JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2000, 51, 2, 333—346

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EXPERIMENTAL INFLAMMATORY BOWEL DISEASE — ROLE OF T CELLS

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Background: Our experiments were aimed to test:

1. which lymphocyte subpopulations participate in mouse colitis, produced by intrarectal (i.r.) deposition of trinitrobenzene sulphonic acid (TNBSA, TNP hapten);
2. the expression of cell adhesion molecules on lymphocytes draining the site of reaction;
3. the influence of mouse haplotype on the development of colitis.

Methods: CBA/J, BALB/c and C57Bl/6 inbred and outbred Swiss Webster strains were used. Mesentheric lymph node (MLN) cells of immunized animals, unseparated or separated into $CD4^+$, $CD8^+$ or $\gamma\delta^+$ and $\alpha\beta^+$ T cell subpopulations or depleted of B lymphocytes, were transferred into recipients which were challenged i.r. with TNBSA. Inflammatory reaction in the colon was confirmed macro- and microscopically and by myeloperoxidase (MPO) level. MLN lymphocyte surface markers were tested cytofluorimetrically using appropriate antibodies.

Results: Sensitization with TNP results in chronic colitis (hapten dose-dependent colon weight gain and cellular infiltrate, significant increase of MPO level) only in CBA/J and BALB/c strains and can be adoptively transferred in a cell-dose dependent manner into syngeneic recipients by T $\alpha\beta^+$ cells of both $CD4^+$ and $CD8^+$ subpopulations. T $\gamma\delta^+$ cells were ineffective and B lymphocytes do not participate in the passive transfer reaction. In MLN the number of T lymphocytes positive for cell adhesion molecules particularly LPAM-1 (V-CAM1) and LPAM-2 increases significantly.

Conclusions: Both $CD4^+$ and $CD8^+$ lymphocytes participate in the development of TNP-induced colitis. High MPO level may suggests that both Th1 and Th2 cells are involved. Colitis is accompanied by a significant accumulation in MLN of T lymphocytes positive for several cell surface adhesion molecules characteristic for memory T cells. Significant differences in susceptibility to develop colitis were found between different strains of mice.

Key words: *Cell adhesion molecules, Experimental Colitis, Myeloperoxidase, Passive transfer, T cell subpopulations, TNP*

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) constitute the most typical examples of chronic idiopathic inflammatory bowel disease (IBD). These are multifactorial disorders whose etiology remains largely unknown and exact pathogenesis is poorly understood. More recently it has been proposed that a common factor in both UC and CD is a breakdown of mucosal immune homeostasis although CD is Th1 dependent while UC is driven by Th2 cells (1, 2). Infection, toxicity and neurologic disorders can also contribute (3) to the development of IBD.

The clinical manifestations of UC and CD might be quite similar, which makes differentiation and diagnosis more difficult. Macroscopic and microscopic manifestations are also similar in both cases. Microscopy of the colon reveals a dense infiltrate of acute and chronic inflammatory cells (neutrophils, eosinophils and lymphocytes) along with evidence of distortion of the crypt epithelium. There are also macrophages and mastocytes present (4—6). To date, the reasons of IBD exacerbations and remissions are not known.

Spontaneous colitis is very rare in laboratory animals, providing these animals are not genetically prepared (selective breeding, transgenic) (7, 8). The most popular method of producing experimental colitis is based on intrarectal introduction of trinitrobenzenesulphonic acid (TNBSA) in ethanol. Ethanol provides epithelial destruction, which is necessary for hapten penetration. After being coupled with mucosal proteins and enterocytes, the hapten becomes immunogenic. While each component alone gives no effect, together they cause the long-standing colitis similar to CD observed in humans (9, 10). The term colitis is used throughout this paper.

In this paper using direct active intrarectal sensitization with TNP and passive transfer, we describe the role of $T\alpha\beta^+$ $CD4^+$ and $CD8^+$ T cells as well as T $\gamma\delta^+$ lymphocytes with respect to colitis generation. We also show that in mesentheric lymph nodes (MLN) draining the site of reaction significantly more lymphocytes than in corresponding location in control animals express cell adhesion molecules that mediate homing of T cells to mucosal tissues.

MATERIALS AND MATHODS

Mice

6—8 week old inbred male CBA/J, BALB/c and C57BL/6 mice or colony bred Swiss Webster mice were used. All animals were from the breeding unit of the Department of Immunology. Each experimental group consisted of at least 4—6 mice and each experiment was repeated two or three

times. Animals were kept under routine conditions with free access to commercial murine food and water.

