

# Characterization of cells of the B lineage in the human adult greater omentum

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## Introduction

The human omentum is a double-layered sheet of fatty tissue in the peritoneum. There are two omenta in humans. The 'fatty apron'-like greater omentum, made up of four layers of peritoneum, is attached from the greater curvature of the stomach to the transverse colon and hangs as an apron from the lower part of the transverse colon. The greater omentum contains numerous adipocytes and lymph nodes. The lesser omentum is attached to the top edge of the stomach, and extends to

## Summary

Peritoneal B cells and their omental precursors play an important role in the immune response of the peritoneal cavity and mucosal surfaces in mice. We have previously shown that peritoneal and mucosal B lineage cells are unlikely to be significantly linked in humans. However, the status of the omentum remains unknown. Here, using immunohistochemistry, we observed that sparse, quiescent B cells and occasional clusters of B cells were present in the omentum and that plasma cells, predominantly with cytoplasmic immunoglobulin G (IgG), were present. We analysed sequences of immunoglobulin genes amplified using reverse transcriptase-polymerase chain reaction (RT-PCR) from the normal human greater omentum, and describe the characteristics of variable region genes used by IgG, IgA and IgM. We focused on the properties of IgV<sub>H</sub>4 and IgV<sub>H</sub>5 families to allow comparisons of like with like between different Ig isotypes and cells from different immune compartments. We observed that the IgM genes were derived from a mixed population with mutated and unmutated immunoglobulin sequences. All IgV<sub>H</sub>4 and IgV<sub>H</sub>5 genes used by IgA and IgG from omental cells showed evidence of somatic hypermutation but the load of mutations was not significantly different to that seen in either the systemic or the mucosal compartments. The trends observed, including the dominance of IgG plasma cells, the IgA1/IgA2 ratio being biased towards IgA1, JH1 usage, and a moderate level of somatic mutations, link omental B lineage cells with the systemic compartment. These observations reinforce previous studies highlighting the difference between human and murine B-cell compartments and their relationship to the mucosal immune system.

**Keywords:** human; omentum; immunoglobulin gene; B cell

the undersurface of the liver. These omenta cover and support blood vessels and various abdominal organs; for example, they attach the stomach to the body wall. The human omentum has been considered as a lymphoid organ because of the presence of clusters of leucocytes, termed 'milky spots', which are most abundant in childhood.<sup>1,2</sup> The fetal omentum is a site of lymphopoiesis.<sup>3</sup> It has been speculated that the human omentum may be analogous to thymic tissue or may be a source of peritoneal macrophages.<sup>4-7</sup> It has also been suggested that the omentum may be involved in immune responses to gut

Abbreviations: H-CDR3, third complementarity-determining region of the immunoglobulin heavy chain; IgV<sub>H</sub>DJ<sub>H</sub>-C<sub>H</sub>, immunoglobulin heavy chain productive/functional transcript.

pathogens.<sup>4</sup> Interest in the latter was increased recently with the report that B cells in the murine omentum are involved in the seeding of the peritoneal cavity with B1 cells.<sup>8</sup> Murine B1 cells in the peritoneal cavity are considered to be a potential source of the immunoglobulin A (IgA) plasma cells that populate the intestinal lamina propria. Although we and others<sup>9,10</sup> have previously shown that human peritoneal B cells are not related to the mucosal B-cell response, the status of the omentum in this context is not known. The aim of this study, therefore, was to characterize the cells of the B lineage in the adult human greater omentum.

## Materials and methods

### *Origin of formalin-fixed, paraffin-embedded blocks of human omentum*

Specimens of the human omentum were obtained from gynaecological surgery with the informed consent of the patients. Only patients with omentum without pathological changes, according to the histopathology report, were included in this study. Formalin-fixed, paraffin-embedded blocks of human omentum were studied from 16 patients. In addition, omental specimens from two female patients were obtained fresh (within 1 hr of surgery). They were snap-frozen and stored in liquid nitrogen until required. The first patient was a 68-year-old with clear cell carcinoma of the right ovary. The second patient was a 50-year-old with pelvic pain, irregular vaginal bleeding and an ovarian cyst, which, on histological examination, proved to be benign.

