INFLAMMATORY BOWEL DISEASE

Related IgA1 and IgG producing cells in blood and diseased mucosa in ulcerative colitis

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Background: Ulcerative colitis (UC) is a chronic inflammatory bowel disease in which the colonic mucosa is infiltrated with plasma cells producing IgG autoantibodies. It is not known whether this represents a local mucosal response which has switched to IgG or a peripheral response which may have been initiated by peripheral antigen which homed to the colonic mucosa. The clonal distribution of IgG secreting cells and isotype switched variants in UC is not known.

Aims: To investigate the clonal distribution of mucosal IgG in UC and to search for related IgG and IgA secreting cells in normal and diseased mucosa and blood in UC. To investigate characteristics which may discriminate between the mucosal and peripheral repertoire in the normal mucosa and in UC.

Patients: Blood and normal and diseased mucosa from two patients with UC were studied.

Methods: Immunoglobulin gene analysis and clone specific polymerase chain reaction were used to study the clonal distribution and characteristics of IgG and related IgA in the mucosa and blood of patients with UC.

Results: The IgG response in the mucosa of UC patients included widespread clones of cells that were present in both the diseased mucosa and blood but that were scarce in normal mucosa. Clonally related IgA class switch variants, all IgA1, were detected but also only in the diseased mucosa and blood. This suggests that these clones home preferentially to the diseased mucosa. We showed that J_H1 usage was characteristic of the peripheral repertoire, and that examples of J_H1 usage were observed in mucosal IgG in UC.

Conclusions: Overall, these data are consistent with a model of UC in which a peripheral response is expressed and expanded in the colonic mucosa.

Ulcerative colitis (UC) is a chronic, relapsing, organ specific, inflammatory disease of the colon which tends to be restricted to the mucosa. In areas of chronic inflammation in UC, the inflammatory infiltrate includes an increase in mucosal plasma cells. Although plasma cells secreting all isotypes are increased in UC, the population with the greatest percentage increase is that secreting IgG, predominantly IgG1.¹ In addition, the ratio of IgA1:IgA2 secreting plasma cells increases.² The higher IgA1:IgA2 ratio and increased proportion of IgG producing cells are characteristics of the peripheral humoral response.³ Evidence suggests that proinflammatory complement fixing IgG autoantibodies, which may arise through cross reactivity with bacterial antigens, are involved in the pathogenesis of UC.^{4–6} IgG antibodies to organ specific autoantigens such as colonic epithelial cells, and mucosal production of more broadly distributed autoantigens such as antineutrophil cytoplasmic antibodies, are consistently identified in the colon in UC.^{4,5,7} It is not known whether this IgG response originates in the periphery or in the mucosa. It is possible that mucosal IgG in UC represents a peripheral response stimulated by peripheral antigen, which isotype switched and then homed to the mucosa. A response to a peripheral antigen may cross react with antigen in the mucosal microenvironment, such as a component of the flora and/or autoantigen from which it is normally segregated by the endothelial barrier,⁸ resulting in mucosal inflammation. Previous studies of factors that influence lymphocyte traffic have demonstrated that homing is distorted in UC,^{9,10} supporting the idea that a peripheral IgG response may be aberrantly recruited to the gut. If this is the case, then characteristics of the peripheral repertoire would be expected in mucosal IgG in UC. In addition, IgG producing

cells and any cells clonally related to them would be expected to be focused to the site of the disease and blood.

Another alternative is that aberrant switching from IgM to IgG rather than to IgA may be induced in the mucosa in UC as a result of the change in cytokine profile.¹¹ IgA and IgG differ considerably in their function. IgA generally has a passive role although it is capable of stimulating polymorphonuclear leucocyte respiratory burst whereas IgG is capable of stimulating polymorphonuclear leucocyte respiratory burst and opsonisation, and efficient complement fixation.^{12,13} If IgG antibodies which are normally absent from the mucosa arose by local isotype switching, then this change in isotype profile may result in tissue damage such as that observed in UC.

