Sequence analysis of immunoglobulin variable region genes that encode autoantibodies expressed by lymphomas of mucosa associated lymphoid tissue

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Abstract

Aim—To determine whether the immunoglobulin genes used by three gastric mucosa associated lymphoid tissue type lymphomas with known autoreactivity are mutated from germline as mutation from germline is an indicator of exposure to a mutational mechanism which characteristically acts on B cells as they undergo a follicle centre response.

Methods—Cell lines established from two cases of MALT type lymphoma secrete autoantibodies recognising follicular dendritic cells (one case) and basement membrane (one case). The immunoglobulin heavy chain variable region genes (IgVH) and light chain variable region genes (IgVL) used by these cell lines, and the IgVH genes from a third case recognising human IgG were sequenced.

Results—All three cases studied had mutated IgVH genes, while the IgVL genes were unmutated.

Conclusion—The presence of mutations in IgVH genes is consistent with the origin of gastric MALT type lymphomas from B cells which have traversed the lymphoid follicle.

Keywords: mucosa associated lymphoid tissue type lymphoma, gastric lymphoma, mutation.

Low grade B cell MALT type lymphomas are thought to be malignancies of marginal zone B cells which surround the mantle zone in normal human spleen and Peyer’s patches.4 Consistent with the phenotype of marginal zone B cells, MALT type lymphomas are predominantly CD5−, IgM+, IgD−, although cases expressing other immunoglobulin iso-types have been observed.5 Most normal human marginal zone B cells have mutated immunoglobulin heavy chain variable region genes (IgVH).6 Mutations in IgVH genes are characteristically acquired as B cells undergo a follicle centre response during the process of affinity maturation.7 In the course of this process in the follicle centre, B cells with mutations in their IgVH genes that result in an increase in the affinity of the encoded immunoglobulin for antigen are expanded, whereas B cells which acquire mutations resulting in loss of affinity are deleted. It is likely, therefore, that normal human marginal zone B cells have been though a follicle centre and may be memory B cells. This is consistent with a study showing that in rats memory B cells colonise the marginal zone.8 The aim of this study was to determine whether the immunoglobulin genes used by three gastric MALT type lymphomas with known autoreactivity9 are mutated from germ-line. If so, this would suggest that these lymphomas are derived from B cells which have been through a follicle centre response.

Methods

CELL LINES AND TISSUES

The immunoglobulin genes used by three cases of low grade B cell gastric lymphoma of MALT type were analysed. Heterohybridoma cell lines produced by fusing cells from cases 1 and 2 with murine hybridoma cell line NSO were characterised in a previous study.1 The heterohybrid clone derived from case 1 (F.26) secreted tumour derived IgMκ, recognising follicular dendritic cells.2 The clone from case 2 (C.26) secreted IgMλ, recognising a venular basement membrane component1. These cell lines were used as a source of RNA for this study. The tissue used for the study of case 3 (IgMκ, recognising IgG) (unpublished observations) was received fresh and was snap frozen in liquid nitrogen and stored at −70°C until required.

Gastric lymphomas of mucosa associated lymphoid tissue (MALT) type may be dependent on the spiral bacterium Helicobacter pylori for growth as eradication of the bacteria results in regression of lymphoma in many cases.1 However, the immunoglobulin expressed by the tumour cells in four cases studied to date recognises single, distinct autoantigens and does not cross react with H pylori.23 The four published cases recognise an epitope associated with IgA and IgM,2 follicular dendritic cells,3 a basement membrane component,3 and IgG (unpublished observations).3 The recognition of distinct autoantigens in each case reported suggests that reactivity with autoantigens may be a significant factor in the pathogenesis of gastric MALT type lymphomas.
A

| 4.30 | CGG CTG CAG CTG CAG GAG TCG GCC CCA GGA CTG GTG AAG CCT TCG GAG ACC CTG TCC CTC ACC |
| 4.30 | TGG ACT GTC TCT GGT GCC TCC ATC AGC AGT AGT AGT TAC TAC TGG GGC TGG ATC CGC CAG CCC |
| 4.30 | CCA GGG AAG GGG CTG GAG TCT GAT TGG GTG AGG ACC TAT TAT AGT GGG ACC ACC TAC TAC AGG CAG |
| 4.30 | TCC TCT AAG AGT CGA GTC ACC ATC TCA GTC GAC AGC TCC AAG ACC TCT CCC CTG AAG CTG |
| 4.30 | AGC TCT GTG ACC GCC GCG GAC AGC GCC GTG TAT TAC TGT GGG AG |
| 4.30 | TCC CCG GCA |

