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γδ T cell receptor-positive cells of the human gastrointestinal mucosa: occurrence and V region gene expression in *Heliobacter pylori*-associated gastritis, coeliac disease and inflammatory bowel disease

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SUMMARY

T cells expressing the γδ heterodimer of the T cell receptor (TCR) were studied with respect to their occurrence and expression of γδTCR variable region (V) genes in the normal gastrointestinal mucosa and in a variety of inflammatory conditions. In controls, γδTCR$^+$ cells were a minority population confined to the epithelial compartment of stomach, small bowel and colonic mucosae. Unlike in the periphery, gastro-intestinal γδTCR$^+$ intraepithelial lymphocytes (IEL) were mainly Vδ1$^+$ (89·98 ± 17·70%); few were Vδ2$^+$ (6·04 ± 13·8%) or Vγ9$^+$ (11·38 ± 10·73%). All γδTCR$^+$ IEL were CD5^{low}; nearly half were CD8$^+$ and the remainder were CD4$^-$CD8$^-$ 'double negatives'. There was no significant change from normal in percentages of γδTCR$^+$ IEL in *H. pylori*-associated gastritis, Crohn's disease and ulcerative colitis. However, in coeliac disease, γδTCR$^+$ IEL were elevated from 2·54% (± 1·71) in controls to 29·6% (± 16·1) in untreated patients ($P < 0·001$) and 18·5% (± 7·2) in treated patients ($P < 0·001$) and more were CD4$^-$CD8$^-$. Otherwise, γδTCR$^+$ IEL phenotypes were little changed: the majority remained Vδ1$^+$Vδ2$^-$Vγ9$^-$ and all were CD5^{low}. These data suggest that increased γδTCR$^+$ IEL are not a generalized response to intestinal inflammation or to stress proteins, although the typical Vδ1$^+$Vδ2$^-$Vγ9$^-$ CD5^{low} phenotype is retained.

Keywords γδTCR$^+$ cells Vδ expression immunohistology double-label immunofluorescence

INTRODUCTION

The majority of T cells in peripheral blood and lymphoid organs express the αβ heterodimer of the T cell receptor (TCR), whereas T cells expressing γδTCR heterodimers represent a minority population of about 5% (reviewed by Raulet, 1989a). As the function of γδTCR$^+$ cells remains elusive, considerable interest has been generated by reports that intra-epithelial T lymphocytes of the murine epidermis and intestinal mucosa are γδTCR$^+$ (Stingl *et al.*, 1987; Goodman & Lefrancois, 1988). However, in the normal human intestinal mucosa, γδTCR$^+$ cells are a minority population of CD3$^+$ intra-epithelial lymphocytes (IEL) and are essentially absent from the lamina propria (Bucy, Chen & Cooper, 1989; Groh *et al.*, 1989; Trejdosiewicz *et al.*, 1989b; Spencer *et al.*, 1989; Halstensen, Scott & Brantdzaeg, 1989; Jarry *et al.*, 1990), although the percentage of γδTCR$^+$ IEL is higher in the colonic mucosa than in the small bowel (Trejdosiewicz *et al.*, 1989b).

It has been suggested that γδTCR$^+$ cells perform an 'autologous surveillance' role (Janeway, Jones & Hayday, 1988), removing damaged cells by recognition of 'stress' or 'heat shock proteins' of highly conserved homology between prokaryotes and eukaryotes (Haregewoin *et al.*, 1989; Raulet, 1989b). The autologous surveillance hypothesis thus predicts that in situations where cell damage occurs, as in an inflammatory response, there should be an increase in γδTCR$^+$ cells. The increase of γδTCR$^+$ IEL in coeliac disease (Spencer *et al.*, 1989; Halstensen *et al.*, 1989) is compatible with this hypothesis. However, the persistent elevation of γδTCR$^+$ IEL in coeliac disease after histological amelioration in patients following a gluten-free diet (Halstensen *et al.*, 1989), and the lack of elevation in patients with other small bowel enteropathies (Spencer *et al.*, 1989; Viney, MacDonald & Spencer, 1990), are not readily explicable by the autologous surveillance hypothesis.

