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Systemic Activation and Antigen-Driven Oligoclonal Expansion of T Cells in a Mouse Model of Colitis

Jennifer L. Matsuda,*† Laurent Gapin,† Beate C. Sydora,‡§ Fergus Byrne,§ Scott Binder,¶ Mitchell Kronenberg,2‡† and Richard Aranda§

Transfer of CD4+CD45RBhigh T cells into immunodeficient mice results in both the expansion of the transferred T cells and colitis. Here we show that colitis pathogenesis requires expression of MHC class II molecules by the immune-deficient host. Analysis of the TCRβ repertoire of the cells found in the large intestine of diseased mice revealed a population with restricted TCR diversity. Furthermore, nucleotide sequence analysis demonstrated the selection for particular CDR3b amino acid sequence motifs. Collectively, these data indicate that the expansion of T cells in the intestine and colitis pathogenesis are likely to require the activation of Ag-specific T cells, as opposed to nonspecific or superantigen-mediated events. There is relatively little overlap, however, when the TCR repertoires of different individuals are compared, suggesting that a number of Ags can contribute to T cell expansion and the generation of a T cell population in the intestine. Surprisingly, many of the expanded clones found in the large intestine also were found in the spleen and elsewhere, although inflammation is localized to the colon. Additionally, donor-derived T cells appear to be activated in both the intestine and the spleen at early time points after cell transfer. Together, these results strongly suggest that disease induction in this model involves either the early and systemic activation of antigen-specific T cells or the rapid dispersal of T cells activated at a particular site. The Journal of Immunology, 2000, 164: 2797–2806.

Inflammatory bowel diseases (IBD) are complex immune-mediated diseases characterized by chronic inflammation of the intestine (1). Although the etiology of IBD remains elusive, numerous studies indicate an important role for T cells in its pathogenesis. Not only are high numbers of activated T cells present in the diseased intestine (2–5), but the levels of proinflammatory cytokines are often elevated (6–8), particularly in subjects with Crohn’s disease. Although the involvement of T cells in IBD is clear, it is not known whether particular Ags from bacteria or other microorganisms play a role in disease induction. Examination of the diversity of activated colitogenic T cells may help reveal whether a specific Ag(s) or superantigen(s) shapes the TCR repertoire during disease, as well as what role these Ags might play in the disease onset and progression. Although the T cell response to a single Ag in some cases may be characterized by the expansion of a particular T cell clone(s) (9, 10), the response to a superantigen would result in the expansion of T cell clones that use the same TCR Vβ gene(s) but with diverse CDR3b sequences (11, 12). Unfortunately, investigation of the TCR repertoire in the site of disease of IBD patients is complicated by the differences in disease manifestation from one individual to another, the numerous genetic and other variables of the human population, and the potential presence of long-lived mucosal T cells before disease onset.

Currently, several mouse models facilitate the study of IBD. One of these models involves the adoptive transfer of purified CD4+CD45RBhigh T cells from the spleen or lymph node into syngeneic or MHC-matched immune-deficient recipients. After homing to the intestine, the donor-derived cells express phenotypic markers characteristic of normal intestinal lymphocytes (13), but within several weeks of the transfer, the recipients show chronic inflammation of the intestine, with greater severity in the colon. The CD4+CD45RBhigh T cells have been shown to preferentially produce proinflammatory cytokines, such as IFN-γ and TNF-α, following transfer, and those cytokines are important for pathogenesis (6). Studies in this model and others have shown that induction of colitis requires the presence of enteric bacteria (13–15). Furthermore, in a related model, T cells from mice prone to spontaneous colitis induce disease following in vitro activation by bacterial Ags and transfer into immune-deficient mice (16). Taken together, these data suggest that cells activated by microbial Ags may play a role in colitis induced by T cell transfer into SCID mice.

Analysis of the TCR repertoire of the immunodeficient recipients in the CD4+CD45RBhigh T cell transfer model provides an opportunity to characterize the T cell population present in the intestine of diseased individuals in a manner not possible in clinical studies on human patients. The advantages of this model include 1) the predictability of disease onset, 2) the use of inbred donors and recipients, and 3) a lack of resident mucosal lymphocytes in the recipients, so that all lymphocytes found in the intestine must have been recently derived from the donor population. Therefore, for this study we have used a variety of techniques, including the use of
of sterile PBS. One aliquot of sorted donor lymphocytes (4 × 10^5) was harvested from stock originally obtained from the University of California-Los Angeles SCID mouse core facility, or purchased from The Jackson Laboratory and maintained at the La Jolla Institute for Allergy and Immunology vivarium. Recipients received adoptive transfers between 7 and 12 wk of age. Donors and recipients were always of the same sex. All mice were housed under specific pathogen-free conditions. MHC class II^−/− mice and Rag2^−/− mice were purchased from The Jackson Laboratory and were intercrossed and maintained for the duration of the study at the vivarium at the University of California-Los Angeles. Small and large intestines were analyzed. Intestines were minced, transferred to 250-ml Erlenmeyer flasks, and shaken three times at 200 rpm for 30 min each time. Intestines were then cut into 0.5-cm pieces, transferred into 250-ml Erlenmeyer flasks, and shaken three times at 200 rpm for 30 min each time. The area under each peak was proportional to the quantity of TCR transcripts. Amplified PCR products were diluted 1/100 with H_2O, and 1 μl was electrophoresed in a 12% acrylamide gel stained with ethidium bromide to monitor the quality and quantity of the reaction products. Each V_b-chain of interest and an antisense oligonucleotide for C_b have been described previously (17). Forty cycles of PCR were conducted in a 9600 Perkin-Elmer Automatic, with each cycle consisting of 94°C for 45 s, 60°C for 45 s, and 72°C for 45 s. Each PCR product was then used as a template for extension, or run-off, reactions with oligonucleotides labeled with fluorescent tag. Fluorescent primers used in this study include an internal C_b primer and primers specific for each of the 12 J_b clones (17). The fluorescent run-off products generated varied in size depending on CDR3 length. Run-off products were subjected to capillary electrophoresis in an automated DNA sequencer (Applied Biosystems, Foster City, CA), and CDR3 size distribution and signal intensities were then analyzed with GeneScan software (Perkin-Elmer). The patterns observed contained up to eight size peaks, each spaced by three nucleotides, corresponding to in-frame transcripts. The area under each peak was proportional to the quantity of TCR transcripts of the corresponding CDR3 length in the sample.

