Virchows Archiv (1994) 424:553–561



ORIGINAL ARTICLE

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# Down-regulation of membrane immunoglobulin-associated proteins, MB-1, B29 and Lyn, in AIDS-lymphomas and related conditions

Received: 16 June 1993 / Accepted: 22 February 1994

**Abstract** B-lymphocytes infected with Epstein-Barr virus (EBV) can proliferate in immunocompromized hosts to form lymphomas (MLs). Similar MLs are produced in mice with severe combined immune deficiency (SCID) by transfusion of human lymphocytes infected with EBV (SCID-EBV-positive BML). Mb-1 and B29 are recently found transmembrane proteins associated with membrane immunoglobulins (mIg) on the surface of B cells. Lyn is a *src* family gene product expressed in B cells submembranously, in association with mIg, possibly through Mb-1/B29 heterodimer. These mIg-associated proteins (Mb-1, B29 and Lyn) are known to mediate antigenic stimulation through mIgs. We noted recently that Lyn is decreased selectively in around a half of SCID-EBV-positive BMLs. We extended this line of investigation to other mIg-associated proteins. Five acquired immunodeficiency syndrome (AIDS)-MLs and ten SCID-EBV-positive BMLs were first analysed by immunohistochemistry for the expression of Mb-1, B29 and Lyn. It was found that in AIDS-MLs, all the mIg-associated proteins were heavily down-regulated. In SCID-EBV-positive BMLs, Mb-1 was down regulated in six of ten, B29 in nine of ten and Lyn in six of ten, whereas no down-regulation was noted in eight EBV-free B MLs that were also maintained in SCID mice. An additional flow-cytometric study of two SCID-EBV-positive and two EBV-negative BMLs showed similar down-regulation in the former cases exclusively. Whereas mIg was also decreased in three of five SCID-EBV positive BMLs, it did not necessarily match the decrease of mIg-associated proteins, which contrasts with the recent finding that mIgs coexist with Mb-1 or B29. Some EBV-encoded proteins may activate host molecules located downwardly; this, in turn, may lead to the suppression of these upwardly-located mIg-associated proteins.

**Key words** AIDS · Lymphoma · Epstein-Barr virus
Tyrosine kinase · Immunoglobulin

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## Introduction

Patients with acquired immune deficiency syndrome (AIDS) have a high incidence of non-Hodgkin's malignant lymphomas (AIDS-MLs): MLs have been observed in 4.5–16% of AIDS autopsies in Western countries [7, 12, 13, 18, 21, 28], while a much higher incidence has been noted in Japanese AIDS patients [19]. Defective T-cell surveillance in AIDS patients seems to permit the proliferation of Epstein-Barr virus (EBV)-transformed B cells, which finally leads to the appearance of lymphoid tumours. Such EBV-associated lymphoproliferative disorders range from polyclonal B-cell hyperplasia to monoclonal diffuse B-MLs [1, 8]. Similar EBV-bearing B-cell tumours occur in patients with congenital immunodeficiency, immunosuppressed organ or bone marrow transplant recipients, or mice with severe combined immune deficiency (SCID) transfused with EBV-bearing human B cells (SCID-EBV-positive BMLs) [8, 23]. Thus, SCID-EBV positive BML is regarded as a good experimental model of AIDS-ML.

Lyn protein, encoded by a member of the *src* gene family *lyn*, is a submembranous protein-tyrosine kinase expressed on human B cells and monocytes/macrophages [30, 31, 32]. It plays a role in membrane immunoglobulin (mIg)-mediated signal transduction by physical and functional association with mIg-associated Mb-1/B29 heterodimer. Mb-1 [24] and B29 [9] are recently found membrane proteins that noncovalently link to mIg on the B-cell membrane and take part in antigen-mediated signal transduction [5, 6, 22]. Antigenic stimulation

on mIgs, or stimulation due to coupling of specific antibody against mIgs, causes the activation of Lyn and other submembranous *src* family gene products through signal transduction mediated by Mb-1 and B29 [2, 4, 10, 33].

Immunohistological study has shown that Mb-1 is expressed intensely on mantle zone B cells, weakly on germinal centre B cells and very intensely on plasma cells [16, 17]. B29 is demonstrated on mantle zone B cells intensely but not on other B cells [17]. Lyn is expressed evenly on germinal centre lymphocytes, mantle zone B cells and macrophages/histiocytes [31]. In B ML biopsies, Mb-1 was reported to be found in 63/64 cases [16], whereas the expression of B29 on B ML has not been reported.

