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Spontaneous Development of Inflammatory Bowel Disease in T Cell Receptor Mutant Mice

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Summary

We describe the spontaneous development of inflammatory bowel disease (IBD) in several immunodeficient mouse strains created via gene targeting in embryonic stem cells. Chronic colitis was observed in T cell receptor (TCR) α mutant, TCR β mutant, TCR β × δ double mutant, or class II major histocompatibility complex (MHC) mutant mice, but not in recombination-activating gene *RAG-1* mutant mice or nude mice kept in the same specific pathogen-free animal facility. This clinical pattern suggests that the disease requires the presence of B lymphocytes and the absence of class II MHC-restricted CD4$^+$ αβ T cells. IBD in the mutant mice has some of the features of the human disease ulcerative colitis. Based on these results, we suggest that dysfunction of the mucosal immune system may underly the pathogenesis of some types of IBD in humans.

Introduction

In the past few years, there has been great progress in our understanding of the development and function of αβ T cells, but much less is known about γδ T cells. In an effort to understand differential functions of αβ and γδ T cells in vivo and to investigate their possible interactions during thymic and peripheral development, we have created several strains of mutant mice by targeting T cell receptor (TCR) genes in embryonic stem cells. Mice with a mutation at the TCR α or TCR β locus (TCR α or TCR β mutant mice) are selectively deficient in αβ T cells (Mombaerts et al., 1991, 1992a), and, conversely, mice with a mutation at the TCR δ locus (TCR δ mutant mice) are selectively deficient in γδ T cells (Itohara et al., 1993). By crossing TCR β mutant mice with TCR δ mutant mice, we generated TCR β × δ double mutant mice, which are deficient both in αβ and γδ T cells (Mombaerts et al., 1992a). By mutating the recombination-activating gene *RAG-1* (Schatz et al., 1989), we produced mice totally deficient in mature T and B lymphocytes (Mombaerts et al., 1992b).

