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Immunoglobulin idiotype expression in reactive lymphoid tissues and B-cell lymphomas

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Summary. In an investigation of the immunoglobulin idiotypic expression of non-tumour and neoplastic B lymphocytes in situ, fresh-frozen specimens of reactive tonsils, lymph nodes and B-cell malignant lymphomas (B-MLs) from Japanese patients were studied immunohistochemically with 39 different anti-idiotype antibodies. In reactive lymphoid tissues, while idiotype-bearing cells were largely distributed sparsely in follicles and perifollicular areas, some were heavily crowded in particular germinal centres (GCs), suggesting the presence of oligoclonal proliferations of B-cells in GCs. Forty-eight out of 100 B-MLs reacted with anti-idiotype antibodies. This proportion was significantly higher than those reported in Western cases (27–36%), indicating that Japanese B-MLs share public idiotypes much frequently than western cases. The idiotypes demonstrated in these lymphomas, in contrast to those not expressed in any B-ML, were found much commonly in non-tumour lymphocytes, suggesting that such public idiotypes as were common in B-MLs were frequently shared by normal B-lymphocytes.

Key words: Immunoglobulin – Idiotype – B-lymphocyte – Lymphoma – Lymph node

Introduction

Human B-cell lymphomas (B-MLs) express a unique monomorphic immunoglobulin (Ig) variable region (idiotype) which has been regarded as specific to each patient (Lowder and Levy 1985; Badger and Bernstain 1986; Houghton and Scheinberg 1986; Lowder 1986; Sikorska 1988). The diagnostic and therapeutic potential of such idiotypes on B-MLs has been investigated intensively. Initially, monoclonal anti-idiotype antibodies for each patient suffering from B-ML were produced laboriously (Hamblin et al. 1980; Miller et al. 1982; Meeker et al. 1985; Rankin et al. 1985). Once a suitable anti-idiotype antibody-producing clone was identified, large quantities of antibodies were produced and employed in individual patients. This method, however, had some disadvantages for therapy: the production of an anti-idiotype antibody for an individual patient, including heterohybridoma production and screening, scaling up, purification and quality control of the product, took approximately 1 year (Rafeld et al. 1985; Carroll et al. 1986; Kon et al. 1987).

The existence of idiotypes shared among a group of patients and recognized by the same anti-idiotype antibody, termed as public idiotypes, has been noted, and it has become possible to construct a library of anti-idiotype antibodies recognizing public idiotypes, thereby making it unnecessary to create a monoclonal antibody for each individual patient. In accordance with this view, Miller et al. (1989), examining 199 anti-idiotype antibodies, were able to select 37 antibodies as public antibodies. Twenty out of these 37 public antibodies were found to react with a total of 49 out of 150 B-MLs (Brown et al. 1989).

The existence of clonal growth of B-lymphocytes in non-tumour lymphoid tissues has been a matter of debate for many years. Recently Valles-Ayoub et al. (1990), by the use of molecular biological techniques, and Grace et al. (1989), using a cytogenetic method and flow cytometry, have shown that small populations of B-lymphocytes in reactive lymphoid tissues are in a single clone, by revealing clonal Ig gene rearrangements (Valles-Ayoub et al. 1990), or specific chromosomal translocations and clonal expression of surface Ig light chains (Grace et al. 1989). Those studies were based on cell suspensions or lysates, whereas no detailed studies have been reported on the monoclonal or oligoclonal proliferation of B-lymphocytes at a histological level (Thielemans et al. 1984; Samoszuk et al. 1987).

The Ig light chains, k and/or λ, have been used widely to demonstrate the clonal growth of B-lymphocytes in tissue sections. This method is effective if clonally proliferating cells predominate in the cellular components in

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a tissue section, but ineffective when such cells constitute only a minor part. The anti-idiotype antibodies can be used as another tool for the demonstration of B-cell clonality. Such antibodies have an advantage in that small numbers of clonal cells can be demonstrated, as very small numbers of non-tumour B-lymphocytes share the same idiotype. Meanwhile, such study has the disadvantage in that the available antibodies are not sufficient to cover the whole range of B-cells. Accepting these limitations, we studied ten reactive lymphoid tissues using available public anti-idiotype antibodies to determine the existence of clonal growth of B-lymphocytes in reactive lymphoid tissues.