Reagents

The following reagents were used: trinitrobenzenesulfonic acid (TNBSA) (Eastman Organic Chemicals, Rochester, NY). Anti-hamster IgG antibody-coated magnetic beads (1μm iron magnetic particles, Advanced Magnetics, Cambridge, MA). Hexadecyltrimethyl-ammonium bromide and o-dianisidine dihydrochloride (Sigma Chemical Co, St.Louis, MO). Myeloperoxidase standard (Calbiochem, San Diego, CA). Low-tox rabbit complement (C) (Pel-Freeze, Brown Deer, WI). Fetal calf serum (FCS) and RPMI 1640 medium (Gibco, Grand Island, NY).

Antibodies

Anti-TCR β mAb (hamster IgG, clone H57—597) was received from Dr. R. Kubo, University of Colorado, anti-TCR δ mAb (hamster IgG, clone UC7—135D5), was obtained from Dr. J. Bluestone, University of Chicago, anti-CD4 (GK—1.5) and anti-CD8 (TIB 211), were a gift from Dr. C.A. Janeway Jr., Yale University. Cells were cultured under standard conditions, and supernatants were used as a source of mAb. These supernatants contained at least 10 μg mAb per ml, as tested by Elisa assay. All fluorochrome (FITC or PE)-labelled antibodies used for cytofluorometry were from Pharmingen (San Diego, CA).

Induction of colitis

Induction of colitis in the distal colon was adapted either from the protocol developed by Morris *et al.* (9), who used single intrarectal (i.r.) hapten application or Elson *et al.* (10, 11) who recommended double treatment. Mice were deprived of food 8 hours before sensitization and anesthetized with ether. To induce colitis, a catheter was carefully inserted into the colon until the tip was 20—40 mm approximal to the anus. Mice in experimental groups received various doses of TNBSA (0.5 to 4.5 mg) in 50% ethanol (ETOH) in a total volume of 50 μl. Control mice received ETOH alone. Animals were then kept in an upside down position for 30 sec. before being returned to their cages. In parallel experiments mice were sensitized twice (at 7 days time intervals), according to the protocol by Elson *et al.* (10, 11). Two weeks after i.r. sensitization animals were tested for colitis or used as donors of immune cells. Colons were harvested, cut in 10 mm long pieces, weighted and then tested for histological changes and myeloperoxidase (MPO) activity. Colon segment mass is expressed in mg/10 mm. In experiments performed on different strains of mice results are expressed as percent increase of colon mass in sensitized mice as compared with animals which received ethanol only.

Grading of histologic changes

Mice were killed, distal colon was removed, opened, washed with saline and spread flat on a paper with mucosa facing up and fixed in 10% buffered formalin. Tissue was oriented to get perpendicular cuts of rectal wall. Routine tissue processing and paraffin embedding were followed by hematoxylin and eosin staining of 3 μm paraffin sections.

An area of alcohol and hapten application was easily recognized histologically, even in cases where colitis was absent. This area was characterized by scarred connective tissue with adhesion of

mucosa to muscularis propria (but otherwise normal and free of colitis) (Score 1). Mild colitis was characterized by a thickening of the lamina propria with minor mononuclear infiltrate and, in some cases, thinning of the mucosa with disoriented and partially distorted glands (Score 2). In moderate colitis, in addition to adhesions of mucosa to muscularis propria there was a decrease in gland density, while a chronic inflammatory infiltrate was present in an abundant lamina propria (Score 3) Severe colitis either showed an ulceration or an inflammation with crypt abscesses acompanied by a more dense inflammatory infiltrate. Polymorphonuclear cells were the predominant infiltrating cells (Score 4).

Measurement of colonic myeloperoxidase (MPO) activity

Colonic MPO activity was used to monitor acute inflammation and was measured by the method of Bradley *et al.* (12). The distal, 10 mm segment of the colon was homogenized in 0.5% hexadecyltrimethylammonium bromide pH 6.0 (50 mg of tissue / 1 ml). The homogenates were freeze — thawed 3 times, centrifuged at 40.000 g and then 0.1 ml aliquots were mixed with 2.9 ml phosphate buffer, pH 6.0, containing 0.167 mg/ml o-dianisidine dihydrochloride and 5×10^{-4}% H_2O_2 and incubated at 25°C for 20 min. The absorbance was measured at 460 nm in 96-well flat bottom plates. MPO activity was expressed in units per 1 cm of colon length (1 U converts at 25°C 1μM of H_2O_2 to H_2O per minute).