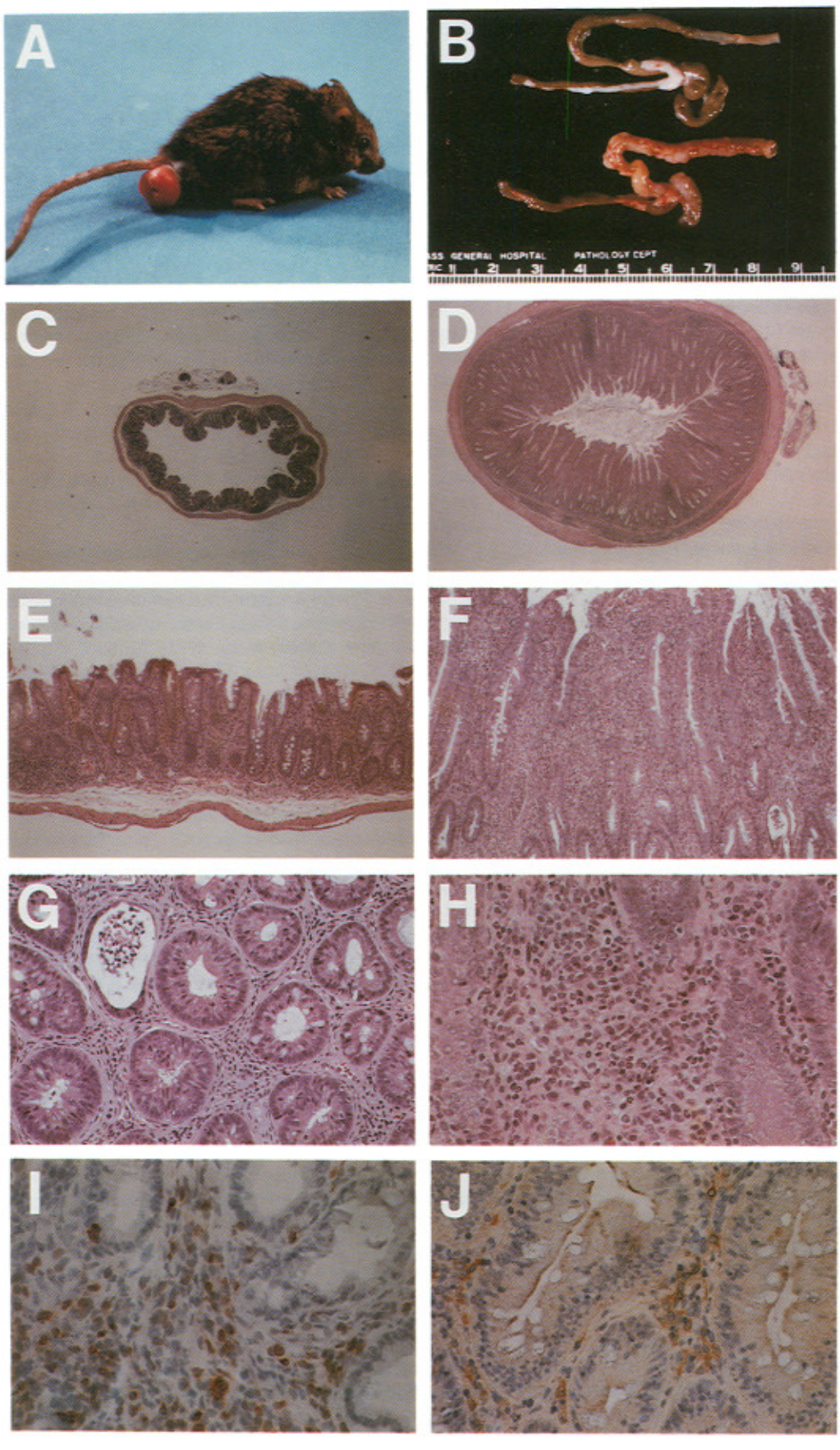
We have previously concluded that αβ and γδ thymocyte development occur in a mutually independent fashion (Mombaerts et al., 1992a; Itohara et al., 1993). Our preliminary analyses of the peripheral lymphoid systems of the various mutant mice suggest that functional αβ T cells develop apparently normally in the absence of γδ T cells (Itohara et al., 1993) and that γδ T cells are present in the peripheral lymphoid organs of αβ T cell–deficient mice (Mombaerts et al., 1992a). γδ T cells may have unique regulatory functions during certain immune responses in vivo (Mombaerts et al., 1993).

In this paper, we document the spontaneous development of inflammatory bowel disease (IBD) with age in TCR α, TCR β, or TCR β × δ mutant mice, as well as in mice with a mutation in the class II major histocompatibility complex (MHC) Aβ gene (Grusby et al., 1991). No such disease was found in wild-type mice, TCR δ mutant mice, *RAG-1* mutant mice, nude mice, or mice homozygous for the lymphoproliferative disease (*lpr*) mutation. All these strains were housed during the same period in the same colony. There was no evidence of the involvement of an infectious pathogen. The macroscopic and histological features of IBD in the mutant mice resemble those of the human disease ulcerative colitis (UC). Thus, TCR mutant mice may provide a useful small animal model for this human disease whose etiology and pathogenesis are not well understood (Podolsky, 1991a, 1991b). We suggest that the pathogenesis of UC in humans may be attributed to dysfunction of the mucosal immune system, such as lack of αβ T cell–mediated suppression of B cells.