Japanese MLs have racial and/or geographical specificities, such as much lower frequency of follicular MLs, much higher frequency of diffuse large non-cleaved/immunoblastic B-MLs, and the existence of a large cluster of HTLV-1 associated T-MLs (Suchi et al. 1979). Accordingly, we also studied the specificities of Japanese B-MLs in their Ig idiotype expression.

Materials and methods

The specimens consisted of ten unfixed, frozen reactive lymphoid tissues including five tonsils and five lymph nodes and 100 B-ML tissues. The five tonsils had been resected from patients with tonsillar hypertrophy and later diagnosed histopathologically as reactive hyperplasia. Non-tumour lymph nodes, all from the cervical region, had been removed from patients with cervical lymphadenopathy. These lymph nodes showed the histopathological features of reactive hyperplasia without additional pathological changes. The B-ML tissues were taken from Japanese patients. The histopathological classification followed the criteria of the Working Formulation (The Non-Hodgkin's Lymphoma Pathological Classification Project 1982) as shown in Table 1. Thirty-nine anti-idiotype antibodies listed in Fig. 1, and six commercially available murine monoclonal antibodies including L26 (Ishii et al. 1986), Leu4, Leu2a, Leu3a (Evans et al. 1981; Ledbetter et al. 1981; Kan et al. 1983) and DRC-1 (Naiem et al. 1983) were employed to determine the cellular components of germinal centres (GCs) in detail and to exclude the possibility of neoplastic process in GCs of reactive tissues. The specificities of anti-idiotype antibodies have been described previously (Brown et al. 1989; Miller et al. 1989). Optimally diluted normal mouse serum was introduced as a negative control to rule out false positivity caused by the second- and third-phase reagents of immunostaining.

Table 1. Lymphomas expressing shared idiotypes, according to histological subtype

Histology	No. positive/total no.	
	Japan	USA*
F	5/19	55/251
DLC	29/57	5/41
DSC	4/7	10/22
IBL	2/7	4/10
SCL	4/5	38/171
SNC	3/5	5/22
Total	48/100	135/517
%	48%	26%

F, Follicular type; DLC, diffuse large cell type; DSC, diffuse small cleaved cell type; IBL, immunoblastic type; SCL, small cell lymphocytic type; SNC, small non-cleaved cell type
* Miller et al. 1990

The frozen sections of these tissues were studied immunopathologically by an avidin-biotin alkaline-phosphatase method (Boenish 1989). This is used to rule out endogenous false reaction which cannot be eliminated by the peroxidase method. The details of the staining procedures have been reported previously (Samoszuk et al. 1987). Briefly, the cryostat section was air-dried and fixed with periodate-lysin-paraform solution for 10 min. After rinsing with 0.05 M TRIS-HCl buffer (pH 8.7), the sections were incubated for 30 min each with the optimally diluted monoclonal antibodies, biotinylated anti-mouse Ig (E413, Dakopatts Copenhagen, Denmark), and avidin-conjugated alkaline phosphatase (D396, Dakopatts) successively, with three washes with TRIS-HCl buffer between each step. The slides were finally treated with the New Fuchsin solution for 30 min and mounted. The New Fuchsin solution was prepared by mixing solution A, containing 0.2 ml of 5% New Fuchsin (Chroma, in 2N HCl), 0.5 ml of fresh 4% sodium nitrate, 100 ml of 0.05 M TRIS-HCl buffer, pH 8.7, and 100 l of 1 M levamisole (L-9756, Sigma) with solution B, containing 0.6 ml *N-N* dimethylsulphonamide and 50 mg Naphthol AS-BI phosphate (N2250, Sigma).

For the quantitative analysis of each idiotype-bearing cell on non-tumour lymphoid tissues, the number of stained cells in ten randomly selected high-power fields (X400) was counted and the mean number was calculated. For the statistical analysis, Cochran-Cox's *t*-test was introduced to determine the difference of the means between different groups.