B

| DP38 | GAG GTG CAG CTG GTG GAG TCT GGC GGA GGC TTT GTA AAG CCT GGG GGG TCC CTG AGA CTC TCC |
| DP38 | TGT GCA GCC TCT GQA TTT CTG AGG GCC TGG TCG AGC TGG GTC CGC CAG GCT CCA GGG |
| DP38 | AAG GGG CTG GAG TGG GTG GCC | CDR1 |
| DP38 | GCA CCC GTG AAA GCC AGA TCC ACC ATC TCA AGA GAT TGT TCA AAA ACC AGC CTG TAT CTG CAA |
| DP38 | ATG AAC AGC CTG AAA ACC GAG GAC ACA GCC GTG TAT TAC TGT ACC ACA GA |
| JP4b | TAC TGG GCC CAG GAG ACC CTG GTC ACC GTC TCC TCA TCC TCA |

C

| DP32 | GAG GTG CAG CTG GTG GAG TCT GGG GGA GGT GTG GTA CGG CCT GGG GGG TCC CTG AGA CTC TCC |
| DP32 | TGT GCA GCC TCT GQA TTT CTG AGG GCC TGG TCG AGC TGG GTC CGC CAG GCT CCA GGG |
| DP32 | AAG GGG CTG GAG TGG GTG GCC | CDR1 |
| DP32 | GTC CCC GTG AAA GCC AGA TCC ACC ATC TCA AGA GAT TGT TCA AAA ACC AGC CTG TAT CTG CAA |
| DP32 | ATG AAC AGC CTG AAA ACC GAG GAC ACA GCC GTG TAT TAC TGT ACC ACA GA |
| JP4b | TAC TGG GCC CAG GAG ACC CTG GTC ACC GTC TCC TCA TCC TCA |

Comparison of VH-JH sequences derived from (A) case 1, (B) case 2 and (C) case 3 with closest match germline sequences. Replacement mutations are shown in upper case; silent mutations in lower case. In case 3 no sequence data were available for codons 1–21 of IgVH or the 3′ end of JH because of the positions of the primers used. All sequences are mutated.

ANALYSIS of cDNA SEQUENCE IN CASES 1 AND 2

RNA from cell lines F.26 and C.26 was isolated and reverse transcribed as described previously. The IgVH genes were amplified by PCR using VH leader region primers10 and a Cµ constant region primer11 under conditions described previously. At least two independent PCR products were purified and sequenced directly as described by Chapman et al.12 Vλ genes were amplified using a mixture of primers specific for framework region 1 and JH.13 PCR products were purified by gel electrophoresis and subcloned into the TA vector (Promega, Madison, Wisconsin, USA). At least six clones from two independent PCR reactions were analysed. Sequence analysis was performed...
using MacVector 4.0 software (IBI, New Haven, Connecticut, USA) and alignment made to EMBL and V-BASE databases.\(^\text{14}\)

**ANALYSIS OF DNA SEQUENCE FROM CASE 3**

As a cell line was not available for case 3, genomic DNA sequences were analysed. DNA was isolated from 7 μm sections of frozen tissue, immunohistochemically stained using an antidiotopic antibody to identify the tumour cells as described by Russell et al.\(^\text{1}\) VH genes, from VH framework region 1 region to JH, were amplified using a nested PCR technique and cloned into the vector pCR-Script SK\(^{\text{+}}\) as described previously.\(^\text{6}\) Clone inserts were amplified using T3 and T7 primers under standard conditions.

