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Repertoire of the $\alpha\beta$ T-cell receptor in the intestine

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Summary: The majority of T cells in the human and mouse intestine express the T-cell receptor (TCR) as an $\alpha\beta$ heterodimer on their cell surface. As the major recognition element of antigens in the context of major histocompatibility complex-derived proteins, an examination of the structure of the $\alpha\beta$ TCR in intestines has provided significant insights into the potential function of these cells and the major determinants that drive their selection. Studies in the human intestine have shown that the repertoires of intraepithelial lymphocytes (IELs), and likely lamina propria lymphocytes, are polyclonal before and shortly after birth, with the repertoire becoming oligoclonal in adults. Similarly, in adult mice the repertoire is oligoclonal, while in the newborn it is polyclonal. Investigations in mice have shown that some T cells may evade thymic selection. The population size and oligoclonality of IELs is influenced by the microbial content of the luminal microenvironment. This microenvironment probably directly determines the TCR repertoire. Studies in human inflammatory bowel disease (IBD) indicate that inflammation further skews the TCR repertoire. We speculate that dominant antigens associated with the pathogenesis of IBD are responsible for such skewing and that identifying the antigenic drivers may shed light on the environmental factors that trigger or potentiate human IBD.

Keywords: T-cell receptor, repertoire, intestines

Introduction

The epithelium of the gastrointestinal tract is the site most exposed to antigens and microbial organisms in vertebrates. Food and the products of its digestion bathe the intestine throughout life. Bacteria and fungi colonize the intestine soon after birth. In mammals, the intestine is seeded with flora from the maternal genital tract to which other bacteria are added during suckling. However, the greatest change in flora coincides with weaning, as food may carry bacteria and fungi. The greater range in nutrients and reduction in maternal antibodies and bacteriostats (inhibitory compounds such as lactoferrin) from milk permit the establishment of a diverse flora around the time of and after weaning. Best estimates predict that this flora changes little in later years (1).

It is crucial that animals are tolerant of luminal antigens and yet retain the capacity to respond to gastrointestinal infection.

The immune system within the intestine must, therefore, be tightly regulated. While part of the regulation will be controlled by the cytokine and chemokine milieu of the intestine, regulation of the T-cell repertoire and thus antigen responsiveness of the resident T cells must also be important, as any disturbance of the 'immune environment' may unleash a disastrous hypersensitivity reaction. The study of the T-cell receptor (TCR) repertoire thus provides some insight into the regulation of responses to luminal and mucosally associated (host and foreign) antigens. This review addresses the $\alpha\beta$ TCR usage in normal intestine and disease states.

The generation of the TCR repertoire by recombination of germ line gene segments has been reviewed elsewhere (2) and is beyond the scope of this review. However, there are several points that bear repetition. The $\alpha\beta$ TCR is a heterodimer with both the α and the β chains having virtually unlimited capacity for rearrangement and the generation of diversity. The TCR has three domains; complementarity determining region 1 (CDR1) and CDR2 are well conserved. CDR3 is the major hypervariable region that is involved with recognition of antigen in the context of major histocompatibility complex (MHC) (2). This diversity arises from the recombination of gene segments from the variable (from at least 44 V α and at least 60 V β segments), joining (from at least 61 J α and 13 J β segments), and diversity (from at least two D β segments) genes as well as from the insertion of non-germ line nucleotides (N-region additions) to create a functional CDR3 domain (3–6). The diverse and extensive recombination events result in generation of a CDR3 loop by which a T cell can then contact and recognize specific peptides, typically, 9–18 amino acids in length, presented in the context of MHC class I or class II molecules.

Each T-cell clone has the same receptor as its progenitor. The $\alpha\beta$ T-cell repertoire is thus determined by the number of receptor combinations and the frequency with which each receptor is expressed. In a polyclonal population, there will be countless different T-cell clones. However, if the repertoire of the population has been subjected to external selective pressures, it will then be skewed. Such pressures may bring about the deletion of clones that are hyperreactive to the MHC of the host in combination with host-derived peptides and thus possess exceedingly high affinity that would otherwise give rise to autoimmunity (negative thymic selection). In contrast, T-cell clones with proper affinity are positively selected. However, skewing may also arise from events that occur extrathymically in the periphery both within regional lymphoid (primary) structures, where education to antigens occurs, and within secondary lymphoid structures, such as the effector sites of the lamina propria and intestinal epithelium. In the secondary

lymphoid structures of the intestines, T-cell clones are further selected by both their ability to enter the intestines, due to the expression of gut-homing molecules (e.g. $\alpha 4\beta 7$ and CCR9), and their ability to recognize cognate antigens (local auto-antigens and microbial antigens), and in the setting of numerous cofactors, such as the local cytokine milieu [e.g. interleukin-15 (IL-15), transforming growth factor- β , and IL-10] and other costimulatory surface receptors (e.g. CD28, programmed death-1, and carcinoembryonic antigen-related cellular adhesion molecule 1) that are present in the mucosal tissues (7). Together, these events lead to enrichment of particular T-cell clones within the mucosal tissues of the intestines.

