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$CD4^+$ T lymphocytes injected into severe combined immunodeficient (SCID) mice lead to an inflammatory and lethal bowel disease

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SUMMARY

Transfer of 2×10^5 congenic or semiallogenic purified TCR$\alpha\beta^+$ $CD4^+$ T cells to SCID mice leads to an infiltration of the recipient gut lamina propria and epithelium with a donor-derived $CD4^+$ T cell subset which induces a lethal inflammatory bowel disease (IBD) in the recipients. In contrast, IBD was not observed in SCID mice transplanted with unfractionated splenic cells. The earliest detectable pathological changes after $CD4^+$ T cell transfer were proliferation and hypertrophy of the entire colonic epithelial layer, including increased mitotic activity, increased expression of epithelial nuclear proliferation antigen, and elongation of the crypts. Later on, massive mononuclear cell infiltration, hypertrophy of all layers of the colon and occasional epithelial ulcerations were observed. At this stage, accumulations of IgA, IgM and small numbers of IgG1-, IgG2- and IgG3-secreting plasma cells were present in the lamina propria of both the small and large intestine. We conclude that low numbers of intraveneously transferred $CD4^+$ T cells induce IBD in SCID mice. In the late stages of $CD4^+$ T cell-induced IBD, the colonic lamina propria becomes infiltrated with macrophages, neutrophils and plasma cells secreting IgA, IgM, and to a lesser degree IgG antibodies which might play an accessory role in the pathogenesis of IBD.

Keywords SCID mouse T cells inflammatory bowel disease

INTRODUCTION

SCID mice lack functional and mature T and B cells due to an autosomal recessive mutation in the gene coding for recombinase [1]. Consequently, the central and peripheral lymphoid organs, including mucosa and gut-associated lymphoid tissues (GALT), completely lack mature lymphocytes, and immunoglobulins are absent from the serum of SCID mice. In a number of experimental settings, we have demonstrated that congenic and syngeneic $CD4^+$ T cells can be engrafted and expanded in SCID mice with no signs of cellular infiltrations or parenchymal damage in the liver, gut, lungs, gonads, or adrenals of recipients examined for up to 4 months post-T cell transfer [2]. The vast majority of engrafted T cells repopulate the GALT, including the intestinal mucosa, the mesenteric lymph nodes and the splenic white pulp, whereas peripheral lymph nodes are not or only very poorly repopulated [3].

Recently, we described the development of inflammatory bowel disease (IBD) in SCID mice transplanted with a full thickness gut wall graft obtained from the ileum or colon of immunocompetent congenic donors. Although T lymphocytes in the GALT of normal mice include both $CD4^+$ and $CD8^+$ TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ T cells [4,5], only $CD3^+CD4^+$ TCR$\alpha\beta^+$ T cells were observed in the GALT of most gut wall-transplanted recipient SCID mice, suggesting a pathogenic role of this T cell subset [6]. The aim of the present work was to study whether an intestinal pathology, similar to that observed in our previous study [6], could be induced by transfer of low numbers of purified lymph node or splenic $CD4^+$ T cells. Here we describe the histo- and immunopathology of intestinal disease development following the engraftment of adoptively transferred, cell sorter-purified, peripheral $CD3^+$ TCR$\alpha\beta^+$ $CD4^+$ T cells in SCID recipients.

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MATERIALS AND METHODS

Mice

C.B-17 *scid/scid* (SCID) mice (H-2^d), congenic C.B-17 +/+ (H-2^d) mice, and BALB/c^{dm2} (H-2^dL^{d-}) (dm2) mice were bred under specific pathogen-free conditions in the animal facility of the University of Ulm, Germany, and the animal facility of the Panum Institute, Copenhagen, Denmark.

Preparation and labelling for FACS analysis of intestinal intraepithelial lymphocytes and lamina propria lymphocytes
Lymphocytes were prepared from the mesenteric lymph nodes and the gut lamina propria of SCID mice engrafted with $CD4^+$ T cells using previously published procedures [6]. In brief, the intestinal tissue was depleted of fat and was cut longitudinally and rinsed in RPMI 1640 culture medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 25 mM HEPES, 100 U/ml penicillin and 100 μg/ml streptomycin. Intestinal intraepithelial lymphocytes (IEL) were obtained by floating the tissue in medium under constant stirring for 30 min at 37°C. Cells in the supernatant were collected and passed through a glass wool column. The cells were centrifuged in a discontinuous 40%/70% Percol gradient (Pharmacia Fine Chemicals, Uppsala, Sweden) at 600 ***g*** for 25 min. Cells from the 40%/70% interface represent the purified IEL population. Lamina propria lymphocytes (LPL) were isolated from intestinal tissue freed of their epithelial layer. The tissue was digested with collagenase type II (Sigma Chemicals, St Louis, MO; C2139) for 45 min at 37°C. Recovered single cells were filtered through glass wool and centrifuged on a discontinuous Percoll gradient as above. More than 90% viable cells with a lymphoid cell morphology were recovered from the 40%/70% interface. The cells were reacted with PE-conjugated anti-CD4 MoAb GK1.5 (Becton Dickinson, Mountain View, CA; no. 1353), and FITC-conjugated anti-CD8α MoAb 53-6,7 (Dianova/Pharmigen, Hamburg, Germany). Non-reactive PE- and FITC-conjugated and isotype-matched MoAbs served as controls for non-specific binding to Fc receptors. Data acquisition and two-colour flow cytometry analyses were performed on a FACScan using Lysis II software (Becton Dickinson).

Labelling and purification of peripheral $CD4^+$ T cells by FACS
$CD4^+$ T cells obtained from inguinal/axillary lymph nodes or spleen were aseptically prepared from groups of 12-week-old dm2 mice. The cells were labelled as above with PE-conjugated anti-CD4 MoAb GK1.5 and FITC-conjugated anti-CD8α MoAb 53-6,7. The $CD4^+CD8^-$ cells were separated by cell sorting using the Epics V flow cytometer (Coulter Inc., Hialeah, FL). Sorted cells (2×10^3–2×10^5/mouse) were injected intraveneously into 4-week-old, sex-matched SCID mice. The sorted cells were routinely reanalysed and showed a purity of >96%.

