T cell repertoire and inflammatory bowel disease

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K CROITORU, DKH WONG, ME BACA-ESTRADA. T cell repertoire and inflammatory bowel disease. *Can J Gastroenterol* 1996;10(2):110-114. The diversity of the T cell receptor repertoire is generated through rearrangement of the variable, junctional and constant region genes. Selection processes in the thymus and periphery serve to eliminate self-reacting T cells, thereby preventing autoimmune disease. The possibility that inflammatory bowel disease (IBD) is an autoimmune disease has led to the search for an auto-antigen. In addition, studies are exploring the T cell receptor repertoire in IBD patients for changes that may provide clues regarding etiopathogenesis. Using monoclonal antibodies to T cell receptor variable-gene products or polymerase chain reaction analysis of variable-gene mRNA expression, the mucosal T cell repertoire has been examined in humans. The intestinal intraepithelial lymphocytes show a significant degree of oligoclonal expansion that may represent local antigen exposure or unique selection processes. This is in keeping with studies that show that murine intestinal intraepithelial lymphocytes undergo positive and possibly negative selection independent of the thymus. In the inflamed human gut, shifts in the T cell receptor repertoire may also reflect recruitment of peripheral T cells to the gut. In one study, a subset of Crohn's disease patients was shown to have an increase in the proportion of variable $\beta 8$ peripheral blood lymphocyte and mesenteric lymph node cells, suggesting a superantigen effect. The authors hypothesized that changes in the functional T cell receptor repertoire can also occur which might be independent of changes in the distribution of T cells expressing variable β T cell receptors. In fact, the authors have shown there is a selective decrease in the cytotoxic function of peripheral variable $\beta 8$ T cells in Crohn's disease. Furthermore, stimulation with the variable $\beta 8$ selective bacterial enterotoxin staphylococcal enterotoxin E failed to increase the cytotoxic function in this subset of Crohn's disease patients compared with controls. This suggests that in Crohn's disease, variable $\beta 8$ T cells have undergone

an alteration in function that may reflect previous exposure to a superantigen-like stimulus. The relationship to the etiology and pathogenesis of IBD remains to be defined.

Key Words: *Inflammatory bowel disease, Mucosal immunology, T cell, T cell receptor*

Cellules T et maladie inflammatoire de l'intestin

RÉSUMÉ : La diversité des récepteurs des cellules T provient du réarrangement des gènes régionaux variables jonctionnels et constants. Les processus de sélection dans le thymus et à la périphérie servent à éliminer les cellules T auto-réagissantes, ce qui prévient la maladie auto-immune. La possibilité que les maladies inflammatoires de l'intestin soient une maladie auto-immune a guidé la recherche sur les auto-antigènes. De plus, les études explorent les récepteurs des cellules T chez les patients atteints de MII pour vérifier la présence d'anomalies qui pourraient en expliquer l'étiopathogénèse. À l'aide d'anticorps monoclonaux dirigés contre les récepteurs des cellules T, les produits variables des gènes ou de l'amplification génique de l'expression des gènes variables a permis l'examen du répertoire des cellules T de la muqueuse chez l'humain. Les lymphocytes intra-épithéliaux intestinaux manifestent un degré significatif d'expansion oligo-clonale qui peut représenter une exposition locale à des antigènes ou des processus de sélection uniques. Cela concorde avec des études qui montrent que les lymphocytes intra-épithéliaux intestinaux murins subissent une sélection positive et possiblement négative, indépendamment du thymus. Dans l'intestin humain enflammé, les changements du répertoire des récepteurs des cellules T peuvent également refléter le recrutement des cellules T de la périphérie vers l'intestin. Dans une étude, des patients d'un sous-groupe atteints de maladie de Crohn ont présenté une augmentation de la proportion des leuco-

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cytes du sang périphérique de la variable $\beta 8$ et des cellules des ganglions lymphatiques mésentériques, ce qui suggère un effet de superantigène. Les auteurs ont émis l'hypothèse que des anomalies du répertoire des récepteurs des cellules T fonctionnelles puissent survenir indépendamment des changements de distribution des cellules T exprimant les récepteurs des cellules T de la variable β . En fait, les auteurs ont démontré qu'il y avait diminution sélective de la fonction cytotoxique des cellules T périphériques de la variable $\beta 8$ dans la maladie de Crohn. De plus, la stimulation à l'aide

de l'entérotoxine bactérienne $\beta 8$ sélective, l'antérotoxine E staphylococcique, a échoué à faire augmenter la fonction cytotoxique chez le sous-groupe de patients atteints de maladie de Crohn en comparaison avec des patients témoins. Cela donne à penser que dans la maladie de Crohn, les cellules T de la variable $\beta 8$ ont subi une altération fonctionnelle qui peut être le reflet d'une exposition antérieure à un stimulus de la part de superantigènes. Le rapport avec l'étiologie et la pathogenèse des MII reste à définir.