Assessment of the TCR repertoire

The literature of the study of TCR repertoire reflects the evolution of molecular methods in immunology. Early work used antibodies, followed by radiolabeled blotting techniques. Subsequently, refined fluorescent labeling techniques were used to complement cloning and DNA sequencing work. It is important to briefly review these with respect to the analysis of $\alpha\beta$ TCR repertoire analyses.

Antibody methods

Early studies of the $\alpha\beta$ TCR repertoire exploited monoclonal antibodies. Typically, these antibodies were raised against the variable region of the TCR β and, to a lesser extent, the TCR α chain, allowing for a determination of variable chain usage.

Antibodies can be used to study prepared tissues to show the location of the T cells expressing a particular variable region. The development of flow cytometry has meant that the expression of particular TCR variable regions by individual cells in suspension, such as intraepithelial lymphocytes (IELs) or lamina propria lymphocytes (LPLs), can be readily quantified. Antibody-based approaches have a role in studying the overall shape of the repertoire or the identification of unique populations of lymphocytes that are monomorphic with respect to their TCR α or β usage. An excellent example of the latter is the invariant natural killer T cell, which is restricted to CD1d and which, in humans, expresses V $\alpha 24$, which can be detected by a monoclonal antibody (or a CD1d tetramer loaded with a glycolipid antigen, α -galactosylceramide) together with multiple different TCR β chains (8, 9). If compared to a reference tissue, skewing of variable chain usage can be assessed. However, antibody approaches also have some limitations, as there are numerous variable chains for which there are no commercially available antibodies.

Southern and Northern blotting

The description of the variable region genes led to the application of Southern and Northern blotting techniques to the analysis of TCR repertoire. Nucleotide probes, radiolabeled or non-radiolabeled, can be designed to anneal to DNA or RNA derived from cell populations or tissues, which enable the TCR α or β chain genomic or transcript levels to be studied. The overall levels reflect the relative abundance of these TCRs within the tissue and provide an indirect repertoire analysis. However, since it is not a measure of the number of cells but only the TCR expression level, it may not accurately reflect a quantitative abundance of particular T-cell clones. Similar to direct probing of tissues, extracted DNA and RNA can also be studied by this technique. Probes can also be used to hybridize with templates that have been amplified by polymerase chain reaction (PCR).

Genomic DNA probes must be designed with care to recognize recombined TCR gene segments and not germ line segments. Furthermore, the probe must avoid the intron that separates the constant from the junctional region. Riboprobes offer an alternative, but they must still be designed with care and, of course, are more difficult to use on fixed tissue in which RNA may have degraded.

In some respects, Southern blotting has the same limitations as antibody approaches. Namely, skewed variable region gene transcripts may be assessed, but expansions of specific clones may be overlooked. However, if the investigation is designed to study a particular clone, then clonotypic probes based on the junctional region may be designed. A further weakness of the early application of blotting was the use of radioisotopes, although newer methods such as digoxigenin-based methods have replaced the necessity for these.

Polymerase chain reaction

PCR has greatly enabled the complexity of the TCR repertoire to be studied. Using primers that flank or incorporate the hypervariable CDR3 enable its length to be studied. Flanking primers are typically designed to anneal to the constant and the variable gene segments of complementary DNA (cDNA). If genomic DNA is studied, then the primers must be designed to the joining and the variable gene segments; this assay requires a panel of primers, as there are 13 joining gene segments for the TCR β chain in humans. Alternatively, the primers must be pooled.

As discussed above, there is some diversity in length of the CDR3; if PCR products are resolved by electrophoresis and imaged, then a series of products, three nucleotide base pairs apart, can be identified. If the repertoire is polyclonal, then the frequency distribution of CDR3 lengths of these products is

Gaussian (10). In contrast, if a particular clone has expanded, then the CDR3 length will be increased as a result of the greater number of cells transcribing it, which will lead to a distortion of the Gaussian distribution. Initially, imaging was undertaken using radioisotopes that were incorporated into the PCR product or into the primer; however, newer non-radioisotope techniques have replaced this approach. These new approaches include the use of fluorescent labeling techniques such as SYBR green incorporation or fluorescent nucleotide incorporation, which allow for the delineation of a spectratype for a particular population of T cells and silver staining of DNA gels (11).

Fluorescently labeled PCR products can be analyzed using MegaBACE DNA Analysis System or similar equipment. The data can be integrated and displayed in graphical form. These data readily demonstrate whether the repertoire is Gaussian in distribution or not (Fig. 1). Furthermore, there is now software to interpret the data mathematically (10).

In silver staining, the DNA binds to silver nitrate and formaldehyde (Silver Sequence staining reagents, Promega, Madison, WI, USA). The polyacrylamide gel is then developed like a photographic negative. This approach has the advantage that specific bands can be seen without a special apparatus. Moreover, these bands can be cut directly from the gel, and the DNA within them can be eluted for further CDR3 sequence analysis.

