

INFLAMMATION AND INFLAMMATORY BOWEL DISEASE

Development of antigen induced colitis in SCID mice reconstituted with spleen derived memory type CD4⁺ CD45RB⁺ T cells

M-N Kweon, I Takahashi, M Yamamoto, M H Jang, N Suenobu, H Kiyono

Gut 2002;50:299–306

See end of article for authors' affiliations

Correspondence to:
Dr H Kiyono, Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka 565, Japan;
kiyono@biken.osaka-u.ac.jp

Accepted for publication
22 May 2001

Background and aims: Enteric bacterial and/or food antigens may be crucial in the development of colitis but little is known of the exact mechanism of antigen specific reactions in this condition. The aim of this study was to determine whether systemically primed antigen specific CD4⁺ T cells containing both CD45RB^{high} and CD45RB^{low} populations participate as a pathogenic subset that in turn leads to inflammatory reactions selectively in the large intestine.

Methods: SCID mice were reconstituted with splenic CD4⁺ CD45RB⁺ T cells or CD4⁺ CD45RB^{low} T cells isolated from donor mice systemically primed with ovalbumin (OVA) plus CFA. The reconstituted mice were then fed OVA for several weeks.

Results: Reconstitution of SCID mice with OVA primed splenic CD4⁺ T cells, containing populations of CD45RB^{high} and CD45RB^{low}, resulted in the development of colitis by 4–5 weeks following repeated administration of oral OVA. Histopathological study revealed thickened wall, inflammatory cell infiltration, crypt elongation, and loss of goblet cells in the large intestine. The CD4⁺ CD45RB^{low} population of cells extracted from the affected large intestine secreted high levels of interferon γ (IFN- γ) and tumour necrosis factor α (TNF- α) at the protein and mRNA levels. Administration of neutralising antibodies to TNF- α , but not to IFN- γ , prevented the development of colitis. Furthermore, adoptive transfer with OVA primed splenic CD4⁺ CD45RB^{low} T cells evoked severe colitis.

Conclusions: These results demonstrate that systemically primed activated/memory CD4⁺ CD45RB^{low} T cells can mediate the development of specific antigen induced colitis in SCID mice, and also that TNF- α is critical in the induction of this type of colitis. Our results contrast with those from studies in some colitis models in which CD45RB^{low} T cells appeared to prevent colitis through secretion of immunosuppressive cytokines.

Manipulation of genes for specific cytokines, cytokine receptors, and immunological molecules can result in chronic inflammation, preferentially in the large but not the small intestine, of experimental animals.^{1–4} Several hypotheses are proposed to explain this regional disease development. Firstly, differences in the microbial environment—for example, the much greater population of bacteria in the colon—may favour the development of colonic inflammation; indeed, experimental colitis has often been abrogated under germ free conditions or after treatment with antibiotics.^{5,6} Secondly, the mucosal immune environment differs between the small and large intestine.^{7–9} For example, the frequency of CD4⁺ and $\alpha\beta$ T cells as well as expression of lymphocyte function associated antigen 2 (LFA-2) and L-selectin are higher in the large intestine than in the small intestine.^{7–9} Thus large intestinal lymphocytes may differ from small intestinal lymphocytes in their immunological responses to oral and/or enteric bacterial antigens. Thirdly, a specific cross talk immune pathway may exist between the systemic compartment (for example, the spleen) and the large intestine. In this regard, our results and those of others have shown that selected populations of T cells, derived from systemic tissue, induced localised inflammatory responses in the large intestine.^{10–12}

The results obtained by different experimental models of colitis indicate that antigens derived from food and/or bacterial flora may be involved in the induction of localised inflammation.^{13–15} For example, CD4⁺ T cells isolated from C3H/HeJBiR mice, which spontaneously develop colitis, had significant proliferative and cytokine responses following exposure to protein antigens of enteric bacteria.¹³ Also, we demonstrated

that colitic T cell receptor (TCR) $\alpha^{-/-}$ mice suffered from food sensitisation, and their colonic T and B cells were reactive to food proteins such as soy bean and wheat¹⁴ whereas TCR $\alpha^{-/-}$ mice fed an elemental diet without antigenic proteins did not develop colitis.¹⁵ The enteric bacterial species present in the TCR $\alpha^{-/-}$ mice fed an elemental diet also differed substantially from those in mice fed a normal diet.¹⁵ Taken together, these results indicate that enteric bacterial and/or food antigens may be crucial in the onset of colitis; however, little is known of the exact origin and role of antigen specific CD4⁺ T cells in the development of inflammation in the large intestine.

Our recent and a separate study demonstrated that adoptively transferred antigen primed splenic CD4⁺ T cells obtained from GFP transgenic donor mice preferentially migrated into the large but not the small intestine.¹¹ These results together with those of other studies¹⁰ suggest that a unique and important immunological cross talk system exists between the spleen and large intestine. This system may influence the development of colitis. Thus a major aim of this study was to determine whether systemically primed antigen specific CD4⁺ T cells containing both CD45RB^{high} and CD45RB^{low} populations participate as a pathogenic subset that

Abbreviations: TNF- α , tumour necrosis factor α ; IFN- γ , interferon γ ; OVA, ovalbumin; TCR, T cell receptor; PBS, phosphate buffered saline; mAb, monoclonal antibody; IL, interleukin; RT-PCR, reverse transcription-polymerase chain reaction; ICAM-1, intercellular adhesion molecule 1; TGF- β , transforming growth factor β ; CFA, complete Freund adjuvant; LFA, lymphocyte function associated antigen; KLH, keyhole limpet haemocyanin.

in turn leads to inflammatory reactions selectively in the large intestine. To this end, SCID mice were reconstituted with splenic CD4⁺ CD45RB⁺ T cells or CD4⁺ CD45RB^{low} T cells isolated from donor mice systemically primed with ovalbumin (OVA) plus Complete Freund Adjuvant (CFA). The reconstituted mice were then fed OVA for several weeks. We found that spleen derived activated/memory CD4⁺ CD45RB^{low} T cells of the Th1 type from OVA primed mice and tumour necrosis factor α (TNF- α) were crucial in the development of colitis in the reconstituted SCID mice after OVA feeding.

METHODS

Mice

BALB/c and SCID mice of the same background were purchased from Japan Clea Co. (Tokyo, Japan). STAT4^{-/-} mice of the BALB/c background were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA). All mice were housed in the experimental animal facility at the Research Institute for Microbial Diseases, Osaka University, and fed sterilised food (certified diet MF; Oriental Yeast Co., Osaka, Japan) and tap water ad libitum. All mice were aged 6–8 weeks at the beginning of the experiments.

