Evidence for Local Expansion of IgA Plasma Cell Precursors in Human Ileum

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IgA plays a crucial role in establishment and maintenance of mucosal homeostasis between host cells and commensal bacteria. To this end, numerous IgA plasma cells are located in the intestinal lamina propria. Whether the (immediate) precursor cells for these plasma cells can expand locally is not completely known and was studied here. The total number of IgA plasma cells in human ileal biopsies was counted. Sequence analysis of IgA VH genes from human ileal biopsies revealed the occurrence of many clonally related sequences within a biopsy, but not between different biopsies. This observation strongly argues for local expansion of IgA precursor cells. By comparing the number of unique sequences with the number of clonally related sequences within a biopsy, we estimated that ~100–300 precursors were responsible for the 75,000 IgA-producing cells that were present per biopsy. These precursor cells must therefore have divided locally 9–10 times. Since all sequences contained mutations and most of the mutations present in clonally related sequences were shared, the IgA precursor cells must have arrived initially as mutated cells in the lamina propria. Our data show evidence for the existence of two waves of expansion for IgA-producing cells in human ileum. The first wave occurs during initial stimulation in germinal centers as evidenced by somatic hypermutations. A second wave of expansion of IgA-committed cells occurs locally within the lamina propria as evidenced by the high frequency of clonally related cells. The Journal of Immunology, 2009, 183: 4871–4878.

Materials and Methods

Human intestinal tissues

After given informed consent (protocol approved by the Medical Ethical Committee of the University Medical Center Groningen) ileal biopsies were taken from patients that were undergoing endoscopy because of different intestinal problems. The intestine of these patients showed no signs of intestinal inflammation upon histological evaluation of the tissues. One patient with diarrhea showed no clinical signs that the diarrhea was of an...
For the analysis of the distribution of Ig H-CDR3 lengths in P1, P2, and P3, 3130 nt on the basis of the sequenced parts of the constant region of IgA. The estimates of the number of B cell precursors and their SDs were calculated using formulas of Chao and Lee (18). The number of observed V\textsubscript{H} gene sequences were similarly divided on the original scale. The transcript of the S-plus program used for estimating the number of precursor B cells is given in supplemental Fig. S1.6.

Results

Number of IgA plasma blasts in ileal biopsies

Immunoperoxidase staining was performed on normal ileal biopsies to identify IgA\textsuperscript{+} cells in the lamina propria. From three patients (P5–P7), three biopsies were analyzed to estimate the total number of IgA\textsuperscript{+} cells present. Using these numbers, we estimated that there are, on average, ~75,000 IgA\textsuperscript{+} cells per biopsy (Table I). cDNA was synthesized from RNA isolated from six biopsies from other patients (P1–P3) which resulted in appropriate β-actin and IgA signals upon RT-PCR. Because of the high number of IgA plasma blast and plasma cells within our biopsies, we assume that the IgA signals that we obtained from our biopsies were strongly dominated by the mRNA from these plasma blast or plasma cells.

Estimation of the number of B cell precursors giving rise to IgA\textsuperscript{+} cells

The number of precursors that generated the above-mentioned IgA\textsuperscript{+} cells can be estimated from the diversity of the IgA repertoire in the ileum. To this end, we sequenced IgA V\textsubscript{H} genes of various biopsies. For each patient, 50 IgA V\textsubscript{H} sequences from ileum were analyzed. In patients P1, P2, and P3, we obtained 25 sequences from proximal and 25 from distal ileal biopsies, while from patient P4 we analyzed 50 sequences from only one biopsy. Of the 200 sequences 183 sequences were productively rearranged from patient P4 we analyzed 50 sequences from only one biopsy. Of the 200 sequences 183 sequences were productively rearranged (Fig. 1 and supplemental Tables S1 and S2).