Histology, counting and immunohistochemistry
The small and the large intestine were removed, fixed in Bouin's solution (picrylic acid, formalin, acetic acid), embedded in paraffin, cut by serial microtomy and stained with haematoxylin/eosin or periodic acid-Schiff (PAS) aurentia. For countings of IEL and LPL in the small intestine, transversely or longitudinally cut villi were examined at ×1000 magnification. Countings were performed on consecutive areas of serially cut specimens. Estimations of the ratio between numbers of epithelial cells and numbers of IEL/LPL were based on countings of a total of 1000–4000 epithelial cells and the numbers of IEL/LPL in the corresponding microscopical fields (see Table 1). For detection of IgA-secreting plasma cells, material from paraffin-embedded sections was incubated with isotype-specific, biotin-conjugated rabbit anti-mouse IgA (Southern Biotechnology Associates, Birmingham, AL), biotin-conjugated rabbit anti-mouse IgM (Serotec, Oxford, UK), and biotin-conjugated rabbit anti-mouse IgG1, IgG2a, IgG2b and IgG3 (Zymed Labs, San Francisco, CA). The antibodies were used in ×100 dilutions. The sections were reacted overnight with antibody or PBS (negative controls) at 4°C. This procedure shows reproducible and optimal binding to immunoglobulin-containing plasma cells of normal intestinal tissue. Endogenous peroxidase activity was quenched by treatment for 10 min with 0·1% freshly prepared H_2O_2 solution. Horseradish peroxidase-conjugated steptavidin (RPN 1231, batch 56; Amersham, Aylesbury, UK) diluted ×100 was used as secondary layer for 30 min at room temperature. Reactivity was visualized by incubation in DAB (KemEnTec, Copenhagen, Denmark) with freshly prepared 0·02% H_2O_2 for 10 min. For quantification of the different subclasses of plasma cells, 20 consecutive microscopic fields of the tunica mucosa were examined per specimen at ×100 magnification.

For detection of cycling cells, an antibody (IgG2a) against proliferation nuclear antigen (PCNA), clone PC10, was obtained from Dako (Copenhagen, Denmark). Prior to labelling for PCNA, histological slides were subjected to two rounds of 5 min in a microwave oven at 600 W in distilled water. The primary binding was revealed with biotin-conjugated anti-mouse IgG2a antibody followed by peroxidase-conjugated streptavidin (see above).

RESULTS

Histology of the normal SCID gut
The histological appearance of the small and large intestine of mutant, adult C.B-17 *scid/scid* mice was indistinguishable from that of adult C.B-17 mice, except for the absence of lymphocytes in the epithelium and in the lamina propria. Numerous clusters of IgA- and IgM-secreting plasma cells were observed in the lamina propria of the C.B-17 intestine, but not in the gut of SCID mice (see below, Table 3).

Repopulation of SCID GALT with adoptively transferred $CD4^+$ T cells
The intravenous transfer of 10^3–2×10^5 purified peripheral dm2 $CD3^+CD4^+$ T cells to SCID mice led within 6–8 weeks to a partial reconstruction of lymphoid cell numbers in the gut epithelium and lumina propria of the small intestine. The numbers of intraepithelial and lamina propria lymphocytes were relatively independent of the number of transplanted T cells, suggesting host-mediated control of donor cell expansion (Table 1). The phenotypes of the engrafted T cells are shown in Table 2. The vast majority of T cells in the GALT expressed the $CD4^+$ single-positive phenotype, but significant numbers of $CD4^+CD8^+$ double-positive T cells were present in the intraepithelial compartment of the small intestine. None of the lymphocytes in GALT expressed the TCR$\gamma\delta$ receptor (data not shown).

Chronic wasting disease in $CD4^+$ T cell-transplanted SCID mice
Seven to eight weeks following the transfer of 2×10^5 purified dm2 $CD3^+CD4^+$ T cells, the recipient SCID mice gradually started to lose weight (Fig. 1). Within the next 2–3 months, the grafted SCID mice exhibited symptoms of severe chronic diarrhoea and intestinal bleedings. Six to eight months post-T cell transfer many of the engrafted SCID mice were dead. In contrast, mice injected with unfractionated congenic or semisyngeneic spleen cells gained normal weight and stayed healthy. These SCID recipients appeared to have their T and B cell subsets phenotypically reconstituted (see Table 3 and [2]). Only weight curves for SCID mice receiving 2×10^7 unfractionated splenocytes are shown in Fig. 1.

Table 1. Lymphoid cell reconstitution in the small intestine of 12 SCID mice following engraftment with varying numbers of dm2 $CD3^+CD4^+$ lymph node T cells for 6–8 weeks

No. of T cells injected per mouse	Number of cells counted		
	Epithelial cells	Lymphocytes	
		% IEL†	% LPL†
0	3075 (2*)	0·1	0·6
10^2	9035 (3)	3·0 ± 0·3‡	3·6 ± 1·0
10^3	12 813 (4)	2·9 ± 0·7	2·6 ± 0·9
10^4	5925 (2)	2·0	3·8
10^5	11 573 (3)	2·6 ± 2·3	4·7 ± 0·2

* Number of mice per group.

† Percent lymphoid cells calculated as: $\frac{\text{no. of lymphoid cells} \times 100}{\text{no. of epithelial cells}}$

‡ S.D. values.

The number of epithelial cells, intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) were counted at ×1000 magnification in serial sections stained with haematoxylin/eosin and the percentages of IEL and LPL were calculated.

Table 2. Phenotypes of intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) in the small (SI) and large (LI) intestine and mesenteric lymph node (M-LNL) of SCID mice 6–8 weeks post-transplantation of purified $CD4^+$ T cells from dm2 mice

$CD3^+$ T subset*	Lymphoid compartments				
	M-LNL	SI-IEL	SI-LPL	LI-IEL	LI-LPL
$CD4^+$	56 ± 19†	23 ± 10	30 ± 15	56 ± 11	75 ± 1
$CD8^+$	<1	<2	<1	<1	<1
$CD4^+CD8^+$	<1	23 ± 17	3 ± 3	8 ± 4	3 ± 2
$CD4^-CD8^-$	<2	<1	<1	<1	<1

* Percent of lymphoid cells in the gate.

† Data represent mean values of six SCID mouse recipients ± s.d. values.