**Analysis of intestine tissue sections**

Tissue samples of ~5 mm were taken from the intestine and fixed in 10% formalin. Fixed tissue was embedded in paraffin; 3-μm sections were prepared and then stained with hematoxylin-eosin. Samples were coded and scored by a pathologist blinded to the conditions under which the experiment was conducted. A previously described scoring system was used for the intestine sections (13). This system incorporates five parameters grading the degree of inflammatory infiltrate in the lamina propria, mucin depletion, epithelial hyperplasia/atypia, number of IEL in epithelial crypts, and number of inflammatory foci per 10 high powered fields. The maximum score is 14; higher scores indicate greater pathology.

**Flow cytometric analysis of lymphocytes**

IEL and LPL were resuspended in PBS staining buffer containing 2% BSA and 0.02% NaN_3. After preincubulation for 15 min at 4°C with the blocking 2.4G2 anti-FcγRII mAb, the cells were stained at 4°C for 30 min with labeled mAb. Samples were then washed three times in PBS staining buffer. Tricolor-conjugated streptavidin was added as secondary staining reagent for the biotinylated mAb followed by two washes in staining buffer. The samples were immediately analyzed at this point, or they were fixed in PBS containing 1% paraformaldehyde and 0.02% NaN_3 and stored at 4°C. mAbs used in this study include CyChrome- or FITC-labeled anti-TCRβ clone H57-597, FITC- or PE-labeled anti-K_b clone AF6-88.5, FITC- or PE-labeled anti-CD44 clone IM7, and PE-labeled anti-CD3 clone H1.2F3. FITC-labeled or biotinylated anti-CD4 clone RM4-4, PE-labeled anti-CD45RB clone 16A (PharMingen), and FITC-labeled anti-CD62L clone MEL-14 (Caltag, Burlingame, CA). Flow cytometric analysis was performed on a Becton Dickinson FACScan 440 flow cytometer at the La Jolla Institute for Allergy and Immunology.

**mRNA extraction and cDNA synthesis**

mRNA was prepared using the QuickPrep micro mRNA purification kit (Pharmacia). The full quantity of mRNA obtained was used for single-strand cDNA synthesis. The mRNA was denatured for 10 min at 70°C, then incubated with (dT)_{15} (5 mM), dNTPs (1 mM each), RNasin (40 U; Promega, Madison, WI), and AMV reverse transcriptase (2 U; Roche, Mannheim, Germany) in the supplier’s buffer at 43°C for 1 h, followed by incubation at 55°C for 10 min.

**PCR amplifications, primer extensions, and data analysis**

PCR amplification was conducted in 50 μl using 1/30 to 1/40 of the cDNA with 2 U of Taq polymerase (Perkin-Elmer, Foster City, CA) in the supplier’s buffer. Sense oligonucleotide primers specific for each of the 23 V_b genes, and antisense oligonucleotides for C_b have been described previously (17). The fluorescent run-off products generated varied in size depending on CDR3 length. Run-off products were subjected to capillary electrophoresis in an automated DNA sequencer (Applied Biosystems, Foster City, CA), and CDR3 size distribution and signal intensities were then analyzed with GeneScan software (Perkin-Elmer). The patterns observed contained up to eight size peaks, each spaced by three nucleotides, corresponding to in-frame transcripts. The area under each peak was proportional to the quantity of TCR transcripts of the corresponding CDR3 length in the sample.

**Cloning and sequencing of select V_b-J_b rearrangements**

Amplified PCR products were diluted 1/100 with H_2O, and 1 μl used as a template for amplification of selected V_b-J_b rearrangements. PCR was performed with the reagents and quantities described above, using a sense oligonucleotide specific for the V_b-chain of interest and an antisense oligonucleotide specific for the J_b-chain of interest. Twenty-five cycles, each at 94°C for 45 s, 60°C for 45 s, and 72°C for 45 s were completed in a 9600 Perkin-Elmer Automatic. PCR products were analyzed on a 2% agarose gel stained with ethidium bromide to monitor the quality and quantity of the reaction products. Each V_b-J_b amplified product was then shotgun cloned with the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Resulting colonies were randomly selected, and cultures were grown. Plasmid DNA was isolated from bacterial cultures using Wizard Plus Miniprep kits (Promega). Sequencing reactions were performed with ABI Prism dRhodamine Terminator Cycle Sequencing Ready reaction kit (Perkin-Elmer) and analyzed on an automated sequencer.