Adoptive cell transfer

Two weeks after i.r. sensitization mice were killed by cervical dislocation and mesenteric lymph nodes were harvested. A single cell suspension was prepared by a gentle teasing between frosted microscopic slides. Cells were then filtered, washed with ice cold PBS and resuspended in RPMI 1640 medium + 5% FCS (positive control) or in supernatants containing appropriate monoclonal antibodies. Usually, 1×10^7 cells were incubated on ice with 1 ml of supernatant for 40 min. Thereafter cells were washed again with PBS and then resuspended in PBS + 2% FCS. Cells incubated with anti-TCRβ (H57) or anti-TCRδ (UC7) mAbs were separated with magnetic beads (see below). Cells preincubated with anti-CD4 or anti-CD8 mAb were treated with diluted rabbit C for an additional 45 min at 37°C. Subsequently isolated cell populations were transferred i.v. into naive syngeneic recipients where the reticuloendothelial system also contributed to depletion. Some mice received mesenteric lymph node cells purified on nylon wool column (B cell depleted). Then 4—6 hours after cell transfer recipient mice were challenged intrarectally with 4 mg of TNBSA in 50μl of PBS and 48 hours later were screened for development of colitis as described above.

Immuno-magnetic bead cell fractionation

Mesenteric lymph node cells opsonized with anti-TCRβ or anti-TCRδ were resuspended in PBS + 2% FCS to get a final concentration 5×10^6/ml and mixed with goat anti-hamster Ig-coated paramagnetic beads at 5—10 beads per target cell. This was followed by incubation on ice for 30 min in a flat vertical 50 ml flasks; then a magnet (Advanced Magnetics, Cambridge, MA) was applied to one side of the flask and both magnetic bead non-adherent and adherent cells were recovered. Mesenteric lymph node cells contained 2% $\gamma\delta^+$ cells and 90% $\alpha\beta^+$ cells, as determined by cytofluorometry.

Removal of B lymphocytes

A nylon wool (Leuko-Pac Leukocyte Filter, Fenval Lab., Deerfield, IL) column was prepared by the method of Julius *et al.* (13) Nylon wool was treated with 1 N HCl, washed extensively with distilled H_2O and then packed into syringe; 0.5 g column was incubated with RPMI 1640+5% FCS at 37°C for 30 min and subsequently 1×10^8 mesenteric lymph node cells were applied to the column. After 1h incubation at 37°C non-adhering T cells were washed out with warm medium. As determined by cytofluorometry (Cytoron Absolute, Ortho) using polyclonal FITC-conjugated anti-mouse Ig. MLN lymphocytes contained approximately 8 percent B cells, while nylon column-eluted population had less than 1 percent B lymphocytes.

Flow cytometry

Five $\times 10^5$ of MLN lymphocytes (in 0.1 ml) from TNBSA+50% ETOH treated or control mice treated with ETOH alone were added to wells of a V bottom 96-well plate kept on ice. Cells were double stained for 30 min with an appropriately diluted FITC-conjugated anti-αβ (H57—597) or anti-γδ (GL3) mAb together with one of appropriately diluted PE-conjugated mAbs against CD3ε, CD4, CD8a, CD11a (LFA-1), CD49d (Integrin α_4 chain), CD54 (ICAM-1) or CD62L (L-selectin). Washed cell pelets were resuspended in 200 µl FACS buffer containing 1% paraformaldehyde, and stored at 4 °C in the dark. FACS analysis was performed on Ortho Cytoron Absolute flow cytometer collecting 1×10^4 cells. Immunocount II was used for data acquisition and analysis.

Statistical methods:

The statistical significance of results was evaluated using the double-tailed Student's t-test and $p < 0.05$ was taken as a minimum level of significance.

RESULTS

Dose-dependence of hapten-induced colitis. Morphological and biochemical criteria.