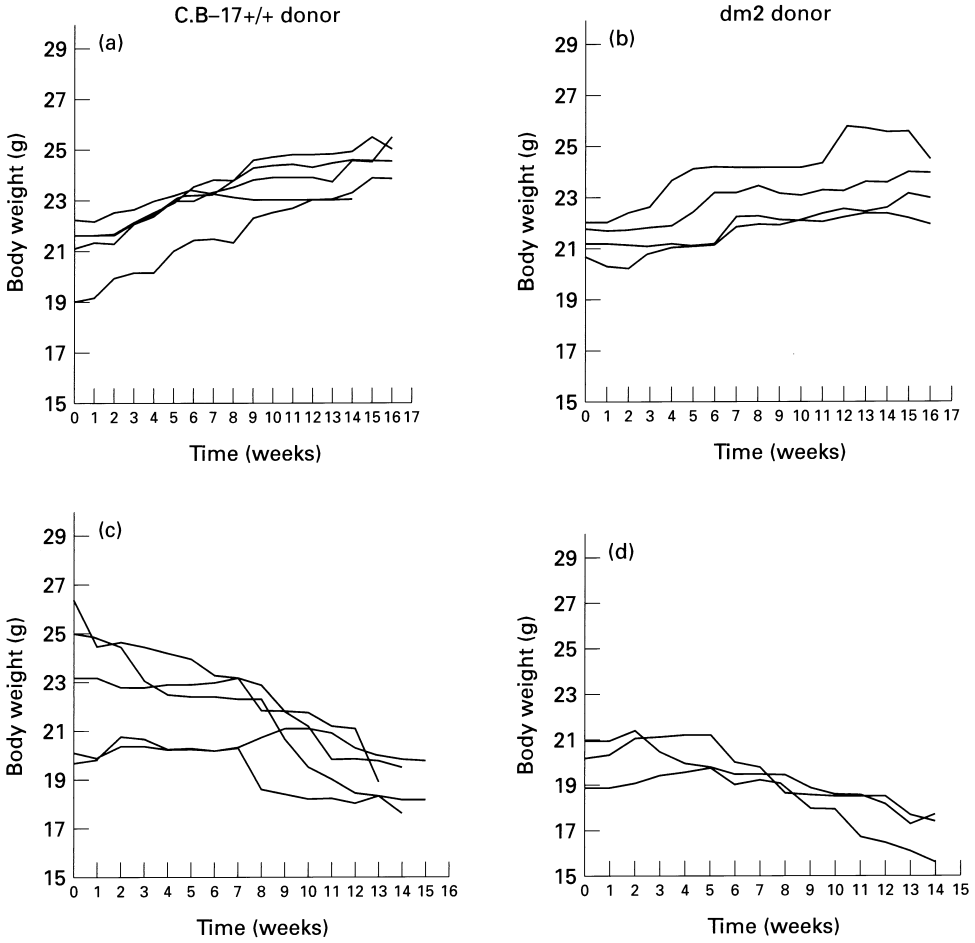


Fig. 1. Body weight curves of SCID mice following engraftment of 2×10^5 $CD4^+$ peripheral T cells or 2×10^7 splenocytes, respectively. Each curve represents one animal. (a) Non-frationated C.B-17 spleen cells (2×10^7 cells/mouse). (b) Non-fractionated BALB/c dm2 spleen cells (2×10^7 cells/mouse). (c) Purified dm2 inguinal $CD4^+$ LNC (2×10^5 cells/mouse). (d) Purified dm2 mesenteric $CD4^+$ LNC (2×10^5 cells/mouse).

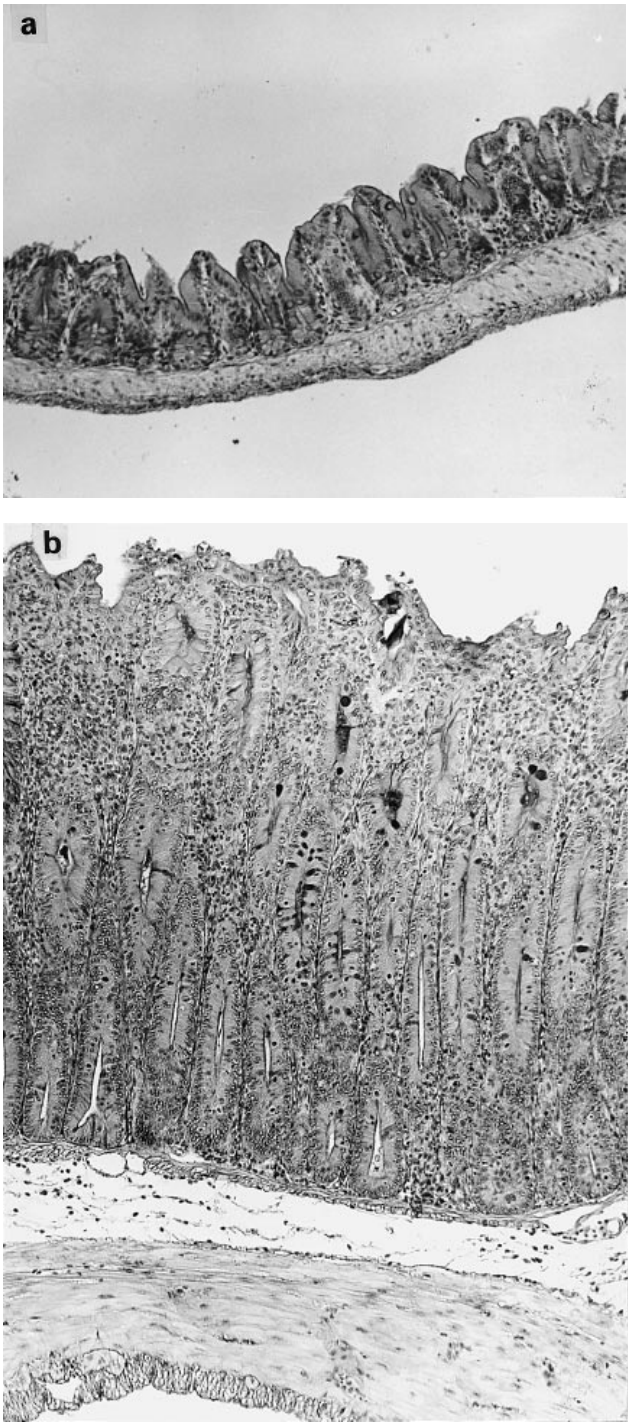


Fig. 2. Large intestine of a normal CD-17 SCID mouse (a) and a SCID mouse transplanted 4 months previously with 2×10^5 $CD4^+$ T cells (b) at the same magnification (×100). Note the pronounced hypertrophia of all layers of the intestine.

