T-cell transfer, as described in the Supplementary Material.

In vivo Tracking of Interstitial Cell of Cajal Stem Cell Homing in Mice With Dextran Sulfate Sodium Colitis

Mice were kept on purified, chlorophyll-free diet optimized for in vivo imaging (OpenSource Diet D1001, Research Diets, Inc., New Brunswick, NJ) for >2 weeks before and throughout the study. 2×SCS2F10 ICC-SCs and strain-matched dermal fibroblasts were labeled with VivoTrack 680 near-infrared dye (Perkin Elmer, Waltham, MA) as per the application notes. Five × 10^6 cells/250 μL PBS/mouse or PBS vehicle were injected intraperitoneally 10 hours before starting DSS administration. After removal of abdominal hair and verification of lack of fluorescence from intestinal contents, live imaging was performed immediately after the injection (day 0) and on days 3, 5, and 8 (day of euthanasia) using the Xenogen IVIS 200 imaging system (Perkin Elmer). At each time point, we also performed postmortem imaging of colons and ceca isolated from 2 additional mice/group.

$Cd4^+$ T-Cell Proliferation Assay

Naïve $Cd4^+$ splenocytes were isolated using magnetic separation kits (Miltenyi Biotec, Auburn, CA) from C57BL/6J mice, as per the manufacturer's instructions. The T cells were stimulated with T-cell activator Cd3/Cd28 mouse Dynabeads (Life Technologies, Oslo, Norway) and cultured with 2×SCS2F10 ICC-SCs irradiated at 3300 Gy. [^{3}H]thymidine (Perkin Elmer) was added on day 4 and proliferation was determined after 18 hours of incubation by measuring the uptake of [^{3}H]thymidine in a scintillation counter and expressed as counts per million. Some of the T-cell proliferation assays (TPAs) were performed using Transwell permeable supports (Corning, Inc., Tewksbury, MA) with a pore size of 0.4 μm separating the activated T cells from the ICC-SCs and preventing their physical contact, while permitting the exchange of soluble factors between the 2 compartments.

Statistical Analyses

Data are expressed as means ± SE and analyzed by Student *t* test, one-way analysis of variance, or nonparametric alternatives.

Results

Interstitial Cell of Cajal Stem Cells Mitigate Dextran Sulfate Sodium Colitis in Mice

We first investigated the immunosuppressive potential of ICC-SCs in a murine model of acute DSS colitis. DSS causes injury to the intestinal epithelium, permitting exposure of the submucosa to bacteria and other luminal antigens, resulting in inflammation with cells typically involved in innate immunity.[19] Administration of 5% DSS resulted in severe colitis manifested by bloody diarrhea, weight loss, shortening of colon length, and histologic features of inflammation, including destruction of epithelium, abnormal crypts, edema, and infiltration of inflammatory cells (Figure 1*A*–*F*). Intraperitoneal administration of 5 × 10^6 2×SCS2F10 ICC-SCs 10 hours before and 4 days after the initiation of DSS exposure prevented the development of severe colitis. Relative to the PBS-injected DSS controls, mice treated with ICC-SCs displayed lower body-weight loss (from day 6 to euthanasia, $P = .03$; Figure 1*A* and *B*) and disease activity scores ($P < .001$; Figure 1*C*); had longer colons ($P < .001$; Figure 1*D*), reduced epithelial damage and less infiltration with inflammatory cells (Figure 1*E*), resulting in a significant decrease in the blinded histologic scores