In order to test further the autologous surveillance hypothesis, we have studied γδTCR$^+$ cells in a number of inflammatory gastrointestinal conditions, including gluten-sensitive enteropathy (coeliac disease), as well as gastritis associated with colonization by *H. pylori*, and inflammatory bowel disease (ulcerative colitis and Crohn's disease), where no aetiological agent has yet been identified. We have further examined expression of γδTCR variable (V) region genes. Here we report

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Table 1. Monoclonal antibodies used in this study

Antibody	Specificity	Class	Source
T cell receptor			
TCRδ1	TCR δ chain	IgG	M. B. Brenner
βF1	TCR β chain	IgG	M. B. Brenner
3/62	Vδ1	IgG	In preparation
TiVδ2	Vδ2	IgG	T. Hercend (Miossec *et al.*, 1990)
TiγA	Vγ9	IgG	T. Hercend (Triebel *et al.*, 1988)
Other T lineage			
UCHT1	CD3	IgG	P. C. L. Beverley
T4	CD4	IgG	Dako (High Wycombe, UK)
OKT4B	CD4	IgM	Ortho Pharmaceuticals (Raritan, NJ)
RFT1γ	CD5	IgG	G. Janossy
RFT1μ	CD5	IgM	G. Janossy
RFT8γ	CD8	IgG	G. Janossy
RFT8μ	CD8	IgM	G. Janossy

that the percentage of intraepithelial γδTCR$^+$ cells is significantly elevated only in coeliac disease, although the γδTCR$^+$ IEL continue to express γδTCR V region gene products typical of gut lymphocytes, rather than of peripheral γδTCR$^+$ cells.

MATERIALS AND METHODS

Patients and tissue specimens

Peroral gastric antral biopsies were obtained from 11 patients, of whom six had histologically diagnosed chronic superficial or atrophic gastritis, were shown to harbour gastric *H. pylori* organisms by histological staining with modified Giemsa stain, were urease positive using the CLOtest (Gist-Brocades, West Byfleet, UK) and were *H. pylori* antibody seropositive by ELISA. The five control patients of similar age and sex were endoscopically and histologically normal, had no histological evidence of *H. pylori* colonization, were urease negative and *H. pylori* seronegative.

Peroral small bowel (jejunum and duodenum) biopsies were obtained from 26 adult patients, of whom 16 had coeliac disease. Of these, seven were untreated and had the typical abnormal small bowel morphology of villous atrophy and crypt hyperplasia; all have subsequently shown a clinical and morphological response to a gluten-free diet. Nine patients had treated coeliac disease; all had shown a response to a gluten-free diet with normal histology or mild partial villous atrophy of their small bowel mucosa. The 10 small bowel control patients of similar age and sex had normal small bowel histology and did not have any evidence of small intestinal disease.

Colonic biopsies were obtained from 21 patients. Six patients had Crohn's disease of the colon; all had areas of active disease at the time of colonoscopy with characteristic histology. Eight patients had ulcerative colitis; seven had total colitis with varying degrees of active inflammation and one had distal active colitis. Biopsies from seven control patients of similar age and sex showed no evidence of histological abnormality and there were no other indications of inflammatory disease.

Biopsy samples were placed into Histocon medium, transported to the laboratory, orientated on cork, coated with OCT compound and snap-frozen in thawing isopentane. Cryostat sections (5 μm) were cut, throroughly air-dried and stored desiccated at −70°C.

Antibodies and double-label immunofluorescence

The primary antibodies used are detailed in Table 1. Antibody 3/62 is a new reagent specific for all isoforms of the Vδ1 gene product, and therefore has a broader specificity than antibody δTCS1, which only recognizes Vδ1 in conjunction with the δ chain junction gene product Jδ1 (Faure *et al.*, 1988; Wu *et al.*, 1988) and probably Jδ2 (Koning *et al.*, 1989). The preparation and characterization of 3/62 will be described in detail elsewhere (manuscript in preparation).

Double-label immunofluorescence was performed as described previously (Trejdosiewicz *et al.*, 1989a, 1989b), using a combination of IgG and IgM class monoclonal antibodies for all analyses (Table 1). After incubation with primary antibody combinations, sections were re-incubated with absorbed, class-specific goat anti-mouse IgG and goat anti-mouse IgM second layers, conjugated to FITC and TRITC, respectively. Immunoconjugates were purchased directly from Southern Biotechnology Associates (Birmingham, AL), or via Sera-Lab (Crawley Down, UK) and pre-assayed for titre and specificity. Specimens were aqueous mounted and examined using a Zeiss Axioplan microscope fitted with wide-aperture immersion objectives, epifluorescent illumination and selective filters for FITC and TRITC. All cell counts were based on double-label experiments, and were performed by two independent observers using a ×40 objective. IEL were enumerated separately. For each biopsy section, all CD3$^+$ IEL were counted.