Results

Intestinal Disease in αβ T Cell–Deficient Mice

Over a period of 2 years, we noticed that many TCR α mutant or TCR β mutant mice showed signs of ill health. After a disease-free period of at least 3–4 months during which they were indistinguishable from their wild-type or heterozygous littermates, they gradually developed chronic diarrhea, leading to a progressive wasting syndrome often associated with anorectal prolapse. Figure 1A shows a typical example of a mouse with terminal stage disease. Mortality was substantial after 6 months of age, and few mutant mice survived beyond 1 year. In sharp contrast, *RAG-1* mutant mice, which were of the same mixed genetic background and were bred and housed in the same two rooms as the αβ T cell–deficient mice, did not show these clinical signs and symptoms. This fact suggested to us that the disease of the αβ T cell–deficient mice was not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the result of an infection in the colony or of an intolerance to the autoclaved food: if this were the case, it seemed paradoxical that *RAG-1* mutant mice, having neither B or T lymphocytes, would be in better health than αβ T cell–deficient mice. Although *RAG-1* mutant mice also rarely survived more than 1 year, they did not have diarrhea but suffered at old age from other diseases, such as Pneumocystis carinii pneumonia and thoracic or abdominal abscesses. Of approximately 1000 *RAG-1* mutant mice, only a few had diarrhea or anorectal prolapse, whereas at least one third of approximately 3000 αβ T cell–deficient mice had diarrhea, frequently associated with prolapse.

Macroscopic examination of diseased TCR α or TCR β mutant mice revealed a marked dilatation and thickening of the rectum and colon, extending continuously up to the cecum. In severe cases, the entire large bowel was affected and markedly shortened (Figure 1B). In milder cases, only the rectum or terminal colon was affected, sometimes with a sharp boundary of macroscopically diseased bowel adjacent to normal bowel. The small intestine, including the terminal ileum, appeared grossly normal. The mesenteric lymph nodes were always enlarged, but there was no consistent splenomegaly. The contents of the colon and rectum were loose, in contrast with the more solid, often beaded appearance of the feces in wild-type or *RAG-1* mutant mice. There was no obvious ulceration of the mucosa, and blood was not seen in the stools.

IBD Resembling UC

Transverse sections of rectum illustrate the marked thickening of the wall (Figures 1C and 1D). The lesions were invariably more severe in the rectum than in the proximal parts of the large intestine. The crypts were elongated and showed evidence of proliferation. In severe cases there was distortion of the crypts, including branching and cystic dilations. A characteristic feature was the pronounced depletion of the mucin-producing goblet cells (Figures 1E and 1F). Occasionally, crypt abscesses (crypts containing a neutrophilic infiltrate) were identified (Figure 1G). A mixed inflammatory cell infiltrate, consisting of lymphocytes, plasma cells, and neutrophilic granulocytes, was present in the lamina propria (Figure 1H) and in severe cases extended into the muscularis mucosae or even into the submucosa. The outer muscle layers were not affected by inflammation. The nature of the lymphocytes in the lamina propria was identified immunohistochemically in a few selected cases. The number of γδ T cells was markedly increased (in a normal large intestine, only a few scattered γδ T cells can be found), and there were many immunoglobulin M (IgM)-, IgG-, or IgA-positive B cells (Figures 1I and 1J). Mucosal ulcerations were rare. There was no evidence of granuloma formation, fibrosis, or transmural fissures. Overall, the macroscopic and histological features of this disease were more reminiscent of the human IBD UC rather than of the other major human IBD, Crohn's disease. Noticeable differences, however, are the absence of marked mucosal ulcerations and of blood in the stools.

An obvious cause for IBD, which we ruled out, is the presence of a pathogenic microorganism in the colony. The mice were kept in specific pathogen-free conditions (see Experimental Procedures for definition) in two dedicated rooms, were given autoclaved food and water, and were handled, apart from one animal care taker, by only one of us (P. M.). Routine examination of sentinel mice as part of the general surveillance program did not reveal the presence of a microorganism known to be pathogenic for mice. In addition, several immunodeficient mice were found to be free of specific pathogens by two veterinary diagnostic laboratories (at the Massachusetts Institute of Technology [MIT] and at the Charles River Breeder Laboratories). We conclude that IBD is unlikely to be caused by infection with a specific pathogenic microorganism. It remains possible that an unidentified opportunistic microorganism is the cause. We do not rule out the possible involvement of commensalic microorganisms in the disease (see Discussion).