Table 3. Immunoglobulin-positive plasma cell subsets in the lamina propria of the small intestine (SI) and large intestine (LI) of normal mice, SCID mice, and SCID mice transplanted with non-fractionated spleen cells or purified $CD4^+$ T cells

		Plasma cell immunoglobulin isotype					
		IgA	IgM	IgG1	IgG2a	IgG2b	IgG3
CD-17	SI	++++*	++++	−	−	−	−
	LI	++++	++++	+	−	+	+
SCID	SI	−	−	−	−	−	−
	LI	−	−	−	−	−	−
Spleen[†]	SI	++++	++++	−	−	−	−
	LI	++++	++++	+	+	+	−
Prol[‡]	SI	+	+	−	+	−	−
	LI	+	−	−	++	−	−
Lesion[§]	SI	++++	++++	++	+	−	−
	LI	++++	++++	++	++	+	++

* Number of plasma cells in the lamina propria per field at ×100 magnification: +, <2 cells; ++, <10 cells; +++, <50 cells; ++++, >50 cells.

[†] Healthy SCID mice fully reconstituted with CD-17 splenocytes [21].

[‡] Proliferative stage of the disease.

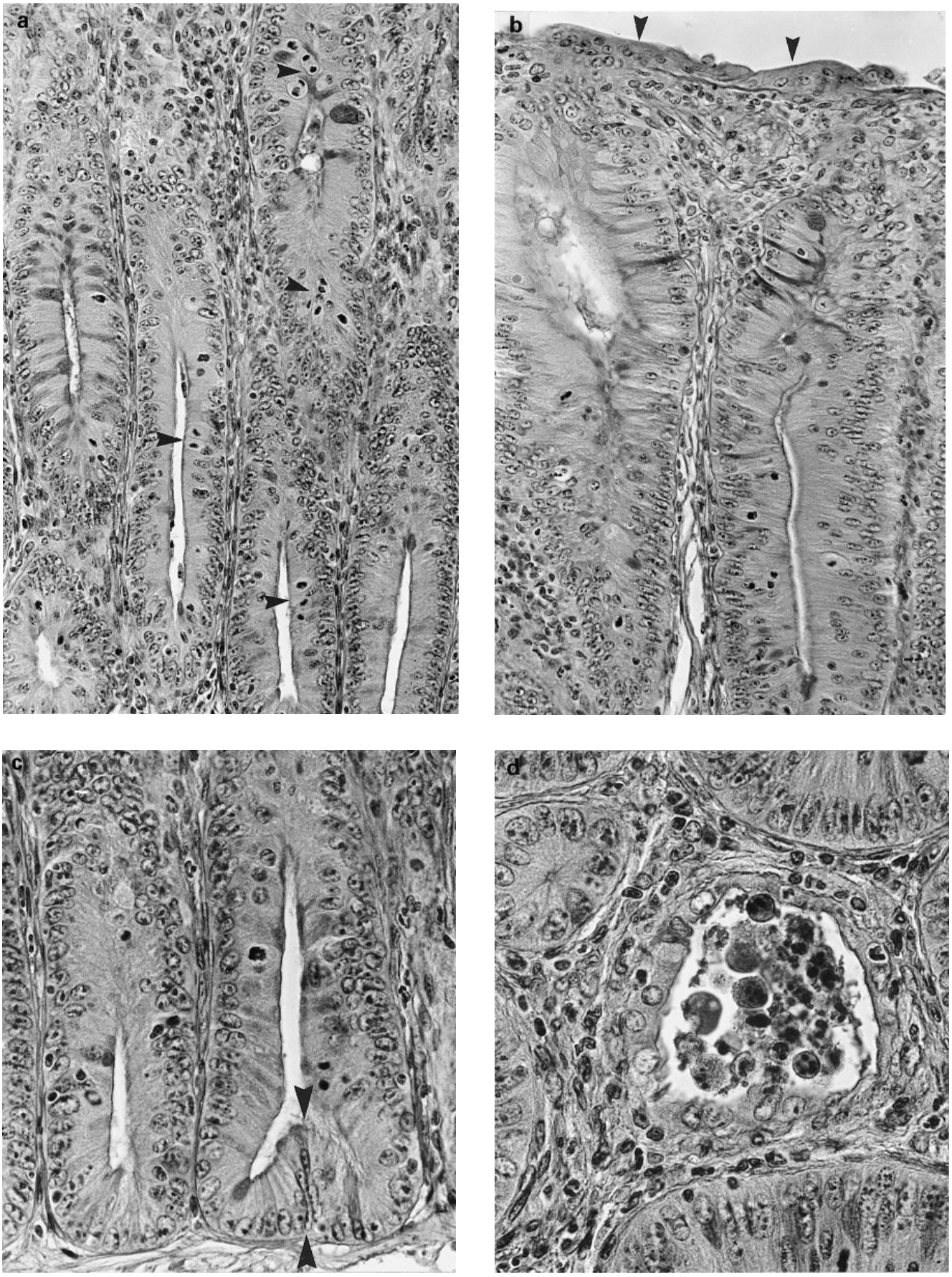
[§] Ulcerative stage of the disease.

Gut histology of the engrafted SCID mice

Samples for histology were recovered from the onset of weight loss and throughout the period of the disease. Figures 2–6 demonstrate the gradual histological changes in the gut of SCID mice intravenously injected with 2×10^5 $CD3^+CD4^+$ peripheral dm2 T cells. In general, the severity of the histopathological changes described below increased with increasing time post-engraftment. The most severe pathological changes including ulcerative lesions were observed only from 16 weeks post-engraftment and onwards. However, even in severely ill animals many areas of the colonic mucosa looked quite normal except for the cellular infiltrations and a slight to moderate epithelial hypertrophy (see below).

