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The lymphatic system controls intestinal inflammation and inflammation-associated colon cancer through the chemokine decoy receptor D6

Stefania Vetrano, Elena M Borroni, Adelaida Sarukhan, Benedetta Savino, Raffaella Bonecchi, Carmen Correale, Vincenzo Arena, Massimo Fantini, Massimo Roncalli, Alberto Malesci, Alberto Mantovani, Massimo Locati, Silvio Danese

ABSTRACT

Background and aims Inflammatory CC chemokines have long been associated with cancer, but unequivocal evidence of a role in clinically relevant models of carcinogenesis is lacking. D6, a promiscuous decoy receptor that scavenges inflammatory CC chemokines, plays a non-redundant role in reducing the inflammatory response in various organs. As inflammation is a key player in the development of inflammatory bowel disease (IBD) and IBD-associated colorectal cancer, we investigated D6 expression in human colitis and colon cancer, and its role in experimental colitis and inflammation-associated colon cancer.

Results In humans, D6 was mainly expressed by lymphatic vessels and leukocytes in the mucosa of individuals with IBD and colon cancer, as well as the mucosa of control individuals. Mice lacking expression of D6 were significantly more susceptible to experimental colitis than wild-type mice and failed to resolve colitis, with significantly higher levels of several pro-inflammatory chemokines. In bone marrow chimeric mice, the ability of D6 to regulate colitis was tracked to the stromal/lymphatic compartment versus haemopoietic cells. Finally, after administration of the carcinogen azoxymethane, D6−/− mice showed increased susceptibility to colitis-associated cancer in the distal segment of the colon compared with wild-type mice.

Conclusions D6 expressed on lymphatic vessels plays a key role in the control of intestinal inflammation and the development of inflammation-associated colon cancer. Our results reveal a new unexpected role for the lymphatic system in the pathogenesis of IBD and intestinal cancer, and candidate chemokines as novel players in tumour promotion and progression.

Leukocyte egress from the circulation is a fundamental component of inflammation. Chemokines are secreted chemotactic cytokines with a fundamental role in guiding leukocytes into tissues. Chemokines can be classified, according to the relative position of cysteine residues, into four families (CXC, CC, CX3C and C) and according to whether they are constitutive/homeostatic or in response could contribute to the chronic intestinal inflammation and the switch in malignancy.
The aim of this study was to investigate the role of D6 in colitis and inflammation-associated colon cancer. We found that D6 expression is increased in the lymphatic vascular bed of colonic sections from IBD patients and patients who have developed colon cancer associated with IBD compared with healthy subjects. In a murine model of dextran sulfate sodium (DSS)-induced colitis, ablation of the D6 receptor resulted in increased production of chemokines and recruitment of inflammatory cells, and in the development of more severe colitis. Experiments in bone marrow chimeras clearly indicated that D6 expressed in the non-haemopoietic compartment had a prominent role in the control of intestinal inflammation. When cancer was chemically induced, the increased susceptibility to inflammation of D6-deficient mice led to more severe malignancy. These results reveal a novel role of the lymphatic system in the control of intestinal inflammation and inflammation-associated colon cancer through the non-redundant chemokine-scavenging activity of the chemokine decoy receptor D6.

**METHODS**

**Patients**

Intestinal tissues were obtained from surgical specimens of patients with Crohn’s disease, ulcerative colitis and IBD-associated colon cancer. Normal areas of the intestine of patients admitted for bowel resection because of colon cancer, polyps or diverticulosis were used as controls. Specimens were formalin-fixed and paraffin-embedded or frozen in Cryoblock Compound (DiaPath, Bergamo, Italy) on dry ice and stored at -80°C. Human studies were approved by the ethics committee of the Istituto Clinico Humanitas.

