INFLAMMATORY BOWEL DISEASE

Systemic administration of the chemokine macrophage inflammatory protein 1 α exacerbates inflammatory bowel disease in a mouse model

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Introduction: Exacerbations of inflammatory bowel disease are thought to be related to concurrent infections. As infections are associated with elevated local and serum concentrations of chemokines, we have determined whether systemic administration of the CC chemokine macrophage inflammatory protein 1 α (MIP-1 α) exacerbates colitis in a mouse model.

Methods: Colitis was induced in Balb/c mice using trinitrobenzene sulfonic acid (TNBS). Starting four days later, animals received daily intraperitoneal injections of recombinant MIP-1 α . On day 7, mice were killed and pieces of colon taken for immunohistology and polymerase chain reaction analysis. The direct effects of MIP-1 α on mucosal T cells and fibroblasts *in vitro* were also investigated.

Results: Systemic administration of MIP-1 α markedly enhanced colitis with mice developing large transmural ulcers filled with granulation tissue. Treatment resulted in increased numbers of CD4 cells infiltrating the colonic lamina propria, increased interferon γ (IFN- γ) levels, and increased transcripts for tumour necrosis factor α (TNF- α) and matrix metalloproteinase 3 (MMP3). Isolated lamina propria lymphocytes from mice with TNBS colitis contained increased numbers of IFN- γ and TNF- α transcripts when stimulated with MIP-1 α *in vitro*. Colonic lamina propria fibroblasts also responded to MIP-1 α with increased proliferation and decreased collagen 1 synthesis but fibroblast proliferation was not seen *in vivo*.

Conclusions: These experiments show that increasing serum concentrations of a chemokine, MIP-1 α , exacerbates immune mediated colitis. The effect seems to be due to the ability of MIP-1 α to boost Th1 responses in the gut wall. Our findings also suggest a potential pathway by which peripheral infections can exacerbate inflammatory bowel disease.

While there are now many and varied strategies to induce remission in patients with inflammatory bowel disease (IBD), the high relapse rates continue to be problematic. There are very few clues as to why some patients remain disease free for prolonged periods while others relapse rapidly. Crohn's patients, whose mucosal biopsies secrete high levels of tumour necrosis factor α (TNF- α), relapse faster than those who secrete low levels of TNF- α ,¹ but this may merely reflect the fact that the former were not really in remission and that definitions of clinical remission are insensitive.²⁻⁴ Indeed, a classic study by Modigliani *et al* showed that the majority of patients in clinical remission after treatment with prednisolone had lesions visible at endoscopy.⁵

There is also a large body of largely anecdotal evidence to suggest that extraintestinal events may trigger relapse in IBD. Acute viral enteritis may trigger relapse symptoms⁶ and epidemiological and microbiological studies suggest that infections with gut pathogens trigger relapse (reviewed by Stallmach and Carstens⁷). Gut infections however cannot be responsible for relapse in the majority of cases because these are relatively rare in developed countries, especially in adults. On the other hand, respiratory tract infections are common in adults and children in the developed world and respiratory infections have been shown to be associated with relapse in paediatric IBD.⁸

Viral infection of respiratory tract epithelial cells *in vitro* and during *in vivo* infection leads to the production of a large number of chemokines such as RANTES, macrophage inflammatory protein 1 α (MIP-1 α), monocyte chemoattractant protein 1, and interleukin (IL)-8,⁹⁻¹³ the function of

which is to attract inflammatory cells from the blood into the tissues. Consequently, elevated concentrations of these chemokines are found in serum, and nasopharyngeal and tracheal secretions of children with respiratory tract infections.⁹⁻¹²

Chemokines however have a much broader role than merely attracting leucocytes into tissues. There is now a large body of evidence to show that the binding of a chemokine to its receptor (a G protein coupled receptor) can modulate leucocyte activity by activating intracellular signal transduction pathways.¹⁴⁻¹⁷