Twenty-one sequences were 100% identical to at least one other sequence, resulting in 162 truly unique sequences. We observed many clonally related IgA-V\textsubscript{H} sequences in all biopsies. These sequences have the same H-CDR3 (i.e., the same VDJ joining), but may have some nucleotide differences within the V\textsubscript{H} gene (Fig. 1 and supplemental Table S3). B cells with V\textsubscript{H} genes with few mutations in the H-CDR3 regions can still be considered to be clonally related, especially when there are also shared mutations within the V\textsubscript{H} region (8).

In addition, 100% identical sequences were obtained from six

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### Table I. Number of IgA plasma cells per biopsy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Biopsy</th>
<th>No. of Sections</th>
<th>No. of Sections</th>
<th>Estimated No. of IgA Plasma Cells</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1</td>
<td>5</td>
<td>99,711</td>
<td>[75,191; 124,232]</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>2</td>
<td>5</td>
<td>133,293</td>
<td>[109,327; 157,262]</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>3</td>
<td>24</td>
<td>170,443</td>
<td>[150,950; 189,933]</td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>1</td>
<td>13</td>
<td>48,868</td>
<td>[40,436; 57,297]</td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>2</td>
<td>17</td>
<td>57,080</td>
<td>[49,512; 64,650]</td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>3</td>
<td>14</td>
<td>72,252</td>
<td>[61,826; 82,679]</td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>1</td>
<td>7</td>
<td>36,288</td>
<td>[21,875; 50,701]</td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>2</td>
<td>7</td>
<td>32,609</td>
<td>[27,322; 37,896]</td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>3</td>
<td>8</td>
<td>21,965</td>
<td>[16,362; 27,566]</td>
<td></td>
</tr>
</tbody>
</table>

* The area of tissue was determined by morphometric analysis. Five to 24 sections were completely counted per biopsy and numbers represent the mean number of plasma cells per biopsy with the 95% CI.

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Infectious nature (no fever, no elevated serum C-reactive protein levels). Three ileal biopsies frozen in Tissue-Tek were taken from the ileum of three patients each (P5–7; Table I) and used to show the presence of IgA\textsuperscript{+} cells by immunohistochemistry. Two biopsies from each patient (P1–P3) were taken from the terminal ileum just before the Bauhin valve (labeled as low) and ~20 cm above the Bauhin valve (labeled as high). From patient P4 only one biopsy was taken from before the Bauhin valve. Biopsies from patients P1–P4 were snap frozen in liquid nitrogen along with RNase out (Invitrogen) and used for RNA isolation.

**Immunohistological staining and counting of IgA plasma cells**

Biopsies from patients P5, P6, and P7 weighed ~7 mg, which yielded ~250 sections of 5-μm thick. IgA\textsuperscript{+} cells were counted from every 10th section from the biopsy of patient P5, while 5–17 randomly selected sections were counted from the other biopsies. To visualize IgA\textsuperscript{+} cells in ileal biopsies, cryostat sections were fixed with acetone and incubated with peroxidase-labeled rabbit anti-human IgA. Peroxidase was revealed by diazino benzidine and hematoxyl staining was used to view nuclei. The surface area of each section (whose IgA-containing cells were counted) was measured using the program Analysis where the area of the intestinal lumen was excluded from the analysis. The number of IgA\textsuperscript{+} cells was counted from nine biopsies taken from three patients (Table I).

**Molecular cloning of IgA V\textsubscript{H} genes**

RNA was isolated from ileal biopsies by the TRIzol method (Sigma-Aldrich) according to the manufacturer’s instructions. Total RNA (15–20 μg) was dissolved in 100 μl of H\textsubscript{2}O. Of this solution, 17.5 μl was used to synthesize cDNA using oligo(dT) primers (Invitrogen) in a final volume of 30 μl. Integrity of the cDNA was analyzed by β-actin PCR. V\textsubscript{H} primers used for samples from P1, P2, and P3 were as described by van Dongen et al. (10). The V\textsubscript{H} primer set for framework (FR) 1 was used in combination with a 3’ Co primer. The Taq error rate determined to be 1 in 3130 in the region of the sequenced part of the constant region of IgA. The product was loaded onto agarose gels and the ethidium bromide-stained band was ok. To confirm the presence of IgA transcripts from all V\textsubscript{H} families, PCR was performed on the cDNA of P4 using individual V\textsubscript{H} 5’ primers with 3’ Co primers separately. PCR products were gel purified using a gel purification kit (Roche), cloned using pCR4 TOPO vector (Invitrogen), and sequenced using an automated sequencing device as previously described (11).