Ethanol alone when applied intrarectally, produces local inflammation of low intensity (histological score **I**). Single treatment with TNBSA 0,5 mg per mouse, has no visible additional effect (histological score I, rarely II). Rectal dose of 1,5 mg and 4,5 mg per mouse results in colon inflammation and increased mass of the distal colon two weeks after sensitization (histological scores II and III or IV respectively). Double sensitization of animals led to a significant distal colon mass increase at all doses tested. The highest dose used (4.5 mg/mouse) was equally effective in mice sensitized either once or twice (*Fig. 1*)

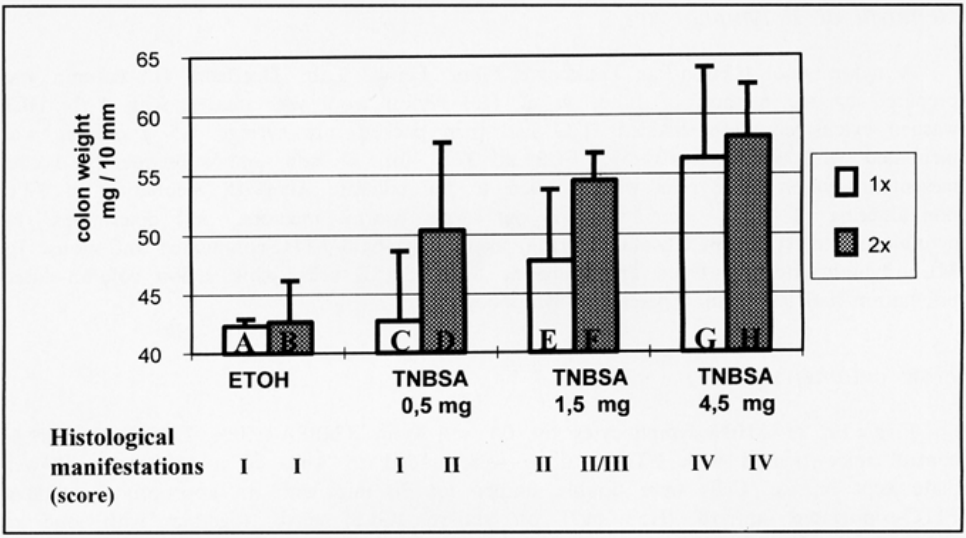


Fig. 1. TNP-induced colitis in mice sensitized once or twice to TNBSA. CBA/J mice were sensitized intrarectally (i.r.) with different doses of TNBSA in 50% ethanol on one or two occasions (at 7 days intervals). Control group mice received i.r. only ethanol (ETOH). Two weeks later, colons of these animals were harvested, cut into 10 mm long pieces, weighted and examined for histological changes. Results are expressed as colon weight / 10 mm in mg $\pm$ SD. The bottom row indicates grading of histological changes in each group. Statistical significance at $p < 0.05$ level: A vs E and G; B vs. F and H.

Activity of colonic MPO was negligible in control animals which received only ETOH (0.064 ± 0.027 U/10 mm) but increased severalfold in mice sensitized with the high (4.5 mg) dose of TNBSA (0.490 ± 0.069 U/10 mm). When lower doses of hapten was used intermediate values of MPO were registered (results not shown) (*Fig. 2*).

Passive transfer of colitis by mesenteric lymph node (MLN) cells.

MLN lymphocytes of mice immunized two weeks earlier were transferred intravenously (i.v.) to naive recipients. After i.r. challenge with hapten (4mg TNBSA) intensity of colitis in recipients as measured 48 hrs later by colon mass increase was dependent on : (a) the number of transferred cells and, (b) intensity of colitis in donors (which was subsequently dependent on the dose of immunizing agent). While $4x10^7$ cells from donors sensitized with 4.5 mg TNBSA transferred a significant reaction into recipients, $4x10^5$ cells produced no significant effect (histologic score III or IV, versus I or II respectively) (*Fig. 3*). On the contrary the transfer of lymphocytes from the group of mice treated with ETOH alone was negative (histologic score I) (data not shown).