Rearranged immunoglobulin heavy chain genes have unique junctional regions where V_H, D, and J_H recombine to form the third complementarity determining region (CDR3). This allows identification of related cells, including isotype switched variants by alignment of CDR3 DNA sequences. Polymerase chain reaction (PCR) primers that are CDR3 specific can also be used with 3' constant region primers to search for clonally related cells, including isotype switch variants.¹⁴ These methods have been used here to analyse the clonal distribution of IgG and isotype switch variants in mucosa and blood in UC. Three families of V_H genes were studied to allow comparisons of "like-with-like" within each family in terms of hypermutation and J_H usage. V_H5 was selected because it is a

Abbreviations: UC, ulcerative colitis; CDR, complementarity determining region; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR; PBL, peripheral blood lymphocytes.

Table 1 Sequences of oligonucleotides used as polymerase chain reaction primers

Oligonucleotide primers	Sequence (5' to 3')
Amplification of IgV _H DJ _H -C _H transcripts	
External 5' primers*	
V _H 1-leader	CCATGGACTGGACCTGGA
V _H 3-leader	CCATGGAGTTTGGGCTGAGC
V _H 5-leader	ATGGGGTCAACCGCCATCCT
Internal 5' primers†	
V _H 1-FR1	CCTCAGTGAAGTCTCCTGCAAGG
V _H 3-FR1	GGTCCTGAGACTCTCCTGTGCA
V _H 5-FR1	GAAAAAGCCCGGGGAGTCTCTGAA
3' C _H primers	
C _μ	CAGGAGACGAGGGGGAA
C _α	GGAAGAAGCCCTGGACGAGC
C _γ	CACCGTCACCGGTTCCG
IgG clone CDR3 specific primers	
Clone 1, patient No 1	CTAAGAAGTAGCAGCACCTG
Clone 2, patient No 1	GCGCCTTTACCCCTACTATCG
Clone 3, patient No 1	CGTCGGGAGGGAGCTTCTATG
Clone 4, patient No 1	GGAAGCAGACACTATGG
Clone 5, patient No 2	GAGGTGGGAGGACTATAGC
Clone 6, patient No 2	CGCGTAGGGGCGGTCTGGC
Clone 7, patient No 2	CGGGTACAGCAGTGGC

*External 5' primers hybridise to the 5' leader sequence.

†Internal 5' primers hybridise to the framework region 1.

small family¹⁵ with a bias towards J_H6 usage in UC,¹⁶ although earlier studies using microdissection could not determine whether this was isotype restricted. V_H1 and V_H3 were studied because of their common usage¹⁷ and because V_H3 is preferentially used by one group of autoantibodies in UC.¹⁸ The distribution of IgA subclasses in UC has previously been analysed using subclass specific antibodies.³ By sequence analysis we are able to determine not only the ratio of IgA subclasses in normal bowel and UC but also the IgA subclasses associated specifically with mucosal IgG.

MATERIALS AND METHODS

Human tissues used

Samples of colonic mucosa from two patients with UC undergoing total colectomy were received in the laboratory within an hour of surgery. Patient No 1 was a male aged 23 years and patient No 2 a female aged 64 years. The samples were immediately snap frozen in liquid nitrogen and stored until required. In addition, a peripheral blood sample was obtained from each patient on the day of surgery. Peripheral blood lymphocytes (PBLs) were isolated using Ficoll-paque research grade (Amersham Pharmacia Biotech Ltd, Little Chalfont, UK) according to the manufacturer's instructions. PBLs were immediately snap frozen and stored in liquid nitrogen until required. Previous studies have shown that mucosal plasma cells with indistinguishable properties (frequency of V_H hypermutation and J_H usage) are present throughout the normal large and small bowel and other mucosal sites such as the

salivary gland.^{16 19 20 21 22 23} However, it has been shown that within this population, it is vitally important to control for the variable and constant region usage when analysing hypermutation and J region usage. Therefore, control IgM and IgA sequences using V_H5 from another study of mucosal plasma cells were used,¹⁹ which were isolated from human duodenum. IgG sequences using V_H5 were generated from the same tissues specifically for use in this study.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from colonic mucosa or PBLs using 1 ml of TRIzol reagent (Life Technologies, Paisley, UK) according to the manufacturer's instructions. One fifth of the total RNA yielded was used for synthesis of single strand cDNA using oligo-dT¹⁹ primer and M-MLV reverse transcriptase (Promega, Southampton, UK) in a final volume of 20 μl.