Crohn's disease and ulcerative colitis are characterized by chronic intestinal inflammation of unknown etiology. It has been proposed that in an individual with the appropriate genetic background, exposure to environmental agents or organisms leads to activation of intestinal immune cells and chronic inflammation. Regardless of whether this results in autoreactivity, it is proposed that the activation of intestinal T cells is critical to the initiation and perpetuation of inflammatory bowel disease (IBD).

T cells recognize antigen via the surface T cell receptor (TCR). Signals initiated through the TCR lead to a functional response that may be characterized by proliferation, cytotoxicity or cytokine production. Under certain conditions there is a lack of response (anergy) or activation of programmed cell death (apoptosis). A number of cellular processes and molecular signals serve to influence the outcome of TCR-mediated T cell activation (1). These processes serve to turn off a T cell response, ie, induce anergy. The mechanisms of anergy induction are related to inappropriate signals or lack of accessory signals.

T lymphocyte responses are also controlled by selection processes that shape the TCR repertoire, so that reactivity to foreign antigens is permitted and reactivity to self-antigens prevented, ie, distinguishing self from nonself. Although selection occurs predominately in the thymus a significant element of extrathymic T cell development has been shown to occur in mice (2). In the intestine, these may serve to limit reactivity to self-antigens and possibly dampen responses to dietary and bacterial antigens presented to the gut. Therefore, the study of the TCR repertoire provides the context in which to consider the role of T cell activation in IBD. If IBD is an autoimmune disease, it is possible that control of T cell responses to foreign and self-antigens may be dysfunctional. Therefore, in the context of IBD, it is possible that the environment in which antigen is encountered, ie, the intestine, can have an important influence on the T cell repertoire and the nature of T cell responses. Identifying the changes in such control mechanisms will help clarify the role of T cells in IBD and may provide clues regarding the nature of disease-specific antigen.

To explore the mechanisms that control the induction of T cell tolerance in the intestine we have studied the function of mucosal T cells in mice. In addition, we have examined the cytotoxic function of T cell subsets in patients with Crohn's disease to determine whether TCR-restricted changes in function may reflect exposure to superantigens.

MUCOSAL T CELL DIFFERENTIATION

Significant differences exist between the mucosal and systemic immune system (3). The mucosal immune system is compartmentalized into distinct anatomical regions. Lymphocytes from mesenteric lymph nodes, Peyer's patches, lamina propria (LPL) and the epithelium differ in morphology, phenotype and function. Collectively these sites are referred to as the gut-associated lymphoid tissue. Antigens and bacterial products within the intestinal lumen all serve to activate mucosal T lymphocytes, as reflected by the unique phenotypes (4). For example, in humans, a majority of mucosal lymphocytes express surface markers associated with 'memory' or activated T cells, eg, CD45RA-low, CD45RO-high (5). In addition to the 'activated' state of mucosal lymphocytes, there is evidence that mucosal T cells in the mouse can develop extrathymically. In fact, the positive and negative selection of the TCR repertoire of thymic-independent intraepithelial lymphocytes (IEL) is thought to occur to some degree within the intestine (6-8). It is possible, therefore, that the local TCR repertoire is shaped to accommodate local antigen exposure.

These issues have been studied best in the IEL, which make up 10% to 15% of the cells in the epithelium (9). Most of these cells express the T cell marker CD8 and either the α/β or γ/δ TCR (10,11). T cells in IEL are unusual in that half lack pan-T cell markers such as Thy1 and CD5 (11,12) and CD8 is expressed as an α/α homodimer rather than the usual α/β heterodimer (11). It has been suggested that these unusual phenotypes identify the extrathymic lineage of T cells (6,7) that can express the γ/δ TCR or 'autospecific' α/β TCR. In mice, autoreactive T cells of either the α/β and γ/δ TCR subsets somehow escape thymic deletion. We have shown that IEL expressing the autoreactive variable $\alpha 6$ TCR in MIs-1^a mice fail to proliferate or secrete interleukin (IL)-2 in response to TCR stimulation. Importantly, this anergic response can be reversed by the addition of exogenous IL-2, suggesting a mechanism by which tolerance in autoreactive mucosal T cells can be overcome. If such a mechanism serves to control the response of autoreactive T cells in vivo then loss of such control can lead to intestinal damage and inflammation.