Immunisation and adoptive transfer

BALB/c mice were primed by the subcutaneous route with 1 mg of OVA (fraction V; Sigma Chemical Co., St Louis, Missouri, USA) or 100 μ g of keyhole limpet haemocyanin (KLH) (Sigma) in 100 μ l of CFA (Difco Laboratories, Detroit, Michigan, USA), respectively. One week after systemic challenge, the spleen was removed aseptically and a single cell suspension was prepared by mechanical dissociation methods, as described previously.¹⁶ FACS separated CD4⁺ CD45RB⁺ T cells or CD4⁺ CD45RB^{low} T cells ($4\text{--}5 \times 10^5$) were resuspended in 200 μ l of phosphate buffered saline (PBS) and adoptively transferred to SCID mice by tail vein injection. One week after the reconstitution, the recipient SCID mice were repeatedly challenged with 50 mg of OVA or 5 mg of KLH by gastric intubation three times a week. Body weight of naive and reconstituted SCID mice was measured every week. After 8–10 oral administrations, the spleen, and small and large intestines were removed aseptically.

Histological evaluation

For the histopathological study, the small and large intestines from recipient SCID mice were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (5 μ m) were stained with haematoxylin and eosin for evaluation of general histopathology changes and stained with periodic acid-Schiff for identification of goblet cells. The degree of inflammation in the microscopic sections of the colon was graded semiquantitatively in a blinded manner using a scoring system of 0 to 4 (0, no inflammation; 1, very mild inflammation; 2, mild inflammation; 3, moderate inflammation; 4, severe inflammation, with infiltration of mononuclear cells, crypt elongation, crypt abscesses, loss of goblet cells, and thickening of the colon wall).¹⁷

Neutralising monoclonal antibody treatment

SCID mice were injected intraperitoneally with 100 μ g of anti-mouse interferon γ (IFN- γ) monoclonal antibody (mAb) (XMG1.2; Pharmingen, San Diego, California, USA), anti-mouse TNF- α mAb (MP6-XT3; Pharmingen), or isotype matched rat IgG2b mAb (R35-38; Pharmingen) once a week for the entire period of the experiments. The mAb treatment was started one week before the adoptive transfer.

Isolation of mononuclear cells

Intraepithelial lymphocytes and lamina propria lymphocytes from the small and large intestine were isolated as described previously.^{14–18} In brief, intestinal tissues were digested using RPMI medium containing 1 mM EDTA and a collagenase

(type IV 0.5 mg/ml of RPMI 1640; Sigma) after removal of Peyer's patches and colonic patches in the 37°C incubator. The single cell suspensions were pooled, washed, and placed on a discontinuous 40% and 70% Percoll gradient (Pharmacia, Uppsala, Sweden). After centrifugation for 20 minutes at 600 *g*, the cells were collected from the interface.

FACS analysis and cell sorting

Isolated mononuclear cells from the spleen, small intestine, and large intestine were preincubated with an Fc blocking mAb (2.4G2; Pharmingen) for 15 minutes on ice. The following Abs were purchased from Pharmingen and used in this study: FITC conjugated antimouse CD4 (L3T4), CD2 (RM2-5), CD11a (CL891F) mAbs, and PE conjugated antimouse CD48 (BCM1), CD69 (H1.2F3), CD44 (1M7), CD54 (3E2), and CD45RB (16A) mAbs. Two colour analysis was performed in flow cytometry analysis using a FACS Calibur (Becton Dickinson, San Jose, California, USA). In some experiments, the flow cytometry sorting separation was performed by FACS Vantage (Becton Dickinson) using FITC conjugated anti-CD4 and PE conjugated anti-CD45RB mAbs.

In vitro OVA specific proliferative responses

Mononuclear cells isolated from the spleen and large intestine of the reconstituted SCID recipients were suspended in RPMI 1640 medium (Sigma) containing 10% heat inactivated fetal calf serum, HEPES buffer, L-glutamine, penicillin, and streptomycin. The cells were cultured in the presence of 1 mg/ml of OVA for four days, as described previously.¹⁶ To measure antigen specific proliferation, 0.5 μ Ci of [³H]thymidine (ICN, Costa Mesa, California, USA) was added for the final 18 hour incubation and the amount of [³H]thymidine incorporation was determined by scintillation counting.

Intracellular cytokine assay

For intracellular cytokine analysis, mononuclear cells isolated from the spleen and the small and large intestines were cultured with complete RPMI medium containing 10% fetal bovine serum, soluble anti-CD28 mAb (37.51, 2 μ g/ml), and recombinant interleukin (IL)-2 in 24 well flat bottomed plates coated with anti-CD3 ϵ mAb (145–2C11, 10 μ g/ml) for 16 hours.^{11,19} GolgiStop (2 μ M/ml; Pharmingen) was added during the final four hours of incubation, and cytoplasmic staining was then performed using Cytofix/Cytoperm Kits (Pharmingen). FITC conjugated antimouse CD45RB (16A) and PE conjugated antimouse IFN- γ (XMG1.2), TNF- α (MP6-XT22), IL-4 (BUD4-1D11), IL-10 (JES5-16E3), and antirat IgG1 (R3-34) mAbs for isotype control were used. Labelled cells were analysed by flow cytometry analysis using FACS Calibur (Becton Dickinson). Unless otherwise indicated, mAbs were purchased from Pharmingen.

Quantitative RT-PCR method

A quantitative reverse transcription-polymerase chain reaction (RT-PCR) was employed for the assessment of cytokine specific mRNA expression by different subsets of CD4⁺ T cells according to a method described previously.¹¹ Total RNA was extracted using TRIzol reagent (Life Technologies, Gaithersburg, Maryland, USA), and 5 μ g/ml of extracted RNA was subjected to RT reaction using Superscript II reverse transcriptase (Life Technologies). The cDNA from 10 ng of RNA was used for each cytokine specific PCR (for example, IFN- γ , TNF- α , IL-4, and IL-10). Rapid cycle DNA amplification was performed by a LightCycler (Boehringer Mannheim GmbH, Mannheim, Germany) with the double strand specific dye SYBER Green I.²⁰ The conditions of the PCR cycle used were as follows: initial denaturation at 95°C for two minutes, followed by 45 cycles of denaturation at 95°C for 0 seconds and combined annealing-extension at 55°C for five seconds and 72°C for 10 seconds. Cycle to cycle fluorescence emission readings were plotted on the computer screen for