### *Immunohistochemical staining*

All immunological reagents were purchased from Dako-Cytomation Ltd (Eli, UK), unless otherwise stated. In order to study the lymphocytes in the human omentum, the following primary antibodies were used on formalin-fixed, paraffin-embedded tissue sections: rabbit anti-human IgA, rabbit anti-human IgG, mouse monoclonal antibody (mAb) anti-human CD20cy (clone L26), rabbit anti-human CD3, and mouse mAb anti-human Ki-67 antigen (clone MIB-1). For single stain procedures, the immunoreactivity of the primary antibodies was demonstrated using an indirect immunoperoxidase method with a peroxidase-conjugated, swine anti-rabbit or rabbit anti-mouse immunoglobulin, as appropriate, and a 3,3'-diaminobenzidine tetrahydrochloride (DAB) substrate. For double stain procedures (e.g. CD20/MIB-1), the reactivity of the second immunostain was visualized using a biotinylated F(ab')<sub>2</sub> fragment of rabbit anti-mouse immunoglobulin followed by an Extravidin<sup>®</sup> alkaline phosphatase conjugate (Sigma-Aldrich Company Ltd, Gillingham, UK). Binding was visualized using fast

blue, which contrasts with the brown DAB precipitate. Slides were washed in tap water without prior counterstain and then mounted in Aquamount (VWR International Ltd, Lutterworth, UK). Furthermore, tissue sections of human tonsil or appendix were processed in parallel to check the sensitivity of all our staining protocols.

### *Total RNA extraction and RT-PCR*

Total RNA was extracted from frozen specimens of omentum, which had first been split into two fragments to look for evidence of related cells in a small environment. Total RNA extraction was carried out using 1 ml of TRIzol<sup>®</sup> reagent (Invitrogen Ltd, Paisley, UK) per sample according to the manufacturer's protocol.

Synthesis of first-strand complementary DNA (cDNA) was carried out as described elsewhere.<sup>9</sup> Functional IgV<sub>H</sub>4 and IgV<sub>H</sub>5DJ<sub>H</sub>-C<sub>μ</sub>, -C<sub>α</sub> and -C<sub>γ</sub> transcripts were amplified using a semi-nested PCR strategy.

The sequences of all oligonucleotides used as sense PCR primers for the amplification of functional IgV<sub>H</sub>4 and IgV<sub>H</sub>5DJ<sub>H</sub>-C<sub>H</sub> transcripts were taken from references.<sup>11–13</sup> Both first- and second-round PCRs were carried out as described elsewhere.<sup>9</sup> cDNA prepared from human tonsil was used as a template in positive control RT-PCR. For all negative control RT-PCRs, the murine Moloney leukaemia virus (MMLV) reverse transcriptase was omitted in the first-strand cDNA synthesis.

### *T-cloning of IgV<sub>H</sub>5DJ<sub>H</sub>-C<sub>H</sub> functional transcripts and DNA sequencing*

PCR products were size-fractionated by electrophoresis in a 3.3% MicroSieve<sup>®</sup> 3 : 1 agarose gel (FMC BioProducts, Flowgen, Lichfield, UK) under 1 × Tris-borate-ethylenediaminetetraacetic acid (EDTA) running buffer and stained with ethidium bromide. IgV<sub>H</sub>4 and IgV<sub>H</sub>5DJ<sub>H</sub>-C<sub>H</sub> PCR products were cloned into the pGEM-T Vector using the pGEM<sup>®</sup>-T Vector System (Promega UK Ltd, Southampton, UK) and *Escherichia coli* JM109 competent cells. Cloned inserts were sequenced by contracted DNA sequencing (Lark Technologies, Inc., Takeley, UK).