The most prominent histolopathological changes following $CD4^+$ T cell transfer were cellular infiltration, proliferation and hypertrophy of all the layers of the large intestine (compare Fig. 2a and 2b) and to a lesser degree the epithelial layer of the small intestine. Numerous mitotic figures were present not only in their normal position at the bottom of the crypts, but also in the upper areas leading to elongation of the entire crypt (Fig. 3a). The high rate of proliferating epithelial cells, particularly in the colon, led to attenuation in the numbers of goblet cells, which were completely absent in many crypt areas (Fig. 3b). Occasionally, dividing crypts were encountered (Fig. 3c). Along with proliferation and hypertrophy, small crypt abscesses containing macrophage-like cells with a strongly eosinophilic cytoplasm were observed (Fig. 3d).

Fig. 3. Larger magnification of the colonic mucosa from a $CD4^+$ T cell-transplanted SCID mouse. (a) Lower part of elongated crypts with increased numbers of mitotic epithelial cells (arrowheads) and goblet cell depletion of mucus and absence of visible goblet cells (×320). (b) Upper part of elongated crypt showing mitotic activity in the epithelium as sign of expanded proliferative compartment. Goblet cells are almost absent in the surve epithelium (arrowheads), which appears flattened (×320). (c) Dividing crypt (arrowheads), suggesting mucosal growth (×400). (d) Horizontal section through crypt abcess with inflammatory cells and debris (×630).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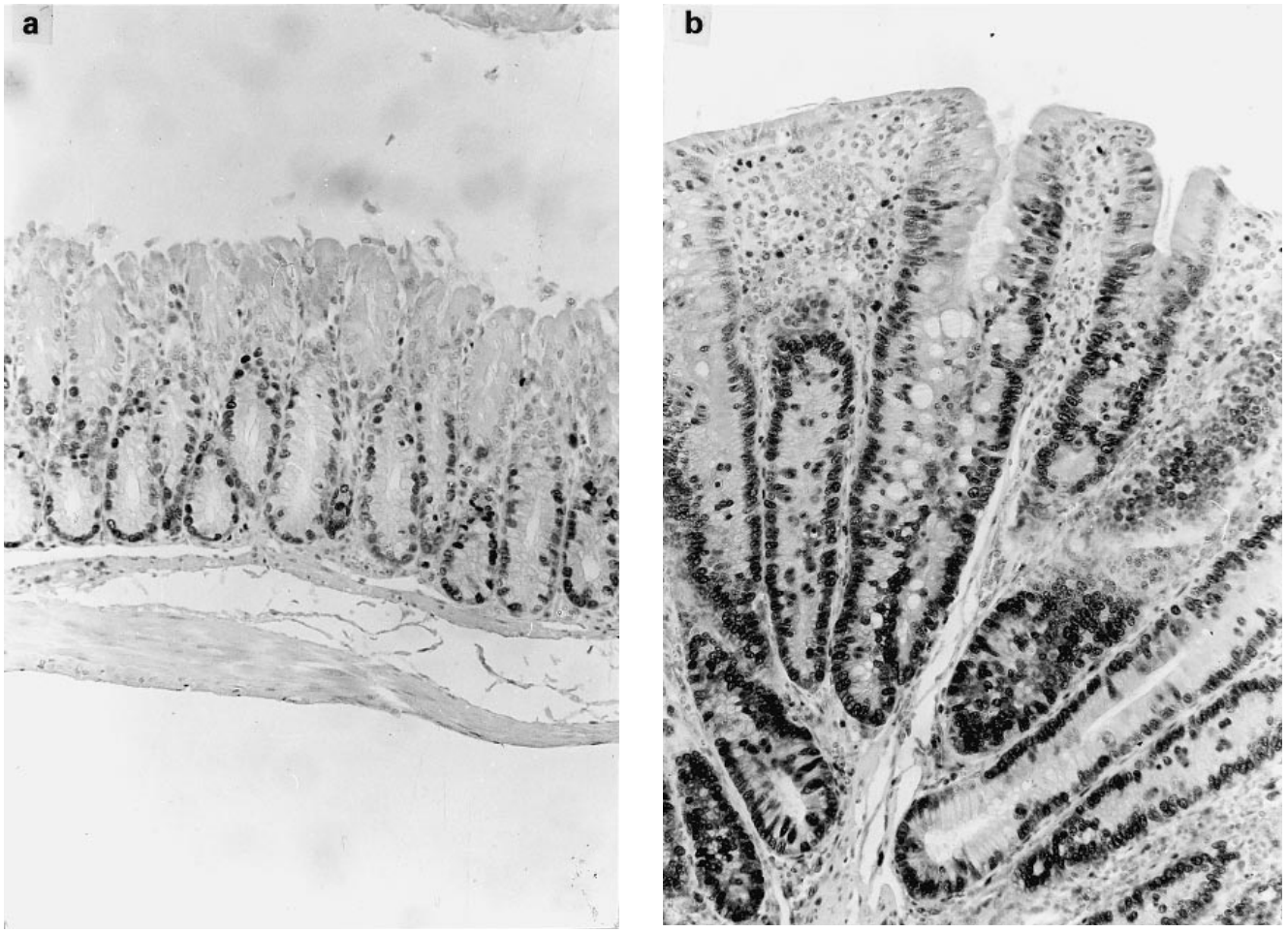