Cloning and sequencing

The amplification of α or β chain transcripts by PCR facilitates the sequencing of nucleotides that encode these chains. To many investigators, this approach is the ultimate means of determining true clonal expansion and frequency for particular T cells, given the overlapping usage of TCR chains by specific populations. Once amplified, the PCR products can be subjected to direct (cycle) nucleotide sequencing, or the products can be ligated into a plasmid and grown in bacteria, and then plasmid DNA from individual bacterial colonies can be sequenced at random. Direct sequencing from PCR products is fraught with problems unless the cDNA of all the isolated T cells has the exact same sequence (i.e. the T cells are monoclonal). Consequently, subcloning using plasmids and bacteria is often preferred to obtain $\alpha\beta$ TCR CDR transcript usage information. The relationship between clonality as determined by spectratyping and that determined by sequence data has received little attention, until a recent study compared the data from the two methods (12). This study has shown that there is remarkable consistency between the spectratype data and that obtained by cloning and sequencing; significantly, spectratyping enables more of the repertoire to be addressed than is feasible by cloning and sequencing.

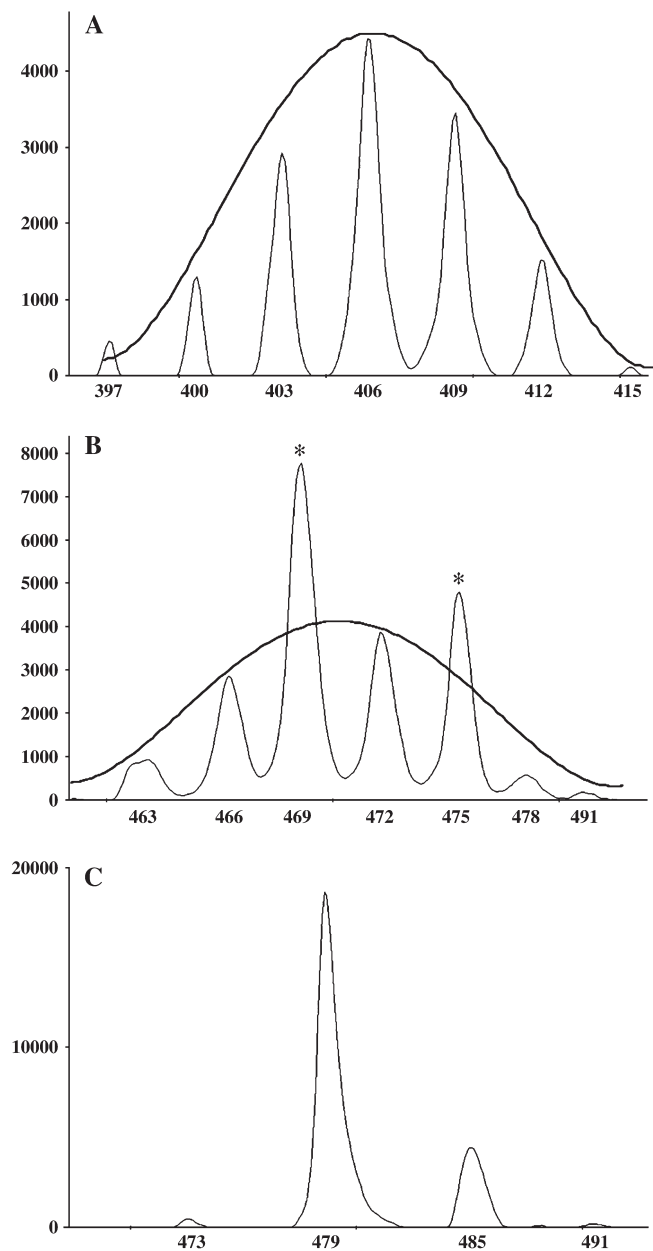


Fig. 1. Examples for spectratype output. (A) Polyclonal repertoire with Gaussian distribution. (B) Some clonal expansions (*) on polyclonal background. (C) Oligoclonal repertoire. Reprinted from Probert CSJ, et al.(12). The effect of weaning on the clonality of $\alpha\beta$ TCR T cells in the intestine of GF and SPF mice (copyright 2006, with permission from Elsevier).

Intestinal $\alpha\beta$ TCR repertoire in health

Human studies: before birth

To prepare the intestine for antigen exposure *ex utero*, the fetus must undergo T-cell seeding into the intestines before birth. In humans, T cells can be identified in the intestine from 14 weeks of gestation. $\alpha\beta$ T cells in the intestine have been the focus of only a few studies.