Nucleotide sequences of IgV<sub>H</sub>DJ<sub>H</sub>-C<sub>μ</sub>, -C<sub>α</sub> and -C<sub>γ</sub> transcripts were analysed with GENEJOCKEY II software (by Philip L. Taylor, distributed by Biosoft<sup>®</sup>, Cambridge, UK) using the V BASE database,<sup>14</sup> which contains all known human germline immunoglobulin heavy- and light-chain gene segments. IgV<sub>H</sub> and J<sub>H</sub> gene segments were assigned manually. The third complementarity-determining regions of the immunoglobulin heavy chain (H-CDR3) were defined as starting at the first nucleotides after the end of IgV<sub>H</sub> gene segments and stopped just before the 'TGGGG' motif in the rearranged J<sub>H</sub> gene

segment. Analysis of the H-CDR3 nucleotide sequences provided evidence of clonal interrelatedness, whereas analysis of the 5' end of the C<sub>H</sub> exons provided evidence of immunoglobulin class switch DNA recombination. For each immunoglobulin transcript, the frequency of somatic hypermutation was computed as follows: (number of base substitutions identified on the IgV<sub>H</sub> gene segment when compared against the unrearranged, germline IgV<sub>H</sub> gene sequence/total number of bases analysed along the IgV<sub>H</sub> gene) × 100. Analysed immunoglobulin sequences are accessible through the GenBank/EMBL/DDBJ nucleotide sequence databases (accession numbers AM233752–840).

#### Statistical methods

Comparisons of the frequencies of point mutations on the IgV<sub>H</sub> gene segments and comparison of H-CDR3 lengths were carried out using a Mann–Whitney *U*-test. Comparisons of the J<sub>H</sub> gene segment usages were carried out using  $\chi^2$  tests in a Microsoft Excel spreadsheet. Observed differences were considered statistically significant at  $P \leq 0.05$ .

## Results

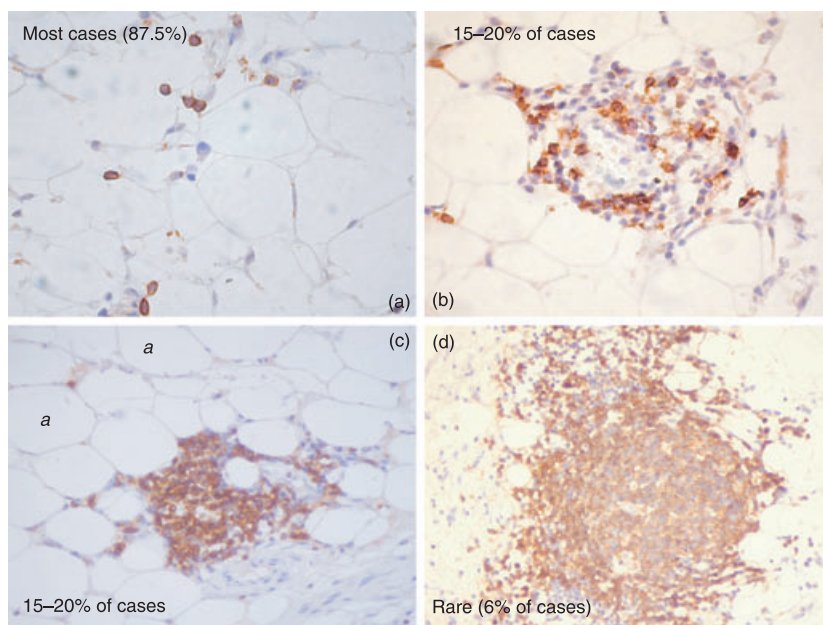
### Immunohistochemical study

Blocks of formalin-fixed, paraffin-embedded omental tissues from 16 women were used for immunohistochemical analysis. We found that 14 cases (87.5%) showed isolated CD20<sup>+</sup> and CD3<sup>+</sup> cells only and three cases (18.8%) showed clusters containing both CD20<sup>+</sup> and