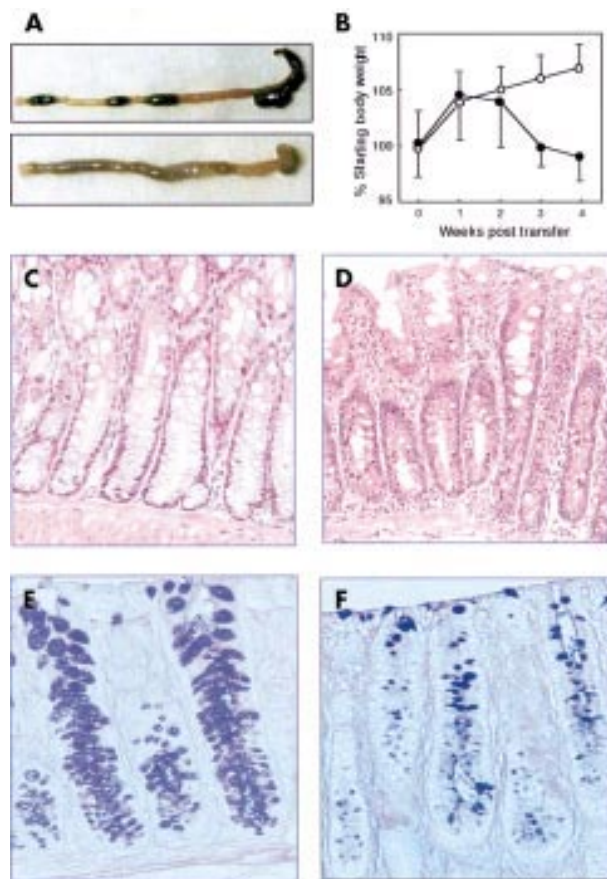


Figure 1 Induction of colitis in SCID mice by reconstitution with ovalbumin (OVA) primed splenic CD4⁺ CD45RB⁺ T cells. BALB/c mice were immunised by subcutaneous injection with OVA in complete Freund adjuvant. One week after systemic challenge, FACS separated CD4⁺ CD45RB⁺ T cells obtained from the spleens were adoptively transferred to SCID mice of the same background mice (5×10^5 cells per mouse). Control SCID mice received splenic T cells from non-primed BALB/c mice. Beginning one week after the reconstitution, the recipient SCID mice were repeatedly challenged with 50 mg of OVA by gastric intubation. After four weeks of oral challenge, tissue sections were taken from the proximal, middle, and distal regions of the large intestine and stained with haematoxylin and eosin (C, D) or periodic acid Schiff staining (E, F). (A) Gross view of the large intestine of control SCID mice without colitis (upper) and reconstituted mouse with colitis (lower). (B) Change in body weight in control (open circle) and reconstituted (filled circle) SCID mice. (C, E) Histology of a control SCID mouse without colitis. (D, F) Histology of the proximal large intestine of a reconstituted SCID mouse with a severe pathological lesion. Periodic acid-Schiff staining shows decreased numbers of goblet cells in the proximal region of the large intestine from a diseased mouse (F) compared with similar tissue from a control mouse (E). Original magnification $\times 100$ –200.

continuous monitoring of PCR product using LightCycler software.²⁰

Data analysis

Data are expressed as mean (SEM) and were evaluated by the Mann-Whitney U test for unpaired samples using a Statview II statistical program (SAS Inc., Cary, North Carolina, USA) designed for the Macintosh computer. *p* values less than 0.05 were assumed to be statistically significant.

RESULTS

Systemically primed splenic CD4⁺ T cells induce weight loss and provoke inflammation in the large intestine of the SCID mice after specific antigen feeding

To investigate whether antigen specific CD4⁺ T cells derived from the systemic immune compartment play a pathogenic

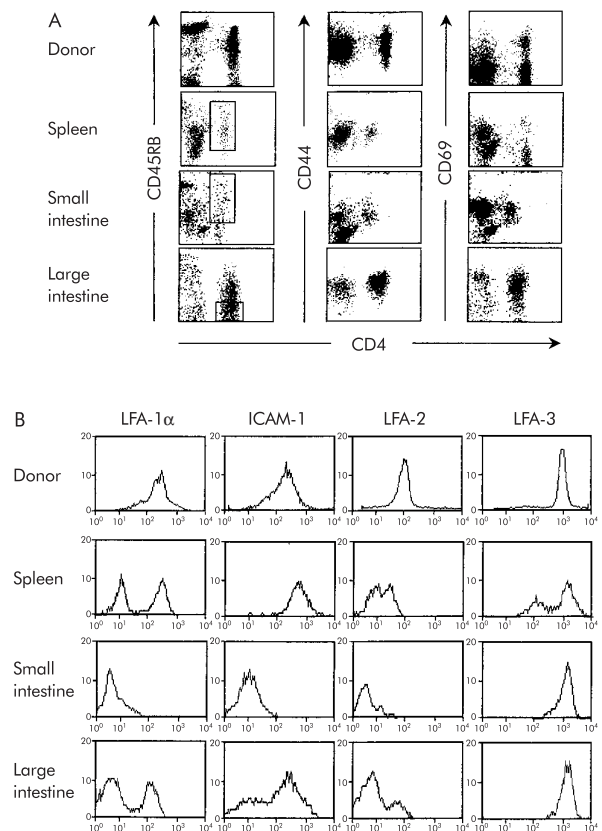


Figure 2 Expression of an activated/memory phenotype (A) and adhesion molecules (B) by the CD4⁺ T cells isolated from spleen, and small and large intestines of the reconstituted SCID mice after oral challenge with ovalbumin. The small boxes in the dot plot of the FACS data indicate the phenotype of the activated/memory type population (for example, CD45RB^{low}, CD44^{high}, and CD69^{high}). These data are representative of three independent experiments containing 3–5 mice per group. Detailed immunisation methods are described in the legend to fig 1. ICAM-1, intercellular adhesion molecule 1; LFA, lymphocyte function associated antigen.

role in the large intestine, splenic CD4⁺ T cells taken from mice systemically primed with OVA containing populations of both high and low expression levels of CD45RB molecule (CD45RB^{high} and CD45RB^{low}) were adoptively transferred to SCID mice which were then fed OVA for four weeks and sacrificed. We found that the mice had lost weight, had loose stools, and had gross colitis (fig 1A, B). Histological evaluation of the spleens and small and large intestines revealed that lesions were restricted to the large intestine with the most severe lesions being located in the proximal colon. Prominent inflammatory cell infiltration, crypt elongation, and crypt abscesses were present (fig 1D), and the number of goblet cells was decreased (fig 1F) compared with the control group (fig 1C, E).