**Materials and Methods**

**Mouse**

Donor (C57BL/6 × BALBcF1 [CB6F1] mouse, purchased from Monkson (Gilroy, CA) and The Jackson Laboratory (Bar Harbor, ME) and maintained either at the University of California-Los Angeles vivarium or the La Jolla Institute for Allergy and Immunology vivarium. Recipients received adoptive transfers between 7 and 12 wk of age. Donors and recipients were always of the same sex. All mice were housed under specific pathogen-free conditions. MHC class II^−/− mice and Rag2^−/− mice were purchased from The Jackson Laboratory and were intercrossed and maintained for the duration of the study at the vivarium at the University of California-Los Angeles. Small and large intestines were analyzed. Intestines were minced, transferred to 250-ml Erlenmeyer flasks, and shaken three times at 200 rpm for 30 min each time. Intestines were then cut into 0.5-cm pieces, transferred into 250-ml Erlenmeyer flasks, and shaken three times at 200 rpm for 30 min each time. The area under each peak was proportional to the quantity of TCR transcripts. Amplified PCR products were diluted 1/100 with H_2O, and 1 μl was electrophoresed in a 12% acrylamide gel stained with ethidium bromide to monitor the quality and quantity of the reaction products. Each V_b-chain of interest and an antisense oligonucleotide for C_b have been described previously (17). Forty cycles of PCR were conducted in a 9600 Perkin-Elmer Automatic, with each cycle consisting of 94°C for 45 s, 60°C for 45 s, and 72°C for 45 s. Each PCR product was then used as a template for extension, or run-off, reactions with oligonucleotides labeled with fluorescent tag. Fluorescent primers used in this study include an internal C_b primer and primers specific for each of the 12 J_b clones (17). The fluorescent run-off products generated varied in size depending on CDR3 length. Run-off products were subjected to capillary electrophoresis in an automated DNA sequencer (Applied Biosystems, Foster City, CA), and CDR3 size distribution and signal intensities were then analyzed with GeneScan software (Perkin-Elmer). The patterns observed contained up to eight size peaks, each spaced by three nucleotides, corresponding to in-frame transcripts. The area under each peak was proportional to the quantity of TCR transcripts of the corresponding CDR3 length in the sample.

**Isolation of intraepithelial and lamina propria lymphocytes**

Mucosal lymphocytes were isolated according to a previously published method (13). Briefly, small and large intestines were removed and carefully cleaned from their mesentery, opened longitudinally, and flushed of fecal content. Intestines were then cut into 0.5-cm pieces, transferred into 250-ml Erlenmeyer flasks, and shaken three times at 200 rpm for 30 min each time at 37°C in HBSS without Ca^{2+} or Mg^{2+} and containing 1 mM DTT (Sigma, St. Louis, MO). The cell suspensions were passed through a 60-μm nylon mesh and pelleted by centrifugation at 1200 rpm. The pellets were resuspended in 20% Percoll (Pharmacia, Piscataway, NJ), layered over a discontinuous 40/70% Percoll gradient, and centrifuged at 900 × g for 25 min. The cells from the 40/70% interface were collected, washed, and resuspended in complete RPMI 1640 medium supplemented with 5% FBS. To isolate lamina propria lymphocytes (LPL), the remaining intestinal tissue was minced, transferred to 250-ml Erlenmeyer flasks, and shaken for 60 min at 37°C in complete RPMI supplemented with 5% FBS containing dispase at 1.5 mg/ml (Sigma). The cell suspensions were collected and passed through nylon mesh, and cells were pelleted by centrifugation at 1200 rpm. The cells were passed over a Percoll gradient and processed as described above for the preparation of intraepithelial lymphocytes (IEL). The cell suspensions were pelleted for a final time for both IEL and LPL, were snap-frozen in liquid nitrogen, and were stored at −70°C until mRNA extraction was performed. None of the fragment length analysis data shown was derived from analysis of a cell population smaller than 1.5 × 10^6 cells.
Consistent with previous studies, RAG⁻/⁻ recipients of CD4⁺CD45RBhigh T cells developed significant weight loss by 6 wk (Fig. 1). Between 4 and 5 wk, the RAG⁻/⁻ recipients showed overt signs of disease, including a hunched over appearance, piloerection of their coat, and loose stool. We reasoned that if pathogenesis depended on Ag-reactive T cells, elimination of the major Ag-presenting molecules for CD4⁺ T cells, MHC class II, would abrogate disease. To investigate this possibility, we generated class II−/− RAG⁻/⁻ mice and transferred CD4⁺CD45RBhigh T cells into these animals in parallel with identical transfers into class II−/− RAG⁻/− recipients. CD4⁺CD45RBlow T cells from the same donor pool, which are known not to induce colitis (18), were concurrently transferred into both class II−/− RAG⁻/− mice and class II−/− RAG⁻/− mice as controls. By 8 wk all RAG⁻/− recipients of CD4⁺CD45RBhigh T cells developed colitis and were sacrificed for analysis. By contrast, class II−/− RAG⁻/− recipients of CD4⁺CD45RBhigh T cells remained healthy and continued to gain weight, even at 18 wk posttransfer, as did both sets of recipients of the CD4⁺CD45RBlow T cell population. Consistent with the lack of weight loss, there was no evidence for inflammation of the intestinal tissue following the transfer of CD4⁺CD45RBhigh T cells to MHC class II−/− immune-deficient mice. The average histologic score for the tissue sections of the class II−/− RAG⁻/− recipients was 4.7 ± 0.6, similar to the 4.0 score obtained when disease-free, class II⁺ recipients of CD4⁺CD45RBlow T cells were examined. This is in contrast to an average score of 12.2 for class II⁺ recipients of CD4⁺CD45RBhigh T cells (13).