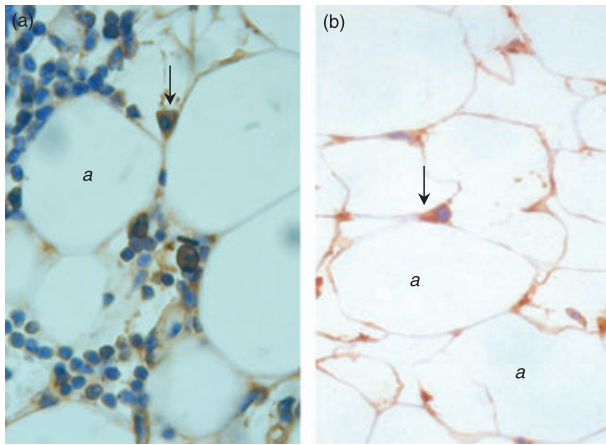
CD3<sup>+</sup> cells but with no identifiable zonation and no germinal centres in the same or in serial tissue sections. A germinal centre-like structure with a small associated T-cell zone was observed in one case only. The range of appearance of CD20<sup>+</sup> B cell-containing structures is illustrated in Fig. 1.

In order to test whether isolated cells and cells in small clusters of lymphoid tissues in human omentum were proliferating, sections of human omentum from five cases (four non-serial tissue sections per case) were single stained for MIB-1 or double stained for MIB-1 and CD20. Another set of 12 tissue sections from the same three cases was subjected to MIB-1/CD3 double staining. In these five cases, expression of MIB-1 antigen could not be detected, either alone or in association with CD20 or with CD3 expression. We therefore concluded that scattered lymphocytes in the human greater omentum were non-proliferative.

Plasma cells expressing IgG, IgA (Fig. 2) and IgM were present in the omental tissue sections. The plasma cells were commonly seen in confined spaces between adipocytes where they appeared 'squashed' into the triangular junctions between adipocytes. The resulting triangular appearance of the plasma cells is not typical. Normally, plasma cells are observed in diffuse stromal connective tissue such as that in the intestinal lamina propria, the bone marrow or the medullary cords of lymph nodes where they have a characteristic oval shape. IgG-expressing plasma cells were most abundant. By extrapolation, having counted plasma cells in known tissue volumes in tissue sections and consulted the histopathology reports providing us with the sizes of the omentum, we estimated that approximately  $3 \times 10^6$



**Figure 1.** Single immunostain using a mouse anti-human CD20 monoclonal antibody on formalin-fixed, paraffin-embedded tissue sections of the human greater omentum, illustrating the variety of microanatomical locations in which B cells were observed. CD20, a pan B-cell marker, appears as brown surface staining. *a*, adipocyte.



**Figure 2.** Single immunostain using a rabbit anti-human immunoglobulin A (IgA) polyclonal antibody on formalin-fixed, paraffin-embedded tissue sections of the human greater omentum. Note the adipocytes (labelled 'a') and their distended vacuoles encompassing lipids. The antibody-secreting cells, with intracytoplasmic IgA (arrowed) and an unusual triangular shape, appear compressed at the junction of the adipocytes.

IgG-containing cells are present in the adult greater omentum.

#### Analysis of IgV<sub>H</sub>DJ<sub>H</sub>-C<sub>H</sub> transcript DNA sequences amplified by RT-PCR from the human omentum

The immunoglobulin gene sequences were analysed to determine whether the plasma cells showed the hallmark of effector cells from mucosal or peripheral lymphoid tissues. A total of 124 immunoglobulin sequences were obtained and analysed, of which 89 were unique IgV<sub>H</sub>DJ<sub>H</sub> gene segment rearrangements. We analysed 37 immunoglobulin transcripts from patient 1 and 52 transcripts from patient 2. Further details of the numbers of different immunoglobulin transcripts analysed per patient are shown in Table 1. Immunoglobulin transcripts PCR-amplified from human duodenal mucosa and splenic red pulp were retrieved from a previous study<sup>15</sup> and were analysed here in order to provide an element of comparison for the mucosal and systemic immune compartments, respectively.