The CD4⁺ CD45RB^{low} population was selectively expanded in the large but not the small intestine

To define the phenotype of mononuclear cells from the spleen, small intestine, and large intestine of the reconstituted SCID mice, flow cytometry analysis was performed using mAbs specific for various surface-membrane molecules (fig 2). Higher numbers of CD4⁺ T cells were found in the large intestine than in the spleen and small intestine of the recipient SCID mice following repeated oral administration of OVA (for example, the frequency of CD4⁺ cell subset: spleen 16%, small intestine 25%, and large intestine 73%). In addition, the large intestinal CD4⁺ T cells contained high

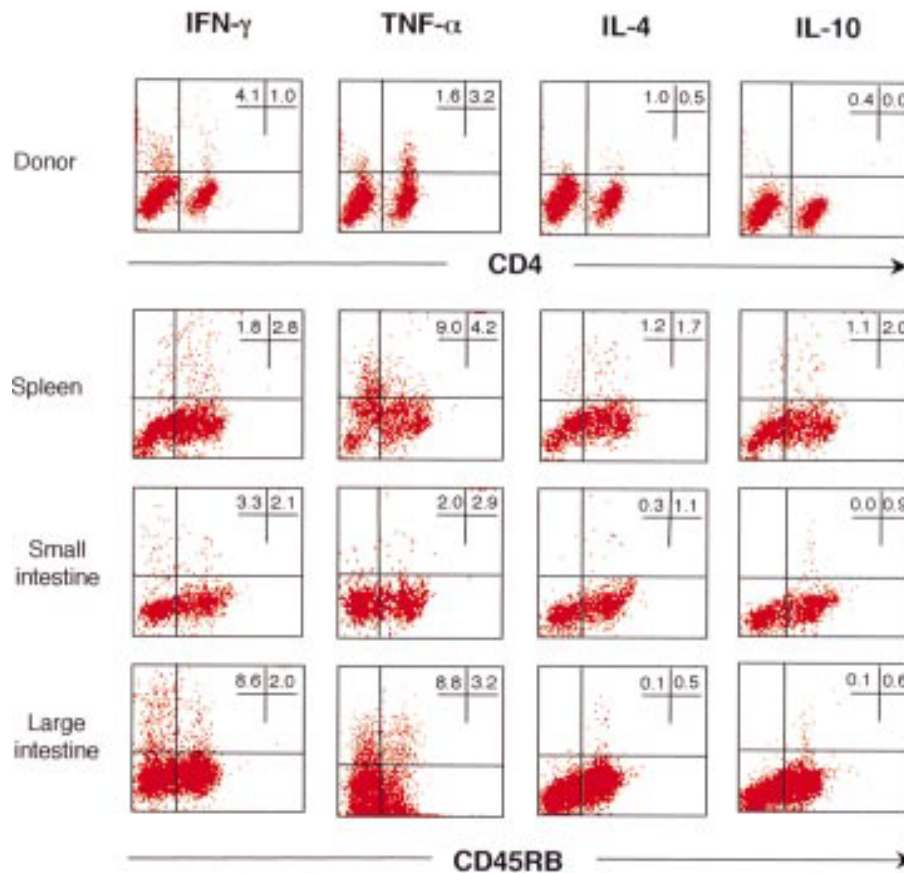


Figure 3 Analysis of cytokine production by ovalbumin primed large intestinal CD4⁺ and CD45RB^{low} T cells. Details are described in the methods section. The main quadrant line represents staining with isotype control monoclonal antibody. These data are representative of two independent experiments containing 3–5 mice per group. TNF- α , tumour necrosis factor α ; IFN- γ , interferon γ ; IL, interleukin.

numbers of CD45RB^{low} CD44^{high} CD69^{high} T cells which are well known phenotypes of activated/memory type T cells (fig 2A). Also, mononuclear cells isolated from the large intestine expressed high levels of LFA-1 α , intercellular adhesion molecule 1 (ICAM-1), and LFA-2 compared with those of the small intestine (fig 2B). Neither large intestinal nor small intestinal mononuclear cells expressed L-selectin, α _{EL} β , or α _{EL} β (data not shown). The expression pattern of adhesion molecules in mononuclear cells isolated from the spleen was similar to that in cells from the large intestine (fig 2B). As it is well established that increased expression of LFA-1, LFA-2, LFA-3, and ICAM-1 is a marker of activated/memory type cells,^{21, 22} our data demonstrate that activated/memory type CD4⁺ T cells preferentially migrated to and were expanded in the large intestine.

CD45RB^{low} subsets of the large intestine produced predominant Th1 type cytokines

To determine the profile of cytokine synthesis of CD4⁺ CD45RB⁺ T cells isolated from the spleen, small intestine, and large intestine of the recipient SCID mice, we performed intracellular cytokine staining and cytokine specific RT-PCR assays. Analysis of cytokine production at the single cell level revealed that a significant number of large intestinal CD45RB^{low} subsets expressed IFN- γ compared with those from the small intestine (fig 3). Furthermore, large numbers of TNF- α secreting cells were also detected in the CD45RB^{low} population of the large intestine and spleen (fig 3). In contrast, no IL-4 or IL-10 secreting cells were detected in the spleen, small intestine, or large intestine of recipient SCID mice (fig 3). When the cytokine specific RT-PCR assays were performed, colonic CD4⁺ T cells were found to possess high

levels of mRNA for IFN- γ and TNF- α ; however, no mRNA for IL-4 and IL-10 was detected (table 1). Taken together, these findings suggest that the development of colitis in reconstituted SCID mice is caused by the spleen derived colonic CD4⁺ CD45RB^{low} T cells producing IFN- γ and TNF- α in the colon.

Administration of neutralising Ab to TNF- α but not to IFN- γ protected the development of colitis in SCID mice reconstituted with systemically primed splenic CD4⁺ CD45RB^{low} T cells

To directly examine the role of Th1 type cytokines in the development of colitis, reconstituted SCID mice were treated

Table 1 CD4⁺ T cells obtained from the large intestines of SCID mice produce predominant interferon γ (IFN- γ) and tumour necrosis factor α (TNF- α)

Cytokines	Location of CD4 ⁺ T cells	Fluorescence units (at 520 nm)*		
		30 cycle	35 cycle	40 cycle
IFN- γ	SP	2.0	10.0	13.0
	LI	8.0	16.5	18.5
TNF- α	SP	ND	ND	ND
	LI	22.5	33.5	40.0
IL-4	SP	ND	ND	ND
	LI	ND	ND	ND
IL-10	SP	ND	ND	ND
	LI	ND	ND	ND

*Reverse transcription-polymerase chain reaction methods were performed by the LightCycler (Boehringer Mannheim). ND, not detectable; SP, spleen; LI, large intestine; IL, interleukin.