Spencer et al. (13) were the first to publish the phenotype of intestinal T cells in the human fetus. Nine fetal ileal samples were studied; there appeared to be a twofold increase in the number of CD3⁺ stained cells in the epithelium between 11 and 19 weeks of gestation. CD8⁺ cells were more common than CD4⁺ cells. At the same time, there was no obvious increase in the number of B cells. Meanwhile in the lamina propria, CD4⁺ cells were more common than CD8⁺ cells. The group extended this work to address TCR repertoire, when 10 years later, Thomas et al. (14) reported on the expression of TCR β chain usage in the small intestine of 10 human fetuses aged between 16 and 24 weeks using immunohistochemistry with antibodies against 14 TCR β variable chains. Stained cells were counted, and usage was expressed as a percentage of CD3⁺ stained cells. These authors noted that there were too few IELs to permit analysis, but LPLs could be studied. Only 1–2% of T cells expressed a particular TCR β chain, although some skewing was noted, especially toward TCRBV8.

Koningsberger et al. (15) also obtained fetal material from the second trimester. The intestinal T-cell repertoire was studied in four fetuses aged 13–23 weeks using PCR. The intestines were obtained, and only a small number of lymphocytes were isolated, a potential limitation of this study. There were multiple TCR β chain transcripts in both the IEL and LPL fractions. The authors concluded that the TCR β repertoire of LPLs was in fact polyclonal and changed little with the age of the fetus. TCR α chains were much less diverse, and IELs appeared to be oligoclonal, even at this early stage of development. Given that the TCR α and TCR β chains combine in the TCR, the polyclonality of the TCR β repertoire means the functional repertoire must also be polyclonal.

Human studies during infancy

Recently, the first detailed description of the $\alpha\beta$ TCR repertoire in human infants has been published (16). TCR β chain usage was studied by PCR of cDNA. TCR β chain diversity was assessed by cloning and sequencing of TCRBV gene segments (TCRBV4, TCRBV6, and TCRBV12) that were obtained from small and large intestinal samples of infants aged 6 days to 12 months. Full mucosal samples and LPLs provided comparable results: all were polyclonal in TCRBV6 and TCRBV12. One child provided particularly interesting data, in that a sterile duplication cyst lay parallel to the ileum. The TCR repertoire of the cyst and the adjacent ileum were polyclonal, and there were no shared clones. These data suggest that seeding of T cells to the intestine is not under any specific antigenic influence but is arrived at by chance.

In contrast, the TCRBV4 repertoire in the small intestine of both the 2-month-old and the 1-year-old children were

monoclonal (16). Moreover, in the large intestine, there was evidence of emerging clonal expansions that correlated with increasing age. An additional sample, from a child aged 56 months, showed a more adult repertoire. Once again, one child gave particularly interesting data: a colon that was defunctioned from birth was similar to that of other colons, with some emerging clones evident. Together, these data suggest that seeding of T cells to the intestine is a stochastic event. The repertoire is later shaped in response to environmental antigens. However, the data from the defunctioned colon may imply that the antigen need not be within the colon lumen itself, merely in the intestine.

Human studies in adults

The first investigation to report TCR usage in the human intestine using PCR was published in 1991 (17). Collaborating with Ellen Ebert, who had refined the technique for large-scale isolation of IELs from the human small intestine, Balk et al. (17) generated a human jejunal IEL cell line. Flow cytometric analysis of the line showed that the phenotype was remarkably similar to that of freshly isolated cells. The authors then addressed the repertoire of the line. Initially, the TCR α chain was studied by 5'-rapid amplification of complementary ends; one chain, TCRAV13S1-AJN, accounted for 19/25 transcripts sequenced. The authors were able to confirm that this was not a culture artifact by looking at other samples and also by addressing TCR β chain gene expression; this time, one sequence, TCRBV12J1S6-LGA, dominated and accounted for 13/27 transcripts. This study indicated that human IELs were oligoclonal, despite their location within the proximal small intestine (jejunum) and adjacency to a large number of potential dietary and microbial antigens.

In 1992, Van Kerckhove et al. (18) reported the TCR repertoire of the human colon using PCR. Twenty-two primers were designed to anneal to the 20 known TCRBV families. These primers were used with constant region primers and the PCR product probes with a further, radiolabeled constant region primer. IEL and LPL fractions were studied from five colonic and one jejunal samples. Among the IEL samples, it was reported that TCRBV 1–3 chain genes were dominant. Moreover, the same genes, TCRBV 1, 2, 3, and 6, were most likely to be dominant in each colonic sample, but TCRBV6 was dominant in the small intestinal sample. To further investigate the clonality, DNA utilizing the TCRBV6 gene from two of the colonic samples was subjected to cloning and sequencing. In both, one clone predominated: in the first, the dominant clone accounted for 13/22 transcripts, and in the second, the dominant clone accounted for 18/28 transcripts. These studies confirmed those reported by Balk et al. (17) and indicated that oligoclonality extended to the normal human colon.