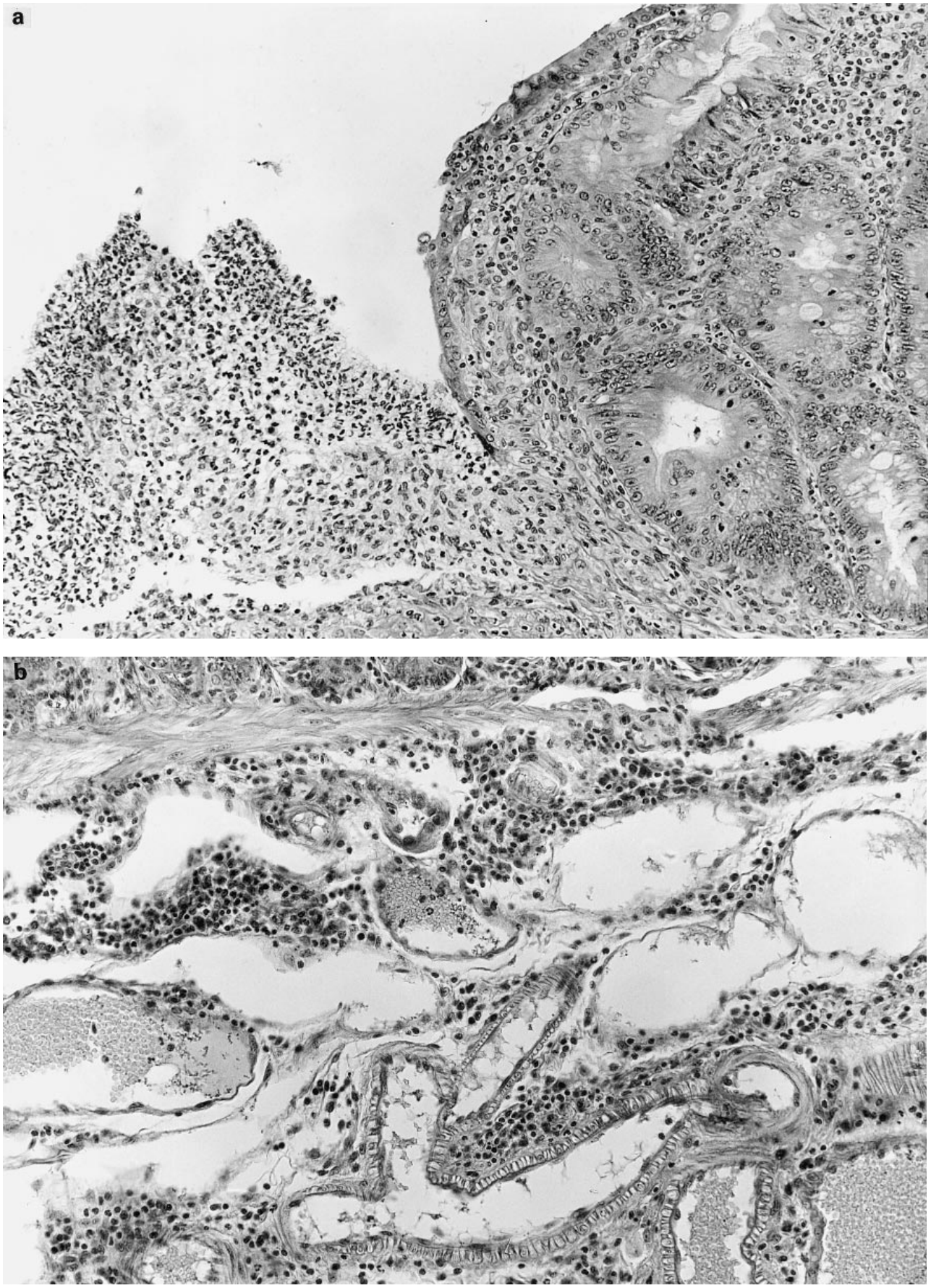
Fig. 4. Proliferation nuclear antigen (PCNA) staining of colonic epithelium in a normal mouse (a) and a SCID mouse (b) engrafted 4 months previously with $CD4^+$ T cells. The colon is in a proliferative stage of inflammatory bowel disease (IBD). PCNA staining is evident also in the upper parts of the crypts in the lamina propria at the proliferative stage. Note the presence of numerous PCNA-positive cells in the lamina propria ($\times$250).

Labelling of cells with anti-PCNA antibody confirmed the abnormally high level of S-phase epithelial cells (Fig. 4a,b). Also, anti-PCNA antibody labelled many of the mononuclear infiltrating cells in the lamina propria of $CD4^+$ T cell transplanted SCID mice. At later time intervals, scattered, large ulcerations were observed in the colon, which became heavily infiltrated with mononuclear and polymorphonuclear cells (Fig. 5a). At this late stage of disease, the tela submucosa showed extensive oedema and cellular infiltration (Fig. 5b). Destructive lesions including ulcerations and crypt abscesses were never encountered in the small intestine. During the proliferative stages of disease development a few scattered accumulations of plasma cells secreting IgA or IgG2a were observed in the intestinal lamina propria (Table 3). At later disease stages numerous clusters of IgA- and IgM-secreting plasma cells were present throughout the lamina propria of the small and large intestine (Table 3). Occasionally, lymphostasis of the central lymphatic villus capillary was observed (Fig. 6). The clusterings in lamina propria of plasma cells were randomly distributed and in particular, no accumulations of plasma cells were observed in the vicinity of the ulcerative lesions in the colon (Fig. 6). As illustrated in Table 3, the levels of plasma cell subsets in normal mice and SCID mice reconstituted with unfractionated spleen cells were similar.

DISCUSSION

The present study shows that transfer of purified non-fractionated $CD3^+CD4^+$ TCR$\alpha\beta$ T cells into SCID mice results in engraftment and expansion of donor $CD4^+$ T cells in the GALT of the recipient and the development of wasting and chronic IBD with a lethal course. In most cases, the onset of disease is 6–8 weeks post-T cell transfer, and the disease runs for 4–6 months, the main symptoms being weight loss and diarrhoea. Major histological changes were marked hyperplasia of all colonic layers and, to a lesser degree, the small intestine. In addition, the colon showed extensive mononuclear cell infiltration, crypt abscesses and large ulcerative lesions. Massive accumulations of IgA- and IgM-secreting plasma cells in the intestinal lamina propria were present at the late stages of the disease.

Fig. 5. Colon of a $CD4^+$ T cell-transplanted SCID mouse. (a) Edge of a superficial ulceration in the colonic mucosa with mono- as well as polymorphonuclear cell infiltration in the lamina propria and ulceration of the epithelium ($\times$250). (b) Extensive oedema and mononuclear cell infiltration of the submucosa ($\times$250).