**Animals**

Ly5.1 and Ly5.2 congenic C57BL/6 mice (Jackson Laboratory, L’Arbresle Cedex, France) and D6-/- mice, generated as described by Jamieson et al., were maintained under pathogen-free conditions. Procedures involving animals conformed with institutional guidelines in agreement with national and international law and were approved by the ethics committee of the Istituto Clinico Humanitas. For bone marrow transplantation, recipient mice were maintained on antibiotic water (800 mg/ml gentamycin; Italfarmaco, Milan, Italy) for 1 week before irradiation. Mice were irradiated with two consecutive doses of 450 rad delivered within 2 h. D6-/- and Ly5.2 C57BL/6 wild-type mice were reconstituted with Ly5.1 C57BL/6 wild-type bone marrow cells (Ly5.1 into D6-/- chimeras). Reverse chimeric mice were also created by lethally irradiating Ly5.1 mice and reconstituting them with either D6-/- (D6-/- into Ly5.1 chimeras) or wild-type bone marrow cells (wild-type into Ly5.1 chimeras). Bone marrow cells from donor mice (4 x 10^6 in 200 ml of saline per mouse) were injected into each recipient mouse through the retro-orbital venous plexus 1–3 h after irradiation. Mice were maintained on antibiotic water for 1 week after irradiation, and...
then placed on regular water. After 4 weeks of bone marrow transfer, mice were monitored weekly for the presence on blood leukocytes of CD45 allelic variants using fluorescently labelled monoclonal antibodies (BD Pharmingen, San Diego, California). Cell counts in whole blood were determined using a cell counter (Beckman Coulter, Milan, Italy).

**Experimental colitis and colitis-associated colon cancer**

In the acute colitis model, 8–12-week-old male mice received 3% DSS (MP Biomedicals, Illkirch, Europe) ad libitum in filter-purified drinking water for 7 days. The DSS solution was changed every 2 days. In the colitis-associated colon cancer model, mice were injected intraperitoneally with 10 mg/kg azoxymethane (AOM; Sigma, St. Louis, Missouri, USA) and kept on regular water for 7 days. After 7 days, mice were subjected to three oral cycles of 1.5% DSS, each characterised by 7 days of DSS exposure followed by 14 days of regular water. Colitis severity was scored using a disease activity index score based on daily evaluation of body weight, diarrhoea and presence of blood in stools. Surviving mice were sacrificed on day 57. Grading of intestinal inflammation was determined as previously described. The colonic mucosa was analysed histologically and three grades of mucosal lesion were identified: glandular intraepithelial neoplasia (indicating small clusters of dysplastic colonic crypts on the mucosal surface), and low grade and high grade adenomas. A single experienced pathologist reviewed all cases blindly.

**Endoscopic damage assessment**

Damage to the colonic mucosa during acute and chronic treatment with DSS was followed in vivo using the experimental endoscopy setup Colorview System, (Karl Storz, Tuttlingen, Germany) as previously described. A modified murine endoscopic index score of colitis severity based on evaluation of colon translucency (0–3 points), presence of fibrin attached to the bowel wall (0–3 points), granular aspect of the mucosa (0–3 points), morphology of the vascular pattern (0–3 points), and

**Figure 2**

Colitis severity is increased in D6−/− animals. Wild-type (WT) and D6−/− mice were administered dextran sulphate sodium (DSS) in drinking water for 7 days, and the development of colitis in both groups was compared. Starting from day 6, the reduction in body weight (A) was more rapid, and the disease activity index (DAI) (B) was more severe in D6−/− mice. At day 7, greater signs of inflammation were visible in the colons of the D6−/− mice as visualised by endoscopy (C and D). After sacrifice (day 8), histology of mucosal sections showed more severe microscopic inflammation in the tissues from D6−/− mice (E and F). D6 transcript levels were detected in murine colon obtained from normal mice (CTRL) and significantly increased in colitis mice (DSS) at sacrifice (day 8), as assessed by quantitative real-time PCR (G). Results represent the amount of D6 transcript over the GAPDH used as a housekeeping gene. Five animals/group in three independent experiments were used. *p<0.01.
Figure 3  Inflammatory chemokine expression and leukocyte recruitment are increased in D6−/− mice. At day 8 after dextran sulphate sodium (DSS) administration, the expression of the inflammatory chemokines CCL3/MIP-1, CCL5/RANTES, CXCL1/KC, CCL2/JE and CXCL2/MIP-2 (A–E, respectively) was compared in D6−/− and wild-type (WT) mice. All chemokines were found to be increased in D6−/− mice as compared with wild-type mice. At day 8 after DSS administration, mucosal tissues from wild-type and D6−/− mice were stained in green for the T cell marker CD3 (F–G, respectively), the macrophage marker CD68 (I–L respectively), the dendritic cell marker CD11c (N, O, respectively) and the B cell marker CD45/B220 (Q, R, respectively). Quantitation of infiltrating leukocytes showed a significantly higher number of T, macrophage and dendritic cells in D6−/− mice as compared to wild-type animals (H, M, P, S; 20× magnification). *p<0.01. Nuclei are stained with Hoechst (blue staining).
the presence of loose stools (0–3 points) was applied, as previously described.23