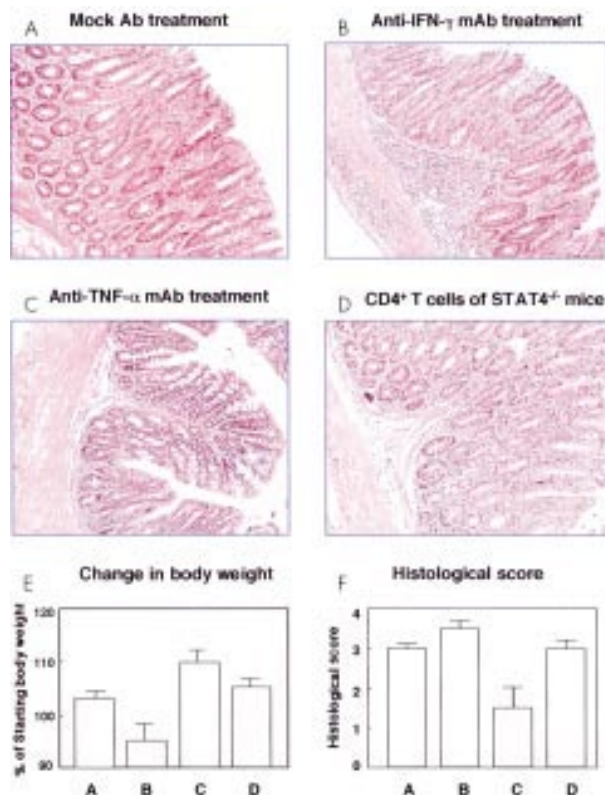


Figure 4 Effects of administering mock antibody (Ab) (A) or neutralising Abs to interferon γ (IFN- γ) (B) or tumour necrosis factor α (TNF- α) (C) to SCID mice reconstituted with splenic CD4⁺ CD45RB⁺ T cells of systemically primed BALB/c mice. Histological sections of the proximal large intestine are shown. (D) Large intestine of SCID mice reconstituted with splenic CD4⁺ CD45RB⁺ T cells of systemically primed STAT4^{-/-} mice. (E) Percentage starting body weight in each group corresponding to those of (A-D). (F) Histological score graded semiquantitatively from 0 to 4. Details of histological score and immunisation schedules are described in the methods and in the legend to fig 1, respectively. Original magnification $\times 100$.

with neutralising Abs to IFN- γ or TNF- α . Neutralising Abs to TNF- α but not to IFN- γ completely prevented clinical and histological evidence of colitis. Histologically, the proximal large intestine of anti-IFN- γ Ab treated SCID mice had prominent crypt elongation, crypt distortion, reduced numbers of goblet cells, and inflammatory cell infiltration (fig 4B, F) whereas these signs of disease were absent in mice treated with anti-TNF- α Ab (fig 4C). Also, mice treated with anti-IFN- γ lost weight but mice treated with anti-TNF- α did not (fig 4E). As expected, colitis was normally developed in the mock Ab treated recipient SCID mice (fig 4A, F). As neutralisation with anti-IFN- γ Ab did not protect against the development of colitis, we next tested whether the STAT4 signalling cascade is involved in the development of colitis. Donor CD4⁺ T cells were obtained from the spleens of STAT4^{-/-} mice following subcutaneous priming with OVA in CFA. Reconstitution of SCID mice with the splenic CD4⁺ T cells resulted in colitis (fig 4D, F), and the level of disease severity was similar to that seen in the SCID mice reconstituted with CD4⁺ T cells from the primed wild type mice (fig 1D). Thus the lack of IFN- γ inducing pathway did not prevent the development of colitis by CD4⁺ CD45RB^{low} T cells. Taken together, our data suggest that TNF- α rather than IFN- γ is crucial for the development of the colitis studied here.

Antigen specific reactions accelerate the development of colitis

To clarify if antigen specific responses are involved in the development of colitis, SCID mice were reconstituted with

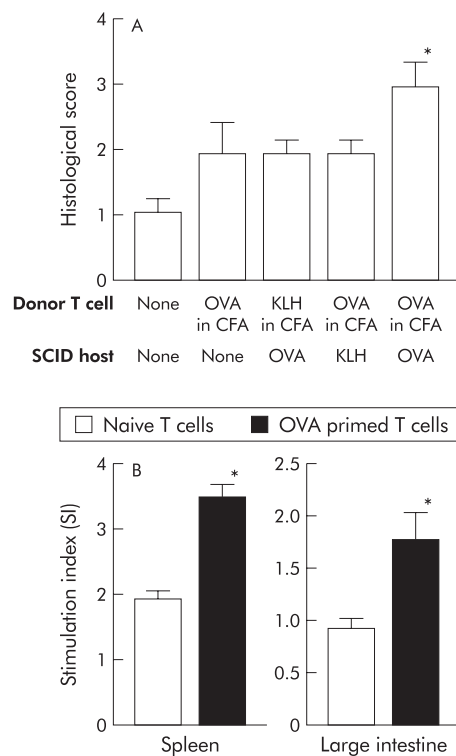


Figure 5 Role of ovalbumin (OVA) specific CD4⁺ T cell responses in the development of colitis. (A) Histopathological score of the proximal large intestine of the reconstituted SCID mice. Donor splenic CD4⁺ CD45RB⁺ T cells were isolated from BALB/c mice systemically primed with OVA or keyhole limpet haemocyanin (KLH) in complete Freund adjuvant (CFA) and then adoptively transferred to each group of SCID recipient via tail vein injection. After one week of the reconstitution, SCID recipients were fed relevant (OVA) or irrelevant (KLH) proteins. Details of histological scoring are described in the methods section. (B) Assessment of OVA specific T cell proliferation by mononuclear cells isolated from SCID mice reconstituted with naive splenic CD4⁺ CD45RB⁺ T cells or OVA primed splenic CD4⁺ CD45RB⁺ T cells. Stimulation index is expressed as [³H]thymidine incorporation by cells incubated with OVA divided by [³H]thymidine incorporation by cells in controls, incubated without OVA. Levels of [³H]thymidine incorporation of control wells were 500–1000 cpm. * $p < 0.05$ versus group of SCID mice reconstituted with naive CD4⁺ CD45RB⁺ T cells.

splenic CD4⁺ CD45RB⁺ T cells isolated from donor mice systemically primed with OVA or KLH in CFA. After four weeks of oral administration with the relevant or irrelevant protein, the severity of colitis was compared. Severe colitis developed in SCID mice reconstituted with OVA primed T cells following oral challenge with OVA (fig 5A). In contrast, mild colitis developed in the reconstituted SCID mice following oral challenge with irrelevant protein or PBS alone; their histological scores were lower than those of SCID mice orally challenged with relevant protein (fig 5A).

To further investigate involvement of antigen specific T cell responses in the development of colitis in SCID recipients, mononuclear cells were isolated from the spleens and large intestines of SCID mice, and OVA specific T cell proliferative responses were then examined. High levels of OVA specific T cell proliferation were seen in the spleen and large intestine of the SCID recipient reconstituted with the OVA primed CD4⁺ CD45RB⁺ T cells following repeated oral administration of relevant antigen—that is, OVA (fig 5B). On the other hand, mice reconstituted with naive T cells had low levels of T cell proliferation. These results further suggest that antigen specific reactions could accelerate the development of colitis.