IEL in humans are similar to those found in mice in that they have cytoplasmic granules and can express the CD5⁺CD8⁺ phenotype (13,14). Although some express the γ/δ TCR, the majority of human IEL express α/β TCR (15).

It is not known whether human IEL contain 'autospecific' TCR or whether they can develop extrathymically.

TCR-TRIGGERED ACTIVATION

TCR molecules are composed of variable, diversity, joining and constant elements, analogous to the immunoglobulin molecule. During T cell differentiation noncontiguous gene segments undergo somatic rearrangement, randomly generating the diversity necessary to deal with the infinite number of foreign antigens (16,17). The self-reactive T cell clones that can emerge during such a random process are eliminated by deletion or functional inactivation. The TCR, which is a disulphide-linked heterodimer and is associated with monomorphic CD3 molecules, is responsible for the recognition of antigen (18).

Antigen or anti-CD3 activation of the TcR/CD3 complex leads to T cell proliferation and cytokine production. Because it is difficult in humans to study antigen-specific cytotoxic T cells, anti-CD3 stimulation allows one to bypass the need for antigen or major histocompatibility complex restriction to study T cell reactivity. In fact, cytotoxicity generated through anti-CD3 stimulation has been used to demonstrate that mucosal T cells have cytotoxic T cell activity (19). The target or physiological role of such cytotoxic cells is unknown (20); however, the ability to study the nature of these T cells can provide new information about T cell function in IBD.

T cell function and response to TCR-mediated activation in the mucosa differ from those seen in peripheral blood lymphocytes (PBL). In an infectious model of intestinal inflammation, Zeitz et al (21) showed that lymphocytes isolated from nonhuman primates infected with *Chlamydia trachomatis* did not proliferate in response to antigen but rather showed an increase in cytokine production. In a second study, anti-CD3 stimulation failed to induce proliferation of LPL or PBL cultured with mucosal tissue supernatants, suggesting that intestinal factors serve to dampen this response (22,23), which possibly explains the low proliferative response of IEL (24). Alternate pathways of mucosal T cell signalling such as anti-CD2 stimulation exist for both IEL and LPL (24,25). Therefore, T cell proliferation alone may not adequately reflect mucosal T cell responses. In addition, T cell responses require appropriate accessory signals such as co-stimulation of CD28 (26).

Furthermore, under appropriate conditions, TCR activation of immature T cells leads to apoptosis or programmed cell death, a part of the negative selection process in the thymus (27,28). Mature peripheral T cells can also be activated to undergo apoptosis (29,30); for example, antigen stimulation of T cells preactivated with IL-2 can lead to programmed cell death (30). Intestinal γ/δ and α/β TCR IEL can undergo apoptosis after TCR stimulation (31). Under certain conditions, anti-CD2 stimulation of activated peripheral T cells can lead to apoptosis (32). Therefore, the outcome of T cell activation by TCR depends on the circumstances surrounding the stimulating event, and there are a number of factors that can influence T cell reactivity.

ALTERATIONS OF MUCOSAL T LYMPHOCYTES IN IBD

Although a number of alterations of the humoral and cellular immune responses have been described in IBD (33,34) it is not clear which are directly related to the pathogenesis or etiology of the disease. Nonetheless, immune activation is a necessary part of the disease. What is known about changes in human T cells in IBD is, for the most part, nonspecific. Increases in IEL numbers have only rarely been described (35). IEL and LPL phenotypes are not overly altered, and although total LPL T cells are increased, the CD4:CD8 ratio is unchanged (33). Data on LPL function are contradictory – some studies have shown that IL-2 production is decreased (36) while others have noted an increase (37). No disease-specific cytotoxic function has been identified (4,33,34). As discussed, antigen-specific T cell responses in IBD are difficult to study. In PBL of IBD patients there is an increase in anti-CD3-induced cytotoxicity (38); however, in LPL there have been conflicting data regarding changes of this activity in IBD (19,39).

With the availability of molecular probes and monoclonal antibodies to TCR, changes in TCR expression have been examined to try to identify specific antigenic stimuli. This examination includes the use of monoclonal antibodies to TCR, variable-gene products and the use of reverse transcriptase-polymerase chain reaction analysis for variable-gene mRNA in chronic inflammatory and autoimmune diseases (40-42). Early studies in IBD failed to show clonal rearrangements at either the α/β or γ TcR loci in LPL (43). More recent work indicates that normal human IEL undergo oligoclonal expansion based on TCR variable-region gene expression (44), but there are few data regarding IBD-specific changes in mucosal lymphocytes (45). Increases in the proportion of T cells expressing TCR variable β -gene product in different chronic inflammatory diseases suggest that superantigens may be involved in the pathogenesis of some of these diseases (40,46).