In this study, we have therefore attempted to model the effects of elevated concentrations of chemokines as a result of infection by direct systemic injection of recombinant protein into mice with trinitrobenzene sulfonic acid (TNBS) colitis. The chemokine we chose to investigate was MIP-1 α (CCL3) as it is well established as being present at elevated concentrations in airway infection.⁹⁻¹² MIP-1 α is a C-C chemokine with three cell surface receptors, CCR1, CCR3, and CCR5.¹⁸ It is made by many different cell types and *in vivo* and *in vitro*, it is a chemoattractant for natural killer cells, monocytes, neutrophils, T cells, and B cells.¹⁸⁻²⁰ Our results indicate that MIP-1 α exacerbates colitis, probably by

Abbreviations: MIP-1 α , macrophage inflammatory protein 1 α ; TNBS, trinitrobenzene sulfonic acid; TNF- α , tumour necrosis factor α ; MMP3, matrix metalloproteinase 3; IBD, inflammatory bowel disease; IL, interleukin; PCR, polymerase chain reaction; LPL, lamina propria lymphocytes; FCS, fetal calf serum; TGF- β , transforming growth factor β ; PBS, phosphate buffered saline; PBT, PBS/Tween 20; BSA, bovine serum albumin

directly augmenting the function of activated Th1 cells in the gut wall.

MATERIALS AND METHODS

Induction of colitis

TNBS (Fluka, Gillingham, UK) was prepared in a 50% ethanol solution diluted to give a final concentration of 2 mg TNBS in 75 μ l total volume. Adult female Balb/c mice weighing more than 20 g were lightly anaesthetised using 200 μ l of a 1/10 aqueous dilution of Hypnorm (Janssen-Cilag, High Wycombe, UK). Colitis was induced by intrarectal administration of 75 μ l of the TNBS solution using a plastic catheter. Control mice received 50% aqueous ethanol only. Mice were checked daily with respect to general condition and body weight. On days 4–6, mice were injected with 0.2 μ g, 2, or 20 μ g recombinant murine MIP-1 α in PBS (Oxagen Ltd). Controls received phosphate buffered saline (PBS) alone. Animals were killed on day 7. This work was carried out with both institutional and Home Office approval.

Immunohistochemistry

Mice were killed and the last 4 cm of the distal colon dissected out. The tissue was opened, washed, and weighed. A small piece was taken for polymerase chain reaction (PCR) and the rest was dissected open, rinsed in PBS, and tightly rolled along the longitudinal axis, then immediately snap frozen in liquid nitrogen and stored at -80°C . Three step avidin-peroxidase staining was then performed on 5 μ m frozen sections, as described previously,²¹ using monoclonal antibodies YTS191 (anti-CD4), Ki67, and HLA-DR (Dako Ltd, Cambridge, UK). Biotin conjugated rabbit anti-rat IgG and goat antirabbit (Dako) were used at 1/50 dilution in Tris buffered saline (pH 7.6) containing 4% (v/v) normal mouse serum (Harlan Seralab, Oxon, UK). Peroxidase activity was detected with 3,3'-diaminobenzidine tetrahydrochloride (Sigma, Poole, U.K.) in 0.5 mg/ml Tris HCl (pH 7.6), containing 0.01 % H_2O_2 (Sigma). The density of positive cells in the lamina propria was determined by image analysis (Zeiss, UK).

RNA extraction and quantitative RT-PCR

Total cellular RNA was isolated from frozen colonic tissues by homogenisation of the tissue in TRIzol (Invitrogen, Paisley, UK) followed by chloroform extraction and isopropanol precipitation. Total RNA was measured at 260 nm by spectrophotometric analysis (Beckman Coulter, Buckinghamshire, UK). Cytokine encoding plasmid (pCMQ2), kindly provided by MF Kagnoff (Department of Medicine, University of California, San Diego, California, USA), was used for quantitative competitive PCR for TNF- α transcripts, as described previously.²¹ To quantify mouse matrix metalloproteinase 3 (MMP3) mRNA levels, we constructed a plasmid that encoded a standard RNA.²²