The TCR repertoire is oligoclonal in the large intestine of diseased mice

We analyzed the TCR repertoire in the large intestine IEL (LIEL) of seven diseased SCID recipients that had received

![FIGURE 1.](image1.png)

**FIGURE 1.** CD4⁺CD45RBhigh T cells do not cause weight loss when transferred into class II−/− RAG⁻/− mice. Donor splenocytes isolated from CB6F1 mice were prepared, stained, and sorted as described in Materials and Methods. Sorted cells (4 × 10⁶) were injected i.p. Recipient mice were weighed on the day of cell transfer and weekly thereafter. The data plotted represent the percentage of original weight as a function of weeks following transfer. The data are the mean ± SD based on the following numbers of mice per group: class II−/− RAG⁻/− recipients of CD4⁺CD45RBhigh T cells, n = 3; RAG⁻/− recipients of CD4⁺CD45RBhigh T cells, n = 2; class II−/− RAG⁻/− recipients of CD4⁺CD45RBlow T cells, n = 2; and RAG⁻/− recipients of CD4⁺CD45RBlow T cells, n = 2. Statistical analysis was performed using Student’s two-tailed t tests to compare the class II−/− RAG⁻/− recipients and the RAG⁻/− recipients of CD4⁺CD45RBhigh T cells at 6 wk. *, p = 0.0027.

![FIGURE 2.](image2.png)

**FIGURE 2.** Analysis of the TCR repertoire in LIEL of an SCID recipient with colitis shows the presence of an oligoclonal population. A. Depicted are the CDR3 profiles from the donor-derived CD4⁺CD45RBhigh T cells pooled from six mice before injection, and the profiles from mouse B for selected Vβ-Cβ PCR amplifications with the indicated Vβ primers. The intensity of fluorescence is represented in arbitrary units as a function of CDR3 length in amino acids, and a CDR3 length of 8 aa is indicated by the dashed vertical line. B, Jβ run-off analysis of the Vβ15 repertoire of the LIEL from mouse B. The Vβ15-Cβ profile, which appears Gaussian, was further analyzed by performing extension reactions using 12 different fluorescently labeled Jβ primers.
peaks, each spaced by three nucleotides, corresponding to in-frame transcripts (Fig. 2A). More detailed analysis of the Vβ repertoire can be achieved by analyzing the CDR3 length distribution with Jβ gene usage taken into consideration. This is achieved by performing 12 separate primer extension reactions on the initial Vβ-Cβ PCR product, with each reaction using a fluorescein-labeled Jβ oligonucleotide specific for a different Jβ gene (17). Analysis of the donor population in this manner further confirmed its polyclonality, as the CDR3 size distribution for each Jβ primer extension reaction was also Gaussian (data not shown).

By contrast, when LIEL of diseased SCID recipients were similarly studied by Vβ-Cβ PCR reactions, clonal TCRβ expansions were commonly observed. Fig. 2A shows representative CDR3 profiles for five Vβs from LIEL of a single SCID recipient (mouse B). There is evidence for predominant single or double peaks for Vβ3, Vβ11, and Vβ18, while Vβ15 shows a Gaussian profile. The CDR3 size distributions for this and one other Gaussian Vβ profile were analyzed for each of the 12 Vβ-Jβ rearrangements to determine whether the populations in question were truly polyclonal. In both cases analyzed, the profiles generated using the fluorescently labeled Jβ primers were not Gaussian. An example of the results of the Jβ extension reactions is shown in Fig. 2B for the Vβ15 repertoire. The normal preferential bias in the recombination machinery to use Jβ2 family members more often than Jβ1 members (23) was not found in this sample. For this Vβ15 repertoire, Jβ1 rearrangements are favored over Jβ2, and Jβ1.5 and Jβ2.4 rearrangements were not detected. Numerous primer extension reactions with different IEL populations demonstrated such preferential reduced Jβ usage, although the Jβ genes that were not detected varied from one instance to the next. Furthermore, the generation of predominant CDR3 lengths other than the typical 8-aa mean by different Vβ-Jβ rearrangements also argued against weak signal strength producing the observed result. Therefore, all data obtained are consistent with an oligoclonal TCR repertoire in the IEL of diseased SCID recipients.

Recipients of the same CD4+CD45RBhigh cells do not share common, expanded T cell clones

Two different donor pools of CD4+CD45RBhigh T cells were used. Each pool was formed by combining spleen cells from six donor mice. Mice A, B, C, and D received cells from one donor pool, and mice E, F, and G received T cells from a second pool generated separately. Fig. 3A shows the complete set of CDR3 profiles for Vβ-Cβ amplifications from mouse E. As noted above, perturbations in the CDR3 profile were commonly found in recipient mice and were categorized as either expansions or pseudo-Gaussian profiles. We define expansions as those profiles with no more than two predominant peaks. If there is a single predominant peak, to be classified as an expansion it must comprise at least 50% of the area under the profile curve, and if there are two predominant peaks,
they must contain at least 70% of the area. We classified profiles as pseudo-Gaussian distributions if they were characterized by a perturbation in the CDR3β profile, i.e., one or more peaks that demonstrate either more or less representation of a given CDR3 than would be expected from a typical Gaussian profile. These classifications were used in the comparison of the Vβ repertoire of the LIEL of seven recipient SCID mice, which are compiled in Fig. 3B. The data indicate that Gaussian profiles were obtained in only a minority of the cases in each recipient. The number of true Gaussian profiles, indicative of polyclonality, is likely to be much less because, as noted above, distributions that appear Gaussian by Vβ-Cβ amplification tend not to be Gaussian following Vβ-Jβ amplification.