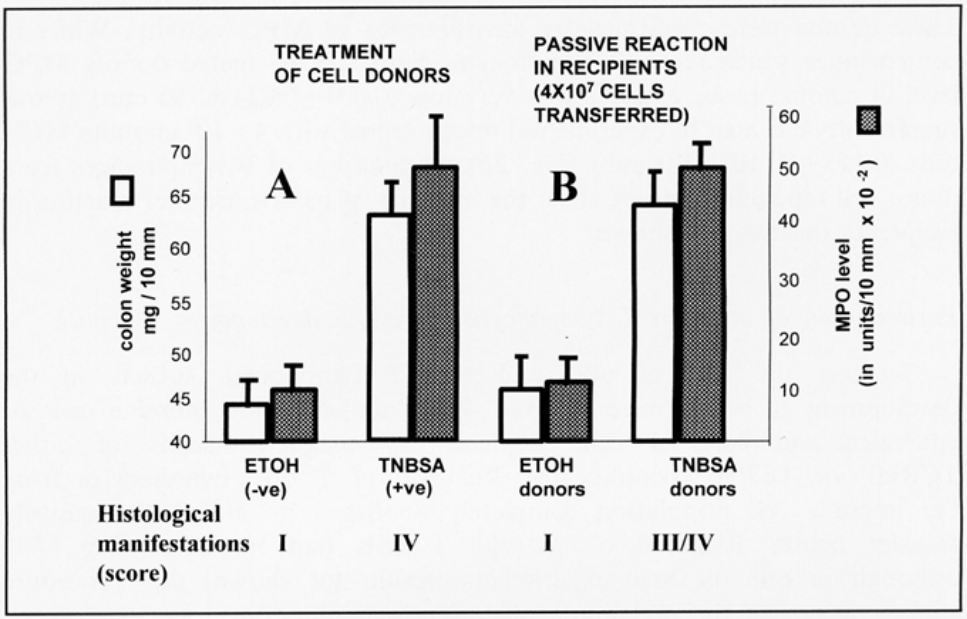


Fig. 2. Passive transfer of colitis and myeloperoxidase (MPO) activity. Donor CBA/J mice were sensitized with a single i.r. dose of 4.5 mg TNBSA as described in legend to *Fig. 1*. Control mice received i.r. ETOH only. Open bars show colon weight / 10 mm in mg ± SD (left ordinate), dotted bars MPO activity in units ± SD (right ordinate). 4×10^7 mesentheric lymph node cells of TNBSA or ETOH — treated donors were transferred i.v. into naive recipients. Four hours after cell transfer animals were challenged i.r. with TNBSA and 48 hr later mice were tested for adoptive transfer of colitis (colon weight and MPO activity). Grading of histological changes in donors and recipients in each group are shown in bottom row.

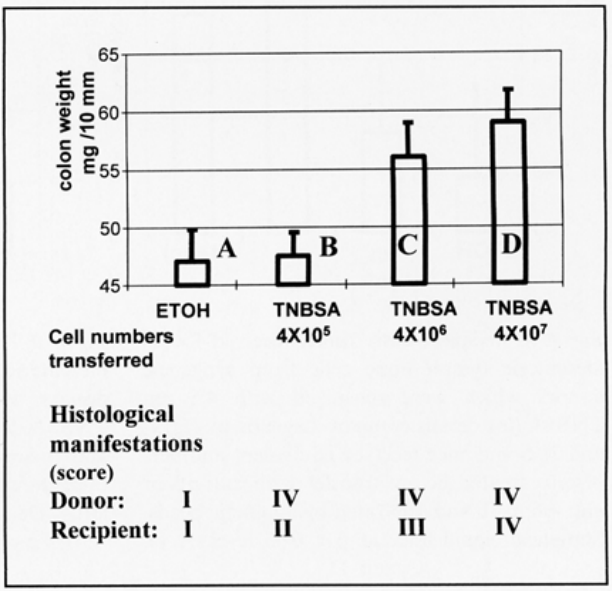
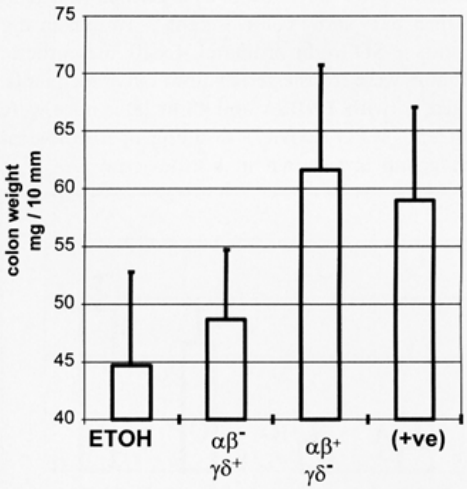


Fig. 3. Dependence of the passive transfer of colitis to TNBSA on cell numbers injected. For details see Legends to *Fig. 1* and *Fig. 2*.