PCR amplification of IgV_HDJ_H-C_H transcript

All reactions were performed using a Hybaid Omnigene PCR machine. The sequences of all PCR primers used for the amplification of Ig_H gene segments are shown in table 1. IgV_HDJ_H-C_μ, -C_α, and -C_γ transcripts were amplified using a semi-nested PCR strategy. A 5 μl sample of the 20 μl reverse transcription product was amplified using 0.5 units of *Taq* DNA polymerase (Promega) in a 50 μl PCR reaction using 100 ng of each primer, 200 μM of each dNTP, and 1.5 mM MgCl₂ in 1×*Taq* DNA polymerase reaction buffer. A hot start of 94°C for seven minutes was performed before addition of the *Taq* DNA polymerase. For the first round of PCR, 30 cycles of 40 seconds at 94°C, 45 seconds at 60°C, and two minutes 40 seconds at 72°C were performed, followed by an additional five minute extension of the PCR products at 72°C. For the second round of PCR, 30 cycles of 40 seconds at 94°C, 45 seconds at 55°C, and two minutes 40 seconds at 72°C were performed, followed by an additional five minute extension of the PCR products at 72°C. The second round PCR reaction used 2 μl of product from the first round PCR as template DNA. PCR primers were manufactured by Genset SA (Paris, France) or Interactiva Biotechnologie GmbH (Ulm, Germany).

Cloning and nucleotide sequencing

PCR products were analysed on a 3.5% NuSieve 3:1 agarose gel (Flowgen, Lichfield, UK) and stained with ethidium bromide. PCR products were cloned into the pGEM-T Vector (Promega), sequenced on both strands using the dye terminator cycle sequencing kit (PE Applied Biosystems, Warrington, UK), and analysed with an ABI 377 automated DNA sequencer (PE Applied Biosystems). Some of the Ig genes were sequenced by Qiagen GmbH (Hilden, Germany) or MWGAG Biotech (Ebersberg, Germany). V_H and J gene segment sequences were analysed using GeneJockey II software and the V BASE Sequence Directory which contains all known human germline heavy and light chain gene segments. J gene segments were assigned manually. An Ig gene rearrangement was considered productive if the V(D)J junction maintained the reading frame into

Table 2 Number of different IgH sequences and rearrangements (in parentheses) studied

	Patient No 1				Patient No 2		Control
	Mucosa			Blood	Mucosa	Blood	Mucosa
	VH1	VH3	VH5	VH5	VH5	VH5	VH5
IgM	7 (7)	7 (7)	11 (6)	8 (7)	17 (11)	8 (7)	16 (14)
IgA	9 (9)	9 (9)	18 (14)	7 (7)	15 (14)	7 (6)	18 (18)
IgG	8 (8)	10 (10)	15 (13)	7 (7)	9 (8)	8 (5)	13 (12)
Total	24 (24)	26 (26)	44 (33)	22 (21)	44 (33)	23 (18)	47 (44)

Figure 1 Alignment of two sets of related sequences identified by CDR3 homology from the diseased mucosa from patient No 2. IgM-1 is related to IgA-1, sharing the same CDR3, and IgA-2 is related to IgG-2, sharing the same CDR3. Each pair of sequences has been diversified by both class switch recombination and somatic hypermutation. All four sequences use V_H5-51 and J_H4B. Dashes indicate identity of the sequences to germline. Upper case letters in the alignments represent replacement mutations and lower case letters silent mutations from germline. The asterisk identifies the RNA splice site and the sequence after the asterisk represents the appropriate constant region sequence. The primers are in bold italics. The underlined bases in the constant region sequence identify the nucleotides that enable the classification of the IgA sequences as IgA1 subclass.

the J segment (inframe) and there were no translational stop codons. Where the reading frame was not maintained into the J gene segment, the rearrangements were designated out of frame. Nomenclature according to the V BASE Sequence Directory has been used. When a group of related genes was found, the mean number of mutations for that group was used in the analysis of the overall frequency of somatic mutations. Because of the nature of the internal V_H1 FR1, V_H3 FR1, and V_H5 FR1 PCR primers used for amplification, somatic mutations could not be detected in the first 70 nucleotides, 69 nucleotides, and 56 nucleotides of the FR1 regions of IgV_H1, IgV_H3, and IgV_H5 gene segments, respectively.