Statistical analysis

Parametric statistics (mean ± s.d.) were used descriptively and non-parametric methods (Mann–Whitney *U*-test) were used to estimate statistical significance.

RESULTS

Expression of γδTCR in the normal gastrointestinal mucosa

Expression of γδTCR was essentially confined to CD3$^+$ cells of the epithelial compartment. However, γδTCR$^+$ cells were infrequent in the gastric and small intestinal mucosa. In the

Table 2. Expression of γδTCR V region gene products by small bowel intraepithelial γδTCR$^+$ lymphocytes

Patient Group	*n*	Vδ1 (%)	Vδ2 (%)	Vγ9 (%)
Normal controls	8	89·98 (±17·704)	6·04 (±13·82)	11·38 (±10·73)
Untreated coeliac disease	7	80·68 (±16·85)	15·65 (±11·07)	4·22 (±3·22)
Treated coeliac disease	8	97·32 (±4·10)	7·93 (±10·33)	2·48 (±4·23)

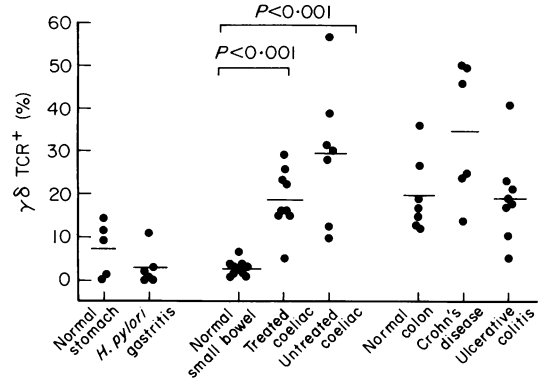

Fig. 1. Scatter plots of the percentages of CD3$^+$ intraepithelial T lymphocytes of the gastrointestinal mucosa which express the γδ T cell receptor (TCR) in controls and patients with inflammatory conditions. Horizontal lines indicate sample means. Statistical significance (Mann–Whitney *U*-test) was as indicated, all other differences were not significant.

stomach, γδTCR$^+$ IEL comprised 7·22% (±6·33) of all CD3$^+$ IEL (range 0–14·3%). In the small bowel, γδTCR$^+$ comprised 2·54% (±1·71) of IEL (range 0·91–6·3%). In the colon, the percentage of γδTCR$^+$ IEL was higher and more variable (19·97±8·73%; range 12·24–36·1%). γδTCR$^+$ cells in the lamina propria were extremely rare (<0·1 %), with none being seen in most biopsy sections.

γδTCR$^+$ cells did not co-express CD4. CD4$^+$ IEL were a 10–20% minority population, all of which were αβTCR$^+$. Throughout the gastrointestinal tract, about half of the γδTCR$^+$ IEL were CD8$^+$; 48·4 (±29·2) in stomach, 42·7 (±31·24) in the small bowel, and 44·3 (±26·78) in the colon. The remainder did not express either CD4 or CD8, and were considered as 'double negatives.' γδTCR$^+$ cells were invariably negative for the CD5 *pan*-T marker and hence scored as CD5^{low}.

Few γδTCR$^+$ IEL were either Vδ2$^+$ (6·04±13·8%) or Vγ9$^+$ (11·38±10·73%); the majority were Vδ1$^+$ (89·98±17·70%; range 52–100%) (Table 2). Vδ1$^+$ and Vδ2$^+$ cells comprised 98·38% (±10·76) of all γδTCR$^+$ IEL. Overall, the percentage of Vγ9$^+$ cells was higher than Vδ2$^+$ cells ($P<0·01$); in two specimens, no Vδ2$^+$ cells were observed, whereas the percentages of Vγ9$^+$ cells were 23% and 7%, respectively. Thus, Vδ1 and Vγ9 expression was not mutually exclusive, although the majority of the Vδ1$^+$ cells were Vγ9$^-$. In some specimens, the counts obtained with the anti-Vδ1 antibody 3/62 were slightly higher than for the anti-*pan* γδTCR antibody TCRδ1. This was thought to reflect the fact that some αβTCR$^+$ cells co-express Vδ1 (Miossec *et al.*, 1990).

Expression of γδTCR in gastrointestinal inflammatory conditions.

H. pylori-*associated gastritis*

In *H. pylori*-associated gastritis, there was no significant change in either percentage (Fig. 1) or in phenotypic distribution of γδTCR$^+$ IEL. Approximately half of γδTCR$^+$ cells were CD4$^-$CD8$^-$ double negatives, the remainder being CD8$^+$, and all were CD5^{low}. As in controls, CD4$^+$ cells were all of the αβTCR$^+$ type. Of the γδTCR$^+$ IEL, cells of the Vδ1$^+$Vδ2$^-$Vγ9$^-$ phenotype remained the dominant population.