**Isolation of RNA and real time RT-PCR**

Colonic RNA was extracted from whole colon with TRIzol (Invitrogen, Milan, Italy) according to the manufacturer’s instructions. First-strand cDNA synthesis (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems, Monza, Italy) from 2 μg of total RNA was conducted according to the manufacturer’s instructions. A Chromo4 Real-Time PCR Detector (MJ Research, Waltham, Massachusetts, USA) was used for quantitative real-time PCR on cDNA (200 ng) using TaqMan Universal PCR Master Mix (Applied Biosystems) and primers specific for murine GAPDH (TaqMan Gene Expression Assay Mm99999915_g1; Applied Biosystems) and murine D6 (TaqMan Gene Expression Assay Mm00445551_m1; Applied Biosystems). Results represent the relative abundance (expressed as percentage of 2-ΔCt, where ΔCt=Ct sample−Ct GAPDH) of mD6 transcript over the total amount of GAPDH.

**Colon organ culture and ELISA assay**

Colon was excised, opened and cut longitudinally into three sections. One section was washed in cold PBS supplemented with penicillin, streptomycin and amphotericin B (BioWhittaker Europe, Cambrex), weighed and incubated at 37°C and 5% CO2 in serum free RPMI 1640 medium supplemented with 0.1% FBS, penicillin, streptomycin and amphotericin B. After 24 h, supernatant fluid was collected, centrifuged and stored at −20°C. The concentration in the supernatants of the chemokines CCL3, CCL5, KC, JE and MIP2 was analysed in duplicates using commercially available ELISA kits (R&D Systems, Minneapolis, USA).

**Immunofluorescence and immunohistochemistry**

Frozen 4 μm sections of colonic mucosa from human and murine tissues were fixed in cold acetone (10 min at −20°C), blocked with PBS containing 3% BSA for 60 min at room temperature, and incubated with primary antibodies. Human tissue sections were incubated for 1 h at room temperature with anti-D6 (1:100 dilution; Santa Cruz, California, USA), anti-D240 (1:80 dilution; Signet, Dedham, Massachusetts, USA) and anti-CD45 (1:50 dilution; Santa Cruz, California, USA), anti-D240 (1:80 dilution; Signet, Dedham, Massachusetts, USA) and anti-CD45 (1:50 dilution; Dako, Milan, Italy), while murine tissue sections were incubated for 1 h at room temperature with anti-CD3 (1:50 dilution; Dako), anti-CD68 (1:200 dilution; BD Pharmingen), anti-CD11c (1:100 dilution; BD Pharmingen), MPO (1:10 dilution; Abcam, Cambridge, UK), β-catenin (1:100 dilution; R&D Systems), Ki-67 (1:200 dilution; Abcam) and CD45/B220 (1.50 dilution; BD Pharmingen). Sections were then incubated for 30 min at room temperature with Alexa Fluor 488-conjugated goat anti-mouse, anti-rat or anti-rabbit IgG and/or Alexa Fluor 594-conjugated goat anti-mouse or anti-rat IgG antibodies (1:1000 dilution; Invitrogen), followed by incubation for 5 min at room temperature with 1 μg/ml Hoechst 33258 (Invitrogen, Milan, Italy). Sections were mounted with FluorSave reagent (Calbiochem, San Diego, California, USA) and analysed with a laser scanning confocal microscope (FluoView FV1000; Olympus, Milan, Italy). Images (1024×1024 pixels) were acquired with an oil immersion objective (60×1.4 NA Plan-Apochromat; Olympus). Infiltrating leukocytes were counted in 10 random fields within inflamed and tumour mucosa of each area under light microscopy at 40×magnification. The proliferative activity represents the percentage of Ki-67-labelled cells/high magnification field. The TUNEL assay was performed on cryostatic-sections using the In Situ Cell Death Detection kit (Roche Diagnostics, Milan, Italy), according to the manufacturer’s recommendations. Apoptosis was quantified as a percentage of apoptotic cells/high magnification field. Immunohistochemical staining for D6 was performed as previously reported.22 Briefly, paraffin-embedded intestinal sections of histologically normal control and IBD-involved mucosa or colon cancer, were cut at 3 mm thickness, deparaffinised, hydrated, blocked for endogenous hydrazine using 3% H2O2/H2O, and subsequently subjected to microwave epitope enhancement using a Dako Target retrieval solution. Incubation with the primary antibody for D6 was carried out for 30 min at room temperature. Detection was achieved using a standard streptavidin-biotin system (Vector Laboratories, Burlingame, California, USA), and antigen localisation was visualised with 3'-3-diamino benzidine (Vector Laboratories). Quantification of D6 expression was performed on immunostained sections by a semiquantitative method (scores from 0 to 5), as previously described.23