A year later, Blumberg et al. (19) extended the study of jejunal lymphocytes, this time reporting the repertoire of IELs and LPLs. Five donors were studied using the method favored by the group (17). Quantification of probed PCR products found that the IEL fraction was very skewed. Like Van Kerckhove et al. (18) a year earlier, they found that the TCRBV6 was among the most dominant, but in some individuals, other TCRBV families were more numerous. Indeed in one subject, 69% of clones appeared to use TCRBV3. Subsequent DNA sequencing found that the TCRBV6 products all arose from a single clone. Clearly that was unusual, and in most cases, dominant clones were less frequent, though in every case the data supported the finding that IELs are oligoclonal. In this study, the group also applied for the first time denaturing and non-denaturing (single-strand conformational polymorphism) gel analyses to study TCR repertoire. Such approaches were forerunners of fluorescently based techniques called spectratyping. The group also reported that LPLs contained expanded T-cell clones. Interestingly, this group was also able to show that some DNA sequences were shared between the IEL and LPL fractions, implying that the T cells traffic between the epithelium and the lamina propria. In general, CD8⁺ LPLs exhibited more oligoclonality than CD4⁺ LPLs, similar to observations made by others with CD8⁺ lymphocytes in the peripheral blood, wherein senescent individuals often exhibited oligoclonal expansions of CD8⁺ lymphocytes (20, 21). However, most clones were not found in both sites, suggesting unique selection events and the uniqueness of each of these anatomic compartments.

Thomas et al. (14) applied a panel of 14 antibodies to study the expression of TCR β chain in adults; four normal ileal samples and six colonic samples were analyzed. In the small bowel, the repertoire was skewed. In three subjects, the dominant TCR β chain was shared by the IELs and the LPLs, but this overlap did not happen in the fourth subject studied. The colonic specimens had too few IELs to justify study, so the authors focused on LPLs. Once again, the TCR β chain usage was skewed, with different chains dominating in different subjects. Another collection of small bowel samples was reported in the same study; they were obtained from patients with Crohn's disease (CD). Using these larger samples, the authors compared the dominant TCR β chain in two sites in seven samples. The sites were up to 1 m apart. In six, a dominant TCR β was found among the IELs at both sites; in the seventh, there was no dominant TCR β chain. In most cases, the dominant chain was different in the two sites, an observation that applied in a similar manner to the LPLs. Furthermore, these authors examined the dominant clones in paired small and large bowel samples in two patients and paired colonic samples in two further patients. In

none of these subjects were the dominant TCR β chains the same in both sites. Although the authors suggest that the regional differences are likely to apply to normal subjects, an effect of the CD cannot be ignored (see below).

The issue of the intestinal distribution of IEL clones was also addressed by Gross et al. (22). Using PCR, clonality in the proximal and distal ends of intestinal resections was investigated. Seven patients were studied: in three both ends were in the small bowel, in two both were in the large bowel, and in the remainder the proximal end was in the ileum and the distal end in the colon. Although all the specimens were obtained at operation, none of the patients had inflammatory bowel disease (IBD). Every sample contained T cells with an oligoclonal repertoire. For six of seven patients, the proximal and distal samples exhibited shared clones; however, for the final patient, no clones were shared between the small and the large intestines. This study provided strong support for a diffuse distribution of oligoclonally expanded T cells in the epithelium of the human intestine.

In 1996, Dogan et al. (23) published their findings after investigating the clonality of T cells in the intestine of the colon. Fragments of tissue were extracted from tissue sections using microdissection. The group investigated clonality by PCR using genomic DNA as the template. For the reasons outlined above, they used primers that anneal to the diversity and the joining genes, thus amplifying the junctional region. The PCR products were finally resolved on a polyacrylamide gel. To demonstrate clonality, cloning and sequencing of DNA was undertaken. In each of the two subjects studied, adjacent areas exhibited shared dominant clones. This study confirmed the presence of oligoclonal expansions of TCR β chains in the normal adult human intestine.

Recently, Bennett et al. (24) have investigated the clonality of LPLs in the human colon. They chose to use antibody staining of isolated cells from 13 patients without IBD. Importantly, they separated CD4⁺ and CD8⁺ T-cell subsets. They found that the TCR usage was skewed compared with that of the peripheral blood, and they concluded that the repertoire of both subsets was oligoclonal. They were unable to demonstrate that the repertoire was skewed in a different manner in different sections of the bowel. However, they also acknowledged that, in their experience, clonal expansions detected by sequencing had been overlooked by the monoclonal antibody approach. From this study, we conclude that the CD4⁺ and CD8⁺ subsets of LPLs are oligoclonal, but the extent to which clones are expanded and shared over different regions of the bowel remains uncertain.

Rodent studies

The year 1984 was an important year for TCR studies. Several key papers were published discussing the generation of the TCR

repertoire in contrast to immunoglobulin (25) and the nature of recombination (26). Such work paved the way for early studies of TCR repertoire in the late 1980s.

