# Immunoglobulin heavy chain variable region family expression in primary cutaneous follicle centre cell lymphomas.

*British Journal of Dermatology* 2001; **144:** 862–865.

# Immunoglobulin heavy chain variable region family expression in primary cutaneous follicle centre cell lymphomas

M.ALAIBAC, A.BELLONI-FORTINA, M.MORI*, B.PIGOZZI, A.PESERICO AND N.PIMPINELLI*

*Institute of Dermatology, University of Padua, Via C. Battisti 206, Padua 35100, Italy*
**Institute of Dermatology, University of Florence, Italy*

Accepted for publication for publication 7 December 2000

**Summary**

*Background* Primary cutaneous follicle centre cell lymphomas (PCFCCL) are the most common type of primary cutaneous B-cell lymphomas. Patients with this disorder develop one or a few clinically indolent tumour nodules, plaques, or papules confined to a circumscribed area. Only limited data have so far been published on the use of immunoglobulin heavy chain variable region (VH) families by these tumours.

*Objectives* Because the definition of the repertoire of VH genes could have significant biological and diagnostic implications, we evaluated a group of patients with PCFCCL for VH family use.

*Methods* DNA obtained from 15 cases of PCFCCL was amplified by polymerase chain reaction (PCR) using a panel of VH family-specific amplimers.

*Results* The pattern of VH family usage was similar to that reported in both normal peripheral blood B cells and the most common low grade non-Hodgkin's lymphomas, where VH region utilization is essentially random with frequent usage of the VH3 family.

*Conclusions* These findings support the hypothesis that PCFCCL may derive from the mature peripheral lymphoid compartment. Our results may also have implications for the routine analysis of B-lymphoid clonality in PCFCCL.

*Key words*: B cells, cutaneous lymphomas, gene rearrangement, IgH repertoire, skin immunology

It is now well established that the diversity for antigen recognition by B cells is generated by rearrangements of the separate immunoglobulin gene segments.[1] The immunoglobulin heavy chain (IgH) gene in the germline state consists of variable (VH), joining (JH) and diversity (DH) gene segments interspersed over hundreds of kilobase on chromosome 14.[2] In the course of normal lymphoid development, the IgH locus undergoes specific somatic recombination that brings a VH gene adjacent to a DH and a JH segment.[3,4] Each individual has about 100–200 germline VH genes, which have been classified into seven gene families on the basis of at least 80% nucleotide sequence homology. Individual families range in size from one (VH6) to greater than 30 (VH3).[3,4] VH family utilization in the mature peripheral lymphoid compartments appears to be essentially random and roughly correlates with estimates of family size.[5,6] Studies on IgH gene rearrangement have shown that human B-cell malignancies may express a biased VH repertoire compared with peripheral blood B lymphocytes.[7–10]

Primary cutaneous follicle centre cell lymphomas (PCFCCL) are cutaneous B-cell lymphomas with an indolent clinical behaviour.[11,12] PCFCCL demonstrate a neoplastic proliferation of follicle centre cells, showing a clinical picture characterized by papules, plaques and/or tumours confined to a circumscribed area with a predilection for the head, neck and trunk. Only limited data have been published so far on the use of VH families by these tumours.[13] Because the definition of the repertoire of VH genes in PCFCCL could have significant biological and diagnostic implications, we evaluated 20 cases for VH family use.

## Materials and methods

Fifteen PCFCCL samples were included in the study. All cases had previously been characterized by careful

Correspondence: Dr Mauro Alaibac.
E-mail: alaibac@ux1.unipd.it

clinical, morphological and immunophenotypic analysis. DNA was obtained from frozen tissue samples according to standard procedures by digestion with Proteinase K and extraction with phenol/chloroform. All samples were analysed to determine DNA concentration by ultraviolet spectrophotometry. DNA from each case was subjected to a set of seven polymerase chain reactions (PCRs), each of which used the common JH amplimer ACCTGAGGAGACGGTGACC for the 3′ end of the JH region and one of the following VH family amplimers:[14] VH1, CCTCAGTGAAGGTCTCCTGCAAGG; VH2, TCCTGCGCTGGTGAAAGCCACACCA; VH3, GGTCCCTGAGACTCTCCTGTGCA; VH4a, TCGGAGACCCTGTCCCTCACCTGCA; VH4b, CGCTGTCTCTGGTTACTCCATCAG; VH5, GAAAAAGCCCGGGGAGTCTCTGAA; VH6, CCTGTGCCATCTCCGGGGACAGTG.