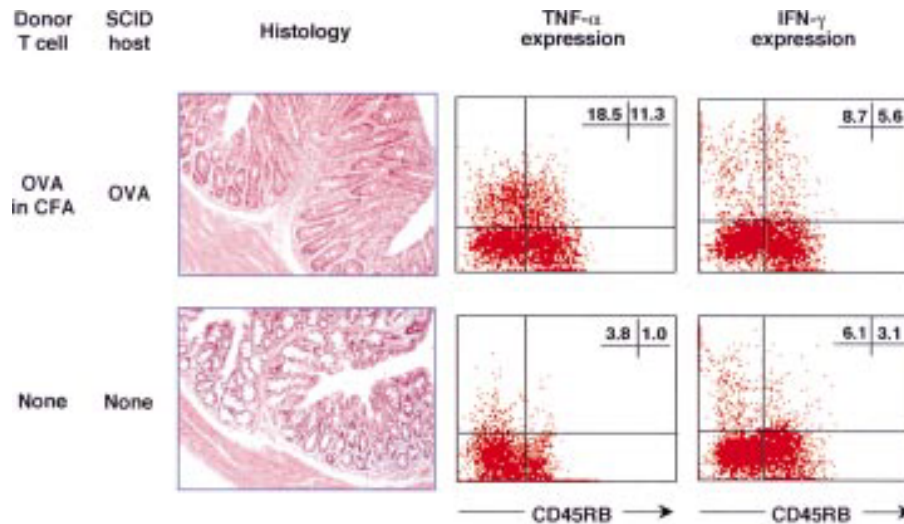


Figure 6 Pathological role of ovalbumin (OVA) primed CD4⁺ CD45RB^{low} T cells in the development of colitis. Histopathological analysis of the proximal large intestine was performed by haematoxylin and eosin staining, and Th1 type cytokine expression by the large intestinal mononuclear cells was determined by intracellular cytokine staining. SCID mice were reconstituted with unprimed or OVA primed splenic CD4⁺ CD45RB⁺ T cells. Beginning one week after reconstitution, SCID mice were repeatedly challenged by gastric intubation with 50 mg of OVA, Original magnification $\times 100$. TNF- α , tumour necrosis factor α ; IFN- γ , interferon γ ; CFA, complete Freund adjuvant.

Reconstitution of OVA primed CD45RB^{low} CD4⁺ T cells leads to colitis in SCID recipients

To directly ascertain a pathogenic role for CD4⁺ CD45RB^{low} T cells in the development of colitis, SCID mice were reconstituted with splenic CD4⁺ CD45RB^{low} T cells isolated from OVA primed mice. Following repeated oral administration of OVA, histological score of the large intestine and intracellular cytokine expression by large intestinal mononuclear cells were examined. The SCID mice adoptively transferred with OVA primed splenic CD4⁺ CD45RB^{low} T cells developed severe colitis characterised by infiltration of inflammatory cells, crypt elongation, loss of goblet cells, and thickened large intestinal wall (fig 6). Most interestingly, increased numbers of TNF- α secreting cells were detected in SCID recipients reconstituted with OVA primed splenic CD4⁺ CD45RB^{low} T cells compared with those of SCID mice adoptively transferred with non-primed CD4⁺ CD45RB^{low} T cells (fig 6). In contrast, no significant difference was seen in IFN- γ secreting cells between the two groups (fig 6). These results further implicate TNF- α in the development of colitis in this model. In summary, TNF- α producing antigen specific activated/memory CD4⁺ CD45RB^{low} T cells play a critical role in the development of colitis.

DISCUSSION

CD4⁺ T cells can be grouped into naive and memory subsets based on expression of CD45RB^{high} and CD45RB^{low}, respectively.²³ These two distinct subsets of CD4⁺ T cells seem to be the key for induction of experimental colitis. Previous studies demonstrated that SCID mice reconstituted with CD4⁺ CD45RB^{high} T cells from naive wild type mice developed colitis.^{12–24–27} It has been proposed that the pathogenesis of this colitis is disturbance in the cytokine balance between the Th1 type cytokines such as IFN- γ or TNF- α and inhibitory cytokines such as IL-10 or transforming growth factor β (TGF- β).⁴ In support of this concept, cotransfer of CD4⁺ CD45RB^{low} T cells with CD4⁺ CD45RB^{high} T cells prevented the development of colitis,^{6,26} a result which suggested that naive spleen derived CD4⁺ CD45RB^{low} T cells contain regulatory T cells producing suppressive cytokines, including IL-10 and/or TGF- β . In contrast, other studies have shown that the adoptive transfer of CD4⁺ T cells containing both CD45RB^{high} and CD45RB^{low} into the SCID mice resulted in a chronic and lethal inflammatory bowel disease.^{28–30}

In the present study, SCID mice receiving spleen derived CD4⁺ CD45RB⁺ T cells from mice systemically primed with OVA lost weight and developed localised inflammation in the large intestine 4–5 weeks following oral administration of OVA (fig 1). As CD4⁺ CD45RB^{low} T cells from the spleens of systemically primed mice were predominantly found in the diseased region of the large intestine (fig 2), we further sought to clarify their cytokine synthesis pattern. When CD4⁺ T cells isolated from the disease region were cultured with anti-CD28 and IL-2 in anti-CD3 precoated wells, high production of IFN- γ and TNF- α was noted (fig 3). Interestingly, reconstitution with OVA primed splenic CD4⁺ CD45RB^{low} T cells into recipient SCID mice resulted in the development of severe colitis and the numbers of TNF- α secreting cells correlated with the severity of colitis (fig 6). Thus it appears that antigen primed splenic CD4⁺ CD45RB^{low} T cells can behave as a pathogenic subset via production of Th1 type cytokine (for example, TNF- α) rather than exerting a suppressive effect in this colitis model.

Uncontrolled activation and proliferation of colonic CD4⁺ T cells is believed to play a critical role in the development of colitis.³¹ This notion is supported by the observation that adoptive transfer of SCID mice with concanavalin A activated splenic CD4⁺ T cells accelerated the development of colitis compared with transfer of non-activated CD4⁺ T cells.³² In addition, colonic CD4⁺ T cells isolated from colitic SCID mice had higher numbers of proliferating cell nuclear antigen positive cells than those from SCID mice without colitis.²⁹ More recently, reconstitution with large (activated) CD4⁺ T cells resulted in early (6–12 weeks) and severe colitis in SCID mice while small (resting) CD4⁺ T cells developed a late onset (12–16 weeks) colitis.^{32,33} In agreement with these data, our present study revealed that activated/memory type CD4⁺ CD45RB^{low} T cells from OVA primed spleen mediated the development of colitis in recipient SCID mice on repeated oral exposure with antigen. It is still unclear how oral antigen can selectively activate colonic CD4⁺ T lymphocytes. Perhaps splenic CD4⁺ pathogenic precursor T cells are activated by systemic challenge with OVA plus CFA to expand and become activated/memory CD4⁺ CD45RB^{low} T cells capable of producing IFN- γ and TNF- α . Also, continuous oral challenge with an identical antigen might preferentially recruit to and further stimulate these activated/memory Th1 type CD4⁺ T cells in the large intestine for the development of colitis. In

support of this view is the observation that colonic CD4⁺ T cells from the reconstituted SCID mice and donor splenic CD4⁺ T cells had an identical phenotype of adhesion molecules (fig 2B). Furthermore, high levels of antigen specific proliferative responses were detected in mononuclear cells isolated from the spleen and large intestine of SCID mice reconstituted with OVA primed splenic CD4⁺ T cells (fig 5B).