Interferon γ (IFN- γ) measurements

Mice were given TNBS and on days 4–6 they were given 2 μ g MIP-1 α . On day 7, the animals were killed and a segment of distal colon was dissected out and snap frozen in liquid nitrogen. Samples were homogenised in ice cold extraction buffer (50 mM Tris HCl (pH 7.4), 10 mM CaCl_2 , 0.05% Brij 35, 0.25% Triton X-100) at maximum speed for 30 seconds using IKA tissue homogeniser (Fischer, Loughborough, UK). Homogenates were centrifuged at 13 000 rpm for 10 minutes at 4°C and the supernatants were removed and assayed for protein concentration (Bio-Rad, Hertfordshire, UK). IFN- γ in the supernatants was then measured using a commercial ELISA (R&D Systems, Abingdon, Oxon, UK) with a lower limit of sensitivity of 2 pg/ml.

In vitro stimulation of lamina propria lymphocytes (LPL)

Four days after TNBS or ethanol, mice were killed and LPL isolated from the colon by first removing the epithelium with EDTA (confirmed by histology) and collagenase digestion, as described previously.²³ The same frequency of T cells (approximately 30%) was seen in cells isolated from ethanol controls and mice with TNBS colitis but the total cell yield was much higher in the latter group. One million cells were cultured in 1 ml RPMI 1640 with 10% heat inactivated fetal calf serum and antibiotics for 48 hours with and without addition of MIP-1 α . At the end of the culture period, cells were harvested from 3 wells/group, pooled, and RNA extracted for PCR. Supernatants were also collected and stored at -70°C and IFN- γ measured by ELISA.

Fibroblast cell culture

Colons were taken from three six week old Balb/c mice, cut open, and vigorously washed in cold PBS. The epithelium and mucus were scraped off with a scalpel blade and discarded. The remaining tissue was minced with two scalpel blades in serum free RPMI culture medium containing antibiotics (penicillin/streptomycin 200 units/200 μ g/ml, gentamycin 100 μ g/ml, and amphotericin B 0.25 μ g/ml) and HEPES (20 mM). Pieces were washed with two changes of medium. Six well plates were seeded with aliquots of the tissue in 1 ml α -MEM containing antibiotics, ribonucleosides, and 10% fetal calf serum (FCS) and cultured in a humidified CO_2 incubator. Half the media was replaced after four days and by day 6, out-growing fibroblasts were observed. Cells were allowed to grow in six well plates with weekly media changes until about day 21 when cells were removed with trypsin-EDTA and placed in 25 cm^2 flasks. Thereafter, cells were trypsinised when confluent and flasks reseeded at 100 cells/ mm^2 .

Cells were grown up to at least passage 8 before there were enough to use in experiments and produced type I and IV collagens, laminin, and expressed smooth muscle cell actin, vimentin, and TGF- β receptors I and II (not shown).

All culture media and supplements were from Invitrogen (Glasgow, UK).

ELISA for matrix proteins and cell proliferation assay

A method was developed to assay deoxycholate insoluble type I collagen produced by fibroblasts grown in 96 well tissue culture plates.²⁴ Cells (passage 10–15) were grown in flasks until just confluent, treated with trypsin-EDTA, washed once in serum containing medium, and then plated

Figure 1 Effect of injection of graded doses of macrophage inflammatory protein 1 α (MIP-1 α) on colonic weight in mice with trinitrobenzene sulfonic acid (TNBS) colitis. Mice received ethanol (EtOH) or TNBS and on days 4, 5, and 6 TNBS treated mice were injected with 0.2, 2.0, or 20 μ g MIP-1 α . There were five mice per group and the experiment was repeated twice with the same results. All mice receiving MIP-1 α had significantly greater colon weights than mice receiving TNBS and vehicle alone ($p < 0.05$).

