SHORT COMMUNICATION

Discordant differentiation antigen pattern in a case of Richter’s syndrome with monoclonal idiootype expression and immunoglobulin gene rearrangement

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Immunophenotyping using monoclonal antibodies (MAbs) has become a widespread tool in the scientific, as well as routine, pathological study of Non-Hodgkin’s lymphoma (NHL). Larger studies have suggested that not only different histologically defined NHL subtypes, but also individual cases of a single NHL subtype, may vary considerably in the expression of leucocyte differentiation antigens (Schuurman et al., 1987). The question, however, whether homogenous or heterogeneous phenotypes correspond to genetically monoclonal or non-monoclonal lymphomas, respectively, has not been investigated so far.

Richter’s syndrome (RS), which is defined as the emergence of a large cell lymphoma (LCL) in the course of a chronic lymphocytic leukaemia (CLL) (Trump et al., 1980), represents a unique condition for the study of this issue. While a number of clearly biclonal RS have been described (McDonnell et al., 1986; Ostrowski et al., 1989; Sklar et al., 1984; Van Dongen et al., 1984), some cases seem to share a common clonal origin despite the heterogenous morphology of LCL and CLL tumors (Bertoli et al., 1987). These cases thus offer an interesting opportunity to study the correlation between monoclonality and differentiation antigen expression. For a detailed analysis of the immunophenotype, a broad panel of MAbs can be used; these recognise lymphocyte surface antigens which have been extensively characterised by the International Leucocyte Typing Workshops (Knapp et al., 1989). In the case of B cell-derived tumours, clonality can be assessed by probing for the variable parts of the immunoglobulin antigen receptor on the protein and the genomic level. Clonal rearrangements of the immunoglobulin genes take place in normal as well as malignant B cells, thus giving rise to unique restriction fragment length patterns and immunoglobulin idiootype expression which both represent exquisite markers for clonality (Arnold et al., 1983; Mayumi et al., 1982).

We investigated the case of patient HK, a 47-year-old male, who presented in 1980 with enlarged lymph nodes, splenomegaly and a white blood cell count of 100,000 μl⁻¹. Histological and immunohistochemical examination of three lymph node biopsies from 1980 to 1982 confirmed the clinical diagnosis of CLL with B cell phenotype. Having received multiple courses of, initially mild and later aggressive, chemotherapy regimens from 1981 to 1986, the patient developed a rapidly progressive disease with fever, massive lymph node enlargement, hepatosplenomegaly, ascites and cachexia in the beginning of 1987, leading to death within four months. Clinical manifestation and progression of the disease in patient HK appeared characteristic of RS, as described in recent surveys (Trump et al., 1980).

At autopsy, a generalised lymphadenopathy, hepato- and splenomegaly and diffuse blastomatus expansion of the bone marrow was seen. In histological examination, the enlarged lymph nodes and the bone marrow both showed infiltration of small lymphocytes consistent with CLL. Furthermore, large pleomorphic cells were found (Figure 1) which had round to irregularly shaped nuclei with one or multiple prominent nucleoli and moderately abundant, strongly basophilic cytoplasm and a high mitotic rate consistent with the histological diagnosis of a high grade malignant lymphoma of the centroblastic, polymorphic subtype (CBp) according to the Kiel classification (Lennert et al., 1978). Diffuse infiltrations by CLL cells were detectable in the splenic red and white pulp, the periporal tracts of the liver and, to a lesser extent, in the lungs. In addition to a focal intraparenchymatous liver infiltration, the CBp was found to infiltrate the kidney interstitium and the peribronchial tissue. The finding of typical admixed CLL and CBp infiltrates confirmed the clinical diagnosis of RS in patient HK. For immunohistochemical studies, cryostat sections of snap frozen tissue specimens were stained by a modified

Figure 1 Lymph node with composite neoplastic involvement. Strands of small lymphocytes are intermingled with large blastic cells characterised by a small rim of cytoplasm, a round blastic nucleus and small to medium sized nucleoli, thus resembling centroblasts. Paraffin section from autopsy tissue, stained with HE (x 87.75). Scale bar = 50 μm.

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peroxidase method as described in detail elsewhere (Möller et al., 1989). The results of three independent stainings performed on bone marrow, lymph node and kidney tissue samples, which are representative for all organs studied, are listed in Table I. Both the CLL and CBp expressed high amounts of HLA-A, B, C and HLA-DR antigens (Figure 2e). B cell origin of CLL and CBp could be demonstrated by positive staining for μ heavy and κ light chain, for CD37 (Figure 2c), CD40 and gp80. Interestingly, the B cell differentiation antigens CD19, CD22 and CD24 (Figure 2a) and CD5 and CD38 antigens were only expressed in CLL, whereas the MHC class II product HLA-DR (Figure 2f) and the B cell related antigen CD23 (Figure 2b) were detectable only in CBp. The non-neoplastic bone marrow cells were mainly composed of CD3 positive T cells, most of them being of the cytotoxic/suppressor type.