Recently, we studied the expression of Lyn on human ML biopsy samples using a recently developed monoclonal antibody Lyn-9 [33], and found that it was commonly expressed in all 24 biopsied B MLs examined. Unexpectedly, we noted that Lyn was decreased or not demonstrated in five of eleven human EBV-bearing B-cell proliferations obtained by transplantation of EBV-bearing B cells into SCID mice (SCID-EBV-positive BMLs). These findings seemed to suggest the presence of some molecular mechanisms that lead to the suppression of Lyn in EBV-positive BMLs (submitted). As AIDS-MLs are believed to be caused by mechanisms identical to those responsible for the SCID-EBV-positive MLs, we considered it worthwhile to determine whether similar down-regulation of Lyn can occur in AIDS-MLs. Also, we were interested to clarify the expression levels of other membrane-associated proteins, Mb-1 and B29 in cases showing down-regulation of Lyn protein. Therefore AIDS-MLs and SCID-EBV-positive BMLs were studied in detail for the expression of Mb-1, B29 and Lyn.

## Materials and methods

Lymphoma tissues obtained from five AIDS patients (AIDS-MLs) were snap-frozen without fixation and stored at −80° C. The patients' age, sex, and the primary sites are listed in Table 1. The histological typing based on the updated Kiel classification [14] is also included in the Table. Specimens from cases 1 and 2 were obtained by biopsy and those from cases 3, 4, 5 at autopsy.

SCID-EBV-positive BML tissues were obtained by resecting tumours maintained in SCID mice. They were initially obtained by engrafting nontumorous human lymphoid tissues (tonsils or lymph nodes) or T-ML tissues obtained from EBV-infected individuals. Tumours, when they developed in the host mice, were excised, cut into pieces and engrafted into other SCID mice for second passage. Tumours that had grown in the second host were analysed for species specificity (mouse or human origin) by immunostaining with anti-human HLA-ABC framework antibody w6/32 and rabbit anti-murine major histocompatibility antigen H-$2^d$ antiserum, and for the presence of EBV-encoded latent proteins LMP1 and EBNA2. B-cell tumours reactive with antibodies against human HLA-ABC framework and LMP1 and/or EBNA2 but not with anti-H-$2^d$ were placed in the category of SCID-EBV-positive BMLs. Ten SCID-EBV-positive BMLs thus obtained were used in the present study. Meanwhile, the SCID-EBV-negative BMLs were obtained by engraftment of EBV-free BML biopsy samples into SCID mice. These were also maintained by serial passage. The tumours obtained from the second passage were checked for their species specificity, Ig class, and the presence of EBV latent gene products, EBNA2 and LMP1. These tumours, bearing human HLA-ABC framework but not the murine histocompatibility antigen H-$2^d$ and Ig heavy and light chains identical to the original ML tissues, and lacking EBV proteins, were placed in the category of SCID-EBV-negative BMLs. Eight SCID-EBV-negative BMLs thus obtained were used as control cases. The histopathology of these MLs were shown in Fig. 1.

Three-step enzyme immunohistochemistry was performed on cryostat sections. The primary antibodies were rabbit polyclonal anti-human Igγ-, α-, μ-, δ -, κ - and λ-chain antibodies (Hoechst, Germany), murine monoclonal antib-Mb-1 antibody (a gift from Dr. David Mason), murine monoclonal anti-B29 antibody (from Dr. David Mason), murine monoclonal anti-Lyn antibody (Lyn9) [33] L26 (CD20, Dakopatts, Denmark) and murine monoclonal anti-leukocyte common antigen (LCA) antibody (NuLPan, Nichirei, Japan). The immunohistological specificities of anti-Mb-1, B29 and Lyn were reported previously [16, 17, 33]. As the second- and third-phase reagents, biotinylated anti-rabbit or anti-mouse Ig (Dakopatts) and peroxidase-labelled avidin-biotin (Dakopatts) were used, respectively. The staining procedure was that of Su et al., with slight modification [31]. Briefly, cryostat sections were fixed with cold acetone and incubated with the first-, second- and third-phase reagents, for 30 min each in a warm moist chamber, and washed with phosphate-buffered saline (pH 7.4) between the steps. Di-aminobenzidine tetrahydrochloride (Sigma, St. Louis) was used as the chromogen.