Figure 1. IBD in αβ T Cell–Deficient Mice

(A) TCR α mutant mouse (10 months old) with terminal IBD. The mouse has a wasted appearance and a large anorectal prolapse.

(B) Gross photographs of dissected large intestine (to the right) and terminal ileum (to the left) of a 7-month-old TCR α mutant mouse (bottom) from a (129/Sv × C3H)F2 background and a 7-month-old immunocompetent (TCR δ heterozygous) mouse (top). Across the bottom is a ruler, shown in centimeters. The large intestine of the mutant mouse is dilated and shortened, and the wall is thickened.

(C) Transverse section of the rectum of a 12-month-old TCR α heterozygous mouse. Routine histology was performed: fixing with formalin, embedding in paraffin, and staining with hematoxylin and eosin. The wall is thin; the mucosa forms broad folds called haustrae. The mucin-containing goblet cells in the epithelium become translucent during this histological procedure. Objective, 1×.

(D) Transverse section of the rectum of a 12-month-old TCR α mutant mouse. The diameter of the rectum is much larger than the normal rectum in (C); the wall is thickened, and the folds have disappeared. Objective, 1×.

(E) Moderate disease. Objective, 10×.

(F) Severe disease. Same mouse as in (D). The crypts are elongated, the goblet cells are mostly gone, and there is an inflammatory cell infiltrate in the lamina propria. Objective, 10×.

(G) Crypt abscess. A collection of neutrophilic granulocytes can be seen inside the lumen of a crypt at the top left corner. Objective, 20×.

(H) Mixed inflammatory cell infiltrate in the lamina propria: neutrophilic granulocytes (multilobular nucleus), lymphocytes (round nucleus and little cytoplasm), and plasma cells (round, cartwheel-like nucleus and more cytoplasm than in lymphocytes). Objective, 20×.

(I) Immunoperoxidase staining with an antibody against TCR δ of the colon of a 6-month-old TCR α mutant mouse with IBD. Whereas wild-type large intestine or nondiseased large intestine of αβ T cell–deficient mice contain very few scattered γδ T cells (data not shown), there is a marked influx of γδ T cells in the large intestine of diseased mice. Objective, 40×.

(J) Immunoperoxidase staining of the colon of the same mouse as in (I), with an antibody against IgA. Objective, 40×.