**Table 1.** Number and origin of IgV<sub>H</sub>4 and IgV<sub>H</sub>5DJ<sub>H</sub>-C<sub>H</sub> transcripts analysed

Patient number	Age (years)	Number of potentially functional IgV <sub>H</sub> DJ <sub>H</sub> -C <sub>H</sub> transcripts analysed					
		V <sub>H</sub> 4-C <sub>H</sub> μ	V <sub>H</sub> 4-C <sub>H</sub> α	V <sub>H</sub> 4-C <sub>H</sub> γ	V <sub>H</sub> 5-C <sub>H</sub> μ	V <sub>H</sub> 5-C <sub>H</sub> α	V <sub>H</sub> 5-C <sub>H</sub> γ
1	68	7*	9	7	3	3	8
2	50	10	18	10	4	5	5
Total		17	27	17	7	8	13

\*Values represent the number of different IgV<sub>H</sub>DJ<sub>H</sub>-C<sub>H</sub> transcripts analyzed, as determined by analysis of H-CDR3 nucleotide sequences.

#### Frequency of somatic hypermutation in IgV<sub>H</sub>DJ<sub>H</sub>-C<sub>H</sub> transcripts amplified by RT-PCR from the human omentum

Figure 3 shows the frequencies of base substitutions in each IgV<sub>H</sub> gene segment for the IgV<sub>H</sub>DJ<sub>H</sub>-C<sub>H</sub>μ transcripts (Fig. 3a), IgV<sub>H</sub>DJ<sub>H</sub>-C<sub>H</sub>α transcripts (Fig. 3b) and IgV<sub>H</sub>DJ<sub>H</sub>-C<sub>H</sub>γ transcripts (Fig. 3c). When data from the omentum were compared, within immunoglobulin isotypes, with a highly homologous group of sequences from the duodenum or splenic red pulp, no significant differences in the frequency of somatic hypermutation were found for any comparison.

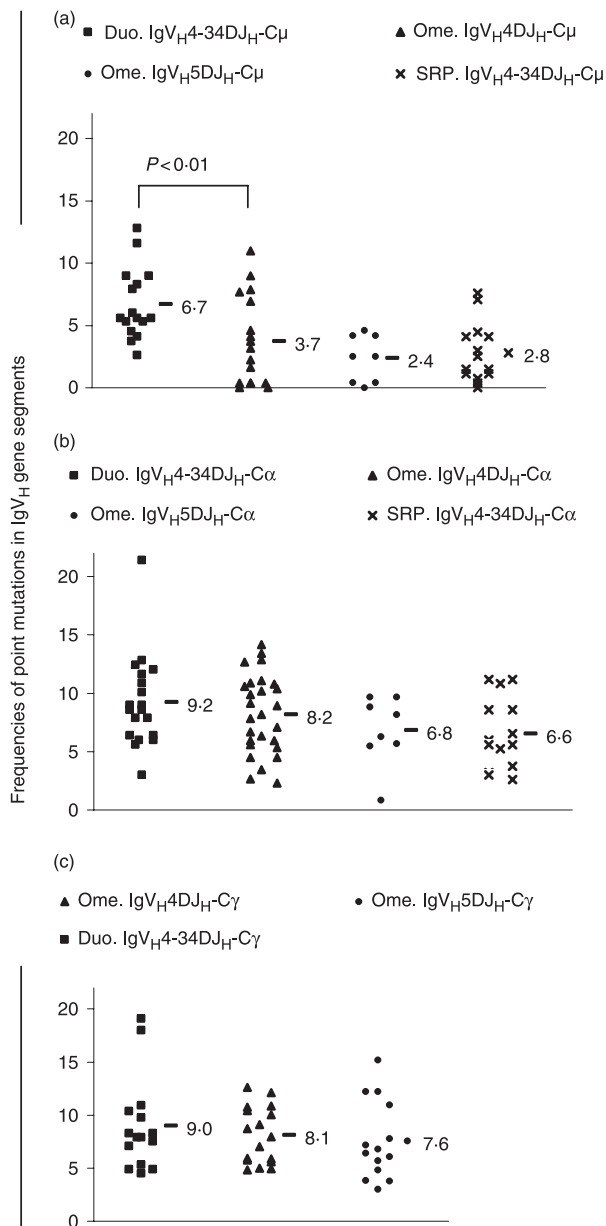
The IgV<sub>H</sub>4DJ<sub>H</sub>-C<sub>H</sub>μ transcripts were a mixed population of unmutated and mutated sequences, presumably representing the presence of naïve and post-germinal centre cells in the omentum. Therefore, although there was a significant difference between the frequency of mutation in the omental sequences and that in other populations of sequences ( $P < 0.01$ ), this may not be of biological significance as it is probably not a comparison of like with like in terms of the composition of the population sampled.