These results were confirmed by measuremens of MPO activity. While in control mice which received lymphocytes from ETOH-treated donors MPO level in colonic tissue extracts was very low (0.084 ± 0.024 U/10 mm), it was significantly elevated in experimental mice injected with 4×10^7 immune MLN cells (0.445 ± 0.110 U/10 mm) (*Fig. 2B*). Elimination of B lymphocytes from donor cell inoculum did not affect the intensity of passive transfer reaction in recipients (results not shown).

Participation of different T lymphocyte subsets in development of colitis

To test the role of $\alpha\beta^+$ and $\gamma\delta^+$ T lymphocyte subsets in the development of colitis mice received $4x10^7$ non-separated immune cells or equivalent numbers of cells depleted by magnetic beads of either TCR$\alpha\beta^+$ or TCR$\gamma\delta^+$ lymphocytes. Removal of T $\alpha\beta^+$ lymphocytes from the immune cell population completely abolished its ability to passively transfer colitis. Removal of $\gamma\delta^+(\alpha\beta^-)$ T cells had no effect (*Fig. 4A*), although in one of three experiments (result not shown) this procedure slightly increased the magnitude of passive immunity.



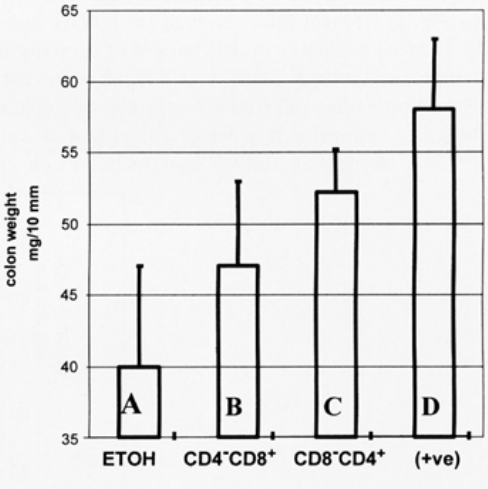


Fig. 4. A. Recipient CBA/J mice received 4×10^7 mesenteric lymph node cells from syngeneic donors which were sensitized with 4.5 mg TNBSA (for details compare Legends to *Fig. 1.* and 2). Some mice received equivalent numbers of cells treated before transfer with anti-$\alpha\beta$ or anti -$\gamma\delta$ mAb and separated by magnetic beads. Statistical significance at $p < 0.05$ level: A vs. C and D.

Fig.4. B. Recipient CBA/J mice received 4×10^7 mesenteric lymph node cells from syngeneic donors which were sensitized with 4.5 mg TNBSA (for details compare Legends to *Fig. 1* and *2*). Some mice received equivalent numbers of cells treated before transfer with anti-CD4 or anti-CD8 mAb and C. Statistical significance at $p < 0.05$ level: A vs. C and D.

In next experiments mice received 4×10^7 non-separated immune cells or equivalent numbers of lymphocytes depleted of either $CD4^+$ or $CD8^+$ cells. Removal of $CD8^+$ T lymphocytes from the immune cell population by anti-CD8 mAb slightly decreased its ability to passively transfer colitis. Removal of $CD4^+$ cells had a more pronounced effect on colitis development and decreased its intensity by over 50 percent (*Fig. 4B*).

Cell surface markers on T $\alpha\beta^+$ cells in mesentheric lymph nodes (MLN)

Intrarectal treatment with ETOH alone does not influence the pattern of cell surface markers distribution on T $\alpha\beta^+$ cells from MLN as compared with non-treated animals. In contrast in TNBSA-immunized mice there is a significant increase of the number of α_4 integrin chain-positive cells (from 35 to 69 percent), characteristic for memory cells. We also found a moderate increase of ICAM-1 positive and L- selectin positive lymphocytes (from 64 to 80 and from 55 to 70 per cent, respectively). The level of LFA-1 positive cells remained unchanged.

Different mouse strains show a different ability to develop colitis

After single i.r. treatment with 4.5 mg TNBSA CBA/J and BALB/c mice developed high grade colitis (135% and 158% of control values respectively, histological score III, IV), while C57BL/6 and outbred Swiss Webster mice were resistant (score I) (*Fig. 5*).