Results

The immune reaction of lymphocytes stained with anti-idiotype antibodies was clear and definite, with the background tissue entirely unstained (Figs. 1, 2). Control slides were entirely unstained, indicating the success in blocking of endogenous enzyme activity. The immune reaction on stained cells was mostly ring-shaped, leaving nuclei unstained. Some plasma cells were stained deeply in their cytoplasm. The staining intensity varied among cells, with moderate reaction in lymphocytes located in the mantle zone, GC and perifollicular area, and the heaviest reaction in plasma cells. These results indicate that both membranous and cytoplasmic Igs are stained.

In non-tumour tonsils and lymph nodes, each anti-idiotype antibody stained a varying number of lymphocytes. As an example, antibody H27-17 stained 15.0 ± 4.1 cells in one high-power view field of tonsils, while another antibody C15-87 stained only 0.06 cells (Fig. 3). These data apparently indicate that the number of stained cells differs greatly among anti-idiotype antibodies. However, the number of stained cells did not differ much among tonsils or lymph nodes. For example, the mean number of cells immunostained with antibody H27-17 was 18.4 in tonsil 1, 14.2 in tonsil 2, 8.6 in tonsil 3, 15.1 in tonsil 4 and 18.9 in tonsil 5 (mean 15.0 ± 4.1). The data show that the number of B-lymphocytes bearing this idiotype did not differ much among patients.

In most of the non-tumour tissues, the immunostained cells were distributed sparsely and evenly. Such stained cells were located in the GC, mantle zone and perifollicular areas, with a slight predominance in the GC. What was peculiar, however, was that in some tissues, the stained cells were heavily crowded in one GC (Fig. 1). In tonsil 1, for example, with antibody H135-32, as many as 250 positive cells in one high-power field

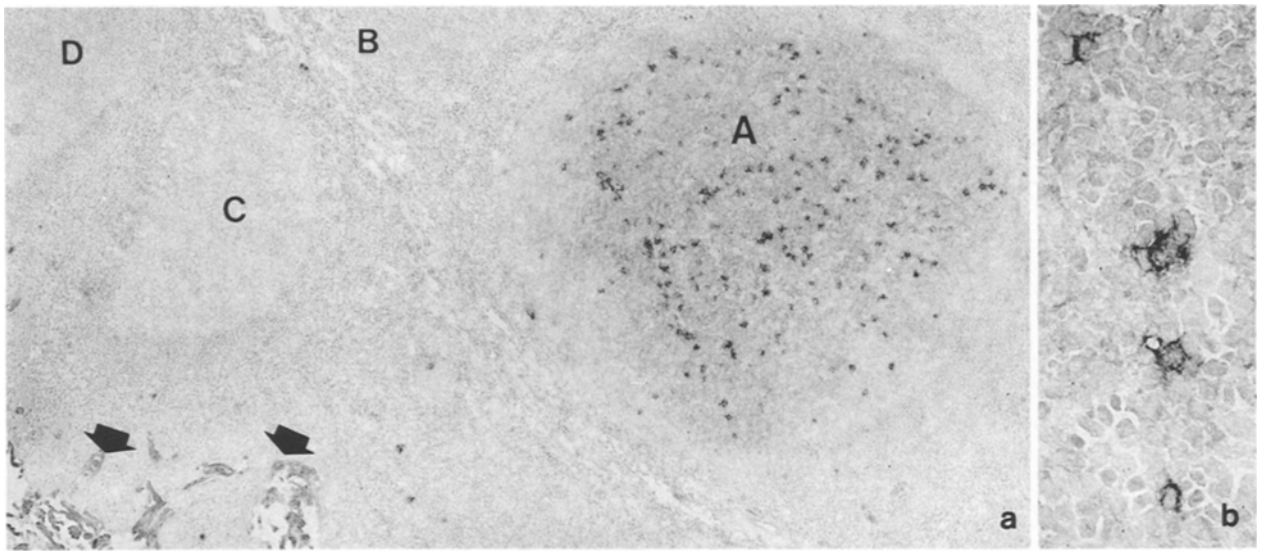

Fig. 1a, b. Immune reaction of a tonsil with an anti-idiotype antibody H135-32. A high concentration of stained cells is noted in a germinal centre (GC) (*A*). Note that the adjacent GCs (*B, C, D*) are not occupied by stained cells. *Arrows*, Tonsillar crypt. **a** Low magnification. **b** High magnification of GC