These amplimers are specific to the first framework region (FR1) of most described members of the individual VH families. Although a VH7-specific amplimer was not employed in this study, it should be noted that the VH1-specific amplimer will also efficiently prime VH7-specific rearrangements.[15] The PCR mixture consisted of 1 U Taq polymerase (Promega, Madison, WI, U.S.A.), a 10 × reaction buffer as supplied by the manufacturer, 0·1 mmol $L^{-1}$ each of deoxyadenosine triphosphate, deoxycitidine triphosphate, deoxyguanosine triphosphate and deoxythymidine triphosphate, 100 ng of each primer, and varying amounts (200 ng to 1 μg) of template DNA in a final volume of 30 μL. The samples were denatured for 5 min at 95 °C and then exposed to 30 cycles of amplification using conditions adapted for each amplimer set as previously described.[9] PCR was performed using a programmable heat block, the Perkin-Elmer Thermocycler (Perkin-Elmer Cetus, Norwalk, CT, U.S.A.). Ten microlitres of the final PCR products were electrophoresed on 2% agarose gel, stained with ethidium bromide, visualized under short wavelength ultraviolet light, and photographed. Strict precautions against cross-contamination were taken. Amplifications without genomic DNA were performed alongside each set of PCRs to control for possible contamination and all results were confirmed on at least two separate occasions. In order to confirm the specificity of the product 2 μL of the PCR product were reamplified using the VH family amplimer and an internal common JH amplimer AGGGT(C/G/T)CCTTGGCCCCAG.

## Results

The usage of VH families was determined in 10 of 15 cases of PCFCCL (Table 1). Although none of the PCR products were sequenced, we are confident they correspond to VH genes as they were of appropriate size and could be further amplified using a universal JH-specific internal primer. The validity of this approach in determining the VH family specificity of clonal rearrangements has been confirmed by nucleotide sequence analysis in other studies.[13–15] Most of the lymphomas expressed heavy chain genes from the VH3 family, whereas several cases showed rearrangements involving the VH4 and VH1 families. In one case PCR products were obtained with two of the VH family-specific primers. It is possible that in this case amplification from both a productive and non-productive allele was being detected. As the PCR products were not sequenced, we could not determine the VH family utilized by the productive allele and, therefore, in this case assignment of VH family usage was not possible.

**Table 1.** VH family repertoire of primary cutaneous follicle centre cell lymphomas

| VH family | VH usage (10 cases) | Family complexity (%) |
|---|---|---|
| VH1 | 2 | 33 |
| VH2 | 0 | 7 |
| VH3 | 6 | 37 |
| VH4 | 2 | 18 |
| VH5 | 0 | 4 |
| VH6 | 0 | 1 |

## Discussion

The overall pattern of VH family usage by PCFCCL appeared similar to that of adult peripheral blood B lymphocytes.[6] Thus, compared with this control B-cell population, VH family usage in PCFCCL is not biased. The majority of PCFCCL expressed VH genes from the largest family, VH3, followed by the VH4 and VH1. Therefore, finding that the largest VH family, VH3 with at least 30 members, was used most often is certainly suggestive of random use. No PCFCCL expressing heavy chain genes from the VH2, VH5, and VH7 families were detected, probably because of the small size of these families relative to the number of cases analysed. The VH1 family was expressed in two cases by PCFCCL. The VH1 family contains about 20 individual germline members, and relative to its complexity is clearly underutilized in PCFCCL. However, the low or absent utilization of the VH1 family has also been observed in

adult peripheral blood B lymphocytes and other B-cell malignancies.[5–10] Taken together, our results show that the VH repertoire of PCFCCL is similar to that associated with the most common low grade non-Hodgkin's lymphomas, where VH region utilization is essentially random, while a biased repertoire is observed in tumours or transformed cells representing early steps of B lymphopoiesis.[5–10] As the use of VH genes reflects the stage of cell maturation in the B-cell line, our results indicate that PCFCCL may derive from the mature peripheral lymphoid compartment. This means that immature B cells are not involved in PCFCCL, a hypothesis in agreement with the data of the other study, which has analysed VH gene expression in PCFCCL.[13] In this previous analysis, four cases of PCFCCL were evaluated: three of these cases expressed VH genes from the VH3 family and one from the VH5 family. In addition to the assignment of VH families, the VH product was cloned and sequenced. The analysis of sequences showed the presence of somatic mutations of rearranged VH genes, which is a characteristic of germinal centres developing in follicles at a time of intense B-cell proliferation during T-cell-dependent antibody responses. Thus, the investigators hypothesized that PCFCCL may be derived from germinal centre cells or their descendants.