**H chain CDR3 (H-CDR3) spectrotyping**

For the analysis of the distribution of Ig H-CDR3 lengths in P1, P2, and P3 patients, PCR were performed by combining the FR3 5’ V\textsubscript{H} primers (10) with a 3’ FAM-labeled Co primer. The fluorochrome-labeled PCR products were size separated in a capillary sequencer polymer and detected by automated scanning with a laser in a Megabase sequencer (Amersham Biosciences). Spectrotypes were analyzed with the Genetic Profiler Program (Amersham Biosciences).

**V\textsubscript{H} gene analysis**

Nucleotide sequences were compared with the IMGT databases of germ-line sequences (12) and V\textsubscript{H} regions were analyzed using IMGT Quest (13) and JoinSolver (14). The lengths of the H-CDR3 domains of translated IgA H chain transcripts were calculated as described previously (15). Replacement over silent mutation (R:S) ratios for FR and CDR were determined (16). Sequences having identical VDJ rearrangements were defined as clonally related or derived from the same B cell (see below).

**Coverage and estimation of B cell precursors**

Coverage of the complexity of the V\textsubscript{H} gene repertoire was calculated by considering the number of clonally related sequences compared with the total number of observed, unique V\textsubscript{H} gene sequences. The probability of picking up unique, new V\textsubscript{H} sequences in each biopsy is related to the number of B cell precursors giving rise to the IgA repertoire. Coverage was calculated as C = 1 − f/ln (in which f is the number of observed unique, individual V\textsubscript{H} sequences and n is the sample size, i.e., the total number of observed V\textsubscript{H} sequences (17). A problem in analyzing coverage is that identical V\textsubscript{H} sequences in one sample could either be derived from the same B cell or from different B cells having identical V\textsubscript{H} genes. A minimal estimate for the coverage was made by excluding identical V\textsubscript{H} sequences from the calculation (so consider them as derived from the same B cell), while a maximum estimate was made under the assumption that also identical V\textsubscript{H} sequences were derived from different B cells.

The estimates of the number of B cell precursors and their SDs were calculated using formulas of Chao and Lee (18). The number of B cell precursors N is estimated by dividing the number of observed V\textsubscript{H} gene sequences D by the sample coverage, with a correction for nonequal class sizes: 

\[
N = \frac{D/C}{1 + \gamma^2(n - 1 - C)/C}
\]

In which γ is an estimate for the coefficient of variation of the relative class frequencies. The 95% confidence intervals (CI) were calculated by transforming the estimated B cell precursor numbers to the natural logarithm scale, calculating 95% CI on this scale and transforming them back to the original scale. The transcript of the S-plus program used for estimating the number of precursor B cells is given in supplemental Fig. S1.6.

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Notes

1. The online version of this article contains supplemental material.
of seven biopsies; only one biopsy did not yield identical sequences. Both clonally related and fully identical sequences were only observed among sequences derived from a certain biopsy, but not in samples taken from one person at distant and proximal ileal sites. In total, we observed 12 sets of clonally related sequences and 11 sets of fully identical sequences (Tables II and III). Within one set of clonally related cells (the sequences P4C32, P4C33, and P4C34), we cannot formally exclude the possibility that P4C34 belongs to a different B cell clone, because only 2 of 16 mutations of this sequence are shared within the VH region with the sequences P4C32-P4C33 (10). We amplified IgA VH genes using a single PCR with the primer. Spectrotypes of IgA VH genes using a single PCR with the described set of FR3-specific primers for all VH sequences that were obtained for multiple members of the same set of clonally related sequences. The number of B cell precursors giving rise to the IgA plasma cells within one biopsy is estimated by dividing the number of observed unique sequences of different B cell precursors by the sample coverage, with a correction for nonequal class sizes.