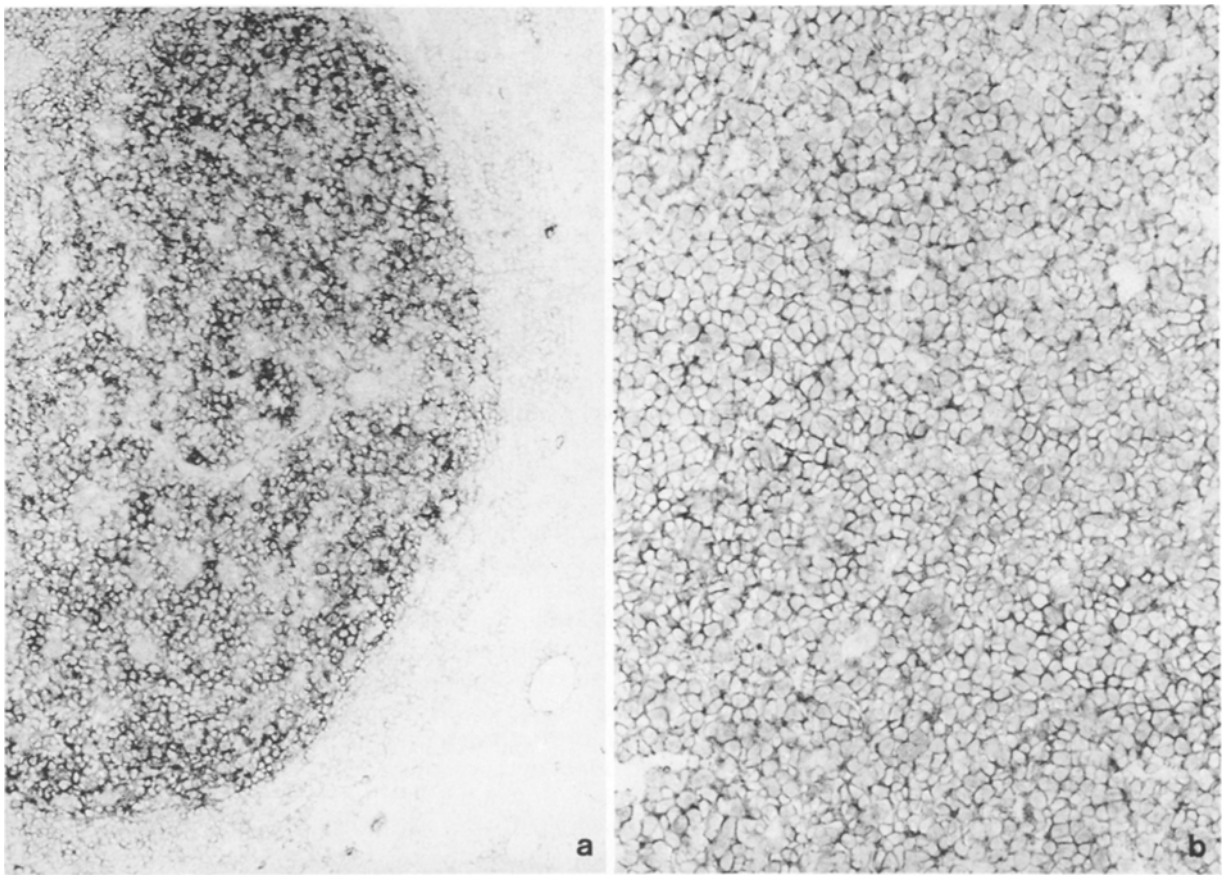

Fig. 2a, b. B-cell lymphomas (B-MLs) immunostained with anti-idiotype antibodies. Antibody L46–49 reacting with a tumour follicle in a case of follicular lymphoma **a**. Antibody S37–48 reacting with a case of diffuse lymphoma **b**

of a GC, constituting 6.2% of all the GC cells, were stained. On the same slide, no cells were shown to be stained in the adjacent GCs (Fig. 1). This apparently shows that a specific B-cell bearing this Ig idiotype is proliferating heavily in the GC. Also, some of the other GCs were found to be heavily populated by two or three different idiotype-bearing cells. This finding indicates that oligoclonal B-cells are proliferating in these GCs. In addition, no histological or immunohistological findings suggestive of neoplastic process were noted on such GCs.