To test whether the observed differences in the expression of leucocyte differentiation antigens correlated with a monoclonal lymphoma in patient HK, two independent strategies of probing for clonality were applied. Firstly, two monoclonal antibodies recognising idiotypic determinants on purified HK tumour immunoglobulin were manufactured using standard methods described previously in detail (Moldenhauer et al., 1983). Specificity of MAbs AHK154 and AHK120 for HK tumour idotype was demonstrated by binding to HK tumour IgM, but not to four unrelated IgM and two IgG (data not shown); and binding to cryopreserved HK tumour cells, but not to a panel of unrelated lymphomas and normal B cells in an ELISA on mildly fixed cells (data not shown). When MAbs AHK154 and AHK120 were applied to immunohistochemical staining of HK tumour tissues, CLL and CBp tumour cells in all organs analysed equally stained for both idiotypic determinants (Figure 2d). In addition, when cryopreserved peripheral blood tumour cells from 1985 were analysed for cell surface expression of idiotypic determinants by flow cytometry all tumour cells (amounting to more than 90 per cent of peripheral blood lymphocytes) again reacted with MAbs AHK154 and AHK120 (data not shown). Thus the idiotypic determinants recognised by the two MAbs had been present in all peripheral blood tumour cells from two years before the clinical onset of RS, and were equally displayed by CLL and CBp cells at autopsy.

To gain further evidence for the clonal origin of CLL and CBp cells, we applied immunoglobulin gene probing to HK tumour material. Genomic DNA from autopsied HK tumour tissues and from HK PBL from 1985 was extracted with standard procedures, digested with appropriate restriction enzymes and blotted onto GeneScreen Plus membranes (NEW, Boston, MA). For hybridisations, a 5.5 kb genomic BamHI/HindIII fragment spanning the human immunoglobulin heavy chain joining region (Arnold et al., 1983) was labelled with a random oligo-prime dinucleotide labelling kit (Boehringer, Mannheim, FRG). An identical rearrangement of both immunoglobulin heavy chain gene alleles was detected in all samples analysed. Following digestion with BamHI and HindIII simultaneously, two rearranged bands of 2.8 and 1.6 kb with equal intensity showed up in addition to a faint band of 4.6 kb which represented residual normal lymphocytes or non-lymphoid tissues with a germline immunoglobulin gene configuration (Figure 3). Equivalent patterns were seen with rearranged alleles and a monoclonal band were produced by digestions with StuI, PstI and BglII enzymes or combinations of these. This pattern indicates a rearrangement of both immunoglobulin heavy chain genes in a single B cell clone which is not an unusual finding in lymphomas (Arnold et al., 1983). It cannot be explained by the presence of two independent B cell clones with a single rearrangement each. In this case the two unrearranged alleles would result in a germline band with an approximately twofold increase in intensity compared with the rearranged bands. Thus a single B cell clone with a rearrangement of both heavy chain alleles was detected in all tumour tissues from 1985 and 1987.

In summary, CLL cells from more than one year before the clinical onset of RS, and autopsy CLL and CBp tumour tissues displayed an identical idotype expression and immunoglobulin gene rearrangement. Given the extremely low probability for a common rearrangement with a shared idotype in two independent tumours, these results clearly demonstrate a common clonal origin of both CLL and CBp tumour cells in the case presented. This is in accordance with three other published cases of clearly monoclonal RS (Bertoli et al., 1987; Michiels et al., 1989; Siegelman et al., 1985).