Design of B cell clone specific CDR3 5' PCR primers

Criteria used for designing the 5' CDR3 primers were: PCR primer length equivalent or above 16 bp, high GC base composition ($\geq 60\%$), lack of homology with either the C_H antisense 3' primer or with any other mammalian sequences (as determined by searching GenBank), lack of internal repeat, and a C or a G at the 3' end of the PCR primer when possible. It was also essential that the clone specific sequence was present 3' of the primer before JH to allow confirmation that the PCR was clone specific by sequencing. Negative controls were first round PCR product IgV_H4DJ_H-C_H amplified from a PBL or tonsil from at least five different patients.

Statistical methods

Comparisons of J gene segment usage were carried out using χ^2 tests. All other data were tested for normality using Microstat software. As the populations compared were normally

distributed, arrays were compared using the Student's *t* test. Observed differences were considered to be statistically significant at $p \leq 0.05$.

RESULTS

A total of 230 sequences were analysed. Details of the number of different heavy chain sequences and rearrangements studied are shown in table 2. We analysed 183 different sequences from two patients with UC, 138 from the mucosa and 45 from blood. Some of these sequences shared the same CDR3 but had single base differences in the V region and could therefore be considered to be part of the same clone which had diversified by somatic hypermutation. Throughout the study, these clones were considered to be a single rearrangement so that 116 different rearrangements from the mucosa and 39 from blood were included. In addition, 47 sequences (44 different rearrangements) from normal intestinal mucosa from other individuals were included for comparison.

Identification of related sequences

Within the related groups of cells sharing the same CDR3, some used different constant regions. Related IgM and IgA1 sequences from the same block of diseased mucosa from patient No 2 were observed, which shared four mutations. In addition, IgM had acquired six further mutations and the IgA 25 further mutations after the switch event. This indicates that the isotype switch event occurred early in the evolution of this clone, and diversification continued independently after the switch event. Related IgG and IgA1 sequences were also

Figure 2 Graphic representation of the number of mutations in IgV_H5 in IgM, IgA, and IgG sequences in normal mucosa and diseased mucosa from a patient with ulcerative colitis (UC). When comparisons are made within isotypes, there were no differences between UC and normal tissues. In UC, as in normal mucosa, IgM had a significantly lower mean frequency of mutations than IgA or IgG, and there was no difference between IgA and IgG.

observed in the same block of diseased mucosa from patient No 2. These sequences shared seven common mutations from the germline. In addition, IgA and IgG had acquired a further seven and 10 mutations, respectively, after the switch event (fig 1). Identical IgM sequences were observed in blood and mucosa from patient No 2, which shared 13 mutational differences from the germline V_H5 sequence from which they originated.

The frequency of V region hypermutation in the V_H5 sequences of each isotype in mucosa in UC (data from patient Nos 1 and 2 pooled) and in control mucosa is illustrated in fig 2. In UC, as previously observed in normal mucosa,¹⁹ the frequency of mutation in IgV_H used by IgM was significantly lower than that observed in IgV_H used by IgA or IgG ($p=0.03$ and $p=0.004$, respectively, using a t test). There was no

significant difference in the frequency of hypermutation in UC and normal mucosa when comparisons within isotypes were made.

J_H usage by IgM, IgA, and IgG in the mucosa and blood in UC

Rearrangements using J_H1 were observed in mucosal IgG in this study (fig 3). This is the first time that J_H1 has been observed in any sample, consisting largely of mucosal plasma cells. J_H1 was not observed in IgM or IgA from the same patients. In the gut, J_H6 was clearly and significantly associated with IgG compared with IgM or IgA in cells using V_H5 in patient No 1 ($p=7.8 \times 10^{-6}$ and 0.0005 by χ^2 for IgG v IgA and IgG v IgM, respectively), and was also significantly higher in IgG than in IgM in blood ($p=0.009$). However, when V_H1 and V_H3 were also studied in patient No 1, this trend towards J_H6 usage specifically by IgG was not apparent (fig 3).

Analysis of clonal distribution of IgG secreting cells and their isotype switch variants in UC

Out of a total of 11 putative clone specific primers tested, designed for specific identification of 11 different IgG secreting clones, seven detected clones against which they were raised reliably, which could be confirmed by sequencing, and were negative in five other preparations of PBLs or tonsils from other individuals.