Expanded peaks of a given CDR3 length that are shared between recipient mice could be indicative of common or public T cell responses in this model of IBD. The fragment length analysis, however, revealed only eight instances in which the predominant rearrangement observed following Vβ-Cβ PCR had a similar length in at least three of the seven recipient SCID mice. These cases are summarized in Fig. 4, and they involve Vβ1, Vβ4, Vβ9, Vβ11, Vβ14, Vβ16, and two predominant rearrangements for Vβ2, one that is 7 and one that is 8 aa in length. Interestingly, with the exception of the Vβ4 and Vβ11 rearrangements, these common lengths were found both in some mice that received donor cells from pool 1 (mice A–D) and some recipients from pool 2 (mice E–G; see Fig. 4).

To determine whether there were, in fact, public clones corresponding to these predominant fragment lengths, Jβ primer extension reactions were conducted on the relevant Vβ-Cβ amplification products. The data indicate (Fig. 4) that there is some sharing of the predominant Jβ used for particular Vβ genes when different mice are compared, but this was not true in all the mice that had a predominant fragment of the same size following Vβ-Cβ amplification. Only three Vβ-Jβ combinations were present in at least three recipient mice. These include, with the CDR3 lengths shown in parentheses, Vβ2-Jβ1.4 (7 aa), Vβ2-Jβ2.1 (8 aa), and Vβ11-Jβ2.1 (9 aa). Both rearrangements involving Vβ2 were selected for further study by nucleic acid sequence analysis on DNA amplified with specific Vβ and Jβ primers and cloned without size selection. The Vβ14-Jβ2.5 (9-aa) rearrangement and the Vβ9-Jβ2.7 (9-aa) rearrangement, each present in only two recipient SCID mice, were also analyzed in this way. Surprisingly, analysis of >50 sequences revealed only one shared sequence between any two recipient mice, involving a Vβ2-Jβ2.1 rearrangement. In mouse F, five of nine of the Vβ2-Jβ2.1 rearrangements sequenced had a CDR3 of 8 aa that coded for the protein sequence TGGNYAEQ. This identical nucleic acid and protein sequence was also found in mouse B, in one of a total of 5 Vβ2-Jβ2.1 sequences that were performed. All five sequences in mouse B had CDR3 lengths of 8 aa. However, a Vβ2-Jβ2.1 sequence found in 3 of 5 cases in mouse B was not identified in the nine sequences in mouse F. Furthermore, in mouse A, none of the 10 Vβ2-Jβ2.1 sequences studied matched those sequences identified in either mouse B or mouse F.

In summary, the analysis of both the Vβ-Cβ and Vβ-Jβ extension reactions provided only a limited number of cases of clones potentially shared between recipients, and the nucleic acid sequence data corroborated these findings and indicated that the majority of expanded Vβ-Jβ rearrangements with the same CDR3 length in different recipients are not identical. Therefore, while we cannot formally exclude the possibility that there are a significant number of clones common to different SCID recipients, the data

![Table 1](https://example.com/table1.png)

### FIGURE 4.
Comparison of Jβ usage in different mice reveals few potentially shared clonal expansions. Jβ extension reactions were performed in different mice to determine the Jβ gene(s) used to generate the expansion of the indicated Vβ rearrangements. These predominant Jβ genes are indicated by filled boxes. White numbers inside the filled boxes indicate the number of sequences identified for that particular Vβ-Jβ rearrangement.

### FIGURE 5.
Similar CDR3β length profiles are found in different locations in recipient SCID mice. Representative profiles for small and large intestine LPL and IEL and from the spleen of three recipient mice are shown. Sequencing results for each of these profiles are shown in Table I. The intensity of fluorescence is represented in arbitrary units as a function of CDR3 length in amino acids.
indicate that the highly expanded clones in one recipient are not likely to be highly expanded in other recipients.

**Lymphocytes in different sites of an individual mouse share similar TCR repertoires**

We analyzed the TCR repertoire of donor-derived cells found in different sites to determine whether there is a unique TCR repertoire in the large intestine, which is the primary site of inflammation. CDR3 profiles from mice A, B, D, and G were generated from IEL and LPL in the small and large intestines and from T cells in the spleen. In total, 28 analyses permitted comparison of the large and small intestinal compartments with the spleen, and an additional 31 analyses allowed comparison between at least two intestinal compartments, with both the LIEL and small intestine IEL (SIEL) represented in each case. Representative data illustrating the CDR3 length profiles for three V\(\beta\) genes are shown in Fig. 5. Comparison of the TCR\(\beta\) repertoire of the lymphocytes found in the large intestine with those of other intestinal compartments and the spleen reveal some similarities in the CDR3 distribution patterns. For V\(\beta\)18, a dramatic expansion of a TCR clone with a CDR3 length of 6 aa in LIEL is likewise present in large intestine lamina propria (LLPL), SIEL, small intestine lamina propria (SLPL), and spleen. Occasionally observed, however, is a less striking TCR clonal expansion in LIEL, as shown in Fig. 5 for V\(\beta\)4 and V\(\beta\)8.3. In the majority of the examples in which we could compare the large and small intestinal compartments, the CDR3 profiles were virtually identical. Exceptions to this tended to exhibit greater CDR3 length diversity in the large intestine than the small intestine.