The results were evaluated as ++, intensely stained; +, stained, ±, borderline; −, unstained. The Ig stained immunohistochemically is referred to in this article as cytoplasmic Ig (cIg), in contrast to mIg determined by flow cytometry, although we consider that immunostaining covers both cytoplasmic and membrane Igs.

Flow cytometry was employed for two purposes, first as a supportive method to confirm the results of immunostaining, and second, to determine mIgs. For flow-cytometric demonstration of Mb-1, B29 and Lyn, two SCID-EBV-positive BMLs and three SCID-EBV negative BMLs were studied. Freshly obtained tumour cells were first suspended in ice-cold phosphate-buffered saline, then washed twice and fixed in 100 μl of buffered formol acetone for permeation [16]. They were incubated with the optimally diluted anti-Mb-1, -B29 or -Lyn antibodies for 60 min, washed twice, and reacted with the fluorescein isothiocyanate (FITC)-labelled goat anti-mouse Ig antibody (Tago, Calif., USA). For the demonstration of mIgs, five SCID-EBV-positive BMLs and eight SCID-EBV-negative BMLs were studied. Their suspended cells were pelleted by centrifugation, mixed with 100 μl of FITC-labelled goat anti-human Ig γ-, α-, μ, or δ-chains (Tago) and incubated for 30 min at 4° C. Fluorescent cells, after being washed twice, were finally analysed on a Spectrum III cytofluorometer (Ortho, USA).

**Table 1** AIDS-associated lymphomas (AIDS-MLs) studied. *IB* immunoblastic, *CB* centroblastic, *M* male, *EBV* Epstein Barr virus

[a] Based on the updated Kiel classification (Lennert and Feller 1992)

| Case | (Age/sex) | Primary site | Histo-pathology[a] | Marker | EBV EBNA2 | EBV LMP1 |
|---|---|---|---|---|---|---|
| 1 | (30/M) | Neck | IB | CD20 | ++ | ++ |
| 2 | (38/M) | Skin | CB | CD20 | ++ | ++ |
| 3 | (37M) | Skin | IB | Igμκ, CD20 | + | + |
| 4 | (41/M) | Adrenal | IB | Igμκ, CD20 | + | + |
| 5 | (44/M) | Liver | CB | CD20 | − | − |