**RESULTS**

**Expression of D6 is increased in colonic sections from patients with IBD**

Expression of D6 has been reported in trophoblast cells, leukocytes, and in the lymphatic endothelium of skin, lung and breast, but no information exists regarding its function in the gut.12 We therefore used confocal fluorescence microscopy to examine the mucosal tissue derived from control individuals, as well as from patients

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*Figure 4 D6 expressed by lymphatic endothelial cells controls susceptibility to colitis. Bone marrow chimeric mice lacking D6 expression on leukocytes (D6−/− in Ly5.1) do not display any differences in body weight loss compared to chimeric mice with normal D6 expression (wild-type (WT) in Ly5.1) (A). In contrast, mice lacking D6 expression on lymphatic endothelial cells (Ly5.1 in D6−/−), show increased susceptibility to colitis after 8 days of 3% dextran sulphate sodium administration compared to Ly5.1 in wild-type mice (B). Data are representative of three repeated experiments. *p<0.05.*

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Intestinal inflammation

with active Crohn’s disease or ulcerative colitis, and colon cancer tissue from patients with inflammation-associated colon cancer. Normal colonic mucosa showed strong vascular lymphatic and mononuclear immunostaining of D6 (figure 1). The specificity of D6 expression by the lymphatics and mononuclear cells was confirmed by co-staining with the lymphatic marker D240 (figure 1A) and the leukocyte marker CD45 (figure 1B). Patients with IBD and colon cancer showed similar expression patterns (figure 1C), and a semi-quantitative scoring of tissue samples showed a significant increase (p<0.05) of D6 expression on lymphatic vessels in ulcerative colitis (1.4±0.1), Crohn’s disease (1.6±0.1) and colitis-associated cancer (1.7±0.1) compared to normal mucosa (0.5±0.1). No differences were observed in D6 expression by intestinal mucosal leukocytes between control individuals and patients with IBD and cancer (not shown).

D6−/− mice show increased susceptibility to experimental colitis

To investigate the functional role of D6 in intestinal inflammation, we used the DSS-induced model of colitis. Both weight loss (figure 2A) and disease activity index (figure 2B) were more severe in D6−/− mice as compared with wild-type mice. D6−/− mice also

Figure 5  Tumour incidence and severity is increased in D6−/− mice. Wild-type (WT) and D6−/− mice were administered azoxymethane followed by three oral cycles of 1.5% dextran sulphate sodium. Disease severity was greater in D6−/− mice, as demonstrated by body weight loss (A) and disease activity index (DAI) (B). Tumour incidence was greater on endoscopy (C) and gross anatomy (D) examination. Histological classification of tumours (E) demonstrated that D6−/− mice showed a significantly higher number of tumours at each grade (GIN, glandular intraepithelial neoplasia; HG, high-grade dysplasia; LG, low-grade dysplasia).

displayed a significant increase in endoscopic damage score (figure 2C,D) and more severe histological inflammation (figure 2E,F). No differences were observed between D6−/− and wild-type mice in the absence of induction of DSS colitis, as assessed by endoscopy and histology (data not shown). Interestingly, as observed in human pathology, colitic wild-type mice also showed a significant increase of mRNA D6 expression in inflamed colon as compared to healthy animals (figure 2G).

**Increased production of inflammatory chemokines and leukocyte recruitment in D6−/− mice**

Since D6 is a decoy receptor for inflammatory chemokines, we next investigated the concentrations of chemokines with a well-recognised role in colitis development in colonic organ cultures obtained from D6−/− and wild-type mice after treatment with DSS for 8 days. As compared with cultures from wild-type mice, colonic mucosa from D6−/− mice produced significantly higher levels of the inflammatory chemokines CCL3/MIP-1α (figure 3A), CCL5/RANTES (figure 3B), CXCL1/KC (figure 3C), CCL2/JE (figure 3D) and CXCL2/MIP2 (figure 3E). No differences in inflammatory chemokines were observed in colonic organ cultures obtained from D6−/− and wild-type mice not treated with DSS (data not shown).