Several recent studies demonstrated that signal transduction via STAT molecules are critical in the development of colitis,³³⁻³⁶ and IL-12/STAT-4 mediated Th1 responses were involved in Crohn's disease.³⁷ Furthermore, mice transgenic for STAT-4 developed severe colitis after immunisation with DNP-KLH plus CFA, suggesting that STAT-4 signalling is a critical intracellular pathway for the pathogenesis of colitis.³⁴ On the other hand, reconstitution with CD4⁺ CD45RB^{high} T cells isolated from STAT4^{-/-} mice showed mild but definite colitis in reconstituted RAG2^{-/-} and SCID mice.³⁶ In agreement with these data, we found that SCID mice that received CD4⁺ T cells obtained from STAT4^{-/-} mice developed colitis at a later time (>20 weeks) compared with colitis in animals that received CD4⁺ T cells from wild type mice.³³ In the present study, the onset of colitis in SCID mice was not influenced by the actions of STAT-4 signalling as adoptive transfer of CD4⁺ CD45RB⁺ T cells from systemically primed STAT4^{-/-} mice resulted in colitis (fig 4). These results suggest that Th1 type proinflammatory cytokines, which have a strong pathogenic effect for the development of colitis, are not controlled by IL-12/STAT4 signalling.

Several studies have attempted to elucidate the role of IFN- γ and TNF- α in the onset of colitis. Previous studies showed that treatment with either anti-IFN- γ or anti-TNF- α Abs protected against the development of colitis in reconstituted SCID mice.¹² In contrast, our present study revealed that administration of neutralising Abs to TNF- α , but not to IFN- γ , protected against the development of colitis. In another study, administration of anti-TNF- α Abs attenuated disease progression in murine³⁸ and human colitis.³⁹ Moreover, colitis did not develop in RAG2^{-/-} mice reconstituted with CD4⁺ CD45RB^{high} T cells obtained from TNF^{-/-} mice even although increased levels of IFN- γ expression were noted in the colons,⁴⁰ and comparably severe colitis was seen in SCID mice reconstituted with CD4⁺ CD45RB^{high} T cells of IFN- γ ^{-/-} mice.³⁶ These studies together with our results provide supportive evidence that neutralisation of TNF- α could be beneficial in the treatment of antigen induced colitis.

Our present results demonstrate that the antigen primed splenic CD4⁺ CD45RB^{low} T cell population is a pathogenic rather than a protective subset when these T cells are continuously exposed to orally administered antigen. These results reveal that spleen derived, antigen specific activated/memory CD4⁺ T cells can mediate the development of localised inflammation in the large intestine. Furthermore, TNF- α produced by these activated/memory CD4⁺ T cells appears to be crucial in the development of this colitis.

ACKNOWLEDGMENTS

This work was supported by Grant-in-Aid for COE Research and Research for the Frontier and Science from the Ministry of Education, Science, Sports, and Culture and Grant-in-Aid for Encouragement of Young Scientists from Japan Society for the Promotion of Science, and grants from the Ministry of Health and Welfare of Japan, and the program for promotion of Fundamental studies in Health Sciences of the Organization for Pharmaceutical Safety and Research of Japan. The authors thank Dr William Brown (University of Colorado, USA, School of Medicine) for his critical review of the paper and editorial assistance.

Authors' affiliations

M-N Kweon, I Takahashi, M H Jang, Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka 565, Japan

M Yamamoto, Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka 565, Japan and Department of Clinical Pathology, Nihon University, School of Dentistry at Matsudo, Chiba 271, Japan

N Suenobu, Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka 565, Japan and Pharmaceutical Division, Pola Chemical Industries, INC, Yokohama 244, Japan

H Kiyono, Department of Mucosal Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka 565, Japan and Immunobiology Vaccine Centre, Department of Oral Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA