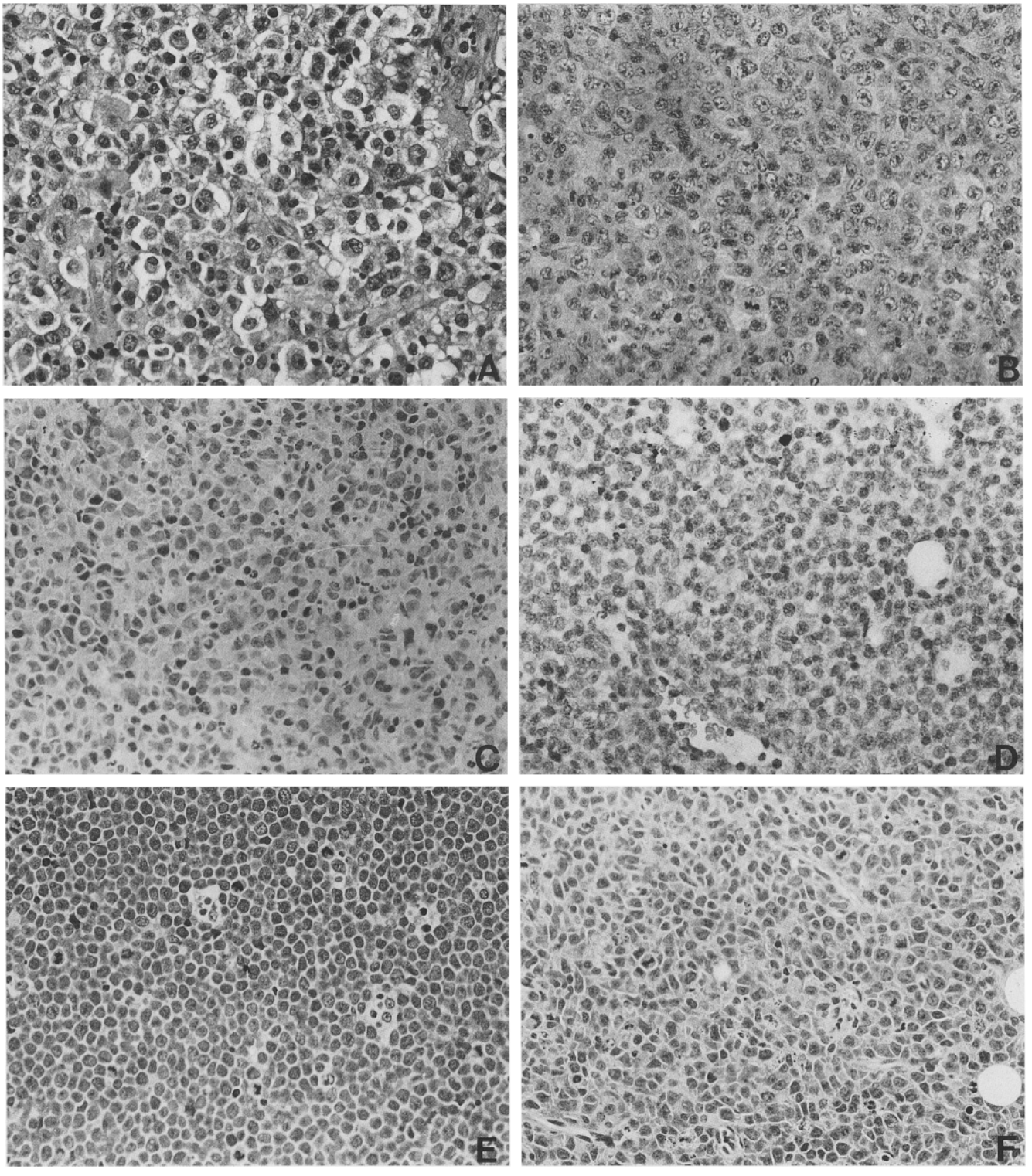


**Fig. 1A–F** Histology of AIDS-associated lymphoma (**A**, corresponding to case number 1 in Tables 1 and 2), Epstein-Barr virus (EBV)-infected human B-cell proliferations maintained in mice with severe combined immune deficiency (SCID); SCID-EBV-positive ML; **B, C** and **D**, corresponding to cases 1, 3 and 9 in Table 3). SCID-EBV-negative ML (**E** and **F**, corresponding to cases c and d in Table 5). Cases **A–F** are also shown in Figure 2, with reference to their immunohistological features

## Results

Immunostaining with anti-Mb-1, anti-B29 and anti-Lyn antibodies was basically clear and easy to evaluate. In normal tonsillar sections, the anti-Mb-1 antibody stained follicular mantle zone cells and plasma cells distinctly, and follicular centre cells rather weakly. The anti-B29 antibody stained mantle zone B cells distinctly but did