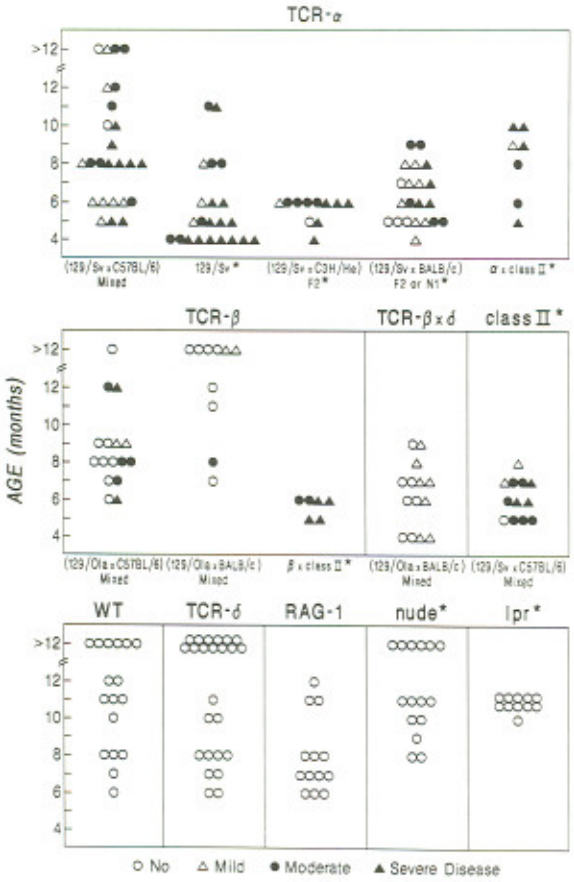

Figure 2. Presence and Severity of IBD in Various Strains of Mutant Mice

Severity of IBD is indicated with four symbols, as explained at the bottom of the figure. The age, rounded to the nearest month, is indicated on the vertical axis. The genetic background is indicated on the horizontal axis. For wild-type mice (WT), a variety of mice heterozygous or wild type for a TCR or *RAG-1* mutation were used; the genetic background includes any of the indicated backgrounds of TCR mutant mice. TCR δ mutant mice were either (129/Ola × BALB/c) or (129/Ola × C57BL/6). Nude mice were either C57BL/6 or BALB/c. *lpr* mice were C57BL/6. N1 means backcross one. Date of birth is between July 1991 and January 1993; date of analysis is between September 1992 and May 1993. Groups indicated with an asterisk represent types of mice, of which virtually all the mice available were used for the purpose of analyzing IBD, except for an occasional mouse who died spontaneously. Several mice with anorectal prolapse did not have any signs of IBD. Focally ulcerative lesions were observed in the rectum of a few *RAG-1* mutant mice; they are most likely due to anorectal prolapse, which has often been observed to be transient.

Presence or Absence of IBD in Various Mutant Mice

After an initial analysis of selected cases, we decided to increase the number of mice and to include other strains of mice bred and maintained in the same rooms. This study was performed retrospectively: most αβ T cell–deficient mice were selected on the basis of diarrhea, anorectal prolapse, poor general health, or all three, whereas for the other strains of mice, age of the mouse, poor general health, or both were criteria for selection. Overall, most of the aging mice that did not die spontaneously were included in the analysis; therefore, the bias of this retrospective study is limited.

Figure 2 lists the presence or absence of IBD in 224 mice, scored histopathologically. IBD was found not only in TCR α or TCR β mutant mice, but also in TCR β × δ double mutant mice, class II MHC mutant mice, TCR α × class II MHC double mutant mice, and TCR β × class II MHC double mutant mice. Equally important are the strains of mice in which no IBD was detected: wild-type mice, TCR δ mutant mice, *RAG-1* mutant mice, nude mice, and *lpr* mutant mice. A possible contribution of the genetic background is suggested by the variation in severity of IBD in a limited number of TCR α mutant mice of various backgrounds. The incidence and severity of IBD was high in inbred 129/Sv mice and in (129/Sv × C3H/He)F2 TCR α mutant mice, but lower in (129/Sv × BALB/c)F2 or N1 mutant mice. Overall, the disease was most severe in TCR α mutant mice and in class II MHC mutant mice, milder in TCR β mutant mice, and mildest in TCR β × δ double mutant mice.

Specificity of Inflammatory Syndrome

We also analyzed other organs of the 224 mice to investigate whether IBD was part of a multiorgan disease. Lung, liver, spleen, kidney, and small intestine were systematically examined by routine histology. In some mice with overt IBD, urinary bladder, uterus, stomach, esophagus, pancreas, and mesenteric lymph nodes were studied as well. Focal mixed inflammatory cell infiltrates were present in the liver of some immunodeficient mice, in particular TCR α mutant mice; these lesions may reflect nonspecific changes as a result of IBD. Many T cell–deficient mice, in particular *RAG-1* mutant mice, showed evidence of P. carinii infection in the lungs. No other pathology was identified. Particularly relevant is the absence of lesions of the small intestine. Given the known association of bowel disease with joint disease in humans, it will be interesting to determine whether the joints are also affected in the mutant mice.