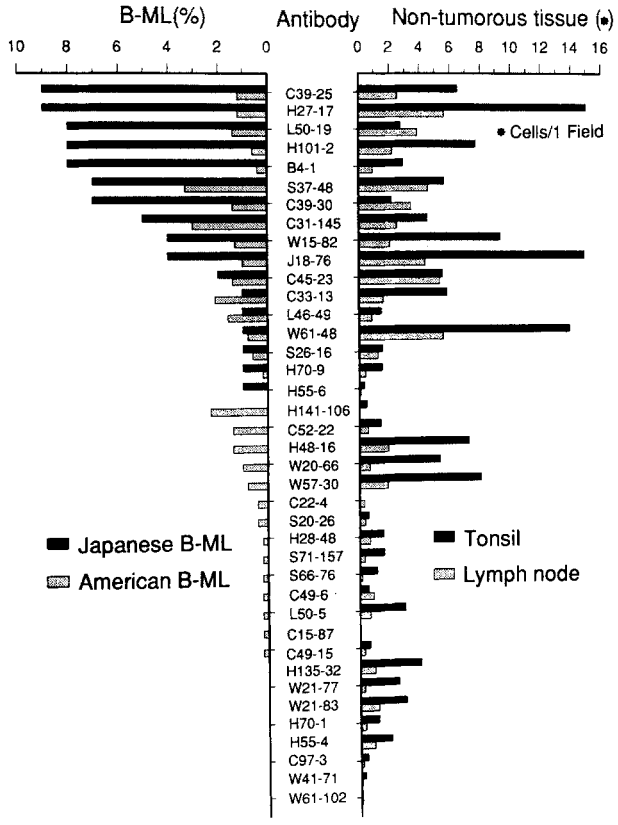

Fig. 3. Reaction of shared anti-idiotype antibodies with B-cell lymphomas (B-MLs) and non-tumour lymphoid tissues. On the left is the percentage of Japanese and American B-MLs stained by each anti-idiotype antibody. On the right is the mean number of immune-stained cells in one light-microscopical high power field (×400) of reactive tonsils and lymph nodes. Each field contains approximately 4000 cells. In the centre are the code names of 39 anti-idiotype antibodies. Data of American B-ML are cited from Miller et al. (1990)

On B-MLs, the immune reaction was easy to recognize. Most of the tumour cells were stained clearly, if the case was positive to a certain antibody (Fig. 2). In follicular lymphomas, a large number of the GC cells were stained (Fig. 2a). In diffuse lymphomas, the reaction was observed in most of the neoplastic cells (Fig. 2b), or not observed at all (not shown).

Forty-eight B-MLs (48%) were found to react with the antibody panel (Table 1). There were only 17 anti-idiotype antibodies which reacted with these MLs, indicating that some antibodies reacted with two or more cases. Actually, there are antibodies that reacted with as many as 9, 8 or 7 B-MLs (Fig. 3). This overlapping suggests the presence of common epitopes in Igs expressed on different B-MLs.

With regard to histopathological subtypes, follicular and immunoblastic lymphomas demonstrated a low positivity (Table 1). The same trend has been noted in American B-MLs (Miller et al. 1989). In Japanese diffuse large cell type B-MLs, however, including diffuse large non-cleaved and cleaved, there is a high positivity rate (51%), a value significantly higher than in American cases, 12% ($P<0.001$, Table 1).

There are some differences in staining intensity among positive cases. Some of diffuse large non-cleaved cell type showed a weak reaction, and the small cell lymphocytic type with plasmacytic differentiation represented the heaviest reaction. However, this result could not be generalized, as some of the large cells were occasionally found to be heavily stained. Rather, there was a prominent difference with different antibodies, that is to say, some antibodies stained positive cells heavily, while the reactions of other antibodies were weak.