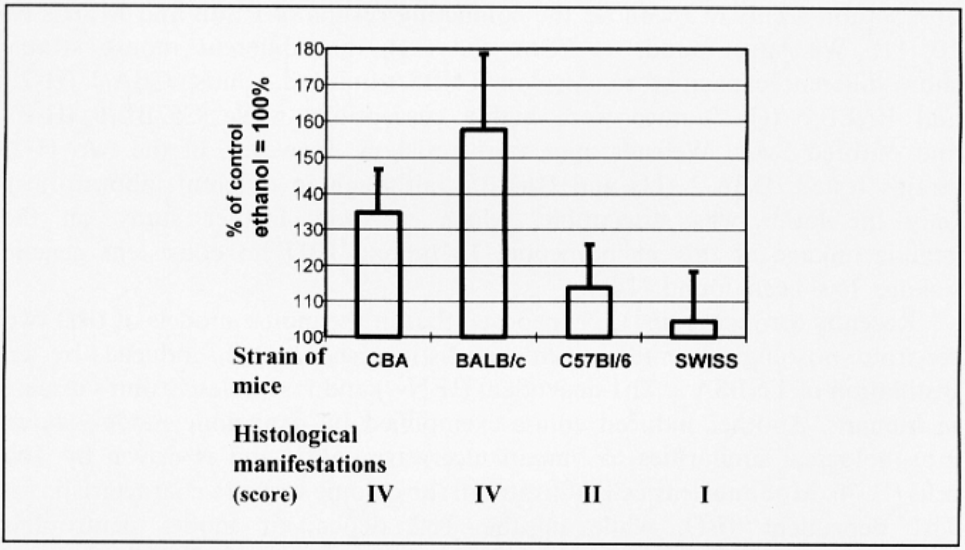


Fig. 5. Induction of colitis by TNBSA in different mouse strains. Inbred CBA/J, BALB/c and C57BL/6 mice and colony bred Swiss Webster mice were sensitized i.r. with 4.5 mg TNBSA. Control mice of each strain were given i.r. ethanol only. At the bottom grading of histological changes is shown.

DISCUSSION

The results of our experiments on the mode of induction and mechanisms of experimental colitis in mice can be summarized as follows:

1. Colitis can be induced in mice by TNBSA applied intrarectally (i.r.), and can be adoptively transferred to naive recipients by mesenteric lymph node (MLN) cells in a dose-dependent manner.
2. Both the $CD4^+$ and $CD8^+$ T $\alpha\beta$ cell subsets, although in different proportions, participate in the pathomechanism of hapten-induced colitis. T $\gamma\delta^+$ cells (i.e. immune cells depleted of T $\alpha\beta^+$ cells) are inactive. Depletion of B lymphocytes from immune cell population does not influence the passive transfer of colitis.
3. In i.r. immunized mice there is a significant increase in MLN of the number of T $\alpha\beta^+$ lymphocytes positive for α_4 chain of integrin molecule and a moderate increase of ICAM-1^+ and L-selectin$^+$ cells.

Active rectal sensitization of mice with TNBSA in ethanol results in chronic colon inflammation, hapten dose-dependent increase in colon mass, and histological manifestations. Similar effects were found by other authors in mice, rats, and rabbits (10). To induce colitis, a single sensitization was as effective as a double one, provided that appropriate doses of hapten were used. This observation seems to reconcile the conflicting results of Elson and Morris (9, 10, 11). We have found, as Elson did (11), that different mouse strains show different capacities to develop TNBSA-induced colitis; CBA/J ($H\text{-}2^k$) and BALB/c ($H\text{-}2^d$) mice were highly susceptible, while C57BL/6 ($H\text{-}2^b$) and outbred Swiss Webster mice were resistant. However, of the two $H\text{-}2^d$ strains tested, DBA/2 (11) and BALB/c (although in different laboratories), only the latter was susceptible, which warrants further study on the genetic linkage of this phenomenon. In human IBD no consistent genetic linkage has been found (14).

Recently Strober *et al.* (1, 2) proposed that in the mouse models of IBD two separate nosological entities can be distinguished. One, induced by i.r. instillation of TNBSA is Th1 dependent (IFN-γ) and resembles Crohn's disease in humans. Another induced colitis exemplified by oxazolone model, shows morphological similarities to human ulcerative colitis and is driven by Th2 cells (IL-4). Mononuclear cell infiltrates in the colonic wall are characteristic for Th1 dependent IBD, while in the Th2 dependent model neutrophils predominate. In our experiments we have found that in TNBSA-induced colitis, particularly in its more severe forms, in addition to mononuclear cells neutrophils constitute a significant part of infiltrating cells. This was also confirmed by a large increase of MPO activity in homogenates of

colonic tissues from i.r. immunized mice. Our results can thus be interpreted to mean that in at least some strains of mice there is no clear cut separation of the two mechanisms and both nosological forms can overlap.