Superantigens activate T cell subsets through binding to a particular variable-region gene product, usually on the β -chain of the TCR (47-49). In mice, in vivo stimulation with the superantigen staphylococcal enterotoxin causes proliferation and alterations in function in variable gene-restricted T cell subsets (50). In Crohn's disease, a subgroup of patients has an increased number of TCR variable β 8 PBL, raising the possibility that superantigens may be involved in activating T cells in IBD (51).

Because the initiation of Crohn's disease may be far removed in time from when most patients are diagnosed, it is possible that remnants of the initiating event may be found in changes in the expression of the TCR repertoire. It is also possible that changes may be detectable in the function of T cell subsets with or without changes in TCR variable-gene expression. Studies of the effect of superantigens on T cells in mice indicate that after initial proliferation of a restricted T cell subset there is loss of function and subsequent elimination through clonal deletion and apoptosis (50,52,53). Furthermore, in the initial stages of disease, patients with

Kawasaki's disease show a short-lived expansion of variable $\beta 2$ and variable $\beta 8$ TCR that, with time, is followed by the deletion of these T cell subsets (54). Therefore, measuring alterations in the proportion of T cells expressing different TCR variable-gene products may fail to detect significant changes relevant to the pathogenesis of Crohn's disease. Further insight into the possibility that a superantigen may be involved in these disorders would be discernible through examining changes in the function of the different T cell subsets as well as changes in TCR expression.

We have examined the cytotoxic activity of T cells bearing specific TCR variable-gene products using monoclonal antibodies specific for individual TCR variable-gene products. This redirected cytotoxicity assay reflects the cytotoxic potential of the T cell subset identified by the monoclonal antibodies used to cross-link the TCR and induce activation (55,56). Our findings indicate that little difference can be detected in the cytotoxic function between controls and Crohn's disease patients by using monoclonal antibodies that recognize five different T cell subsets. On the other hand, the cytotoxic activity of variable $\beta 8$ T cells in PBL was significantly decreased in patients with Crohn's disease. This was not due to changes in the total cytotoxic activity measured with anti-CD3 or to a decrease in the proportion of the CD8⁺ T cells expressing the variable $\beta 8$ TCR. Therefore, whatever the factor(s) involved in this change, it has seemed to affect a common TCR variable-gene expression selectively, which suggests that these patients may have been exposed to a stimulus that behaves like a superantigen. To explore this possibility, we examined the effect of staphylococcal enterotoxins B and E on the cytotoxic activity of variable $\beta 8$ T cells. The variable $\beta 8$ selective, staphylococcal enterotoxin E, failed to increase the cytotoxicity of variable $\beta 8$ T cells in Crohn's patients despite an appropriate proliferative response.

Recently, intestinal epithelial cells have been shown to present superantigens to mucosal T cells (57), although the degree to which this was variable-region selective was not indicated. Staphylococcal enterotoxins have been shown to

affect human T cell function (48,58,59) and can induce anergy without interfering in the cytotoxic function of T cell lines (60,61). Therefore, TCR activation can influence these effector functions independently of one another. The mechanism by which alterations in cytotoxic function of variable $\beta 8$ ⁺ T cells may contribute to the pathogenesis of Crohn's disease is unclear. The superantigen responsible for such changes in Crohn's disease is not necessarily of bacterial origin, although it is tempting to suggest that the increased intestinal permeability associated with chronic inflammation allows exposure to bacterial products. Alternatively, in autoimmune disease T cells have been shown to be resistant to tolerance induction (62). It is possible that the presence of variable $\beta 8$ T cells with altered cytotoxic function may represent a clone of T cells that have escaped tolerance induction, leaving only these functional changes as a marker of this abrogated attempt at tolerance (62).

CONCLUSIONS

The cause of IBD – Crohn's disease and ulcerative colitis – is unknown. It is likely that the intestinal immune system plays an important role in the pathogenesis and possibly the etiology of IBD. T lymphocytes, which are an intrinsic part of the immune response, would therefore be a critical component of the chronic inflammation of IBD. The intestinal lymphocytes, in particular, may hold the clues that will explain the cause of these diseases. Knowing where to search for these clues in the study of T cells remains a challenge.

Advances in the cellular and molecular biology of T cells continue to open new avenues for investigation of the role of T cells in the pathogenesis of autoimmune diseases. In particular, identification and isolation of TCR molecules and their genes, and the specific monoclonal antibodies that allow us to detect these products, provide new tools that can be used in dissecting T cell specificity in diseases of unknown etiology. As new advances are being made in our understanding of mucosal T cell function and differentiation in animal models, opportunities to explore new avenues in human mucosal T cells will emerge.

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