Adult coeliac disease

In virtually all patients with coeliac disease, there was a striking increase in γδTCR$^+$ IEL in surface, crypt and glandular epithelia. Of the treated patients, only one had a value (4·9%) that overlapped the normal range; the remainder had values ranging from 14·8% to 28·8% (mean of whole group 18·5±7·24%). In untreated patients, the range was 9·3–57·0% (mean 29·6±16·1%). The difference between controls and each patient group was highly significant ($P<0·001$). Although untreated patients showed a greater mean increase of γδTCR$^+$ IEL relative to treated patients, the difference was not statistically significant (Fig. 1). There was no clear relationship between the percentage of γδTCR$^+$ IEL and either the degree of pathological damage to the mucosa or the length of time on a gluten-free diet: high γδTCR$^+$ IEL percentages were noted in several treated patients of essentially normal morphology.

As in the normal mucosa, γδTCR$^+$ T cells in coeliac disease did not co-express CD4 or CD5 and all were either CD8$^+$ or double negatives. However, percentages of γδTCR$^+$ IEL which were CD8$^+$ were reduced to 28·25% (±8·98) and 23·79% (±12·06), respectively, in the treated and untreated patient groups, compared with 42·7% (±31·24) in the controls. Taking into account the double negatives, the overall percentage of CD4$^+$ IEL was reduced from 15·47% (±12·40) in controls, to 6·97% (±4·82) in treated patients and 6·57% (±3·89) in untreated patients ($P<0·05$). Percentages of γδTCR$^+$ IEL expressing Vδ1 and Vδ2 were not significantly altered compared with controls (Table 2), although consistently fewer Vγ9$^+$ cells were found in coeliac mucosa ($P<0·05$). As in normal controls, Vδ1$^+$ plus Vδ2$^+$ cells accounted for >95% of all γδTCR$^+$ IEL.

Inflammatory bowel disease

In all inflammatory bowel disease specimens, the phenotypic distribution of γδTCR$^+$ IEL remained unaltered: all were CD5^{low}, CD4$^-$, CD8$^+$ or CD8$^-$, and mainly Vδ1$^+$Vδ2$^-$Vγ9$^-$. In three out of six patients with Crohn's disease, there was an increase in the percentage of γδTCR$^+$ IEL (range 45–50%), although overall, the results (34·57±15·74%) were not statistically significant compared with controls (19·74±8·73%) (Fig. 1). Of the three biopsies with elevated γδTCR$^+$ IEL, one was

from an histologically normal area in a patient with quiescent disease, one was from an histologically abnormal area with active inflammation, although the patient was clinically in remission, and one was from actively inflamed tissue from a patient in relapse.

There was no difference in the percentage of γδTCR$^+$ IEL in ulcerative colitis (19·15 ± 10·56%) compared with controls (19·74 ± 8·73%). There was no clear relationship between percentages of γδTCR$^+$ IEL and the presence of active disease. Only one patient, who had an actively inflamed mucosa with total colitis, showed a percentage (40·9%) of γδTCR$^+$ IEL falling outside 3 s.d. of the remainder of the group. However, the lowest value observed (4·9%) was also from an area of actively inflamed mucosa of another patient.

DISCUSSION

This study of inflammatory conditions of the gastointestinal tract demonstrates that increased γδTCR$^+$ IEL are not specifically associated with inflammation. Only in coeliac disease was there a significant increase in the presence of IEL T cells expressing γδTCR. In this respect, our results fully confirm and extend the observations of Spencer *et al.* (1989), who first reported an increase in γδTCR$^+$ IEL in coeliac disease, and those of Halstensen *et al.* (1989), who also reported the persistence of γδTCR$^+$ IEL in coeliac disease patients following a gluten-free diet. Although we were unable to study abnormal small bowel mucosae from patients with conditions other than coeliac disease, other investigations have shown that there is no increase in γδTCR$^+$ IEL in a variety of non-coeliac conditions affecting the small intestine (Spencer *et al.*, 1989; Viney *et al.*, 1990).