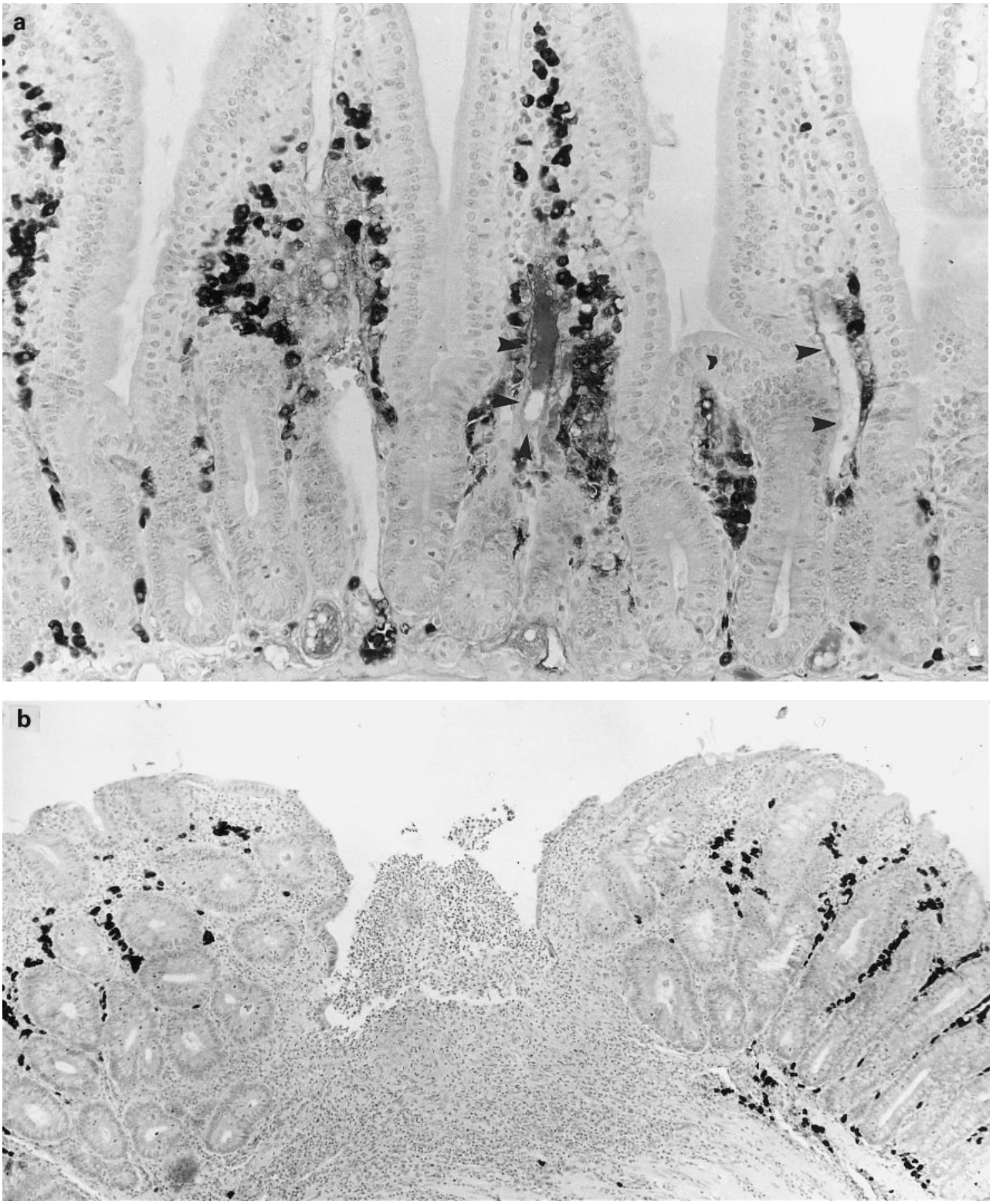


Fig. 6. (a) Accumulation of IgA-secreting plasma cells in the lamina propria of the villi in the small intestine of a $CD4^+$ T cell-transplanted SCID mouse. The central villus capillary appears dilated, indicating lymphostasis (arrowheads) (×250). (b) Ulceration of the colonic mucosa of a $CD4^+$ T cell-transplanted SCID mouse. Aggregations of IgA-secreting plasma cells in the lamina propria of the large intestine. Note the absence of plasma cells in the vicinity of the ulcerative lesion (×100).

The present observations extend previous histopathological findings in SCID mouse recipients of congenic $CD4^+$ T cells [7–9]. In previous studies the histopathology of the colon was found to be dominated by hyperplasia and mononuclear cell infiltrations, whereas ulcerative lesions, crypt abscesses and accumulations of IgA^+ and IgM^+ plasma cells were not documented. The protracted course of disease development and the histopathological changes observed in the present study are comparable to the development of IBD induced by the grafting of a piece of gut wall from immunocompetent mice onto the back of SCID mice [6], but contrasts the very acute disease development and lethal course of IBD following adoptive transfer of $CD4^+$ immature T cells to SCID mice [7]. These workers demonstrated that only purified virgin $CD4^+$ $CD45RB^{high}$ T cells were capable of inducing IBD, whereas unfractionated $CD4^+$ T cells (as used in the present study) or T cells of the memory $CD4^+CD45RB^{low}$ phenotype in fact prevented disease development. The reason for discrepancies regarding the disease-inducing T cell subset and the progression in disease development between the present and previous studies is unexplained. Differences in exposure to environmental pathogens in the studies do not appear to play a role, since the experimental animals in all these studies were raised in a specific pathogen-free environment.

Initially, panintestinal epithelial hyperplasia and proliferation were prominent features in $CD4^+$ T cell-induced IBD. Disregarding the number of injected $CD4^+$ T cells, both the epithelium and lamina propria of the small intestine were quantitatively equally repopulated, reaching numbers 6 weeks post-cell transfer of approximately 50% the normal levels in the mouse gut [10]. At this time, the gut epithelium and lamina propria, and in particular the colon, were infiltrated with T cells displaying the $CD4^+$ phenotype. At later times, the more severe histopathological changes were located in the large intestine and were dominated by cellular inflammation, crypt destruction and ulceration. An essentially similar intestinal pathology was observed in recipient SCID mice heterotopically transplanted with a piece of whole gut wall from congenic, immunocompetent donor mice [6]. However, in this latter study the disease-inducing donor T cell subset was not identified.