To analyze the random distribution of the ileal IgA+ cells, the H-CDR3 lengths were analyzed by performing spectrotype analysis. With this method, Ig gene PCR products from polyclonal B cell populations from normal tonsils, bone marrow, and peripheral blood give a typical fluorescence peak pattern resembling a Gaussian (normal) distribution curve of the lengths of the products (10). Deviations from Gaussian distributions have been used to detect the presence of clones in suspected lymphoproliferative diseases (10). We amplified IgA VH genes using a single PCR with the described set of FR3-specific primers for all VH gene families (10) in combination with a labeled Cc primer. Spectrotypes of IgA H-CDR3 lengths from ileal samples revealed no clear dominancy of particular B cell clones. Patterns from samples taken at the proximal and distal ileum from the same volunteer showed similar patterns (Fig. 2). The range of H-CDR3 lengths in these ileal biopsies was 39–84 nt, which is in accordance with the variation in lengths of the H-CDR3 regions observed in the sequenced samples (24–81 nt). We also analyzed the spectrotypes of IgA expressed by peripheral blood B cells of healthy volunteers. Similar to the spectrotypes of the ileal samples, there was no clear dominancy of a particular B cell clone in the peripheral blood (data not shown).

### Table II. Number of B cell precursors for ileal IgA plasma cells: minimal coverage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sequenced</th>
<th>Productively Rearranged Sequences</th>
<th>No. of Sets</th>
<th>Unique Sequences</th>
<th>Minimal Coverage (%)</th>
<th>Estimated No. of B Cells</th>
<th>Estimated SD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1H</td>
<td>25</td>
<td>20</td>
<td>2 sets (1 × 2, 1 × 3)</td>
<td>15</td>
<td>25</td>
<td>94</td>
<td>67</td>
<td>[24; 382]</td>
</tr>
<tr>
<td>P1L</td>
<td>25</td>
<td>22</td>
<td>1 set (1 × 2)</td>
<td>20</td>
<td>10</td>
<td>231</td>
<td>230</td>
<td>[33; 1620]</td>
</tr>
<tr>
<td>P2H</td>
<td>25</td>
<td>16</td>
<td>1 set (1 × 3)</td>
<td>13</td>
<td>12.5</td>
<td>135</td>
<td>138</td>
<td>[18; 1002]</td>
</tr>
<tr>
<td>P2L</td>
<td>25</td>
<td>22</td>
<td>2 sets (2 × 2)</td>
<td>17</td>
<td>8</td>
<td>100</td>
<td>73</td>
<td>[31; 401]</td>
</tr>
<tr>
<td>P3H</td>
<td>25</td>
<td>21</td>
<td>2 sets (2 × 2)</td>
<td>20</td>
<td>17</td>
<td>132</td>
<td>88</td>
<td>[36; 486]</td>
</tr>
<tr>
<td>P3L</td>
<td>25</td>
<td>24</td>
<td>2 sets (2 × 2)</td>
<td>32</td>
<td>14</td>
<td>373</td>
<td>269</td>
<td>[91; 1526]</td>
</tr>
<tr>
<td>P4</td>
<td>50</td>
<td>37</td>
<td>2 sets (1 × 2, 1 × 3)</td>
<td>32</td>
<td>14</td>
<td>373</td>
<td>269</td>
<td>[91; 1526]</td>
</tr>
</tbody>
</table>

*a Minimal coverage was calculated in which 100% identical sequences are considered to be derived from the same B cell.

*b The number of sets is indicated, with the number of sets × the number of members within that set between brackets.

*c Estimation of the number of B cell precursors giving rise to the IgA plasma cells within one biopsy was done by comparing the number of unique VH sequences with the VH sequences that were obtained for multiple members of the same set of clonally related sequences. The number of B cell precursors giving rise to the IgA plasma cells within one biopsy is estimated by dividing the number of observed unique sequences of different B cell precursors by the sample coverage, with a correction for nonequal class sizes.