Among the 224 mice, there were three tumors: a rectal adenocarcinoma in an 11-month-old TCR α mutant mouse, a benign adenomatous polyp in the colon of a 6-month-old TCR β × class II MHC double mutant mouse, and a $CD4^+CD8^+$ thymic lymphoma in a 7-month-old TCR β mutant mouse. Apart from this study group, moribund mice were frequently subjected to necropsy over the 2 year period, and no obvious tumors were detected by macroscopic inspection. Thus, the spontaneous tumor incidence in this population of several thousand immunodeficient mice was very low.

Clues to the Pathogenesis of IBD

Comparison of the lymphoid system of the strains of mice in which IBD was or was not identified could provide clues to the pathogenic mechanisms of the disease. Table 1 lists the deficiencies in lymphocyte subsets in the various mutant mice. The common denominator in mice with IBD is that they have B cells but are deficient in αβ T cells, in particular class II MHC-restricted $CD4^+$ αβ T cells. An important comparison is between TCR β × δ double mu-

Table 1. Lymphocyte Subsets in Various Mutant Mice

Strain	$CD4^+$ αβ T Cells	αβ T Cells	γδ T Cells	B Cells	IBD
Wild type	+	+	+	+	No
TCR δ	+	+	−	+	No
nude	(−)	(−)	(−)	+	No
lpr/lpr	+	+	+	+	No
RAG-1	−	−	−	−	No
TCR α	−[a]	−[a]	+	+	Yes
TCR β	−	−	+	+	Yes
TCR β × δ	−	−	−	+	Yes
Class II MHC	−[b]	+	+	+	Yes
TCR α × class II	−	−	+	+	Yes
TCR β × class II	−	−	+	+	Yes

Plus, present; minus, absent; minus in parentheses, very few.
[a] Except for a minor population of $CD4^+$ dull TCR β^+ cells (see Mombaerts et al., 1992a).
[b] Except for a small population of $CD4^+$ αβ T cells, whose development and function are unclear.

tant mice (IBD positive) and *RAG-1* mutant mice (IBD negative): the difference between these two types of mice is the presence of B cells in the former and their absence in the latter.

Discussion

UC-like Disease

The only pathology frequently observed in our αβ T cell–deficient mice is inflammation of the large intestine (cecum, colon, and rectum). The selective involvement of the large intestine and the histological features (loss of goblet cells, elongation of crypts, crypt microabscesses, and inflammatory cell infiltrate in the lamina propria) are reminiscent of the human disease UC. A noticeable difference is the absence of obvious ulcerations in the diseased intestine of the mice. Together with Crohn's disease, in which terminal small intestine and proximal large intestine are usually affected and whose histological characteristics are quite distinct, UC is described as an idiopathic IBD (Podolsky, 1991a, 1991b). The inflammation in TCR mutant mice is not part of a multiorgan inflammatory syndrome such as that described recently in transforming growth factor β1 mutant mice (Shull et al., 1992; Kulkarni et al., 1993). Screening by two veterinary diagnostic laboratories failed to reveal the presence of a microorganism known to be pathogenic for mice. Therefore, the TCR mutant mice could serve as small animal models for the study of UC in humans. The understanding of this disease, which affects about 1 in 1000 people in the Western world, has been hampered by the lack of a suitable animal model (Podolsky, 1991a, 1991b).