Much effort was expended, in the 1990s, toward the understanding the phenotype of IELs. Bonneville et al. (27), Goodman and Lefrancois (28), and De Geus et al. (29) each led publications showing the presence of $\gamma\delta$ cells among IEL populations. Takagaki et al. (30) reported that the $\gamma\delta$ IEL population had a diverse repertoire. Interest in $\gamma\delta$ cells grew further when Bandeira et al. (31) studied germ-free (GF) mice and suggested that the presence of such cells in the intestine was independent of microbial colonization. The same group also studied nude mice and concluded that $\gamma\delta$ cells may undergo extrathymic development (32).

At the same time, the study of the intestinal $\alpha\beta$ TCR T cells was progressing, much of it led by Delphine Guy-Grand. Rocha et al. (33) reported the distribution of TCRBV usage in the intestine and observed disparity between the repertoire of CD8 $\alpha\beta$ and CD8 $\alpha\alpha$ T cells. The CD8 $\alpha\beta$ T cells had a skewed repertoire, in which some TCRBV families were not expressed, in contrast to the CD8 $\alpha\alpha$ T cells in which none were missing. The authors speculated that CD8 $\alpha\alpha$ $\alpha\beta$ TCR T cells may be undergoing extrathymic development, in that those cells expressed TCRBV families that were usually deleted in the thymus and that were believed to be potentially autoreactive. As such, these cells bear similarities to $\gamma\delta$ cells. Poussier et al. (34) drew similar conclusions in 1992 and have further suggested a relationship of the CD8 $\alpha\alpha$ -bearing T cells to the pathogenesis of IBD. Others have, however, challenged such a suggestion that an extrathymic pathway of T cells occurs in the intestines (35, 36). Recently, it has been observed in chickens that CD8 $\alpha\alpha$ cells may leave the thymus early in development but only acquire CD8 $\alpha\alpha$ expression in the intestine (37). Similarly, Eberl and Littman (38) have reported that all $\alpha\beta$ T cells in the intestine are the progeny of CD4⁺CD8⁺ thymocytes.

In 1994, Regnault et al. (39) used PCR to investigate the repertoire of CD8 $\alpha\beta$ $\alpha\beta$ TCR⁺ IELs by CDR3 length analysis using fluorescent J β -specific primers. Cloning and sequencing of TCRs was also performed. The repertoire of CD8 $\alpha\beta$ T cells was oligoclonal. In addition, they observed that the same dominant clones were found in contiguous pieces of small intestine, as reported by Gross et al. (22) in humans. Furthermore, the individual inbred mice, from the same litter and maintained in the same environment, did not share dominant clones.

This work led to a series of papers investigating the factors that influence repertoire development in rodents. In 1996, Regnault et al. (40) reported the repertoire of specific pathogen-free (SPF)

and GF adult mice: both sets of mice were oligoclonal. This outcome led the authors to conclude that bacterial flora did not influence the repertoire. However, bacteria colonization of the intestine by bacterial flora leads to the proliferation of IELs (41), and the findings of Regnault do not appear to take account of the fact that in GF mice, there are fewer IELs.

Studies in rats have also found that the $\alpha\beta$ TCR-bearing population of IELs substantially expands during bacterial colonization (42). Furthermore, the TCR repertoire of IEL clones changes when GF animals undergo colonization: specifically, the repertoire of CD8⁺ IELs of adult GF rats was apparently random (43–45). In contrast, the repertoire of SPF and colonized ex-GF rats was skewed, and expansion of particular clones was evident in adults (43–45). These studies suggest that both the number and oligoclonal repertoire of $\alpha\beta$ T cells are determined by the microbial milieu.

These data suggest that equivalent and random seeding of CD8⁺ IEL clones occurs in the intestine of GF or SPF rats, which are then subjected to antigenic pressure by the developing flora, thus molding an adult oligoclonal IEL population. This is supported by the findings of Butcher group (46), that recent thymic emigrants home to the intestine and undergo postthymic differentiation proliferation that is influenced in part by the microenvironment. Collectively, these data suggest that the repertoire of $\alpha\beta$ TCR cells is shaped by the intestinal microenvironment.

TCR repertoire analysis in human IBD

Similar to other chronic immune-mediated disorders, there are increased numbers of T cells present at the sites of intestinal inflammation in the two dominant forms of IBD, Chron's disease (CD) and ulcerative colitis (UC). Although recent advances in genetics have strongly indicated an innate immune recognition defect in IBD, especially in CD, there is a very robust adaptive immune response that still takes place. This adaptive response is primarily characterized by both an influx and an expansion of the $\alpha\beta$ TCR⁺ cells.

