Aryl Hydrocarbon Receptor-Induced Signals Up-regulate IL-22 Production and Inhibit Inflammation in the Gastrointestinal Tract

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BACKGROUND & AIMS: The pathogenesis of inflammatory bowel disease (IBD) is believed to involve an altered balance between effector and regulatory T cells. Aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor that mediates the toxicity of dioxins, controls T-cell responses. We investigated the role of AhR in inflammation and pathogenesis of IBD in humans and mouse models. **METHODS:** AhR expression was evaluated in intestinal tissue samples from patients with IBD and controls by real-time polymerase chain reaction (PCR) and flow cytometry. Intestinal lamina propria mononuclear cells (LPMCs) were activated in the presence or absence of the AhR agonist 6-formylindolo(3, 2-b)carbazole (Ficz). Colitis was induced in mice using trinitrobenzene sulfonic acid (TNBS), dextran sulfate sodium (DSS), or T-cell transfer. Mice were given injections of Ficz or the AhR antagonist 2-metyl-2H-pyrazole-3-carboxylic acid; some mice first received injections of a blocking antibody against interleukin (IL)-22. Cytokines were quantified by real-time PCR and flow cytometry. **RESULTS:** Intestine tissue from patients with IBD expressed significantly less AhR than controls. In LPMCs from patients with IBD, incubation with Ficz reduced levels of interferon gamma (IFN)-γ and up-regulated IL-22. Mice injected with Ficz were protected against TNBS-, DSS-, and T-cell transfer–induced colitis; they had marked down-regulation of inflammatory cytokines and induction of IL-22. Mice given AhR antagonist produced more inflammatory cytokines and less IL-22 and developed a severe colitis. Neutralization of endogenous IL-22 disrupted the protective effect of Ficz on TNBS-induced colitis. **CONCLUSIONS: AhR is down-regulated in intestinal tissue of patients with IBD; AhR signaling, via IL-22, inhibits inflammation and colitis in the gastrointestinal tract of mice. AhR-related compounds might be developed to treat patients with IBDs.**

Keywords: Crohn's disease; Colitis; T-helper cells; Immune Regulation.

The etiology of Crohn's disease (CD) and ulcerative colitis (UC), the major forms of inflammatory bowel diseases (IBD) in humans, remains unknown, but evidence suggests that IBD results from the interaction of genetic and environmental factors that ultimately promote an abnormal immune response leading to organ damage.[1]

Deregulation of various components of the immune system can be seen in the gut of patients with IBD, but hyperactivity of T cells with excessive production of cytokines is perhaps the major immunologic stigmata of these disorders.[1] Such an abnormal T-cell response is in part due to a defective activity of counterregulatory mechanisms and is directed against components of the luminal bacterial flora.[1,2] Consistently, strategies aimed at restoring the balance between inflammatory and anti-inflammatory factors have already been tested with success in both patients with IBD and experimental models of colitis.[3–6]

Aryl hydrocarbon receptor (AhR), a transcription factor ubiquitously expressed in vertebrate cells, mediates a range of cellular events in response to halogenated aromatic hydrocarbons and nonhalogenated polycyclic aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin.[7] Further ligands are small synthetic compounds and natural chemicals, including derivatives of tryptophan, such as 6-formylindolo(3, 2-b)carbazole (Ficz).[8,9] AhR is present in the cytosol in an inactive form, bound to several cochaperones.[7] After binding with ligands, AhR dissociates from the chaperones and translocates into the nucleus, where it binds to its dimerization partner AhR nuclear translocator, and the AhR/AhR nuclear translocator complex initiates transcription of genes with promoters containing a dioxin-responsive element consensus sequence.[7]

Pioneering studies in AhR-deficient mice have emphasized the role of AhR in the development and functions of various organs. AhR-deficient mice exhibit a spectrum of hepatic and skin defects as well as abnormalities in vascular and hematopoietic development.[10] More recent studies have shown that AhR controls specific immune responses. AhR is highly expressed by T-helper (Th)17 cells, and activation of AhR results in expansion of Th17

Abbreviations used in this paper: AhR, Aryl hydrocarbon receptor; DSS, dextran sulfate sodium; Ficz, 6-formylindolo(3, 2-b)carbazole; IFN, interferon; IL, interleukin; LPMC, lamina propria mononuclear cell; Th, T-helper; TNBS, trinitrobenzene sulfonic acid; TNF, tumor necrosis factor.

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cells and enhanced production of Th17 cytokines.[11–13] Consistently, studies in mice with experimental autoimmune encephalomyelitis, a Th17 cell–driven autoimmune inflammatory disease of the central nervous system that serves as a disease model for multiple sclerosis in humans, showed that AhR activation causes earlier onset of the disease and more severe pathology.[14] AhR also controls Th1/Th2 cell–associated immunity. Mice given a synthetic ligand of AhR, the compound M50367, exhibit reduced Th2 cell responses and enhanced production of interferon (IFN)-γ,[15] whereas AhR-deficient mice seem to have more Th1 and Th2 cells.[16] AhR activation in T cells can also regulate production of interleukin (IL)-22,[17,18] a cytokine that can exert either inflammatory or protective effects in various organs.[18–22] Therefore, depending on the cell context analyzed and type of agonist used, AhR-driven signals could differently modulate Th cell response and act as initiators or attenuators of tissue-damaging T cell–dependent inflammatory processes.