#### Lengths of H-CDR3 in IgV<sub>H</sub>DJ<sub>H</sub>-C<sub>H</sub> transcripts amplified by RT-PCR from the human omentum

The mean H-CDR3 lengths were 40.4, 40.2 and 34.8 nucleotides, respectively, for the V<sub>H</sub>4DJ<sub>H</sub>-C<sub>H</sub>μ, -C<sub>H</sub>α and -C<sub>H</sub>γ transcripts and 41.4, 40.5 and 37.2 nucleotides, respectively, for the V<sub>H</sub>5DJ<sub>H</sub>-C<sub>H</sub>μ, -C<sub>H</sub>α and -C<sub>H</sub>γ transcripts. No significant differences in H-CDR3 length were identified either within the data sets from the omentum or when they were compared with immunoglobulin sequences from the duodenal mucosa or splenic red pulp.

#### J<sub>H</sub> gene segment usage

The J<sub>H</sub> gene segment usage in immunoglobulin transcripts amplified by RT-PCR from the human omenta is illustrated in Table 2. Out of a total of 90 immunoglobulin transcripts analysed, the J<sub>H</sub> gene segment usage found can be sorted as follow: J<sub>H</sub>4 > J<sub>H</sub>5 > J<sub>H</sub>6 > J<sub>H</sub>3 > J<sub>H</sub>1 > J<sub>H</sub>2. Two potentially functional immunoglobulin transcripts



**Figure 3.** Frequencies of base substitutions observed in the rearranged  $IgV_H$  gene segment of human  $IgV_{H4}$  and  $IgV_{H5DJ_H-C_H}$  transcripts isolated from the human adult greater omentum (Ome.), duodenal mucosa (Duo.) and splenic red pulp (SRP.). Each point on the scatter plot represents the number or mean number of point mutations for an individual  $IgV_HDJ_H-C_H$  transcript. When more than one nucleotide sequence was obtained for a given immunoglobulin (Ig) transcript (as determined by analysis of the CDR3 sequence), the average number was plotted as a single point to avoid data distortion. For each set of data, the average or mean number of point mutations is shown as appropriate. The numbers of base misinsertions resulting from *Taq* DNA polymerase errors were not subtracted from the values. For  $IgV_{H4DJ_H-C_H}$  transcripts, statistically significant differences in the frequencies of point mutations between sets of data obtained from the duodenal mucosa and the omentum are shown. (a)  $IgV_{H4DJ_H-C_H}$  transcripts; (b)  $IgV_{H4DJ_H-C_H}$  transcripts; (c)  $IgV_{H4DJ_H-C_H}$  transcripts.

**Table 2.**  $J_H$  gene segment usage in  $IgV_{H4}$  and  $IgV_{H5DJ_H-C_H}$ ,  $-C_H$  and  $-C_H$  transcripts amplified by RT-PCR from the human adult greater omentum

$J_H$ gene segment	Human omentum			Total
	$IgV_{H4} + 5-IgM$	$IgV_{H4} + 5-IgA$	$IgV_{H4} + 5-IgG$	
$J_{H1}$	0	1	1	2*
$J_{H2}$	0	0	0	0
$J_{H3}$	2	6	7	15
$J_{H4}$	9	13	13	35
$J_{H5}$	10	6	4	20
$J_{H6}$	3	10	5	18
Total number of immunoglobulin transcripts	24	36	30	90