Patient No 1

In patient No 1, four samples of mucosa (two samples of diseased mucosa and proximal and distal resection margins) and PBLs were analysed. The distribution of cells related to four IgG secreting cells from a sample of diseased mucosa was analysed using 5' clone specific heavy chain CDR3 primers used together with 3' primers in either C γ or C α to identify cells which were related to the original Ig sequence which were either IgG or IgA. The distribution of related IgG and IgA+ cells are shown in table 3.

All four IgG secreting clones were present in both available blocks of diseased mucosa in this patient. They were each also

Figure 3 Pie charts illustrating J_H usage: J_H1=green; J_H2=yellow; J_H3=white; J_H4=black; J_H5=blue; J_H6=red. (A) J_H usage in IgM, IgA, and IgG genes using V_H5, isolated from mucosa and blood of patients (P1, P2) with ulcerative colitis (UC) and normal control mucosa. Identification of J_H1 in IgG in UC is the first ever identification of J_H1 usage in the mucosa. There was significantly more J_H6 usage in IgG than in IgM in blood and mucosa of patient No 1. (B) J_H usage in IgM, IgA, and IgG genes using V_H1 and V_H3 in patient No 1. The bias towards J_H6 usage seen in association with V_H5 was not seen in association with V_H1 or V_H3. However, two further examples of J_H1 usage by mucosal IgG in UC were observed, one each in V_H1 and V_H3.

Table 3 Distribution of IgG clones and related IgA switch variants using CDR3 clone specific primers

Distribution of cells related to IgG clones 1–7											
Patient No 1									Patient No 2†		
IgG-clone 1		IgG-clone 2		IgG-clone 3		IgG-clone 4					
IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG-clone 5	IgG-clone 6	IgG-clone 7	
Sample											
Diseased colon											
1*	+	+	+	–	+	–	+	–	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+
3	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
4	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Normal mucosa											
1	–	–	+	–	–	–	–	–	–	–	–
2	+/-	–	–	+	+/-	–	+/-	–	–	–	–
3	NT	NT	NT	NT	NT	NT	NT	–	–	–	–
4	NT	NT	NT	NT	NT	NT	NT	–	–	–	–
PBL	+	+	+	–	+/-	+	–	+/-	+	+	–

*Sample from which the original IgG sequences were derived.

†No IgA+ cells related to IgG were detected by this method in patient No 2.

+, strong unambiguous band; +/-, weak band.

NT, no tissue: two blocks of each of normal and diseased mucosa only for patient No 1.

Figure 4 Confirmation of clonal relationship between genes identified by CDR3 specific primers in patient No 1 by sequencing. IgG clone 1, the original sequence against which the primers were designed, is aligned to V_H32 and J_H6. Aligned to this are the sequences of the polymerase chain reaction products generated using the clone specific primers. Samples tested were two areas of diseased mucosa designated sample 1 and sample 2, and peripheral blood lymphocytes (PBL). After the RNA splice site, marked with an asterisk, the products generated with the C_γ primer were aligned to the C_γ sequence and products generated with the C_α primer were aligned to C_α. The bases that determine the IgA1 subclass designation are underlined. The related IgG and IgA sequences all share considerable homology in the CDR3 before they begin to align with J_H6. There was a single base difference in the related IgG genes which do not have the T to C mutation. The positions of the primers are shown in bold italics.

detected in one of the two blocks of normal mucosa, and three of the four were identified in blood. IgA switch variants of each clone were detected in the diseased mucosa and blood but not in the normal mucosa. Identity was confirmed by sequencing (fig 4). The exclusive use of IgA1 in the switch variants is compared with the overall frequency of IgA subclasses observed by sequence analysis in the blood and mucosa in these patients and normal controls (table 4).