Because the tissue damage in IBD occurs in areas that are massively infiltrated with distinct subsets of Th cells,[23,24] we investigated whether AhR activation controls Th cell–derived cytokine production and pathogenic responses in the gut.

Materials and Methods

Mucosal Samples

Mucosal biopsy samples were obtained from involved colonic and ileum areas of 19 patients with active CD undergoing endoscopy (median age, 39 years; range, 28–59 years). In 5 of these 19 patients with CD, paired biopsy specimens were taken from both involved and uninvolved mucosal areas. Nine patients with CD were receiving corticosteroids, and the remaining patients were treated with mesalazine. Intestinal specimens, taken from 8 patients with moderate to severe CD undergoing intestinal resection for a severe disease poorly responsive to medical treatment, were used to isolate lamina propria mononuclear cells (LPMCs). Colonic mucosal samples were also taken from 16 patients with active UC who were undergoing endoscopy (median age, 37 years; range, 26–52 years). Four of these patients were receiving corticosteroids, and the remaining patients were treated with mesalazine. In 4 of these patients with UC, biopsy specimens were taken from both inflamed and uninflamed mucosa. Healthy controls included colonic mucosal biopsy samples from 17 patients with irritable bowel syndrome, as well as macroscopically and microscopically unaffected colonic specimens from 9 patients undergoing colonic resection for colon cancer (median age, 46 years; range, 35–69 years). Ethical approval was obtained from the local ethics committee.

Protein Extraction and Western Blotting Analysis

All reagents were from Sigma-Aldrich unless specified (Milan, Italy). Total proteins were extracted from biopsy specimens taken from 5 healthy controls, 6 patients with UC, and 6 patients with CD. Total proteins were also prepared from colonic specimens taken from control (ETOH-treated) and colitic (trinitrobenzene sulfonic acid [TNBS]-treated) mice treated or not with Ficz and killed at day 5. For the detection of AhR, proteins were separated on a 10% sodium dodecyl sulfate/polyacrylamide gel electrophoresis gel. A commercially available monoclonal mouse anti-AhR antibody (1 μg/mL; Abcam, Cambridge, England) followed by horseradish peroxidase–conjugated rabbit anti-mouse immunoglobulin (Ig) G antibody (final dilution 1:10,000; Dako, Glostrup, Denmark) was used, and the reaction was detected with a Dura chemiluminescence kit (Pierce, Rockford, IL). After detection of AhR, blots were stripped and incubated with a mouse anti-human β-actin antibody (final dilution, 1:5000) followed by horseradish peroxidase–conjugated goat anti-mouse antibody (final dilution, 1:20,000; Dako). A computer-assisted scanning densitometry was used to analyze the intensity of the immunoreactive bands.

LPMC Isolation and Culture

LPMCs were isolated as described elsewhere[25] and used to characterize AhR or cultured in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 U/mL). Human cells were preincubated with either Ficz (final concentration, 2–200 nmol/L; Alexis, Milan, Italy) or dimethyl sulfoxide for 1 hour and then stimulated with activating CD3/CD28 antibody-coated beads (Miltenyi Biotec, Calderara di Reno, Italy) for 4 to 24 hours.

TNBS-Induced Colitis

TNBS in 50% ethanol was administered to 6- to 8-week-old female Balb/c mice as previously described.[26] Two or 3 mg of TNBS was used for studies with Aryl antagonist or Ficz, respectively. Controls consisted of mice treated with 50% ethanol. AhR antagonist (2-methyl-2H-pyrazole-3-carboxylic acid, CH223191; Calbiochem, Nottingham, England) was dissolved in phosphate-buffered saline and injected intraperitoneally (10 μg/mouse) 1 day before TNBS administration, whereas Ficz (1 μg/mouse) was injected intraperitoneally 1 day after TNBS administration. Ficz was dissolved in dimethyl sulfoxide at a final concentration of 0, 1 μg/μL, and 10 μL of this solution and was then mixed with 140 μL phosphate-buffered saline. Weight changes were recorded daily, mice were killed at day 5, and tissues were collected for histology, RNA analysis, protein analysis, and LPMC isolation. The colitis histologic score was assigned as described elsewhere.[26] In additional studies, mice were killed at different time points (day 0–4) and colonic tissue was used for epithelial cell and LPMC isolation as described elsewhere.[26] For assessing cytokine expression, mice were killed at different time points. To determine whether IL-22 mediates the therapeutic effect of Ficz, a neutralizing murine anti–IL-22 (100 μg/mouse; R&D Systems, Minneapolis, MN) or nonrelevant control antibody (100