To determine whether the similarities observed by fragment length analysis actually correlated to shared clonal expansions, we performed J\(\beta\) primer extension reactions to determine the primary rearrangement that contributed to the clonal expansion. Additionally, nucleic acid sequence analyses were conducted on V\(\beta\)-J\(\beta\) PCR products from different parts of the intestine and spleen of the same recipient mice. Table I shows a compilation of

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<td>2.1</td>
<td>2/3</td>
<td>1/5</td>
<td>2/2</td>
<td>2/5</td>
</tr>
</tbody>
</table>

*Translated amino acid sequences of expanded clones are shown. Amplifications were done using the indicated V\(\beta\) and J\(\beta\) primers from the recipient mice indicated on the left. Primer pairs were chosen based upon fragment length analysis showing the presence of a predominant V\(\beta\)-J\(\beta\) CDR3 length in one or more regions of the intestine. However, the V\(\beta\)-J\(\beta\) amplification products were shotgun cloned without any selection for rearrangements of the predominant size. The predominant or expanded sequence is indicated, and the representation or fraction of the total sequences from various sites that have the predominant sequence are indicated.

*ND, not done.

**FIGURE 6.** Patterns of sharing of clonal sequences in the various organs of an SCID recipient. On the left is shown the CDR3 fragment length profile for rearranged V\(\beta\)15-J\(\beta\)1.1 sequences obtained from five sites in recipient SCID mouse B. Translated amino acid CDR3 sequences were obtained from the sequencing of random clones generated without size selection of the V\(\beta\)15-J\(\beta\)1.1 amplification products. Sequences obtained for each compartment are listed within the rectangles in order of increasing CDR3 length, with the CDR3 length shown on the left. All sequences with the predominant CDR3 length of 10 aa are underlined, and N-D-N amino acids are shown in bold type. The number of times a given sequence was detected is indicated by a number in parentheses following the sequence; the absence of a number indicates a sequence detected once. Sequences are arranged in columns according to their distribution: unique to one site, shared only between the spleen and either LIEL or LLPL, or only observed in two or more intestinal compartments.
these CDR3β protein sequences and the frequency with which they were detected in various compartments, and Fig. 6 presents a detailed analysis of Vβ15 rearrangements from a single mouse. Strong clonal expansions, as detected by a predominant fragment length in different locations of the same recipient, in fact gave identical sequences. For example, the 6-aa-long Vβ18-Jβ1.1 rearrangement that was predominant in all compartments tested from mouse B (middle row, Fig. 5) corresponded to only one nucleotide sequence (13 of 13) in all five compartments (Table I). Based upon these data, we conclude that a single clone was predominant among the Vβ18 rearrangements found throughout this mouse. Similar examples of such widely distributed and prevalent clones include the Vβ8.1 and Vβ8.3 rearrangements from mouse A and the Vβ4 rearrangements from mouse D (Table I).

The fragment length distribution for Vβ15-Jβ1.1 rearrangements in recipient mouse B was analyzed in detail; it provides an example of a more complex pattern of clonal expansion and fragment length sharing in different sites than was exhibited for several other rearrangements (Table I). There is a clear clonal expansion of a 10-aa-long CDR3 in SIEL and SLPL (Fig. 6, left). While a 10-aa CDR3 length is readily detected in the spleen, LIEL, and LLPL, it is less predominant due to the presence of two or three other CDR3 size peaks (Fig. 6). Using run-off reactions with Jβ-specific primers, the rearrangement of Vβ15 to Jβ1.1 was determined to be the major rearrangement responsible for the 10-aa CDR3 TCRβ clonal expansion (data not shown). DNA sequences obtained from 37 clones resulting from amplification with Vβ15- and Jβ1.1-specific primers, but not selected for the 10-aa size, also are shown in Fig. 6. The sequence data are arranged in rows according to increasing CDR3 length and also in columns according to the distribution of the different sequences in this SCID recipient. Fourteen different sequences were obtained, and three patterns for the distribution of the sequences emerged, including sequences that were found to be shared between the spleen and intestine (middle column, three different sequences in 12 total clones), sequences found in various parts of the intestine only but not in the spleen (right column, three examples), or sequences unique to either the spleen (left column, four total) or unique to one part of the intestine (left column, four total). In this case, sequences shared between the large intestine and spleen were not found in the small intestine, but this pattern was not typical (Table I).

The expanded clone with a 10-aa CDR3 had the sequence RETGGPNTEV. Not surprisingly, it was abundant in the small intestine (4 of 6 sequences), but while it was not found in the spleen (0 of 16 sequences) it also could be found in the large intestine (3 of 15). Two other Vβ15 CDR3 sequences that were detected only in the intestinal compartments corresponded to the 7- and 8-aa CDR3β lengths predicted by fragment length analysis. Other sequences, listed in the middle column of Fig. 6, were shared only between the spleen and the LIEL or LLPL. It should be noted that 10-aa-long CDR3 regions with protein sequences different from those of the predominant clone in the small intestine were found, including one sequence found in spleen, LIEL, and LLPL and one found in spleen only. These data demonstrate that clonal identity, even in different locations in an individual, cannot be strictly inferred from shared CDR3 lengths. Additional data in Table I show the sequence and prevalence of expanded clones in different sites. The degree of diversity may be somewhat higher overall in the LIEL and spleen, but the data illustrate the dissemination of prevalent clones throughout both the large and small intestines and the spleen.