Patient No 2

In patient No 2, eight samples of mucosa and a sample of blood were available for study (table 3). Of the eight samples of mucosa, four were of diseased colon, taken at approximately 10 cm intervals. The additional samples were of normal colon, caecum, and two samples of the normal ileum. Three clones of IgG originally identified in the most proximal sample of disease mucosa could be traced using clone specific primers. All three clones were present in all of the blocks of diseased mucosa and two of the three were identified in blood. The

Table 4 Analysis of IgA subclasses

	IgA1	IgA2	%IgA1
Control mucosa	8	10	44
Mucosa patient No 1	6	7	46
Mucosa patient No 2	10	4	71
Blood patient No 1	6	1	86
Blood patient No 2	5	2	71
IgA related to mucosal IgG in UC	5	0	100

UC, ulcerative colitis.

identity of the clones was confirmed by sequencing. The clone was not identified in any of the normal tissues from this patient. The clone specific PCR using the C α region was not specific and therefore IgA switched variants could not be detected in this patient using CDR3 primers. However, related IgG and IgA expressing cells did exist in this patient because they were detected by sequence alignment (fig 1).

DISCUSSION

Using clone specific primers, we have observed clones of IgG producing cells in UC that are widespread throughout the diseased mucosa and blood but are relatively rare in normal mucosa. Their rarity in normal mucosa is unlikely to be due to the relative number of cells sampled from the normal and diseased mucosa because this method produced strong unambiguous results when diseased mucosa was analysed and a clean negative when most samples of normal mucosa from the same individual were studied. Mucosal cells traffic via the blood and the presence of these cells in blood *per se* does not allow us to conclude that they are of peripheral origin. However, the fact that they were relatively rare in normal mucosa from the patient suggests that the IgG secreting cells observed are not part of the normal population of precursors of plasma cells that home to mucosal sites. These observations suggest a model of UC in which the IgG secreting clones migrate via blood and home specifically to the site of disease in UC but not to the normal mucosa. This scenario is consistent with the observed ability of mucosal lymphoid cells in UC to bind to both mucosal and peripheral venules in adherence assays, and expression of peripheral node addressin and vascular adhesion protein 1 by endothelium in areas of diseased mucosa in UC.^{9,10} These observations together suggest that the barriers that normally exclude plasma cell precursors of peripheral origin from homing to the gut are broken down in UC, allowing invasion of the mucosa with cells producing proinflammatory immunoglobulins with

potential to cross react with components of the flora and mucosal autoantigens.

Assuming that migration of the aberrant IgG expressing population observed in UC is regulated by receptor ligand systems that facilitate the movement of cells into peripheral sites, these plasma cells would also be expected to localise to peripheral sites. There is evidence for this in that patients with UC may also suffer from peripheral antibody mediated problems including arthritis, autoimmune thyroid disease, and autoimmune haemolytic anaemia.²⁴

The observation of related IgG secreting cells in diseased mucosa and blood in UC does not necessarily lead to the conclusion that this response is of peripheral origin as mucosal responses are known to disseminate via blood. However, exclusion of these cells from normal intestinal mucosa suggests that these cells are not components of a normal mucosal response. To pursue this question further, we examined other features that discriminate between the mucosal and peripheral B cell responses. There is a difference in the relative use of the IgA subclasses in the peripheral and mucosal repertoires; IgA₁ being the dominant subclass in the periphery.³ We were interested to identify and characterise any IgA switch variants of the IgG+ clones. An example of related IgG1 and IgA1 sequences was identified by alignment of CDR3 sequences. These shared seven mutations but were individually diversified. The IgA sequence may have arisen by isotype switching from the IgG sequence as the α_1 constant region is 3' of γ_1 . Alternatively, they may have both arisen from the same IgM+ precursor as the V_H of IgM may undergo hypermutation before the class switch event.^{19,20} The fact that we were able to identify these clonally related sequences suggests that this clone is relatively common. Four further IgA sequences, all IgA₁, which were clonally related to IgG, were identified using clone specific primers. Like the IgG+ clones, these were identified in the diseased mucosa and blood only, suggesting that their migration is restricted by the same criteria. The fact that all IgA sequences related to IgG were IgA1 is evidence that this response is peripheral in origin. The Ig genes isolated from inflamed mucosa were not from blood, either through contamination or from blood vessels, because the Ig secreting tissue plasma cells and their immediate precursors have substantially more RNA than cells in blood. Thus plasma cells contribute most substantially to total RNA. Consistent with this, as discussed below, the IgM sequences from these preparations were mutated. If they were derived from blood, many would be unmutated sequences from the virgin peripheral blood B cell population.