Investigations into TCR repertoire analysis of the intestinal T-cell response in IBD have greatly improved over the past few decades as newer and better PCR techniques have developed (Table 1). Initially, using immunofluorescence with a panel of TCRBV-specific antibodies, investigators compared TCR usage between IBD subjects and non-IBD controls. Posnett *et al.* (47) observed an increase in a TCRBV8 population of CD subjects, while others found no difference in the average frequencies of TCRBV usage of the T cells in the lamina propria, including both CD4⁺ and CD8⁺ T cells, between the two groups studied (47–

49). These findings are consistent with the presence of dominant and non-dominant TCRBV usage naturally in humans, and the recognition that this technique lacked the sensitivity to determine whether expansions were truly present in the different populations. However, from these initial studies, some differences were observed, primarily in CD LPL compared to the CD peripheral blood lymphocyte (PBL) TCRBV usage, which was not seen in the UC or control groups (48, 49). Consistent with this latter point, comparison of active and inactive UC PBL samples did not identify significant differences in TCRBV in another study (50).

Using the more sensitive semiquantitative PCR technique (Fig. 2; Courtesy of Dr. Victor M. Morales), repeat TCR analysis of IBD subjects indicated that each individual had a unique LPL and PBL repertoire (51, 52). The differences in TCRBV usage, primarily in the CD4⁺ T-cell LPL population, were consistent with a specific antigenic immune response (52). This response contrasted with the one that would be observed with a super-antigen response, where the same TCRBV would be identified in all individuals (53). Furthermore, the expansions of these CD4⁺ T cells were greater in the inflamed versus the non-inflamed CD tissue sites (52, 54). One attempt was even made to identify potential antigen(s) from CD homogenate tissue fractionation, but this approach proved unsuccessful (55).

Further evidence for CD-specific T-cell clones was found when Nakajima *et al.* (56) used single-strand conformational polymorphism to study T-cell clones in the earliest lesions associated with CD, the so-called aphthoid ulcers. They found that there were specific clonal expansions in early CD ulcers. Moreover, in one individual, specific clones were found in separate ulcers but not in the intervening non-diseased tissue, suggesting expansions of specific T-cell clones in early IBD lesions.

The new ability to better detect clonal expansions in T-cell populations led Chott *et al.* (57) to examine the TCRBV usage in nine UC subjects. They also observed significant and unique expansions within the LPL populations, very similar to the CD results (57). However, the clonal expansions appeared to be greater in the CD8⁺ T cells rather than in the CD4⁺ T cells. In addition, they identified a common TCRBV expansion in five of the nine subjects involving TCRBV3 and BJ1.6 (57). The finding of a common expansion indicated a potential shared and dominant antigenic response in the UC subject population. CDR3 sequence analysis confirmed the expansions within this TCRBV, and a follow-up investigation noted the presence of this motif in association with increased UC activity (58). The dominant antigen was never identified, but new state-of-the-art approaches utilizing TCR cloning into hybridomas and peptide matrix libraries may now make that possible (59, 60).

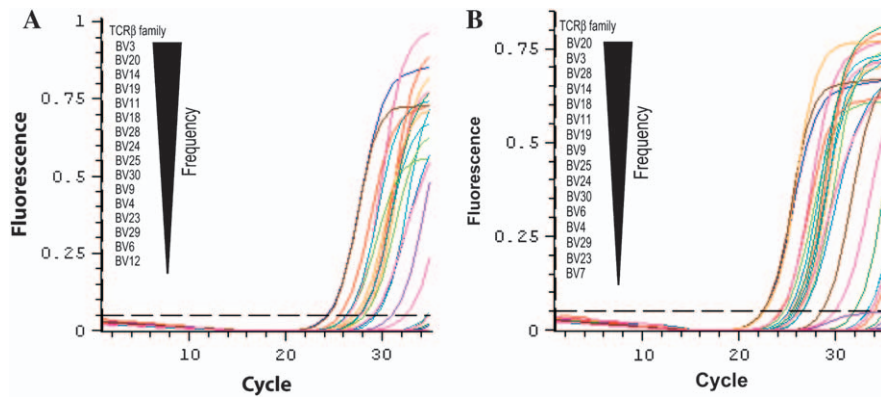


Fig. 2. Example of quantitative PCR approach to TCRBV repertoire analysis. TCR analysis notes specific shifts in TCRBV usage when grossly involved (A) and non-/less involved (B) IBD tissue sites are compared by quantitative PCR (QPCR) within the same CD subject. QPCR was performed using 28 individual TCRBV primers combined with a TCRB constant region primer and SYBR green fluorescent dye

incorporation. Analysis of melting curves indicated a single product, and quantitative amounts were normalized to β -actin, with the results listed in order of predominant levels or 'frequency' of TCR usage for the samples. Only TCRs that amplified sufficiently are shown, but there are obvious increases (e.g. BV19) or decreases (e.g. BV28) in specific TCRBVs, while others remain at a relatively constant level of frequency.