Because inflammatory chemokines are fundamental mediators of leukocyte recruitment, we next investigated leukocyte infiltration in the gut. After 8 days of DSS treatment, D6−/− colitic...
mice showed a significant increase in the number of CD3+ T cells (figure 3G,H), CD68+ macrophages (figure 3L,M), CD11c+ dendritic cells (figure 3O,P) and CD45/B220+ B cells (figure 3R,S) in the inflamed mucosa as compared with wild-type mice (figure 3E,I,N,Q). No differences in leukocyte infiltrate were observed in healthy untreated D6−/− and wild-type mice (data not shown).

**The severity of colitis is controlled by D6 expressed on the lymphatic endothelial cells**

To investigate the relative contribution of D6 expressed on lymphatic endothelial cells or leukocytes to the increased severity of experimental colitis observed in D6−/− mice, we performed a series of experiments using bone marrow chimeras. The role of D6 expressed by the haemopoietic compartment was determined by reconstituting irradiated wild-type mice with bone marrow from D6−/− or D6-competent mice. These chimeric mice, which lack D6-expressing haemopoietic cells but express D6 in the stroma, developed a DSS colitis that was indistinguishable from that observed in wild-type mice and in irradiated wild-type mice reconstituted with wild-type bone marrow (figure 4A). In the reciprocal set of experiments, the role played by lymphatic D6 was determined by reconstituting irradiated

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**Figure 7** Epithelial characterisation in colon cancer. Fluorescence micrographs of colons in wild-type (WT) and D6−/− mice stained respectively for β-catenin (green staining; A–B), Ki-67 (green staining; C–D) and TUNEL (green staining; E–F). Nuclei are stained with Hoechst (blue staining). Images are representative of at least 10 wild-type and 10 D6−/− mice. Magnification: 50×.
Ly5.2+ D6−/− mice with bone marrow from Ly5.1+ wild-type mice. These chimeric mice, in which D6 is expressed on haemopoietic cells but not on endothelial cells, developed colitis in response to DSS similarly to mice that completely lacked D6−/−, but more severely than that developed by wild-type mice or wild-type mice reconstituted with wild-type bone marrow (figure 4B). Consistent with a more severe clinical course, histological inflammation was more severe in mice lacking D6 expression in the stromal/lymphatic compartment (data not shown). Taken together, these data suggest that D6 expressed in the lymphatic compartment has a prominent role in the control of intestinal inflammation.

**DISCUSSION**

Inflammation-associated colon cancer in D6−/− mice

We next investigated whether there was an increase in the overall recruitment of inflammatory cells in the chronic colitis associated-cancer model. Compared with wild-type mice (figure 6A,D,G,L), D6−/− colitic mice showed a significant increase in the number of CD3+ T cells (figure 6A–C), CD68+ macrophages (figure 6E, F), CD11c+ dendritic cells (figure 6H,I) and CD45/B220+ B cells (figure 6M,N) in the cancer mucosa.

In addition, since it has been reported that the β-catenin pathway is implicated in human colon cancers,10 and that it is mutated in the AOM model of experimental cancer, we investigated its expression in wild-type and D6−/− mice. As shown in figure 7, β-catenin was easily detected in both groups of wild-type (figure 7A) and D6−/− (figure 7B) mice, and it displayed a clear shift from the membrane towards a cytoplasmic and nuclear localisation, a phenomenon that was more evident in the D6−/− mice (figure 7B), consistent with higher tumour incidence. Furthermore, as compared to wild-type mice (figure 7C,E), D6−/− displayed a significantly higher number of Ki-67-positive epithelial cells (18.7±1.7 vs 47.6±8.9, respectively; p<0.01) (figure 7D) and TUNEL activity (figure 7F) (16.5±1.1 vs 24.4±1.1, respectively; p<0.001).

**Intestinal inflammation**

D6 is an atypical non-signalling chemokine decoy receptor that internalises and degrades a broad panel of pro-inflammatory CC chemokines responsible for the recruitment of different leukocyte subsets through the activation of the conventional chemokine receptors CCR1 to CCR5. Different experimental models of chronic inflammation in skin and lung have demonstrated that inappropriate clearance of inflammatory chemokines in the absence of D6 lead to excessive and prolonged inflammatory reactions.20 27 We now report D6 expression on both lymphatics and intestinal leukocytes in the gut, similarly to what has been reported for other tissues.20 29 When the functional role of D6 in intestinal inflammation was investigated in the DSS model of colitis, clinical, endoscopic and histological parameters indicated that the susceptibility to colitis of D6−/− mice was significantly increased as compared to wild-type animals. In addition, consistent with the chemokine scavenger function of D6, we also found that intestinal D6 controls the bioavailability of several inflammatory CC chemokines. We also found increased levels of the CXC chemokines CXCL1/KC and CXCL2/MIP-2, possibly related to the increased mucosal inflammation that occurs in D6−/− mice and to the previously described ability of chemokines to regulate expression of other chemokines.30