### Table III. Number of B cell precursors for ileal IgA plasma cells: maximal coverage

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sequenced</th>
<th>Productively Rearranged Sequences</th>
<th>No. of Sets</th>
<th>Unique Sequences</th>
<th>Maximum Coverage (%)</th>
<th>Estimated No. of B Cells</th>
<th>Estimated SD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1H</td>
<td>25</td>
<td>21</td>
<td>2 sets (1 × 2, 1 × 4)</td>
<td>15</td>
<td>29</td>
<td>111</td>
<td>82</td>
<td>[27; 467]</td>
</tr>
<tr>
<td>P1L</td>
<td>25</td>
<td>24</td>
<td>3 sets (3 × 2)</td>
<td>18</td>
<td>25</td>
<td>84</td>
<td>43</td>
<td>[32; 228]</td>
</tr>
<tr>
<td>P2H</td>
<td>25</td>
<td>24</td>
<td>6 sets (3 × 2, 2 × 3, 1 × 4)</td>
<td>8</td>
<td>67</td>
<td>23</td>
<td>7</td>
<td>[13; 43]</td>
</tr>
<tr>
<td>P2L</td>
<td>25</td>
<td>24</td>
<td>4 sets (4 × 2)</td>
<td>16</td>
<td>34</td>
<td>60</td>
<td>24</td>
<td>[28; 134]</td>
</tr>
<tr>
<td>P3H</td>
<td>25</td>
<td>25</td>
<td>3 sets (2 × 2, 1 × 5)</td>
<td>16</td>
<td>35</td>
<td>102</td>
<td>62</td>
<td>[32; 336]</td>
</tr>
<tr>
<td>P3L</td>
<td>25</td>
<td>24</td>
<td>2 sets (2 × 2)</td>
<td>20</td>
<td>17</td>
<td>132</td>
<td>88</td>
<td>[36; 486]</td>
</tr>
<tr>
<td>P4</td>
<td>50</td>
<td>41</td>
<td>3 sets (3 × 2, 2 × 3)</td>
<td>29</td>
<td>30</td>
<td>143</td>
<td>64</td>
<td>[61; 345]</td>
</tr>
</tbody>
</table>

*a See footnotes b and c to Table II for explanations of the column headings. Maximum coverage was calculated by considering all sequences to be derived from different B cells.
There was no difference in patterns when amounts of cDNA were serially diluted. The resulting patterns were similar within a range of 200-25 ng of cDNA, with only slightly lower signals in the diluted cDNA samples (data not shown). Although the spectrotypes data show that the distribution of B cell precursors is not skewed toward a few dominant B cell clones, sometimes a larger peak with a particular H-CDR3 length can be detected. Simulations of expected spectrotypes with different distributions showed that our observed spectrotypes fitted well with an equal distribution of a limited number of B cell precursors (data not shown).

IgA plasma cells in normal ileum express predominantly VH1 family genes

For analysis of VH gene family usage, we analyzed sequences from samples of patients P1, P2, and P3 that were amplified with a mixture of all different VH gene family-specific primers. More than 40% of the unique IgA sequences utilize VH1 family genes, followed by VH4 and VH3 (Fig. 3). Analysis of the usage of individual VH genes showed that VH1–02, VH1–18 and VH1–69 were among the highest used (>10%), which is in accordance with the observed preference for the VH1 gene family. Individual VH genes that were used between 5 and 10% were VH2–05, VH3–23, VH4–59, and VH5–51. The VH genes VH3–21 and VH4–34 that are often described to be present within mucosal tissues were among the lowest used (supplemental Fig. S2).