Table 1 Immunophenotypic marker studies on HK tumour samples

| Antigen | Clone | Reactivity in CLL | CBp |
|---------|-------|-------------------|-----|
| CD2     | OKT11 |                   | -   |
| CD3     | Leu4  |                   | -   |
| CD4     | OKT4  |                   | -   |
| CD5     | OKT1  | +                 | -   |
| CD8     | OKT8  |                   | -   |
| CD38    | OKT10 |                   | -   |
| CD10    | J5    |                   | -   |
| CD19    | HD37* |                   | +   |
| CD20    | 1F5   |                   | +   |
| CD21    | B2    |                   | -   |
| CD22    | HD39* |                   | +   |
| CD23    | HD50* |                   | -   |
| CD24    | VIB-E3|                   | +   |
| CD37    | HD28* |                   | -   |
| CD40    | G28-5 |                   | +   |
| CD53    | HD77* |                   | +   |
| HLA-A,B,C| W6/32 |                   | +   |
| HLA-DR  | ISCR3 |                   | +   |
| HLA-DP  | BT/21 |                   | +   |
| HLA-DQ  | TGU2  |                   | -   |
| Inv. Chain | VIC-Y1 |               | +   |
| CD11c  | LeuM5 |                   | -   |
| CD13   | My7   |                   | -   |
| CD14   | BEAR2 |                   | -   |
| CD15   | LeuM1 |                   | -   |
| CD30   | Ki-1  |                   | -   |
| Vimentin | V9  |                 | +   |
| gp80   | G28-8 |                   | +   |
| HK-Idiotype | AHK154.3* |           | +   |
| HK-Idiotype | AHK120.8* |              | +   |
| Lambda | 1-155-2 |        | -   |
| kappa | NHV361* |                   | +   |
| IgM    | NLIH205* |               | +   |
| IgG    | 8a4   |                   | -   |

* Antibodies produced in our laboratory. MAbs to differentiation antigens are described in 'Leucocyte Typing IV' (Knapp et al., 1989).
globulin gene heavy chain rearrangement, they displayed a distinct pattern of differentiation antigen expression. The phenotype of CLL cells with expression of pan-B markers CD19 and CD20, B-restricted markers CD22 and CD24 and expression of CD5 is in accordance with the data obtained in larger studies (Schuurman et al., 1987). CBp cells displayed an immunophenotype (vimentin−, CD21−, CD30−, CD38+) which is highly suggestive of a follicular centre cell stage of maturation. Thus, morphological as well as immunophenotypic criteria indicate a transition from chronic lymphatic leukaemia to a polymorphic centroblastic lymphoma.

The transition from CLL to CBp may have been caused by mutational events not involving the immunoglobulin heavy chain variable region. Translocations juxtaposing the immunoglobulin light chain or heavy chain genes to a number of oncogenes are commonly found in certain types of B cell lymphomas. Such mutations were shown to lead to a deregulation of the oncogenes and may, in part, be detectable as rearrangements of the translocated gene sequences. Translocations joining the bcl-2 oncogene to immunoglobulin genes are found in 85 per cent of lymphomas with follicular centre cell type and, in very rare cases, in CLL (Adachi et al., 1989) and are thought to give rise mainly to indolent, slowly progressive lymphomas with nodular architecture (Yunis et al., 1987). Translocations involving the c-myc oncogene together with immunoglobulin genes are found in the highly malignant Burkitt lymphomas and are associated with a high rate of cell proliferation (Taub et al., 1982). In addition,
CBp tumour cells was analysed for a possible rearrangement of the c-myc gene which might indicate a translocation. Using a 300 bp PstI-fragment from the second exon of the c-myc gene, identical patterns of restriction enzyme fragments were obtained when DNA from both histological subsets of HK lymphoma and DNA from control normal lymphocytes was digested with PstI, SstI, Smal and Xhol enzymes (data not shown). Thus no evidence for a translocation involving c-myc or other genetic events resulting in a clonal evolution towards high grade malignancy can be provided in the case presented.

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### Table II Clonality in published cases of Richter’s syndrome

| Authors | Cases | Ig Isotype in CLL/LCL | Ig gene re-arrangement | Phenotypic differences (losses in LCL) |
|---------|-------|-----------------------|------------------------|--------------------------------------|
| Splinter | 1     | μλ/μκ                 | N.T.                   | N.T.                                 |
| Delsol  | 1     | μλ/μκ                 | N.T.                   | N.T.                                 |
| Chan    | 4     | 1 identical           | 3 divergent            | 3/4: CD5                             |
| Michiels| 1     | μλ/μκ                 | monoclonal            | No                                   |
| Bertoli | 1     | μλ/μκ                 | monoclonal            | IgD                                  |
| Ostrowski| 1   | μλ/—                  | bclonal                | No                                   |
| McDonnell| 1    | μλ/μκ                | bclonal                | CD21                                 |
| Van Dongen | 1 | μλ/μκ               | bclonal                | CDS                                 |
| Sklar   | 3     | 2 identical           | 3 bclonal              | N.T.                                 |
| Siegelman| 7   | 6 identical           | 5 bclonal              | N.T.                                 |

*Including B cell lymphomas with two histological types without involvement of CLL.
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