It is of interest that the percentage of γδTCR$^+$ IEL does not appear to be raised in response to inflammation *per se*, as suggested by the findings in *H. pylori*-associated gastritis and in inflammatory bowel disease. Our findings in inflammatory bowel disease are supported by previous reports, where no increase in the percentage of γδTCR$^+$ IEL has been found. Halstensen *et al.* (1989) found no increase in a single case of ulcerative colitis studied. Spencer *et al.* (1989) reported no increase of γδTCR$^+$ in the gut of children with Crohn's disease, although no data or patient numbers were given. Finally, Ullrich *et al.* (1990) found no increase in two patients with Crohn's disease, but also none in the one coeliac patient studied.

Previous reports have shown that the majority of γδTCR$^+$ IEL of the small bowel are δTCS1$^+$ (Spencer *et al.*, 1989; Halstensen *et al.*, 1989). However, antibody δTCS1 recognizes Vδ1 only when co-expressed with Jδ1 (Faure *et al.*, 1988; Wu *et al.*, 1988) and possibly Jδ2 (Koning *et al.*, 1989). Using antibody 3/62, which recognizes Vδ1 irrespective of chain junction region (Jδ) gene expression, we have found even higher percentages of Vδ1$^+$ cells: in several specimens, all γδTCR$^+$ cells were Vδ1$^+$. This suggests that intestinal Vδ1$^+$ IEL do not exclusively use the Jδ1 (and Jδ2) gene products. From our data, it is clear that this phenotypic distribution of γδTCR$^+$ IEL is not unique to the small intestine, but is also found in the gastric and colonic mucosae.

The predominance of Vδ1$^+$Vδ2$^-$ Vγ9$^-$ cells in the entire gastrointestinal mucosa argues that gastrointestinal γδTCR$^+$ cells are a population distinct from peripheral γδTCR$^+$ cells, which are mainly of the Vδ1$^-$ Vδ2$^+$Vγ9$^+$ phenotype (Triebel *et al.*, 1988; Miossec *et al.*, 1990). Further evidence for this hypothesis stems from the observations that intestinal γδTCR$^+$ IEL are CD5^{low} (Trejdosiewicz *et al.*, 1989b; Jarry *et al.*, 1990), whereas peripheral γδTCR$^+$ are CD5^{high} (Groh *et al.*, 1989).

The significance of reduced CD5 expression by γδTCR$^+$ cells remains unknown. It is well established that a subset of intestinal CD8$^+$ IEL show reduced expression of CD5 (Selby *et al.*, 1983; Malizia *et al.*, 1985; Trejdosiewicz *et al.*, 1989a, 1989b; Ebert, 1989; Jarry *et al.*, 1990). Although an increase in the percentage of IEL of the CD5^{high} phenotype has been reported in untreated coeliac disease (Selby *et al.*, 1983; Malizia *et al.*, 1985), our data show that this is not due to any increase of γδTCR$^+$ cells, which remained CD5^{low} in all coeliac specimens.

Our results do not support the proposed role of γδTCR$^+$ cells in autologous surveillance by virtue of cross-reactivity between prokaryotic and eukaryotic stress proteins (Janeway *et al.*, 1988; Haregewoin *et al.*, 1989; Raulet, 1989b). The relative abundance of γδTCR$^+$ cells in the normal colon may be due to the microbial flora. However, sensitization to bacterial antigens should result in an increase of γδTCR$^+$ cells in *H. pylori*-associated gastritis, whereas none was found. Similarly, sensitization to eukaryotic stress proteins should result in increased γδTCR$^+$ cells in all inflammatory conditions with epithelial damage, whereas none have been observed except in coeliac disease. Thus, it seems unlikely that gastrointestinal γδTCR$^+$ IEL respond exclusively to either stress proteins of damaged epithelium or bacteria. It has also been suggested that some γδTCR$^+$ cells recognize CD1 antigens (Porcelli *et al.*, 1989; but see Faure *et al.*, 1990). Although colonic epithelium is CD1a$^+$ (Jack *et al.*, 1989), there is no expression of CD1a in either normal or coeliac small bowel epithelia, as judged by immunohistological staining with antibody NA1/34 (Selby *et al.*, 1983; and unpublished observations). Thus, epithelial expression of CD1 may account for the higher percentage of γδTCR$^+$ IEL in the colon, but does not account for the increase in coeliac disease.

Many questions remain to be answered regarding the function of gastro-intestinal γδTCR$^+$ IEL. Although our results provide no answers, they do suggest that γδTCR$^+$ IEL are a distinct population of cells and are not increased purely in response to an inflammatory process. It will be of great interest to see whether increased γδTCR$^+$ IEL are eventually shown to be specific for, and even pathogenic in coeliac disease.

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