Figure 2 Histological analysis of tissues from ethanol (EtOH) controls, mice given trinitrobenzene sulfonic acid (TNBS), and mice given TNBS and macrophage inflammatory protein 1 α (MIP-1 α). These preparations show the thin colonic mucosa in ethanol controls. In mice given TNBS and vehicle, the mucosa is thickened and areas of ulcerations are seen (arrow). In the two examples from different animals given TNBS and 2 μ g MIP-1 α , large ulcers filled with granulation tissue are all that remains of the mucosa. Haematoxylin-eosin, original magnification $\times 100$.

at 2000 cells per well. Cells were allowed to adhere for five hours at 37°C before gently washing three times with serum free medium. Cells were starved overnight in 50 μ l of α -MEM containing 0.5% FCS (starvation medium) and then this medium was replaced with 50 μ l starvation medium containing MIP-1 α (1–1000 pg/ml), TGF- β (1000 pg/ml; R&D), or 10% FCS as positive control (six replicates per treatment). Incubations were stopped at day 0 (after overnight starvation) and four days of culture by washing three times in PBS and air drying for two hours. Duplicate wells on each plate were left cell free and while cells were air drying, a standard curve was prepared for mouse type I collagen (kind gift from Mr T Simms, Southmead Hospital, Bristol, UK). Type I collagen standard (250 ng in 10 μ l 0.05 M HCl) was added to the first well and then serial dilutions (1:3) were made thereafter with a final volume (10 μ l) remaining in the well. Plates were vortexed gently to distribute the matrix standards and then allowed to air dry for two hours. Cells were permeabilised with PBS containing sodium deoxycholate (0.1%) and EDTA (2 mM) for 10 minutes, washed three times in PBS/Tween 20 (0.05%) (PBT), and then all wells were blocked with 1% BSA in PBT (100 μ l) for one hour. After one wash in PBT, 1:4000 rabbit anti-type I collagen (50 μ l) (Novotec, Paris, France) was added in PBT/BSA (1%). Plates were incubated for one hour and washed four times in PBT before incubation with biotinylated donkey anti-rabbit antibody (1:25000; Stratech Scientific Ltd, Soham, Cambridge, UK). StreptABComplexes were prepared as per the manufacturer's instructions and then used in the ELISA at 1:50 dilution. Trimethyl benzidine in pH 5.0 citrate buffer (100 μ l) was used as substrate and incubated for 30 minutes. The reaction was stopped with 2 M H₂SO₄ (25 μ l) and plates were read within 30 minutes at 450 nm. On a separate plate, parallel sets of wells were prepared for cell counting (six replicates). Plates were incubated identically and then cells were washed and air dried as above. Cells were fixed with

100% methanol for 10 minutes before staining with filtered Harris's haematoxylin for 10 minutes, followed by a tap water wash and air drying. Plates were inverted and cells counted using the 2.5 $\times$ objective. The number of cells per field was counted manually and results are expressed as the mean (SEM) of the average number of cells per well calculated from six replicates. Type I collagen results are expressed as pg collagen/100 cells. As data are derived from independent wells, no standard error can be calculated

Statistical analysis

The significance of differences between means was determined using the paired *t* test and the Mann-Whitney U test. A *p* value of <0.05 was considered as significant.

RESULTS

Systemic administration of MIP-1 α exacerbates the lesions in TNBS colitis

In the first experiments, Balb/c mice with TNBS colitis were injected with MIP-1 α (0.2, 2.0, or 20 μ g/mouse) on days 4, 5, and 6 and killed on day 7. At necropsy, the most striking feature was the very large size and weight of the distal colon in MIP-1 α treated mice, which was dependent on the amount of MIP-1 α injected (fig 1). The colon was markedly thickened and adherent to adjacent loops of small bowel and the abdominal wall. Histological analysis of the tissues (fig 2) showed that ethanol controls had essentially normal colonic structure. Mice given TNBS had a thickened mucosa, enlarged colonic lymphoid follicles, and the occasional small ulcer. However, animals given MIP-1 α showed massive areas of ulceration containing granulation tissue. Adjacent intact mucosa was grossly distorted and showed large lymphoid follicles. Ethanol controls given the full dose range of MIP-1 α or normal mice given MIP-1 α showed no change in colon weight (negative data, not shown).