To determine in a more detailed manner which lymphocyte subpopulations participate in mouse IBD we performed passive transfer of TNP-induced colitis by injecting MLN cells to naive recipients. Transfer of immunized cells caused a significant colonic inflammatory response in recipients challenged i.r. with homologous hapten, the intensity of which (increased colon mass, histopathology, MPO level) depended on the cell number transferred and intensity of colitis in lymphocyte donors. Removal of T $\alpha\beta^+$ lymphocytes from the immune cell population by magnetic beads completely abolished its ability to passively transfer colitis, while removal of T $\gamma\delta^+$ cells had no effect, although in one experiment this procedure slightly increased the magnitude of passive immunity. T $\gamma\delta^+$ cells play a significant immunoregulatory role (15), and their participation was shown in tolerance induced by orally administered antigen (16) and in one of the models of IBM (17) thus our observation deserves a more thorough study.

T $\alpha\beta^+$ lymphocytes in MLN draining the site of TNP-induced inflammatory bowel reaction have, as shown by us, a significantly increased proportion of cells positive for integrin α_4 subunit as compared with control mice. The α_4 chain is a part of two heterodimers α_4 β_1 (VLA-4 /LPAM-2) and α_4 β_7 (LPAM-1) with somewhat different cell distribution but overlapping ligands (ICAM-1 or ICAM-1 and MadCAM-1, respectively). Both integrins are particularly rich in memory T cells and are principal surface proteins that mediate homing of T cells, including $CD8^+$ cytotoxic T cells, to mucosal tissues e.g. Payer's patches and/or intestinal lamina propria, and to endothelium at peripheral sites of inflammation (18—21). Our original data substantiate at the morphological and functional (passive transfer of colitis) levels that MLN are the main site of homing of lymphocytes positioned in the lower intestinal tract.

All these observations support a notion that T lymphocytes alone, especially T $\alpha\beta^+$ cells, may be the primary agents of colitis initiation although they do not indicate directly whether Th1 or Th2 pathology or both were responsible for the observed effects. They show, in addition, that also $CD8^+$ T cells, although to a lesser extent, contributed to the passive transfer of colitis in mice. It was suggested that enterocytes are capable of presenting antigens in vivo to intraepithelial $CD8^+$ lymphocytes (21). Even though removal of B cells from transferred cell inoculum did not affect the passive reaction, it has been reported that TNBSA deposited in the colon significantly increases the number of Th2 dependent B cells producing anti-TNP antibodies, including IgM and IgG1 isotypes which presumably participate in the pathomechanisms of hapten

induced colitis (11). These cells accumulate mainly in mucosa and in caudal lymph nodes (11) but also in mesenteric lymph nodes which were used by us as a source of immune cells. These data are in line with our previous experiments which show that TNP-substituted syngeneic cells when given parenterally generate few, if any cytotoxic T cells. However, CTL responses can be significantly augmented and are easily demonstrated in vivo, when TNP-derivatized cells are coupled with anti-TNP antibody (particularly of IgM and IgG1 isotypes) before being injected into recipients (22). We contend that in vivo generation of TNP-anti-TNP cell bound immune complexes, independent of their intrinsic ability to produce inflammation, could lead to development of anti-hapten CTL responses mediated by $CD8^+$ T cells, which in turn contributes to damage of target cells. The issue of participation of $CD8^+$ in pathomechanism of colitis was dealt with by several authors and our functional results add one more parameter to understanding the pathology of colitis (23, 24). In summary although we regard the clinical course of TNP-induced experimental colitis in CBA/J mice as being due to the damaging effects of both $CD4^+$ (possibly Th1 as well as Th2) and $CD8^+$ lymphocytes the role of other cells, particularly neutrophils, producing free oxygen radicals should not be underestimated (25).

The animal model of colitis only simulates the pathologic processes which may be the cause of IBD in humans. However, there is a great chance that therapeutic procedures worked out on experimental models would be effective in clinical trials and open new perspectives in the development of therapeutical means (26).

Acknowledgements: This work was supported by grant from Comittee of Scientific Research (Warsaw, Poland) to M.S.

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R e c e i v e d: October 28, 1999
A c c e p t e d: March 27, 2000

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