Forty percent of the intestinal IgA sequences have somatic mutations with R:S ratios significantly different from random mutations

The presence and nature of somatic mutations in obtained IgA sequences were analyzed by comparison to these sequences with
Figure 2. Spectrotype analysis of IgA V\textsubscript{H} genes. IgA V\textsubscript{H} genes were amplified by using a set of FR3-specific primers in combination with a FAM-labeled primer for the constant region of IgA. Labeled PCR fragments were size separated on a Megabase Sequencer and analyzed with the Genetic Profiler Program. The x-axis presents the size of the fragments in bp, while the y-axis presents the fluorescent intensity of the FAM label. Spectrotypes were analyzed from the biopsies that were also used for V\textsubscript{H} gene analysis. Those biopsies where taken from the terminal ileum just before the Bauhin valve (labeled as L) and ~20 cm above the Bauhin valve (labeled as H).
The number of IgA⁺ plasma cells in biopsies taken from human ileum were determined by counting immunohistologically stained sections. Approximately 75,000 IgA plasma cells are present in a biopsy. This number is in line with the observation by Paniangau et al. (20). They estimated the number of IgA plasma cells in the human gut per so-called "mucosal tissue unit." This unit was defined on the basis of 6-µm-thick tissue sections through a 500-µm wide area (i.e., a volume of 3000 µm³) of the mucosa at full height, from muscularis mucosa to the tip of the villi. Paniangau et al. (20) found that there are ~86 IgA plasma cells per mucosal tissue unit. The mean number of IgA plasma cells in our nine counted biopsies was 172 cells/mm² in tissue sections with a thickness of 5 µm (i.e., a volume of 5000 µm³). The volume of our tissue sections is therefore ~1.67 times (5000/3000) the volume of the mucosal tissue unit used by Paniangau et al. (20). When we converted our IgA plasma cell counts in terms of mucosal tissue units, we observed on average 103 IgA plasma cells per mucosal tissue unit, which is in the same order of magnitude compared with the findings of Paniangau et al. (20). The precursor cells must therefore have divided 9–10 times to give rise to the 75,000 IgA plasma cells present in the biopsies. This expansion must take place locally, because it is extremely unlikely that such large numbers of clonally related cells migrate specifically to a small defined region in the gut after dividing elsewhere in the body.