Figure 3 (A) Macrophage inflammatory protein 1 α (MIP-1 α) increases the number of CD4 and CD8 cells in intact mucosa of animals given trinitrobenzene sulfonic acid (TNBS) and MIP-1 α . Mice received ethanol (EtOH) or TNBS, and on days 4, 5, and 6 TNBS treated mice were injected with 0.2 (M0.2), 2.0 (M2), or 20 μ g (M20) of MIP-1 α . There were five mice per group and the experiment was repeated twice with the same results. All mice receiving MIP-1 α had significantly greater numbers of CD4 and CD8 cells than mice receiving TNBS and vehicle alone ($p < 0.05$). (B) Illustration of the increase in T cells in the colon of mice given TNBS and 2 μ g MIP-1 α . Immunoperoxidase with anti-CD4, original magnification $\times 200$. (C) Illustration of the absence of inflammatory cells from the ulcers induced by TNBS and MIP-1 α . Towards the left of the image is granulation tissue which almost completely lacks cells expressing MHC class II (macrophages). The right of the panel shows deeper layers of the gut wall where positive cells can be seen. Immunoperoxidase with anti-MHC class II, original magnification $\times 200$.

Systemic administration of MIP-1 α increases inflammatory cells, cytokines, and MMPs in TNBS colitis

Immunohistology for T cell and macrophage markers was used to study the lesions in TNBS and TNBS mice given MIP-1 α . The areas containing large ulcers showed granulation tissue containing virtually no T cells or macrophages (fig 3C). However, these were abundant in the deeper submucosa. Adjacent regions of intact mucosa in animals treated with MIP-1 α showed greater numbers of CD4 cells than TNBS controls (fig 3A, B). IFN- γ was also measured in tissue homogenates. Ethanol controls had a mean of 47 (1) pg/200 μ g colonic protein ($n = 5$). In mice with TNBS colitis given vehicle alone, this increased to 62 (6.9)/200 μ g colonic protein ($n = 5$; significantly greater than the ethanol control, $p < 0.004$). Mice given TNBS and MIP-1 α had even greater levels of IFN- γ (78 (10.7) pg/200 μ g protein; $n = 5$), which was significantly greater than TNBS mice given vehicle control ($p < 0.05$).

TNF- α transcripts were also measured and these were increased in mice with TNBS colitis compared with ethanol controls. However, in mice treated with MIP-1 α , even higher levels were seen (fig 4).

Finally, we also measured MMP3 transcripts as there is good evidence that it is an important end stage mediator of ulceration in the gut. These were increased in mice with TNBS colitis and further increased in mice given MIP-1 α (fig 4).

Effects of MIP-1 α on mucosal T cells

It has already been demonstrated that MIP-1 α can signal to T cells and increase Th1 activity.²⁵ We therefore gave mice TNBS or ethanol and four days later isolated colonic LPLs. Cells were then stimulated with low doses of MIP-1 α overnight and TNF- α and IFN- γ transcripts measured. As expected, there were higher numbers of transcripts for both cytokines in cells from mice with TNBS colitis than cells

taken from ethanol controls. Remarkably however, MIP-1 α markedly increased Th1 cytokines in cells from TNBS at a very low dose, although at a higher dose an effect was seen

Figure 4 Macrophage inflammatory protein 1 α (MIP-1 α) treatment increases tumour necrosis factor α (TNF- α) transcripts (A) and matrix metalloproteinase 3 (MMP3) transcripts (B) when given to mice with trinitrobenzene sulfonic acid (TNBS) colitis. Mice received ethanol (EtOH) or TNBS, and on days 4, 5 and 6 TNBS treated mice were injected with 2.0 μ g of MIP-1 α . There were five mice per group and the experiment was repeated once with the same results. All mice receiving MIP-1 α had significantly greater numbers of TNF- α and MMP3 transcripts than mice receiving TNBS and vehicle alone ($p < 0.05$).