It is now apparent that J_H segment usage varies between the mucosal and peripheral repertoires. We have accumulated published data on J_H1 usage for comparison with J_H1 usage in

Table 5 Usage of J_H1 in mucosal and peripheral repertoire

Peripheral			Mucosal		
No of J _H 1s/No of rearrangements in sample (%)	Isotype sampled	Site sampled (ref)	No of J _H 1s/No of rearrangements in sample	Isotype sampled	Site sampled (ref)
1/17 (5.9)	IgM	Spleen ²⁰	0/21	IgM	Gut+SG ²⁰
1/15 (6.7)	IgA	Spleen ²⁰	0/27	IgM	Gut ¹⁹
1/18 (5.6)	IgG	Spleen ²⁵	0/25	IgA	Gut+SG ²⁰
1/29 (3.4)	IgA	Blood ²¹	0/34	IgA	Gut ¹⁹
1/99 (1)	ND	Blood ²⁶	0/19	IgG	Gut ¹⁹
1/71 (1.4)	ND	Blood ¹⁷	0/13	IgG	Gut (this study)
3/235 (1.3)	IgM	Blood ²⁷	0/68	IgA	Gut ²¹
0/84 (0)	ND	Blood ²⁸	0/33	ND	Gut ²³
			0/84	ND	Gut ²²
Grand total 9/568*			Grand total 0/324*		

SG, salivary gland.

* χ^2 comparisons: 9/568 vs 0/324; $p=0.023$.

IgG in UC (table 5). All of the heavy chain sequences from the mucosal repertoire analysed were isotype switched or mutated, or both, and had therefore probably all undergone selection for specificity or functional viability at some time in their history. None used J_H1 . When the population of B cells from the blood was considered as a whole, which includes naïve cells and memory B cells, J_H1 usage was observed. In UC, three examples of J_H1 usage were observed, all in association with IgG. This is consistent with selection of the IgG in UC according to the criteria that shape the repertoire in the periphery.

We have previously observed that J_H6 is preferentially associated with the plasma cell repertoire in UC.¹⁶ It has since been implied by analysis of IgA producing cells that J_H6 is associated with the peripheral immune response.²¹ We have therefore examined J_H6 usage in three different V_H families, in the mucosa and blood in UC. There was a clear and significant association between J_H6 and IgG in V_H5 expressing B cells in both patients studied, but not in genes using V_H1 or V_H3 . Consistent with this, there was no evidence of J_H6 bias in V_H3 genes used by anticolon antibodies in UC in another study.¹⁸ Although the bias towards J_H6 usage in IgG in UC is not common to all families, the fact that this bias was clear in V_H5 , and has been associated with the peripheral repertoire in another study, is consistent with the concept that the IgG originated from a peripheral response.

Analysis of hypermutation showed that, as in normal bowel, all isotypes were predominantly highly mutated, with IgA and IgG significantly more highly mutated than IgM.^{19, 20} None of these populations in UC differed significantly from isotype matched controls from normal mucosa. It has been shown previously that Ig μ genes used by peripheral B cells have a lower frequency of mutation than those used by mucosal B cells.²⁰ In a sample of 13 IgG sequences from the spleen that used V_H6 , Varade *et al*²⁵ showed that IgG sequences had an average of 14 mutations from germline (range 2–25), which is lower than that observed in the sample of IgG producing cells in normal bowel or those from the colon in UC described in this study. However, as the site of expansion of the IgG producing cells in UC is not known, it is possible that once localised in the gut, a response of peripheral origin might be subjected to the same mechanisms which introduce the high load of mutations in V regions observed in the Ig genes used by mucosal plasma cells. It is possible that the IgG expressing cells that were identified in the blood and mucosa in this study arose via a clonal expansion in the mucosa, and were also destined for extravasation in the mucosa.

We therefore propose that the proinflammatory antibody response in the mucosa in UC includes clones of IgG/IgA1 cells which expand within and disseminate through the diseased mucosa via the blood. Evidence suggests that this response originates in the periphery. It is almost certain that the bacterial flora is involved in the pathogenesis of UC, and in animal models of disease, local T cell responses are also implicated.⁶ It is not clear therefore whether the recruitment of proinflammatory autoreactive immunoglobulins to the mucosa in UC is a primary cause of disease or a consequence of the other immunological imbalances. However, it is highly likely that the antibody response is a significant factor in the evolution of mucosal inflammation and peripheral symptoms in UC. This study has increased our understanding of how this occurs.

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