REFERENCES

- 1 Elson CO. Experimental models of intestinal inflammation. New insights into mechanisms of mucosal homeostasis. In: Ogra PL, Mestecky J, Lamm ME, *et al*, eds. *Handbook of mucosal immunology*. San Diego: Academic Press, 1999:1007-24.
- 2 Braegger CP, MacDonald TT. Immune mechanisms in chronic inflammatory bowel disease. *Ann Allergy* 1994;**72**:135-41.
- 3 Strober W, Ehrhardt RO. Chronic intestinal inflammation: an expected outcome in cytokine or T cell receptor mutant mice. *Cell* 1993;**75**:203-5.
- 4 Powrie F. T cells in inflammatory bowel disease: protection and pathogenic roles. *Immunity* 1995;**3**:171-4.
- 5 Taugrog JD, Richardson JA, Croft JT, *et al*. The germ free state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *J Exp Med* 1994;**180**:2359-64.
- 6 Morrissey PJ, Charrier K. Induction of wasting disease in SCID mice by the transfer of normal CD4⁺/CD45RB^{hi} T cells and the regulation of this autoreactivity by CD4⁺/CD45RB^{lo} T cells. *Res Immunol* 1994;**145**:357-62.
- 7 Boll GA, Rudolph S, Spiess S, *et al*. Regional specialization of intraepithelial T cells in the murine small and large intestine. *Scand J Immunol* 1995;**41**:103-13.
- 8 Beagley KW, Fujihashi K, Lagoo AS, *et al*. Differences in intraepithelial lymphocyte T cell subsets isolated from murine small versus large intestine. *J Immunol* 1995;**154**:5611-19.
- 9 Seibold F, Seibold-Schmid B, Cong Y, *et al*. Regional differences in L-selectin expression in murine intestinal lymphocytes. *Gastroenterology* 1998;**114**:965-74.
- 10 Camerini V, Sydora BC, Aranda R, *et al*. Generation of intestinal mucosal lymphocytes in SCID mice reconstituted with mature, thymus-derived T cells. *J Immunol* 1998;**160**:2608-18.
- 11 Kweon MN, Yamamoto M, Kajiki M, *et al*. Systemically derived large intestinal CD4⁺ Th2 cells play a central role for the development of STAT6-mediated allergic diarrhea. *J Clin Invest* 2000;**106**:199-206.
- 12 Powrie F, Leach MW, Mauze S, *et al*. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RB^{hi} CD4⁺ T cells. *Immunity* 1994;**1**:553-62.
- 13 Cong Y, Brandwein SL, McCabe RP, *et al*. CD4⁺ T cells reactive to enteric bacterial antigens in spontaneously colitic C3H/HeJ mice: increased T helper cell type 1 response and ability to transfer disease. *J Exp Med* 1998;**187**:855-64.
- 14 Takahashi I, Kiyono H, Hamada S. CD4⁺ T-cell population mediates development of inflammatory bowel disease in T-cell receptor alpha chain-deficient mice. *Gastroenterology* 1997;**112**:1876-86.
- 15 Kishi D, Takahashi I, Kai Y, *et al*. Alteration of V β usage and cytokine production of CD4⁺ β T cells by elimination of *Bacteroides vulgatus* prevents colitis in TCR α -chain-deficient mice. *J Immunol* 2000;**165**:5891-9.
- 16 Kweon MN, Fujihashi K, VanCott JL, *et al*. Lack of orally induced systemic unresponsiveness in IFN- γ knockout mice. *J Immunol* 1998;**160**:1687-93.
- 17 Ehrhardt RO, Ludviksson BR, Gray B, *et al*. Induction and prevention of colonic inflammation in IL-2-deficient mice. *J Immunol* 1997;**158**:566-73.
- 18 Fujihashi K, McGhee JR, Kweon MN, *et al*. γ/δ T cell-deficient mice have impaired mucosal immunoglobulin A responses. *J Exp Med* 1996;**183**:1929-35.
- 19 Openshaw P, Murphy EE, Hosken NA, *et al*. Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. *J Exp Med* 1995;**182**:1357-67.
- 20 Wittwer CT, Ririe KM, Andrew RV, *et al*. The LightCycler: a microvolume multisample fluorimeter with rapid temperature control. *Biotechniques* 1997;**22**:176-81.
- 21 Mackay CR. Homing of naive, memory and effector lymphocytes. *Curr Opin Immunol* 1993;**5**:423-7.
- 22 Sanders ME, Makgoba MW, Sharrow SO, *et al*. Human memory T lymphocytes express increased levels of three cell adhesion molecules (LFA-3, CD2, and LFA-1) and three other molecules (UCHL1, CDw29, and Pgp-1) and have enhanced IFN- γ production. *J Immunol* 1988;**140**:1401-7.
- 23 Lee WT, Yin XM, Vitetta ES. Functional and ontogenetic analysis of murine CD45R^{hi} and CD45R^{lo} CD4⁺ T cells. *J Immunol* 1990;**144**:3288-95.
- 24 Aranda R, Sydora BC, McAllister PL, *et al*. Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4⁺, CD45RB^{high} T cells to SCID recipients. *J Immunol* 1997;**158**:3464-73.

- 25 **Morrissey PJ**, Charrier K, Braddy S, *et al*. CD4⁺ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified CD4⁺ T cells. *J Exp Med* 1993; **178**:237–44.
- 26 **Powrie F**, Leach MW, Mauze S, *et al*. Phenotypically distinct subsets of CD4⁺ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int Immunol* 1993; **5**:1461–71.
- 27 **Powrie F**, Correa-Oliveira R, Mauze S, *et al*. Regulatory interactions between CD45RB^{high} and CD45RB^{low} CD4⁺ T cells are important for the balance between protective and pathogenic cell-mediated immunity. *J Exp Med* 1994; **179**:589–600.
- 28 **Rudolphi A**, Boll G, Poulsen SS, *et al*. Gut-homing CD4⁺ T cell receptor alpha beta⁺ T cells in the pathogenesis of murine inflammatory bowel disease. *Eur J Immunol* 1994; **24**:2803–12.
- 29 **Claesson MH**, Rudolphi A, Kofoed S, *et al*. CD4⁺ T lymphocytes injected into severe combined immunodeficient (SCID) mice lead to an inflammatory and lethal bowel disease. *Clin Exp Immunol* 1996; **104**:491–500.
- 30 **Rudolphi A**, Bonhagen K, Reimann J. Polyclonal expansion of adoptively transferred CD4⁺ alpha beta T cells in the colonic lamina propria of scid mice with colitis. *Eur J Immunol* 1996; **26**:1156–63.
- 31 **Simpson SJ**, Hollander GA, Mizoguchi E, *et al*. Defects in T-cell regulation: lessons for inflammatory bowel disease. In: Kagnoff M, Kiyono H, eds. *Essentials of mucosal immunology*. San Diego: Academic Press, 1996:291–305.
- 32 **Bregenholt S**, Reimann J, Claesson MH. Proliferation and apoptosis of lamina propria CD4⁺ T cells from scid mice with inflammatory bowel disease. *Eur J Immunol* 1998; **28**:3655–63.
- 33 **Claesson MH**, Bregenholt S, Bonhagen K, *et al*. Colitis-inducing potency of CD4⁺ T cells in immunodeficient, adoptive hosts depends on their state of activation, IL-12 responsiveness, and CD45RB surface phenotype. *J Immunol* 1999; **162**:3702–10.
- 34 **Wirtz S**, Finotto S, Kanzler S, *et al*. Chronic intestinal inflammation in STAT-4 transgenic mice: characterization of disease and adoptive transfer by TNF- α plus IFN- γ -producing CD4⁺ T cells that respond to bacterial antigens. *J Immunol* 1999; **162**:1884–8.
- 35 **Takeda K**, Clausen BE, Kaisho T, *et al*. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity* 1999; **10**:39–49.
- 36 **Simpson SJ**, Shah S, Comiskey M, *et al*. T cell-mediated pathology in two models of experimental colitis depends predominantly on the interleukin 12/signal transducer and activator of transcription (Stat)-4 pathway, but is not conditional on interferon gamma expression by T cells. *J Exp Med* 1998; **187**:1225–34.
- 37 **Monteleone G**, Biancone L, Marasco R, *et al*. Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology* 1997; **112**:1169–78.
- 38 **Kojouharoff G**, Hans W, Obermeier F, *et al*. Neutralization of tumour necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice. *Clin Exp Immunol* 1997; **107**:353–8.
- 39 **Derkx B**, Taminiau J, Radema S, *et al*. Tumour-necrosis-factor antibody treatment in Crohn's disease. *Lancet* 1993; **342**:173–4.
- 40 **Corazza N**, Eichenberger S, Eugster HP, *et al*. Nonlymphocyte-derived tumor necrosis factor is required for induction of colitis in recombination activating gene (RAG) 2^{-/-} mice upon transfer of CD4⁺ CD45RB^{hi} T cells. *J Exp Med* 1999; **190**:1479–92.



Browsing made easy

Collections

With a single click Collections allows you to find all articles that have been published in your chosen subject. Select from over 200 clinical and non-clinical topic collections and/or cross search other specialist journals, the BMJ and Cochrane Reviews

www.gutjnl.com