Figure 5 Macrophage inflammatory protein 1 α (MIP-1 α) enhances cytokine secretion by colonic lamina propria lymphocytes (LPL) from mice with trinitrobenzene sulfonic acid (TNBS) colitis. LPL from mice with TNBS colitis (day 4) or ethanol (EtOH) controls were cultured with MIP-1 α for 48 hours and RNA transcripts measured. In control cultures, LPL from mice with TNBS colitis made more TNF- α and interferon γ (IFN- γ) transcripts than LPL from mice given ethanol. Addition of MIP-1 α however markedly increased transcripts, especially in cells from mice with TNBS colitis. No statistics are shown because the data were obtained from three pooled wells/group to give enough RNA to measure for quantitative reverse transcription-polymerase chain reaction. However, the experiment was done twice with the same results.

on both ethanol and TNBS mice derived lymphocytes (fig 5). This result was also confirmed by measuring IFN- γ protein. Unstimulated supernatants of mucosal mononuclear cells produced 36 pg/ml IFN- γ , which did not increase when MIP-1 α was added. Unsurprisingly, supernatants from mice with TNBS colitis produced more IFN- γ (172 pg/ml) than ethanol controls, which increased even further when cells were costimulated with 1 pg/ml MIP-1 α to 214 pg/ml.

Several experiments were also carried out to determine if MIP-1 α was mitogenic for either normal colonic LPL or LPL from mice with TNBS colitis. While ConA induced a weak proliferative response, MIP-1 α had only a very modest response on proliferation (not shown.).

Effects of MIP-1 α on gut fibroblasts

CCR5 is expressed on fibroblasts²⁶ and so we reasoned that a massive expansion of fibroblasts might contribute to the

Table 1 Macrophage inflammatory protein 1 α (MIP-1 α) induces gut fibroblast proliferation and reduces collagen I production		
MIP-1 α (pg/ml)	Cells/well	Collagen/100 cells
0	985 (56)	1953
1	1117 (199)	1750
10	1446 (165)***	1418
TGF- β 1 (1 ng/ml)	1657 (288)***	1556

Cell number results are shown from an average of six wells, each seeded with 1000 cells. Collagen values are shown as a single point because wells were pooled to give enough collagen to measure.
***p<0.0001.

large ulcers in MIP-1 α treated mice. We therefore determined if MIP-1 α was mitogenic for fibroblasts (table 1). Again, at very low doses, MIP-1 α significantly increased fibroblast numbers and decreased collagen synthesis. However, when we used Ki67 to determine if fibroblasts were proliferating in the ulcers, there was no evidence of any cell division, although extensive proliferation of epithelial cells at the ulcer edges were seen (fig 6). It was also noticeable that in mice with TNBS colitis, large numbers of dividing cells were also seen in the muscle layers.

DISCUSSION

We have shown here that systemic administration of MIP-1 α markedly worsened experimental IBD in mice. The effect was dose dependent and appeared to be due to the ability of MIP-1 α to boost T cell responses in the gut wall. Enhanced T cell activity results in increased local levels of IFN- γ and TNF- α which then probably increases MMP production by lamina propria fibroblasts, leading to extensive mucosal degradation.

MIP-1 α is produced by many different cell types but particularly by activated macrophages. When produced at a

Figure 6 Ulcer beds do not contain proliferating cells, as evidenced by Ki67 staining. (A) Ki67 staining of an ethanol control is shown and staining is restricted to the base of the crypts. (B) In mice with trinitrobenzene sulfonic acid (TNBS) colitis, there is crypt lengthening and an increase in the number of Ki67+ cells. Proliferating cells are also obvious in the muscle layer (arrow). (C) In ulcer beds of mice given TNBS and macrophage inflammatory protein 1 α (MIP-1 α), proliferating epithelial cells are obvious at the edge of the ulcer (arrowed) but Ki67+ cells are virtually absent from the ulcer bed itself. Immunoperoxidase with Ki67, original magnification $\times$ 100.

site of infection or inflammation, it acts as a chemotactic agent which attracts lymphocytes, neutrophils, and monocytes into the tissues. However, it also leaks into the circulation and it can be detected in the serum of normal individuals at very low levels, and at increased levels in patients with infections.^{27–30} Systemic effects include inhibition of proliferation of haematopoietic stem cells,³¹ and a non-aggregating variant of MIP-1 α has been tested in patients for its ability to protect stem cells during chemotherapy.³² It also mobilises dendritic cell precursors and haematopoietic stem cells into the circulation.^{33 34}

We chose three doses of MIP-1 α : 10, 100, and 1000 μ g/kg. These doses are in the range shown by others for in vivo activity in mice.^{31 35} In addition, in humans, subcutaneous administration of a bolus at 100 μ g/kg leads to peak plasma levels of approximately 1000 pg/ml between four and six hours later.³² At a lower dose of 30 μ g/kg, peak plasma levels are approximately 500 pg/ml. It is rather difficult to determine if these values correspond to levels found in the circulation of patients. In patients with a non-defined local inflammatory disease, plasma concentrations of MIP-1 α have a mean value of 25 pg/ml, with some patients showing up to twice that level.^{27 30} However, there is not a great deal of information on plasma MIP-1 α in different infectious and inflammatory conditions.