Development of UC-like disease in αβ T cell–deficient mice suggests, but obviously does not prove, involvement of the immune system in UC in humans. Evidence for an immunopathological, possibly autoimmune, origin of this disease has been weak to date and includes linkage to certain human leukocyte antigen alleles (Podolsky, 1991a) and loss of interleukin-2 (IL-2)-producing $CD4^+$ T cells in the diseased gut (Kusugami et al., 1991). The presence of IBD in class II MHC mutant mice and the absence of IBD in *RAG-1* mutant mice (Table 1) suggest that the disease in mice is caused by inappropriate control of B cells due to the lack of class II MHC–restricted $CD4^+$ αβ T cells. γδ T cells may contribute to the disease, as the disease appears to be more pronounced in TCR β mutant mice than in TCR β × δ double mutant mice. The absence of IBD in nude mice could be explained by the observation that many αβ T cells persist in the gut of these mice (for a review see Rocha et al., 1992). The notion that the lack of $CD4^+$ αβ T cells causes the disease is supported by the development of IBD in IL-2-deficient mice (Sadlack et al., 1993 [this issue of *Cell*]). Since IL-2 is primarily produced by $CD4^+$ αβ T cells, the disease may be caused by a reduction of the IL-2 level in the gut. However, an alternative cause not directly related to the intestinal IL-2 level but related to the lack of T helper cell 1 (Th1) function must also be considered.

It has been speculated that aberrant expression of class II MHC in epithelium of the large intestine could be a cause of IBD in humans (Podolsky, 1991a). Class II MHC molecules are expressed in colonic and rectal epithelia of diseased TCR mutant mice (data not shown). However, aberrant antigen presentation by epithelial cells is unlikely to be an important element in pathogenesis, as TCR α or TCR β × class II MHC double mutant mice, as well as class II MHC mutant mice by themselves, develop IBD. Therefore, class II expression is presumably a byproduct rather than a cause of the inflammation.

The implication of our hypothesis is that IBD could be the result of mucosal insult by autoantibodies and therefore could be regarded as an autoimmune disease. The autoantibodies would be directed most likely against a luminal antigen, derived from food, microorganisms, or both. Thus, loss of oral tolerance to dietary or microbial antigens as a result of a lack of αβ T cell–mediated suppression of B cells could lead to an autoimmune attack against the intestinal epithelium, possibly due to cross-reactive antibodies. This suppression could either be general or antigen specific. The selective involvement of the large bowel could be explained by its highly antigenic environment, caused by the high microbial load. This initial insult may lead to a vicious circle of tissue destruction and release of intracellular epithelial antigens, followed by more

mice homozygous for the *lpr* mutation were purchased from The Jackson Laboratory. Class II MHC (Aβ) mutant mice (Grusby et al., 1991) were bred at MIT.

The mice were housed in two rooms in the animal facility of the Center for Cancer Research at MIT. The microisolator cages were housed on metal racks. The cages were changed weekly, and the mice were handled either with forceps or with gloves, both dipped in a chlorhexidine solution between cages. Food and water were given ad libitum. The food (Autoclavable Rodent Breeder Chow #5013, Purina Mills, St. Louis, Missouri) was bulk autoclaved at 250°F for 20 min with a 10 min drying cycle. Distilled water was placed in bottles, and the baskets wrapped with autoclave paper were autoclaved on a liquid cycle at 250°F for 25 min. The bedding (laboratory grade Northeastern pine shavings, Northeastern Products Corporation, Warrensburg, New York) was bulk autoclaved at 250°F for 20 min with a 10 min drying cycle. Cages were sanitized in a cage washer at 180°F.

Health Status

Sentinel DBA/2J mice were analyzed every 2 months by the Division of Comparative Medicine at MIT. Evaluation included gross postmortem examination. Serology was negative for minute virus of mice, sendai-virus, murine encephalomyelitis virus, mouse hepatitis virus, ectromelia, pneumoniavirus of mice, reovirus type 3, lymphocytic choriomeningitis virus, epizootic diarrhea of infant mice virus (also called mouse rotavirus), and Mycoplasma pulmonis. At the onset of this project, sentinel mice were seropositive for epizootic diarrhea of infant mice virus, but they were seronegative for the remainder of the 2 year period (this virus causes lesions of the small intestine). No ecto- or endoparasites were detected. Rectal swabs were negative for Citrobacter freundii and Salmonella spp. Lung and nares cultures were positive for Pasteurella spp. Over the 2 year period, approximately 12 immunodeficient mice were analyzed at MIT. Intestinal cultures were negative for Campylobacter spp., C. freundii, Salmonella spp., and endoparasites (Cryptosporidium spp., Sephacia obvelata, and endoparasite nitrate flotation test). In some immunodeficient mice, direct smear of the colonic content revealed the presence of trichomonas, but these protozoa are not known to be pathogenic for mice.