\* $J_{H1}$  gene segment usage in the omentum (two of 90) compared with the intestinal mucosa and parotid salivary gland (zero of 324) from Thoree *et al.*<sup>16</sup>.  $P = 0.007$  ( $\chi^2$  test). There was no significant difference in  $J_{H1}$  usage when compared to PBMC and spleen.

encompassing a  $J_{H1}$  gene segment were identified: one  $IGHV4-34DJ_H1-C_H2$  transcript and one  $IGHV4-31DJ_H1-C_H2$  transcript. Out of 324 different immunoglobulin rearrangements amplified from normal intestinal mucosa in previous studies from our own laboratory and others,<sup>16</sup> the  $J_{H1}$  gene segment was not observed. Therefore, the  $J_{H1}$  gene segment was more frequently rearranged in the human omentum than in the intestinal mucosa ( $P = 0.007$  by  $\chi^2$  test). In contrast, when the  $J_{H1}$  gene segment usage was compared between the human omentum and the peripheral compartment [peripheral blood mononuclear cells (PBMC) and spleen], compiled from seven studies, nine sequences out of 568 examples of  $J_{H1}$  usage were observed, which is not significantly different from the two examples in 90 sequences observed here.<sup>16</sup>

#### Identification of clonally related immunoglobulin transcripts based on their unique H-CDR3 DNA sequences

Examples of two clonally related immunoglobulin transcripts from the omentum are illustrated in Fig. 4. Both immunoglobulin transcripts carry the imprint of somatic hypermutation. When the pattern of point mutations was further analysed, 13 mutations were found to be shared between these two immunoglobulin transcripts (10 replacement mutations and three silent mutations), whereas two and three mutations were unique to these sequences. Of note, when the 99 bases of the  $IgC_H$  exon between the splice site and the primer were analysed, one transcript displayed a 99% match with the  $C_H2$  exon while the other transcript displayed a complete match with the  $C_H1$  exon. There are seven base differences between the  $C_H1$  and  $C_H2$  exons over this sequence.



harbour a putative reservoir of intestinal lamina propria plasma cell precursors analogous to the developmental lineage in the peritoneum/omentum in mice. B cells were sparse in the human omentum and occurred predominantly as isolated cells or in small, unstructured clusters. We found no evidence that omental lymphocytes were in cell cycle. Based on both these observations, we propose that the human omentum in adults is unlikely to be a significant source of B-cell or plasma cell precursors for export.

We found that the human greater omentum contains an IgG-containing plasma cell population. We estimated the size of this population at around  $3 \times 10^6$  IgG-containing cells. In comparison, the size of the immunoglobulin-positive cell population in the human bone marrow has been estimated at  $2.3 \times 10^{10}$  cells,<sup>17</sup> of which 51–66% have been estimated to contain IgG.<sup>18,19</sup> The splenic immunoglobulin-positive cell population has been estimated at  $4.7 \times 10^8$  cells,<sup>17</sup> of which 90% have been estimated to be IgG positive.<sup>20</sup> Therefore, our estimate demonstrates that the human omental IgG-containing cell population is minor, being only approximately 0.02% of the size of the bone marrow IgG-containing plasma cell population and 0.7% of the size of the splenic IgG-containing cell population.

The frequencies of point mutations acquired in the  $V_H$  segment of  $IgV_{H4}DJ_{H-C}\mu$  transcripts were significantly lower in the omentum than in the normal small intestinal mucosa ( $P < 0.01$ ).<sup>12,15,21</sup> However, this comparison is biased by the contribution of  $IgM^+$  cells harbouring rearranged but unmutated  $IgV_{H4}$  gene segments, presumably from naïve B cells. However, when other immunoglobulin isotypes were compared, differences in the frequencies of point mutations between  $IgV_H$  genes from the omental and the small intestinal plasma cells were no longer apparent. The frequencies of point mutations in the  $IgV_{H4}$  segment from omental B cells class-switched to IgA or IgG were not significantly different when compared with those observed in the small intestinal plasma cell population or splenic red pulp, the average frequencies of point mutations being intermediate between those two sites. Similarly, analysis of the H-CDR3 lengths did not yield any statistically significant indication of whether the omental immunoglobulin sequences were from cells of mucosal or peripheral origin.