In mice, MIP-1 α signals to cells through three G protein coupled receptors, CCR1, CCR3, and CCR5.¹⁸ It is not entirely clear if T cells in either normal or inflamed mouse colon express these receptors, although based on the knowledge that CCR5 transcripts are high in the transfer model of colitis, that CCR5 is expressed at high levels on Th1 cells, and TNBS colitis is a Th1 model, it is highly likely that this is the case.^{18 36} The therapeutic effects of MET/RANTES which blocks CCR5 signalling in rat TNBS colitis also supports the notion that T cells in the gut in colitis are CCR5+.³⁷ Likewise, CCR5 null mice are somewhat protected from DSS colitis.³⁸

In the context of the present study however, we would suggest that exacerbation of colitis by systemic administration of MIP-1 α is largely due to its direct effects on T cells. While we cannot exclude other cell types, signalling through CCR5 does not appear to increase neutrophil activity and effects on macrophage function, while demonstrable, are rather modest.^{13 39 40} We were able to show that cells taken from the colon of mice with TNBS colitis rapidly increased IFN- γ and TNF- α transcripts when stimulated with MIP-1 α in culture. MIP-1 α co-stimulates human T cells activated with anti-CD3, increasing proliferation, and IL-2 production.⁴¹ Using T cells transgenic for an ovalbumin specific TcR, Karpus *et al* showed that MIP-1 α preferentially induced IFN- γ production.²⁵ Mice given ovalbumin and MIP-1 α by the nasal route also showed increased Th1 responses in the spleen and Peyer's patches.⁴² The molecular basis for the costimulatory activity of MIP-1 α has also been demonstrated. When added to human T cell lines, MIP-1 α activates STAT1 and STAT3.⁴³ One possible mechanistic link would be through the Th1 specific transcription factor t-bet, which is activated via STAT1 signalling.⁴⁴ STAT-1 signalling also markedly enhances anti-CD3 driven Th1 activation in human lamina propria T cells.⁴⁵ Signalling through CCR5 on T cells also activates the tyrosine phosphatase SHP1, SHP2, and the Src related kinase Syk.⁴⁶ These are intimately involved in T cell receptor signalling and their activation through CCR5 as well as through the TcR will enhance T cell cytokine production.

While we feel that the data from the literature as well as many of the studies reported here are consistent with the effects of MIP-1 α on TNBS colitis being through T cells, given the widespread cellular distribution of MIP-1 α receptors, we cannot rule out a role for other cell types. Our original hypothesis to explain these results was that MIP-1 α caused a

massive proliferation of fibroblasts in ulcer beds, and indeed MIP-1 α was shown to be mitogenic for colonic fibroblasts, albeit at a rather modest level. However, when we used Ki67 to look at proliferation in ulcer beds, there was essentially no staining, which effectively negates this hypothesis.

While these studies are in a mouse model, they have some implications for IBD, especially Crohn's disease. MIP-1 α immunoreactivity is increased in IBD.^{47 48} and so locally produced MIP-1 α could ligate CCR5 on T cells and boost local T cell responses, as well as function as a chemotactic agent to draw inflammatory cells into the colon wall. Very recently it has also been shown that MIP-1 α is increased in the colon of rats with TNBS colitis, and that a blocking antibody reduces neutrophil infiltrate.⁴⁹ At the same time, the major implication of this work is that peripheral infections, by increasing plasma chemokines, may reactivate gut inflammation. This work therefore puts forward a mechanistic basis for the observation that relapse of IBD often follows infection.

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