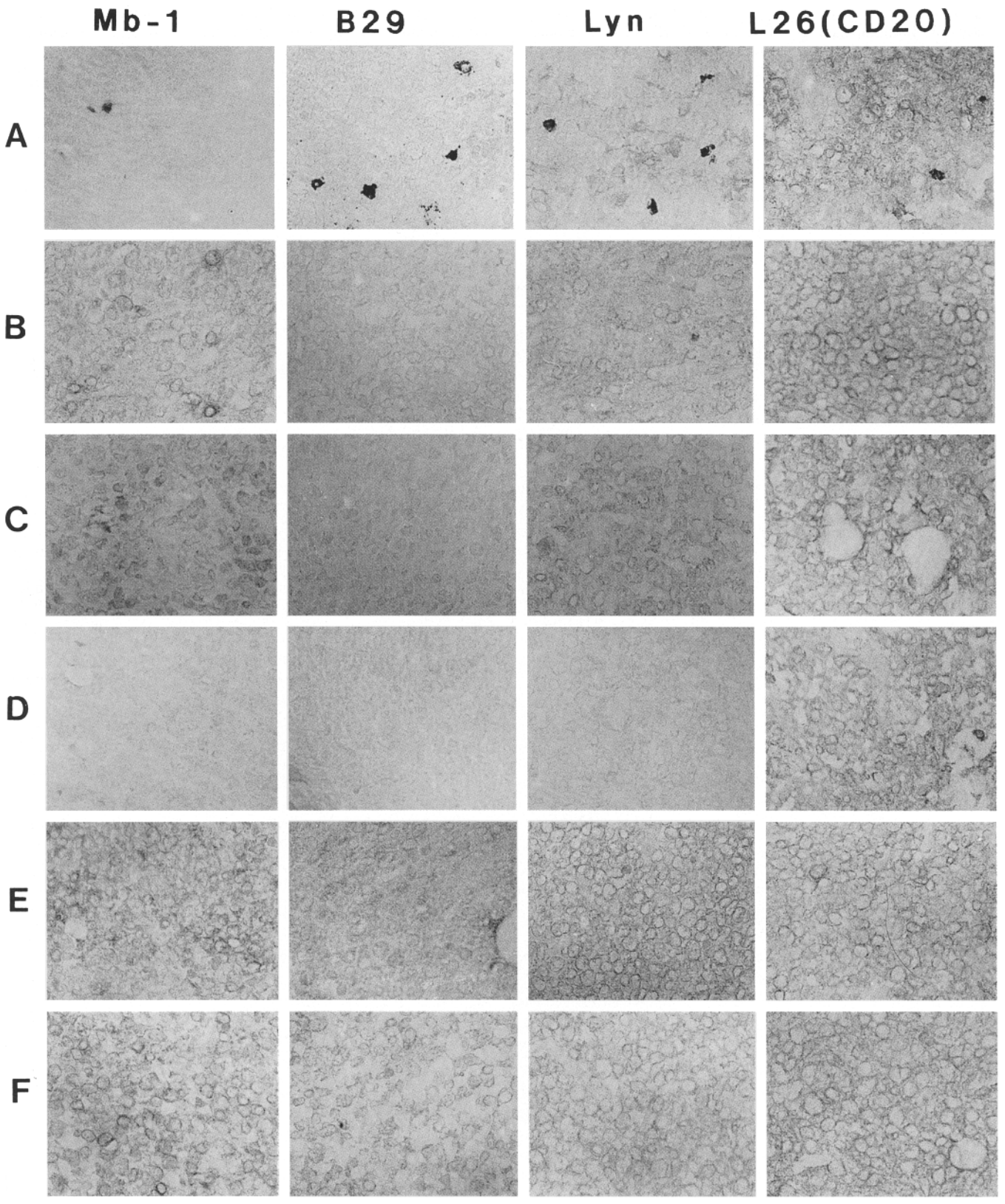


**Fig. 2A–F** Immunohistological demonstration of membrane immunoglobulin (mIg)-associated proteins, Mb-1, B29 and Lyn. **A–F** corresponding to Figure 1. Low expression of mIg-associated proteins is obviously noted in AIDS-ML (**A**), and SCID-EBV-positive BMLs (**B, C, D**) but not in SCID-EBV-negative BMLs (**E, F**)

**Table 2** Expression of MB-1, B29, and Lyn proteins, as were shown by immunostaining in five cases of AIDS-MLs. (± borderline staining, ++ intensely stained)

| Case | Mb-1 | B29 | Lyn | L26[a] |
|---|---|---|---|---|
| 1 | – | – | ± | ++ |
| 2 | – | – | – | ++ |
| 3 | – | – | – | ++ |
| 4 | – | – | – | + |
| 5 | – | – | – | ++ |

[a] L26 (CD20) was used as a kind of positive control antibody

not stain germinal centre B cells or plasma cells. These staining patterns matched those reported previously for these antibodies [16, 17]. The anti-Lyn antibody stained mantle zone B cells and germinal centre B cells equally, with an additional intense reaction on histiocytes/macrophages.

None of the five cases of AIDS-MLs were stained by anti-Mb-1 or anti-B29 antibody, although all were clearly stained by L26 (Table 2, Fig. 2). When SCID-EBV-positive BMLs were stained with anti-Mb-1, the results varied somewhat among cases: four of ten cases were totally unstained, two of ten showed very weak (borderline) staining or staining of only a minor portion (1%) of the neoplastic cells, two of ten showed 30–70% staining, and two of ten showed staining of all the tumour cells (Table 3). This is in contrast to control SCID-EBV-negative BMLs, where all the neoploastic cells were distinctly stained (most of the neoplastic cells intensely stained in six of eight cases, weakly stained in two of eight, and borderline or unstained in none of eight; Table 4). For B29, the difference in immunostaining between SCID-EBV-positive BMLs and EBV-negative BMLs was much more obvious: B29 was demonstrated in only one of ten SCID-EBV-positive BMLs whereas it was observed clearly in all eight SCID-EBV-negative BMLs (Tables 3, 4).