### Table II. Protein sequence similarities in the N-D-N regions of the CDR3 suggest an Ag-driven process of clonal selection and expansion

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Vβ</th>
<th>CDR3</th>
<th>Jβ</th>
<th>Detection Frequency</th>
</tr>
</thead>
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<tr>
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<td>CAS</td>
<td>SRTANSDY</td>
<td>TFG b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAS</td>
<td>SRAANSDY</td>
<td>TFG</td>
</tr>
<tr>
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<td></td>
<td>CAS</td>
<td>HDDWVSYEQ</td>
<td>YFG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAS</td>
<td>SDWVSYEQ</td>
<td>YFG</td>
</tr>
<tr>
<td>A</td>
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<td>RDGSN</td>
<td>LYF b</td>
</tr>
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<td>RDDSNGT</td>
<td>LYF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CGA</td>
<td>RASSNGT</td>
<td>LYF</td>
</tr>
<tr>
<td>B</td>
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<td>CGA</td>
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<td>FFG</td>
</tr>
<tr>
<td></td>
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<td>WRDRGNTEV</td>
<td>FFG</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>CGA</td>
<td>RDQGNANTEV</td>
<td>FFG</td>
</tr>
</tbody>
</table>

* Analysis of all sequences generated in this study allowed for the identification of sequences with similar N-D-N regions which are compiled in this table. N-D-N regions are shown in bold type. Detection frequencies are determined by the number of sequences with the listed CDR3 sequence out of the total number of sequences generated for that particular Vβ-Jβ combination in the mouse indicated, irrespective of the site from which the sequence was obtained. For Vβ15 in mouse B, all sequences identified and their distribution in the various sites can be found in Fig. 6.

* Reiterative sequences that also appear in Table I.
CD4+CD45RB$^{\text{high}}$ T cells appear activated in both spleen and intestine early after transfer

The data obtained from both fragment length measurements and nucleotide sequence analysis indicate that there is a significant degree of sharing of the TCR repertoire of expanded clones between various compartments of the intestine and the spleen of a diseased recipient. This suggests that activated or memory T cells should be found in both the spleen and the intestine. We therefore used flow cytometry to phenotype donor-derived T cells in SCID recipients at relatively early time points following cell transfer. Suspensions of spleen cells and IEL were analyzed 7 or 11 days following transfer of CD4+CD45RB$^{\text{high}}$ T cells from CB6F1 mice (H-2b $\times$ H-2k) into C.B-17 (H-2b) SCID mice. This F1 into immune-deficient parental strain transfer system permits the marking of donor-derived (H-2b) T cells that can be distinguished from the leaky production of T cells by the SCID host. In this transfer there is neither a graft-vs-host nor a host-vs-graft response. Colitis generally does not become severe until around 40 days after transfer. Donor-derived T cells (K$^b$ TCR$^b$) were detected by multicolor flow cytometry, and expression of the activation markers CD69, CD45RB high -induced colitis is characterized by the presence of CD4+CD45RB$^{\text{high}}$ T cells. Donor T cells in the recipients were gated on by selecting K$^b$ TCR$^b$, and this population was analyzed for expression of the indicated markers. For comparison, the CD4+CD45RB$^{\text{high}}$ cells from the two naive CB6F1 spleen were gated on. The percentage of CD4+CD45RB$^{\text{high}}$ cells staining positive for the indicated activation markers from the two naive CB6F1 spleen were as follows: CD62L$^+$ cells were 84.6 and 87.6%, CD69$^+$ cells were 11.1 and 10.2%, and CD44$^+$ cells were 8.5 and 10.1%. Representative data are shown from one of three mice analyzed. For the LIEL, the percentage of donor cells that were CD62L$^+$ ranged from 0.4 to 4.3%, that of CD69$^+$ cells ranged from 47.9 to 69.4%, and that of CD44$^+$ cells ranged from 88.4 to 99.1% of the K$^b$ TCR$^b$ cells. In the spleens of SCID recipients, the percentage of donor cells that were CD62L$^+$ ranged between 4.4 and 56.8%, CD69$^+$ cells comprised 34.8–56.3% of the gated population, and CD44$^+$ cells comprised 94.3–99.0%.

**Discussion**

We made use of the CD4+CD45RB$^{\text{high}}$ T cell adoptive transfer model to analyze the possible role of Ag stimulation in the mucosal localization of T lymphocytes. We considered three models for expansion and intestinal homing by the transferred T cells. First, it is possible that T cells expand in the intestine by nonspecific mechanisms. Consistent with this, it has been reported that LPL are more readily activated through CD2 than through the CD3/TCR complex (24, 25). Second, a superantigen might be responsible. Third, TCR stimulation by peptides presented by MHC class II molecules might cause T cell activation and intestinal localization. The data presented here and in a previous publication (13) argue strongly in favor of this third model.