The  $V_H-C\alpha1/V_H-C\alpha2$  ratio differs between peripheral blood (90% IgA1) and the intestinal mucosa (60% IgA2).<sup>22</sup> In the present study, 68.6% of the  $V_HDJ_{H-C}\alpha$  transcript sequences were spliced to the  $C\alpha1$  exon, whereas 31.4% were spliced to the  $C\alpha2$  exon. Therefore, as previously reported in human splenic red pulp<sup>15</sup> and peritoneal B cells,<sup>9</sup> the ratio of IgA subclasses was biased towards IgA1 in the human omentum. Analysis of the  $J_H$  gene segments identified usage of the  $J_{H1}$  gene segment, which is associated with the peripheral B-cell repertoire.

Out of a total of 90 potentially functional, mutated  $IgV_HDJ_{H-C}H$  rearrangements, two examples of  $J_{H1}$  were observed in the omentum. This frequency was significantly higher than the  $J_{H1}$  gene segment usage reported in the parotid salivary gland and the intestinal mucosa but not significantly different from that in PBMC and the spleen.<sup>16</sup>

The group of clonally related  $IgV_{H4}DJ_{H6-C}\gamma$  transcripts, which includes IgG1- and IgG2-switched variants, is of interest. These sequences showed a similar pattern of point mutations on their productively rearranged IGHV4-4 gene segment. The 13 shared point mutations shown in Fig. 4 were accumulated either before any immunoglobulin class-switch DNA recombination, or at the  $IgV_{H4-C}\gamma1$  stage, as the  $C\gamma1$  exon is located upstream of the  $C\gamma2$  exon in the human constant  $Ig_H$  chain locus.<sup>23</sup> The identification of these immunoglobulin sequences supports the concept that clonally related cells of the B lineage can be disseminated within the omentum. There are two possible, non-mutually exclusive explanations for the presence of clonally related cells within the omentum. Either these cells might be derived from a distant site and arrive through the omental blood vessels and/or clonally related cells might be derived from the omental lymphoid aggregates or milky spots. Although such organized structures are rare and were not sampled by the biopsies taken from the omentum of patient 2, this does not preclude their existence. Such groups of clonally related DNA sequences are commonly observed at mucosal sites over large distances, although they are not exclusive to this compartment. Families of related immunoglobulin sequences have been observed in the following human tissues: the tonsil,<sup>24</sup> spleen,<sup>25</sup> subcarinae,<sup>26</sup> parotid salivary gland,<sup>15</sup> colonic mucosa,<sup>27</sup> paediatric and adult duodenal mucosa<sup>12</sup> and nasal mucosa.<sup>28</sup>

In conclusion, the human adult omental cells of the B lineage analysed in this study included quiescent, isolated and clustered B cells and isolated plasma cells, the IgG isotype being the more abundant. The mixed population of naïve B cells and plasma cells was reflected in mutated and unmutated  $IgV_{H4}DJ_{H-C}\mu$  sequences. However, IgA and IgG sequences were somatically mutated. Although the omental  $IgV_{H4}DJ_{H-C}\alpha$  transcripts used to determine the IgA1:IgA2 ratio were not significantly different from their mucosal and systemic counterparts, we observed a bias towards IgA1 in omental cells of the B lineage, as reported in human splenic red pulp and peritoneal B cells, and examples of  $J_{H1}$  rearrangement that are rare in the intestinal mucosal were seen. Therefore, it is unlikely that human omental cells of the B lineage are significant components of the intestinal immune system, either as a source of effector cells or as a significant site of localization of effector cells. Rather, the adult human omentum is likely to be a minor site of antibody production generated by immune responses predominantly in the systemic compartment.

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