The results of our study may also have implications for the diagnostic detection of clonal IgH VDJ region gene rearrangements by PCR. In a previous investigation, we have shown that it is possible to demonstrate the clonality of PCFCCL by PCR using a single pair of universal primers, which match conserved areas at the 3′ end of the third framework region (FR3) of most VH gene segments and at the 3′ end of all JH regions.[16] Using this approach, we were able to demonstrate clonal IgH VDJ rearrangements in only 50% of the cases investigated. These results were indicative of a high false negative rate, most likely determined by the use of a single universal VH amplimer. In the present study, we have demonstrated clonal IgH VDJ gene rearrangements in 11 of 15 cases of PCFCCL (about 80%) using the above-mentioned panel of VH family-specific amplimers. However, the use of the VH family primers for the routine analysis of B-lymphoid clonality is technically cumbersome, as it involves seven different PCRs on each test sample. As the application of a PCR-based method needs to be both technically simple and have a low false negative rate, our results on the usage of VH family by PCFCCL suggest initial screening of these neoplasms with the VH3 family-specific FR1 primer and the universal VH FR3 primer. As already mentioned, rearranged IgH genes in PCFCCL are known to sustain a high level of somatic mutations. This may in part explain the failure to prime with the universal VH FR3 primer utilized in our previous investigation[16] and similarly may cause a failure to prime with the VH family-specific FR1 primers. Therefore, the use of both VH FR3 and VH FR1 primers in PCFCCL known to accumulate somatic mutations should significantly reduce the false negative rate in screening these neoplasms.

## Acknowledgments

This work was supported by the Italian Ministry of University and Scientific Research (ex 60% funds). We thank Anna Michelotto for technical assistance.

## References

1 Blackwell TK, Alt FW. Immunoglobulin genes. In: *Molecular Immunology* (Hames BD, Glover DM, eds). Oxford: IRL Press, 1989: 1–60.
2 Cook GP, Tomlinson IM. The human immunoglobulin VH repertoire. *Immunol Today* 1995; **16**: 237–42.
3 Walter MA, Surti U, Hofker MH, Cox DW. The physical organization of the human immunoglobulin heavy chain gene complex. *EMBO J* 1990; **9**: 3303–13.
4 Silberstein LE, Rao SP. VH-mediated mechanisms in normal and neoplastic B cell development. *Immunol Res* 1998; **17**: 253–9.
5 Guigou V, Cuisnier A, Tonnelle C *et al.* Human immunoglobulin VH and VK repertoire revealed by *in situ* hybridization. *Mol Immunol* 1990; **27**: 935–40.
6 Bahler DW, Campbell MJ, Hart S *et al.* Ig VH gene expression among human follicular lymphomas. *Blood* 1991; **78**: 1561–8.
7 Humphries CG, Shen A, Kuziel WA *et al.* A new human immunoglobulin VH family preferentially rearranged in immature B-cell tumors. *Nature* 1988; **331**: 446–9.
8 Logtenberg T, Schutte MEM, Inghirami G *et al.* Immunoglobulin VH gene expression in human B cell lines and tumors: Biased VH gene expression in chronic lymphocytic leukemia. *Int Immunol* 1989; **1**: 362–6.
9 Deane M, Norton JD. Immunoglobulin heavy chain variable region family usage is independent of tumor cell phenotype in human B lineage leukemias. *Eur J Immunol* 1990; **20**: 2209–17.
10 Deane M, Norton JD. Preferential rearrangements of developmentally regulated immunoglobulin VH1 genes in human B lineage leukemias. *Leukemia* 1990; **5**: 646–50.
11 Santucci M, Pimpinelli N, Arganini L. Primary cutaneous B-cell lymphoma: a unique type of low-grade lymphoma. Clinicopathologic and immunologic study of 83 cases. *Cancer* 1991; **67**: 2311–26.
12 Willemze R, Kerl H, Sterry W *et al.* EORTC classification for primary cutaneous lymphomas: a proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer. *Blood* 1997; **90**: 354–71.
13 Aarts WM, Willemze R, Bende RJ *et al.* VH gene analysis of primary cutaneous B-cell lymphoma: evidence for ongoing somatic hypermutation and isotype switching. *Blood* 1998; **92**: 3857–64.

14 Baker BW, Deane M, Gilleece MH *et al.* Distinctive features of immunoglobulin heavy chain variable region gene rearrangement in multiple myeloma. *Leuk Lymphoma* 1994; **14**: 291–301.
15 Mortari F, Newton JA, Wang JY, Schroeder Hw Jr .The human cord blood antibody repertoire. Frequent usage of the VH7 gene family. *Eur J Immunol* 1992; **22**: 241–5.
16 Alaibac M, De Paradiso A, Lena M *et al.* Detection of clonality in cutaneous B-cell lymphomas by polymerase chain reaction gene amplification. *Eur J Dermatol* 1995; **5**: 741–2.