Previously, we showed that T cells in the intestine of the diseased SCID mice have an activated phenotype, and it was shown that a normal bacterial flora is required for the efficient establishment of T cells in the host intestine (13, 26). It remained possible, however, that bacteria have a nonspecific activating effect on the transferred T cells. Here we show that expression of MHC class II molecules by the host is required for colitis pathogenesis, consistent with a role for TCR-mediated recognition in this process. Furthermore, we used an extremely sensitive technique, fragment length analysis, to achieve the first detailed description of the TCR repertoire in the intestine of mice with colitis. We find that the TCR $\beta$-chain repertoire in IEL and LPL of SCID mice with CD4+CD45RB$^{\text{high}}$-induced colitis is characterized by the presence of
predominant or expanded clones. If a superantigen were involved, by contrast, we would expect a predominant Vβ with diverse CDR3 lengths. We did not find evidence for a predominant Vβ following PCR amplification, however, and in an earlier publication flow cytometric analysis did not reveal the presence of a predominant Vβ in the intestinal T cells of diseased SCID recipients (27). Similarly, comparative TCR repertoire studies on twins discordant for Crohn’s disease argued against the participation of superantigens in IBD in humans (28). Nucleic acid sequence analysis of T cells in the immune-deficient recipients demonstrates the presence of rearrangements that share amino acid motifs within the N-D-N region of the CDR3 of the β-chain, providing further evidence for the Ag-driven selection or expansion of particular T cell clones. The presence of related clones with similar β-chain N-D-N sequence motifs in an individual argues strongly against a random or homeostatic proliferation of lymphocytes in the “empty” SCID mouse as the explanation for a shared repertoire in different sites. Although these data taken together provide cogent evidence for an Ag driving the population of the host intestine with T cells, it remains possible that some of the T lymphocytes present in the mucosa are bystander cells.

Comparison of the TCR repertoire between recipients of cells from the same donor pool revealed a surprising paucity of shared or public clones in the intestine. Although this finding does not provide support for the hypothesis that there is a single predominant Ag in colitis pathogenesis, it does not exclude this possibility. It has been found that there is a relatively low level of sharing of the TCR repertoire even in immununized inbred mice (29), which might be due to stochastic elements in the Ag receptor gene rearrangement and selection processes. In addition to differences in the naive repertoire, recent studies demonstrate that small differences in the time of encounter with Ag are critical to the preferential expansion of particular T cell clones (30). Therefore, it remains possible that exposure to similar sets of Ags can generate different responses, even in genetically identical individuals.

Analyses of human IBD patients have provided some evidence for shared clones in peripheral blood CD4+ lymphocytes of identical twins concordant for Crohn’s disease (31) and for shared sequence motifs in activated T cells and CD8+ T cells of the intestinal mucosa from unrelated individuals with IBD (32, 33). Similar to the results from our analysis of diseased mice, however, in all these cases private or individual TCR expansions greatly out-number the public clones identified.

An oligoclonal TCR repertoire has been reported in both normal mouse and human IEL and LPL (34–36). Although a few reports do not agree with this finding (27, 37), in these cases a detailed study of CDR3 sequences was not conducted. Interestingly, the limited diversity of the TCR repertoire in the intestine of inbred mice with colitis and the striking differences between recipient mice precisely parallel reports on the TCRβ repertoire of IEL from normal mice (36, 38). These similarities suggest that the process of establishing a T cell population in the SCID intestine may in part reflect processes that form the repertoire of IEL and LPL in normal mice.

Predominant clones in the large intestine of an individual tend to be found in other regions of the body as well. The CDR3 length profiles of the LIEL showed a great deal of similarity to those in other regions of the intestine, including LPL, as well as the spleen, in any given animal studied. Sequence analysis confirmed the presence of systemic clonal expansions, and flow cytometric data demonstrate that CD4+ T cells in the intestine and the spleen tend to have an activated phenotype, even at relatively early time points following cell transfer. These data are consistent with either a systemic activation of T cell clones or a rapid and systemic distribution of T cells that have been activated in a particular site. Dendritic cells in the intestinal mucosa have been shown to be capable of migrating to sites outside the intestine (39, 40), providing a possible mechanism for the systemic presentation of intestinal Ags that might be relevant for colitis pathogenesis, such as those from bacteria. Alternatively, if there were a single site for the initial activation of the transferred T cells, for several reasons that site is unlikely to be either the epithelium or lamina propria of the intestine. First, there are data indicating that transferred T cells require antigenic stimulation to migrate to the intestine (41). Second, work in mice with joined circulatory systems, or parabiotic partners, demonstrates that IEL mix poorly between the parabiotic partners, although LPL recirculate to some extent (42, 43). Therefore, a putative site of initial activation would probably be in some organized lymphoid tissue. As Peyer’s patches were difficult to visualize in the recipients, this site is perhaps the mesenteric lymph nodes or the spleen.

The systemic distribution of predominant clones in the recipient mice stands in contrast to the localization of disease to the large intestine. The large intestine may become inflamed because of the higher bacterial load or because local factors in this environment might be more permissive of the Th1 responses that drive colitis. Additionally, we cannot formally exclude the possibility that subtle differences in the TCR repertoire in the large intestine might be critical for pathogenesis. These differences for the most part do not encompass the highly expanded clones, and therefore, we consider this possibility unlikely. It is more likely that the T cells in the large intestine are slightly more diverse than those in the small intestine simply because it is the site of disease. Once disease is initiated by activated clones, other T cells could be recruited into the large intestine nonspecifically by the production of cytokines and other inflammatory mediators. Additional experiments will be required to determine where the pathogenic T cells are initially activated in the CD4+ CD45RBhigh transfer model and why inflammation is concentrated in the large intestine.

It is not known whether the systemic distribution of activated T cells in the CD4+ CD45RBhigh transfer model also is characteristic of pathogenic T lymphocytes in human IBD. Expanded clones have been found among the CD4+ T cells in peripheral blood of patients with Crohn’s disease (31), however, and these clones persist for at least 1 yr. The relevance of the expanded clones in PBL for disease pathogenesis has not yet been established, but these data are consistent with the possibility that pathogenic cells in IBD are not localized exclusively in the intestine. Based upon these findings, we speculate that analysis of the diversity of the activated T cells in peripheral blood of IBD patients could shed insight into the diversity and specificity of pathogenic T cells.

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References