Bone marrow chimera experiments revealed that D6 expressed on the stromal compartment had a prominent role in the clearance of inflammatory chemokines and in the development of colitis, as compared to D6 expressed on the leukocytic compartment. Since among stromal elements only lymphatic endothelial cells express D6, these results point to a central role of lymphatic endothelial cells in the regulation of intestinal inflammation.

D6 in lymphatic endothelium has been suggested to scavenge inflammatory CC chemokines in tissues and to act as a gate keeper preventing excessive transfer of chemokines to lymph nodes, although it has been argued that the distance between lymphatics in tissues would make scavenging by lymphatic endothelial cells inefficient.31 In agreement with a gate keeper function, D6−/− mice show increased levels of CC chemokines in lymph nodes29 and lymph node hyperplasia in response to inflammatory signals.20 29 32 The finding of a pivotal role of lymphatic expression of D6 in reducing intestinal inflammation is consistent with a direct scavenging function at this level, although an indirect regulatory pathway centred on lymph node-mediated orchestration of inflammation could also play a role. In vivo evidence indicates that D6 expression is increased under inflammatory conditions, both in human diseases and in experimental models (Jamieson et al20 and this report). Interestingly, however, in vitro evidence indicates that D6 expression is not under the direct control of inflammatory mediators; conversely, it is induced by anti-inflammatory stimuli.32 Taken together with growing evidence from gene-targeted animals of a protective role of D6 in several inflammatory conditions,16 20 29 32 these observations support the notion that D6 is induced during inflammatory reactions to contribute to resolution of inflammation through the control of chemokine availability. However, its upregulation in experimental and human IBD is unlikely to be sufficient to remove the excess inflammatory chemokines in the inflamed gut.

Since ulcerative colitis dramatically increases the risk of developing colon cancer, we investigated the role played by D6 in the development of inflammation-associated colon cancer. We found that intestinal D6 is crucially involved in tumour formation and growth, as D6−/− mice displayed increased
tumour incidence and size. Since tumour formation, including colon cancer, is promoted by leukocyte infiltration, we next investigated the composition of CD3, CD68 and CD11c cells. Concomitantly with increased tumour incidence and growth, we found an increase in the leucocyte infiltrate that sustains tumour growth in D6−/− mice. Moreover, epithelial cells in D6−/− mice showed a significant increase in cell turnover and in β-catenin nuclear translocation.

Inflammation and cancer are linked.34 Inflammatory CC chemokines have long been associated with cancer35 and are a candidate target for therapeutic intervention.36 However, unequivocal genetic evidence for a role in carcinogenesis in a clinically relevant system is missing. This gap in knowledge may well be due to the “robustness” of the chemokine system, and in particular CC chemokines and their receptors, in attracting mononuclear phagocytes. While the present study was in progress, Nibbs et al reported that D6−/− mice show increased susceptibility in the DMBA/TPA skin carcinogenesis model.37 D6−/− shows a psoriasis-like pathology upon TPA painting, sustained by T cells and inflammatory cytokines.20 Psoriasis represents an exception in the general scenario of cancer-related inflammation, in that patients with this disorder are protected against skin cancer development. It was therefore important to assess the role of inflammatory CC chemokines in a clinically relevant model of carcinogenesis.39 The results presented here, which take advantage of the unique capacity of D6 to recognise and scavenges most inflammatory CC chemokines, provide unequivocal evidence in a model representative of human colitis-associated cancer, of the non-redundant role of these mediators in orchestrating cancer-promoting inflammation.

In conclusion, these observations demonstrate that the decoy receptor for inflammatory chemokines D6 expressed on lymphatic vessels plays a key role in the control of intestinal inflammation and in the development of inflammation-associated colon cancer. In addition, our results reveal the lymphatic system as a new cellular compartment with an unexpected role in the pathogenesis of IBDC. We confirm the established link between colonic inflammation and intestinal cancer, and show candidate chemokines as novel players in tumour promotion and progression.

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Competing interests None.

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Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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