PCR reaction conditions comprised 30 cycles with an annealing temperature of 45°C. Prior to sequencing, 5 μl of the PCR products was treated sequentially with exonuclease 1 and shrimp alkaline phosphatase, and sequenced using the Sequenase PCR product sequencing kit (Amersham International, Little Chalfont, UK). Sequence analysis was carried out using Wisconsin GCG software and V-BASE. Germline matches were made using the EMBL database.

**Results**

**CASE 1**

The immunoglobulin heavy chain used by the F.26 cell line, which secreted the immunoglobulin from case 1, used VH4.30 and JH5b (figure, panel A). This sequence has 13 mutations (five replacement and eight silent) compared with the published germline sequences of VH4.30 and JH5b. All mutations in VH were situated in the framework region. One silent mutation was situated in JH. The light chain from this immunoglobulin molecule was a precise germline match to Vk 02/012/DPK, Jk1.

**CASE 2**

The immunoglobulin heavy chain used by the C.26 cell line, which was derived from the tumour cells in case 2, used VH3 family member DP38 and JH4b (figure, panel B). The sequence was mutated from the published germline sequence, with four replacement mutations in VH and one in JH. Of the four mutations in VH, three were in the framework region. The light chain used by case 2 was an exact match to germline \(\lambda\) chain gene LV801.

**CASE 3**

The same heavy chain sequence was obtained in five of six clones sequenced using DNA from case 3. This sequence was in frame in CDR3 and contained no stop codons and was therefore thought to be the allele used by the tumour cells. The IgVH DP32 (VH3 family) is associated with JH3. This sequence has 12 mutations compared with the published germline sequence (seven replacement and five silent) (figure, panel C). Four replacement mutations occurred in the framework region and three in the complementarity determining regions.

**Discussion**

All of the IgVH genes sequenced are mutated when compared with the closest match germline sequences. This suggests that the IgVH genes have been exposed to the mutational mechanism, which is characteristically active in the germinal centre.\(^\text{7}\) This is consistent with the origin of MALT type lymphomas from marginal zone B cells, which also harbour mutated immunoglobulin genes, suggesting that they are post-follicular.\(^\text{8}\) Although the databases from which the matched germline sequences were derived are considered to represent the complete repertoire, we cannot exclude the possibility that some apparent mutations are due to polymorphic variation. However, this is unlikely to account for all of the variation observed. The base changes were observed in repeat sequences and therefore were not due to PCR error. In addition, the presence of mutations in JH in cases 1 and 2 would be unusual if the VH were unmutated.

The processes involved in the removal of B cells recognising autoantigens is not clearly understood. However, it has been shown that B cells with specificity for soluble antigens die when they bind soluble autoantigen in the germinal centre.\(^\text{15}\)\(^\text{16}\) This group of soluble antigens could theoretically include IgG, the autoantigen recognised by case 3. The hypothesis that B cells recognising soluble anti-gens are eliminated in the follicle centre is supported by the analysis of IgVH genes used by postimmunisation rheumatoid factors. This analysis provides evidence for the deletion of putative high affinity mutated variants.\(^\text{17}\) The autoreactivity observed in the cases of MALT type lymphoma studied herein suggests that the postfollicular precursors of these lymphomas were unresponsive to, or were not subjected to, such a process of deletion in the germinal centre. They may have been able to avoid deletion through neoplastic transformation.

Immunoglobulin derived from MALT type lymphomas differs from the polyreactive immunoglobulin expressed by cases of chronic lymphocytic leukaemia. The former is produced by mutated IgVH genes and recognises single autoantigens. The latter, however, is encoded mostly, but not exclusively, by germline IgVH genes.\(^\text{15}\) This autoreactivity seems to have a different aetiology in these different lymphoma groups. The frequency with which auto-reactivity is associated with lymphoma suggests that cross-linking of tumour derived immunoglobulin by autoantigen may be a significant factor in the pathogenesis of lymphoma.

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**Acknowledgements**

We are grateful to C Ian Mockridge for help with the sequencing. This work was supported by Tenerus and the Wessex Medical Trust (CJC), the Leukaemia Research Fund (DKD-W) the Cancer Research Campaign (TH).

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