There were 17 antibodies that reacted with at least one B-ML, while the remaining 22 antibodies did not react with any of the present Japanese MLs. The mean number of normal B-cells stained with the former group of antibodies was 5.98 ± 4.79 on reactive tonsils, and 2.78 ± 1.83 on reactive lymph nodes, while the number in the latter group was 2.07 ± 2.25 and 0.61 ± 0.55 respectively. With the use of Cochran-Cox's t-test, the difference of these values is significant at $\alpha < 0.001$ in lymph node and $\alpha < 0.01$ in tonsil, indicating that such idiotypes as were found in B-MLs were expressed commonly in non-tumour B-lymphocytes as well.

Discussion

A small proportion of the B-lymphocytes in reactive lymphoid tissues was recently reported to proliferate clonally. Such knowledge has come mainly from molecular and cytogenetic studies (Grace et al. 1989; Valles-Ayoub et al. 1990). Of we wish to confirm the presence of such clonal growth of B-cells at a histological level the Ig idiotype can be introduced as an useful tool. However, such analysis has remained at a preliminary level, offering no detailed insights into these problems; earlier studies have simply shown the presence of very small numbers of each idiotype-bearing cells dispersed in lymphoid tissue. What was unusual in the present study was that a heavy concentration of specific idiotype-bearing cells was noted in a few GCs. This finding apparently shows that clonal proliferation of B-lymphocytes, if present, would take place in such GCs, a new observation.

Another finding unique to this study is that the idiotypes found on B-MLs were also commonly observed in non-tumour B-lymphocytes. This is easy to understand when we consider that neoplastic transformation can occur in any B-cell, regardless of idiotypic specificities, and thus B-cells sharing common idiotypes are more likely to be found to be neoplastic. This finding differs from previous descriptions which have emphasized that no apparent correlation exists between B-ML and non-tumour B-lymphocytes in terms of idiotypic expression (Brown et al. 1989; Miller et al. 1989). On closer observation of these former reports, it is evident that their consideration of observations was superficial and described in one or two lines only. The use of intensive statistical analysis might have persuaded these authors to come to the same conclusion as us.

The present report has shown that as many as 48% of Japanese B-MLs react with anti-idiotype antibodies. This value is significantly higher than that of previous reports. The reported reactivity, based on a panel of antibodies which largely overlapped with that used in

the present study, was 33% (Miller et al. 1989), and according to recent data based on the present antibody panel, 26% (Miller et al., unpublished observations). Meanwhile, the reactivity reported by Chatterjee et al. (1990) based on European MLs was 36%. Hence, the high incidence in the present study was a surprise, especially when we consider that these antibodies were prepared from American B-MLs. On the analysis of histological subtype, such a gap between Japan and Western countries was found to be largely attributable to the difference in histological subtype. In fact, 51% of our diffuse large cell type were stained, whereas only 12% of this subtype in American cases were positive. As diffuse large cell type is the most common histopathological subtype in Japanese MLs (Suchi et al. 1979), comprising 50% of the present cases, it undoubtedly increased the positivity rate of all of our data.

Why is there such a difference (51% and 12%) between these two groups? One possibility is that the Japanese diffuse large cell type tumour is more differentiated and much more Ig production occurs. The other may be that the sensitivity of immunostaining is higher in the present method. The comparison of data with the use of the same methods will be needed to settle this problem.

In practical application, immunophenotyping of Ig idiotype will be effective in the diagnosis of B-ML cases with scanty neoplastic B-cells. Such B-MLs are not rare in routine surgical specimens. However, we have to point out some disadvantages at the same time. One is that the present anti-idiotype antibodies are effective only on fresh frozen sections (unpublished data). The second is that the present panel of antibodies can cover only 48% of Japanese B-MLs; thus negative results do not rule out the possibility that the tissue is affected by B-ML.

Finally, if anti-idiotype antibody therapy were to be introduced for the treatment of Japanese B-MLs, much better outcomes might be expected, because of the high incidence of cases sharing public idiotypes to which ready-made anti-idiotype antibodies are available. It remains to be ascertained whether this reactivity would be further increased by the introduction of antibodies produced from Japanese B-MLs.

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