We have previously shown that the dominant T cell subset of the GALT of $CD3^+CD4^+$ T cell-engrafted SCID mice displays the phenotype of a mucosa seeking, activated T cell, expresses the homing receptor LPAM-1 for Peyer's patches high endothelial venule cells, and lacks expression of the CD45RB molecules present on naive or virgin T cells [3]. A similar activated memory T cell phenotype was observed in the above mentioned study of SCID mice transplanted with whole gut wall from congenic and syngeneic immunocompetent donors [6]. The relatively high frequency of $CD4^+CD8^+$ small intestinal IEL observed in the present study (Table 2), as well as the observation that many of the mononuclear cells in the lamina propria expressed nuclear proliferating antigen, may also reflect cellular activation. The activating stimulus for the engrafted T cell subset is most probably derived from the intestinal microbacterial flora. However, the extremely diverse $TCR\alpha\beta$ reportoire in the GALT of $CD3^+CD4^+$ engrafted SCID mice [3] argues against the involvement of putative superantigens in the process of T cell activation in the SCID recipients. The hypertrophied epithelial cells and the increased epithelial surface area due to proliferation and lengthening of the crypts observed in the early disease stages may result in a supranormal level of epithelial MHC class II/peptide expression and act as an abnormally strong stimulus for the engrafted intraepithelially localized $CD4^+$ T lymphocyte subset. Moreover, epithelial hyperplasia and lack of mature goblet cells may render the epithelial surface 'leaky' for intestinal antigens. Finally, the engrafted cell population may be devoid of important down-regulatory cell subsets, as indicated by the lack of both $TCR\alpha\beta$ $CD8^+$ cells and $TCR\gamma\delta^+$ cells in the GALT of the $CD4^+$ T cell SCID recipients [5].

Intestinal mucosa-associated T cells have been demonstrated to be both stimulatory and destructive for gut epithelial cells *in vitro*, activities which are mediated by T cell-secreted IL-2, interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) [11,12]. *In vivo*, an increase in gut epithelial cell turnover and crypt hyperplasia followed by mucosal atrophy was observed in allogeneic graft-*versus*-host disease (GVHD) [13]. The role of IFN-γ and TNF-α in disease development is supported by the observations that IBD induced by transferred $CD4^+$ T cells could be prevented by treatment of recipient SCID mice with Th2-derived IL-10, which is antagonistic to Th1 responses, or with anti-IFN-γ antibody, IFN-γ being an important Th1-derived lymphokine [14]. Taken together, these data strongly suggest that the inflammatory reactions in the gut, following adoptive transfer of $CD4^+$ T cells to SCID mice, are initiated by an abnormally up-regulated activity of the Th1 subset in response to gut-derived antigens.

Local cytokine release from activated donor T lymphocytes and host macrophages together with an enhanced intestinal antigen influx might also provide a powerful local activation stimulus of immature B cell subsets. In our normal stock of SCID mice, immunoglobulin 'leakiness' is not present until the age of 10 months. However, $CD3^+CD4^+$ T cell transplanted SCID mice become 'leaky' for serum IgM already 6–8 weeks post-transplantation [15,16]. In these experiments we found that 'immunoglobulin leakiness' was induced more easily by transfer of $CD4^+CD8^-$ thymocytes than of peripheral $CD4^+$ T cells. In preliminary experiments we have observed that IBD is easily induced by $CD4^+CD8^-$ thymocytes.

The present study showed that the lamina propria of the entire gut of $CD3^+CD4^+$ T cell transplanted SCID mice accumulated IgA and IgM as well as a few IgG1- and IgG2a-secreting plasma cells during the lesional disease stage. In our earlier investigations it was clearly demonstrated that the IgM leakiness after transfer of $CD4^+$ T cells is derived from the host, as judged by the immunoglobulin allotype [16]. Because of the purity and peripheral source of the transferred $CD4^+$ T cells we strongly believe, but do not yet have the definitive proof, that all classes of plasma cells detected in the present study are host-derived. If this assumption is correct, T cell-induced B cell 'leakiness' in SCID recipients may result from a cytokine- and antigen-driven expansion of ontogenetically early B cell lineages (Ly-1 B; $CD5^+$ cells: reviewed in [17]). In the mouse, Ly-1 B cells are responsible for the major contribution of IgA-secreting plasma cells of the intestinal lamina propria and the IgM-secreting plasma cells in the spleen. In addition, Ly-1 B cells exhibit a limited immunoglobulin repertoire, express auto- and anti-bacterial specificities and, in particular, anti-thymocyte reactivity, a self-reactivity also observed in serum of 'immunoglobulin leaky' SCID mice [16]. Potential T cell-reactive antibodies produced in the lamina propria of the gut might mimic natural regulatory cytokines, and lead to an unbalanced secretion of inhibitory and stimulatory cytokines.

A chronic hypertrophic, inflammatory or ulcerative bowel

disease has recently been described in 'knock-out' mice lacking genes encoding cytokines IL-2 [18] or IL-10 [19], or lacking TCRα or TCRβ-chain [20]. In all these studies up-regulated and abnormal B cell responses were suggested to play an important part in IBD pathogenesis.

Although the present data clearly show that the immune cell responsible for initiation of the histopathological changes in the gut of SCID recipients belongs to a peripheral $CD4^+$ T cell subset, it is uncertain whether the initial epithelial cell hyperplasia was in part caused directly by cytokine effects mediated by the engrafted T cells, or by stimulatory 'leaky' self-reactive antibodies. However, the late lesional disease stage appeared, in addition to donor-derived lymphocytes, to be dominated mainly by host-derived cells such as macrophages, neutrophils and plasma cells. This stage was associated with accumulations of IgA- and IgM-secreting plasma cells as well as infiltrations of mononuclear and polymorphonuclear cells. A cytokine-driven switch at this stage from a protective IgA isotype to 'destructive' immunoglobulin isotypes such as IgM, IgG1 and IgG2 might lead to local antibody–antigen deposits, activate complement, and induce infiltrating macrophages and neutrophils to release inflammatory and epithelial cell-damaging substances. In line with this suggestion, it was recently demonstrated in human ulcerative colitis, that plasma cells accumulate in the lamina propria and secrete anti-brush border IgG1 autoantibodies which activate complement and may be instrumental in the epithelial ulcerative lesions [21].

Future experiments are aimed at an understanding of the role of individual Th1- and Th2-derived lymphokines as well as host-derived monokines and antibodies in the development of $CD4^+$ T cell-induced IBD in SCID mice.

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