In addition, one immunocompetent mouse from each room, two TCR α mutant mice from one room, and one TCR α mutant mouse from the other room (all three mice had severe IBD) and one *RAG-1* mutant mouse from each room were examined by the Diagnostic Lab of the Charles River Laboratories (Wilmington, Massachusetts). The serology of the two immunocompetent mice was negative for the same viruses as described above and, in addition, for K virus, polyomavirus, mouse adenovirus, mouse cytomegalovirus, hantaanvirus, Encephalitozoon cuniculati, and Carbacillus. Cecal cultures were negative for Pseudomonas spp. and Salmonella spp. Colon cultures were negative for C. freundii, biotype 4280. Nasal and lung cultures were negative for Bordetella bronchiseptica, Corynebacterium kutscheri, Klebsiella pneumoniae, Klebsiella oxytoca, M. pulmonis, Pasteurella multocida, Pasteurella pneumotropica, Pseudomonas spp., Staphylococcus aureus, Streptococcus pneumoniae, β-hemolytic Streptococcus, group B Streptococcus, and group G Streptococcus. No ecto- or endoparasites were detected, except in one immunodeficient mouse whose gut contained Hexamastix spp., which is not known to be pathogenic for mice.

Histological Analysis

Tissue samples were isolated by one of us (P. M.) in a standard fashion. For rectum samples, the last 1 cm of the large bowel was taken; for colon samples, a 1 cm section of the middle colon was isolated. The cecum was removed separately. For small intestine samples, the terminal 2 cm of the ileum was dissected. Samples of the left lung, of the right lobe of the liver, and of the left kidney were taken as well. Tissue samples were fixed in 10% buffered formalin, and paraffin-embedded tissue sections were stained with hematoxylin and eosin using standard techniques.

Immunohistological Analysis

Tissue samples were frozen in OCT compound (Ames, Elkhart, Indiana), frozen in liquid nitrogen, and stored at −70°C. Sections (4 μm thick) were air dried for 2 hr, fixed in acetone for 7 min, air dried again, and stained by the avidin–biotin complex method (Cerf-Bensussan et al., 1983). The sections were incubated with antibodies in optimal dilution, followed by incubation with biotinylated secondary antibodies and a 1:100 dilution of avidin-biotinylated peroxidase complex (Dako, Santa Barbara, California). Each incubation was followed by three washes in phosphate-buffered saline. Endogenous peroxidase activity was blocked by a 30 min incubation in 0.3% hydrogen peroxide in phosphate-buffered saline. Endogenous biotin was blocked by sequential incubations with avidin and biotin (Vector, Burlingame, California). The tissue sections were stained by incubation in a solution of 3-amino-9-ethylcarbazole (Aldrich, Milwaukee, Wisconsin), post-fixed in 2% paraformaldehyde, counterstained with hematoxylin, and mounted in Glycergel (Dako). Antibodies used were as follows: for CD3ε, purified YCD3.1 at 1:50 followed by rabbit anti-rat (Vector) at 1:100; for TCR δ, purified 3A10 (Itohara et al., 1989) at 1:5 followed by goat anti-hamster (Vector) at 1:10; for TCR β, biotinylated H57-597 (PharMingen, San Diego, California) at 1:5 followed by goat anti-hamster (Vector) at 1:20; for IgA, polyclonal antiserum (Boehringer Mannheim, Indianapolis, Indiana) at 1:4 followed by goat anti-rabbit (Vector) at 1:100. Secondary antibodies were biotinylated.

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