Given the prior observations of differences in the PBL populations of IBD subjects and the well-recognized trafficking of T cells into and out of the mucosal compartment, PBL TCR repertoire analysis was carried out among CD, UC, inflammatory (diverticulitis), and normal control samples (61). TCR and CDR3 analysis detected expansions and skewing in all three inflammatory conditions in both CD4⁺ and CD8⁺ T cells, while normal controls demonstrated the typical Gaussian distribution of the CDR3 (61). Furthermore, sequence analysis of archived tissue and repeat blood samples in CD observed that these TCR expansions were persistent over a year and identifiable in both locations (61). These results strengthened the argument for persistent dominant antigenic responses in IBD.

almost identical pattern of TCRBV usage was observed, and CDR3 analysis also confirmed the presence of some similar sequences in the CD4⁺ T cells (61). Consistent with this notion of HLA-defined TCR repertoire responses, detailed sequence analysis of the *in vitro*-expanded, IL-2 responsive or activated T-cell populations from the intestines of IBD, diverticulitis, and normal control individuals revealed distinct private and public patterns of TCRBV CDR3 usage in both CD4⁺ and CD8⁺ T cells (63). The more dominant private response indicated a personal TCR/MHC response to a particular set of antigens, whereas a public response most likely indicated a shared TCR/MHC response, as defined by Cibotti et al. (64). The presence of privately skewed responses in normal controls is consistent with the constant state of T-cell activation normally present in the mucosal compartment and has been similarly noted by another group of investigators (65).

The observation that human leukocyte antigen (HLA) genotype and subsequent MHC phenotype could shape the TCR repertoire within an individual was also examined in the context of IBD (61, 62). In the case of a set of identical twins with CD, an

Newer methodologies are evolving in the area of TCR/CDR3 repertoire analysis and antigen identification (59, 60, 66, 67).

Table 1. Summary of major TCR usage studies in human IBD

Author, year	Site	Important TCRBV usage	Method	Notes
Posnett et al. (1990) (47)	PBL	8	Mab	Possible superantigen effect
Gulwani-Akolkar (1994) (12)	PBL	5, 1, 10, 11, 19, 18	PCR	Twins study noting similar patterns
Shalon et al. (1994) (48)	PBL, LPL	5, 6, 8, 12	Mab	Altered TCR use in CD but not UC
Qiao et al. (1994) (49)	LPL	2, 3, 5, 6, 8, 19, 22	Mab	Increased proliferative responses of LPL
Prindiville et al. (1996) (55)	LPL	2, 5, 6, 14, 17, 20	PCR	Tissue homogenate reactivity
Gulwani-Akolkar et al. (1995) (51)	PBL, LPL	6, 20	PCR	Blood: tissue, \uparrow variability in CD
Gulwani-Akolkar et al. (1996) (54)	LPL	2, 6, 7, 11, 13, 15, 16	PCR	\uparrow oligoclonality in active CD sites
Chott et al. (1996) (57)	LPL	2, 6, 8, 7 and BV3-JIS1 motif	PCR	Shared TCR pattern
Probert et al. (1996) (61)	PBL, Tissue	2, 4, 6, 8, 17, 18	PCR	Persistence of TCRs, twins study
Nakajima et al. (1996) (56)	LPL	1, 3, 5, 1, 6, 7, 8, 9, 15	PCR-SSCP	Early CD aphthoid lesions
Saubermann et al. (1999) (63)	LPL	3, 12, 17, 22	PCR	Public and private patterns of TCR use
Probert et al. (2001) (58)	LPL	BV3-JIS1 motif	PCR	TCR motif association with UC activity
May et al. (2002) (65)	PBL, LPL	1, 3, 8, 13, 14, 18	PCR	TCR colonic distribution patterns

Mab, monoclonal antibody; PCR-SSCP, poly chain reactions-single strand conformation polymorphisms.

Since the sequencing of the human genome, the ability to reclassify all known TCRBV and TCRAV families has been accomplished, allowing greater accuracy in identification of TCR expansions. Recent investigations using this more sensitive and specific quantitative real-time PCR approach to CD tissue analysis is underway (L. J. Saubermann, personal communication) and is being combined with advances in molecular biology aimed at deciphering the dominant antigens associated with IBD.

Overall, these studies suggest that private and public T-cell responses can be observed in both the intestines and peripheral blood of humans with IBD. These studies suggest the existence of disease-specific antigens in the pathogenesis of this disease and have been mirrored by the detection of similar oligoclonal expansions in various mouse models of colitis, including studies in the CD4⁺CD45RB^{hi} adoptive transfer model (68) and the severe combined immunodeficient model of spontaneous colitis (69).

Conclusion

The TCR of the intestine in health is shaped in infancy. *In utero* and shortly after birth, the repertoire is polyclonal. Subsequently, clonal expansions occur, giving rise to an oligoclonal repertoire in both the lamina propria and the epithelium. These events appear to be under the influence of luminal bacteria.

In IBD, there is evidence for further perturbation of the naturally occurring oligoclonal repertoire of the mucosal environment. It remains unclear whether this restriction in TCR usage is driven by responses to particular pathogen(s), autoimmune epitopes, or if it is secondary to the damage in the mucosal barrier and an influx of microbial/environmental antigens. More work is needed in this area in order to define the antigenic stimuli and the role of the oligoclonal TCR repertoire restriction in IBD.

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