In our samples, we did not observe clonally related sequences between different biopsies from the same individual. In apparent contrast, a number of studies reveal that clonally related IgA⁺ B cells can be found at multiple distant sites in different parts of the gut (8, 21–23). The experimental approaches in these studies have in common that only a very restricted population of IgA plasma cells was analyzed: e.g., by amplification of a single selected VH gene (VH4–34) (22), selection of particular H-CDR3 lengths (8), or detection of Ag-specific IgA production after vaccination (23). Among our sequences we did not find clonally related sequences from different sites, but this does not necessarily contradict the above-mentioned findings. This might be due to our sample size and/or to the fact that we did not look to only one particular VH gene (such as VH4–34), but to potentially all VH genes. Dunn-Walters et al. (22) observed that clonally related cells at distant sites share sometimes more mutations with each other then the local members of the same clone. These clones must therefore have been mutated before spreading to distant sites. This notion is in line with our sequence data, showing that within clones, the majority of mutations are shared, implying that mutated plasma cell precursors migrate into the intestinal lamina propria before acquiring additional mutations. The plasma cell precursor cells are likely generated during a first wave of T cell-dependent expansion in germinal centers of gut-associated lymphoid tissues. During this proliferation in germinal centers, the V genes of the B cells undergo SHMs, resulting in (memory) B cells that are selected to bind with higher affinity to the Ag that initiated this response (24). These memory B cells leave the germinal centers and enter via the efferent lymphatics into the circulation. From there they can home...
back into the intestinal lamina propria, where they finally differen-
tiate to IgA-secreting plasma cells (25). We speculate that after
this first wave of expansion in germinal centers, a second wave of
expansion occurs after arrival of the isotype-switched precursors
of IgA plasma cells in the lamina propria. Whether these precursor
cells are already switched to IgA is not clear. More than 80% of
the plasma blasts and plasma cells in the peripheral blood express IgA
and express the adhesion molecule β7 integrin and the chemokine
receptor CCR10, both involved in homing to mucosal sites (26).
Alternatively, the mucosal IgA plasma cells could be derived from
classical isotype-switched memory B cells or from somatically hy-
permuted IgM* IgD+CD27+ B cells, which are thought to be
involved in T cell-independent humoral immune responses (27).
Based on our coverage data, we estimate that the second wave of
(local) expansion must involve the aforementioned 9–10 cell di-
visions. There is evidence that IgA+ (memory) cells are present in
the human intestinal lamina propria (28). These cells have still the
capacity to divide and may be responsible for the second wave of
expansion. We do not know what the driving forces are for this
proliferation and the time frame in which this expansion takes
place. Furthermore, little is known about the life span and turnover
rate of IgA plasma cells in human intestinal tissue due to the fact
that, for obvious reasons, BrdU incorporation studies are difficult
to perform in humans. The proliferation marker Ki-67 (as well as
other markers related to cell division) is for these studies of limited
usage because it binds only to actively dividing cells and does not
give information on life span and turnover rate. We stained ideal
biopsies with Ki-67 and observed that the vast majority (>90%) of
IgA plasma cells were unlabeled (data not shown). The virtual
absence of staining of the lamina propria suggests that the clonal
expansion of IgA plasma cells takes place over a relatively long
period of time. The signals that are required for this local expa-
sion in the lamina propria may differ from those needed for their
expansion in germinal centers. Possibly, these precursor cells can
expand independently from cognate T cell help as suggested by the
T cell-independent IgA production observed in T cell knockout
mice (29) and by the recent observation of a large number of IgA-
producing cells in the lamina propria of CD40−/− mice (6). In this
context, it should be noted that some bacterial ligands can mimic
CD40L signaling (30), normally provided by activated CD4+ T
cells. Importantly, ligation of TLRs by microorganisms can result in
T cell independent proliferation and differentiation of B cells (31).
TLR-mediated activation of murine B cells also leads to up-
regulation of the BAFF family receptor TACI (transmembrane
activator calcium modulator and cyclophilin ligand interactor (32).
The cytokines BAFF and APRIL may induce T cell-independent
CSR after binding to TACI on B cells (33). BAFF and APRIL are
produced by many different cells such as neutrophils, macro-
phages, and dendritic cells. Recently, He et al. (7) showed that human
colonic epithelial cells can secrete APRIL in response to
TLR stimulation to drive local IgA isotype switching. Further-
more, these authors observed activation induced deaminase-positi-
ve B cells within the lamina propria (7). This enzyme is essential
both for SHM and CSR. Our findings indicate that within clones
the majority of the mutations in the V H genes are shared between
the various members of the clone. The additional mutations ac-
quired during the second expansion phase in the lamina propria do
not need per se, however, to be the result of SHM. When only a
few (2–3) mutations are found on top of mutations shared by the
clonc, this can be due to normal mutation rates. However, in those
cases where more than 10 additional mutations are found, we can-
not exclude the possibility of ongoing SHM. The lack of some kind
of selective microenvironment in the lamina propria (e.g., absence
of follicular dendritic cells) could explain why we found no evi-
dence of Ag selection in many of our IgA V H genes. In addition,
the accumulation of somatic mutations (in the absence of proper
Ag selection) may also result in polyreactive and/or autoreactive
Abs despite expressing highly substituted V(D)J genes (34).

In conclusion, we provide evidence that a major part of human
IgA-producing cells in the ileum are generated in different phases.
In the first phase, T cell-dependent activation and multiplication of
precursor B cells for IgA plasma cells takes place in germinal
centers of the gut-associated lymphoid tissue. These isotype-
switched IgA+ (memory) B cells subsequently settle in different
regions of the mucosal tissue and undergo a second phase of local
expansion. This expansion (of already selected and committed) of
plasma cell precursors is likely T cell independent and possibly the
result of direct interaction of products from intestinal microorgan-
isms and these B cells. This expansion phase more likely provides
the individual with higher numbers of those specificities that are
required for homeostasis with the intestinal flora at the mucosa.

Disclosures

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