Immunostaining with anti-Lyn, unlike that with anti-Mb-1 or -B29, showed one case of AIDS-ML to be weakly stained. Exact evaluation of the immune reaction in this case was not easy because the reaction was also observed on histiocytes/macrophages that had infiltrated the tissues intensely. However, the other four AIDS-MLs were judged to be unstained after repeated experiments. In SCID-MLs, this type of false-positive reaction was eliminated because human cells in resected tissues were composed exclusively of neoplastic B-cells and did not include human histiocytes/macrophages, while murine (host) cells were not stained by these antibodies. As a result, the SCID-EBV-positive BMLs, when stained with anti-Lyn antibody, showed a positive reaction in four of ten cases, a borderline reaction in four of ten and no reaction in two of ten. Again this value is in sharp contrast to the SCID-EBV-negative BMLs, all eight of which were intensely stained (Tables 3, 4), suggesting the presence of a specific decrease in SCID-EBV-positive BMLs.

To substantiate the immunohistological results, two SCID-EBV-positive BMLs and two EBV-negative BMLs were studied by flow cytometry. The results correlated well with the immunostaining, as shown in Table 5 and Figs. 3, 4. In one case of EBV-positive BML (case 1 on Table 3), about 30% of the tumour cells were stained by immunohistology (Fig. 2), whereas on flow cytometry, the staining pattern of this case exhibited a broad plateau, extending on both the right (positive) and left (negative) sides (Fig. 3). The reaction of this case with anti-B29 was negative, both on immunostaining (Fig. 2) and flow cytometry (Fig. 3), whereas the reaction with anti-Lyn was at a borderline level using both methods. In the remaining three cases also, the immunohistological and flow-cytometric results correlated well (Figs. 2, 3, Table 5).

The cIgs of the SCID-EBV-positive BMLs, as determined by immunostaining, consisted of Igγ, six; Igμ, two; Igα, one and polyclonal, one (Table 3). Among the control SCID-EBV-negative BMLs, there were four cIgγ-bearing, three Igμ- and one Igα-bearing tumours. As shown in Table 3, the cIg classes of SCID-EBV-positive BMLs did not seem to be related to the expression level of Mb-1, B29 or Lyn. Thus, Mb-1, B29 and Lyn can be expressed or down-regulated, in any cIg class.

**Table 3** Expression of cytoplasmic immunoglobulin (*cIg*), membrane immunoglobulin (*mIg*), membrane immunoglobulin-associated proteins Mb-1, B29 and Lyn, and CD20 (L26) on Epstein-Barr virus (EBV)-bearing human B cell tumours developed in severe combined immunodeficiency (SCID) mice (SCID-EBV-positive BMLs). (± Stained, – unstained, *nd* not done)

| Case | cIg[a] | mIg[b] | Mb-1[a] | B29[a] | Lyn[a] | L26[a] |
|---|---|---|---|---|---|---|
| 1 | γκ | $\underline{\gamma}^c$ | ++,+ (30%) | – | ± | ++ |
| 2 | ακ | $\alpha\underline{\delta}^c$ | – | – | – | ++ |
| 3 | Poly-clonal | nd | – | – | ± | ++ |
| 4 | μγ | μδ | ++ (100%) | ++ (100) | ++ (100%) | ++ |
| 5 | γλ | nd | + (1%) | – | ++ (100%) | ++ |
| 6 | γλ | nd | – | – | – | ++ |
| 7 | μκ | nd | – | – | ++ (100%) | ++ |
| 8 | γκ | nd | ++,+ (70%) | – | ± | ++ |
| 9 | γλ | $\underline{\gamma}$ | ± | – | ± | ++ |
| 10 | γκ | $\underline{\gamma}$ | ++ (100) | – | ++ (100%) | ++ |

[a] Determined by the immunostaining
[b] Determined by flow cytometry
[c] Immunoglobulin heavy chains with underlines imply weak expression

**Table 4** Expression of Mb-1, B29, and Lyn, as demonstrated by immunostaining, on eight cases of B cell lymphomas (BMLs) maintained in SCID mice (SCID-EBV-negative BMLs). (Human BMLs lacking the EBV genome or viral proteins have been maintained in SCID mice. The tumour cells obtained from these mice were studied for the expression of Ig-associated proteins Mb-1, B29 and Lyn, together with CD20 (L26) and CD45RA+RO (LCA)

| Immunostaining | Mb-1 | B29 | Lyn | L26 | LCA |
|---|---|---|---|---|---|
| Intensely stained | 6/8 | 4/8 | 8/8 | 8/8 | 3/8 |
| Stained | 2/8 | 4/8 | 0/8 | 0/8 | 5/8 |
| Borderline or unstained | 0/8 | 0/8 | 0/8 | 0/8 | 0/8 |

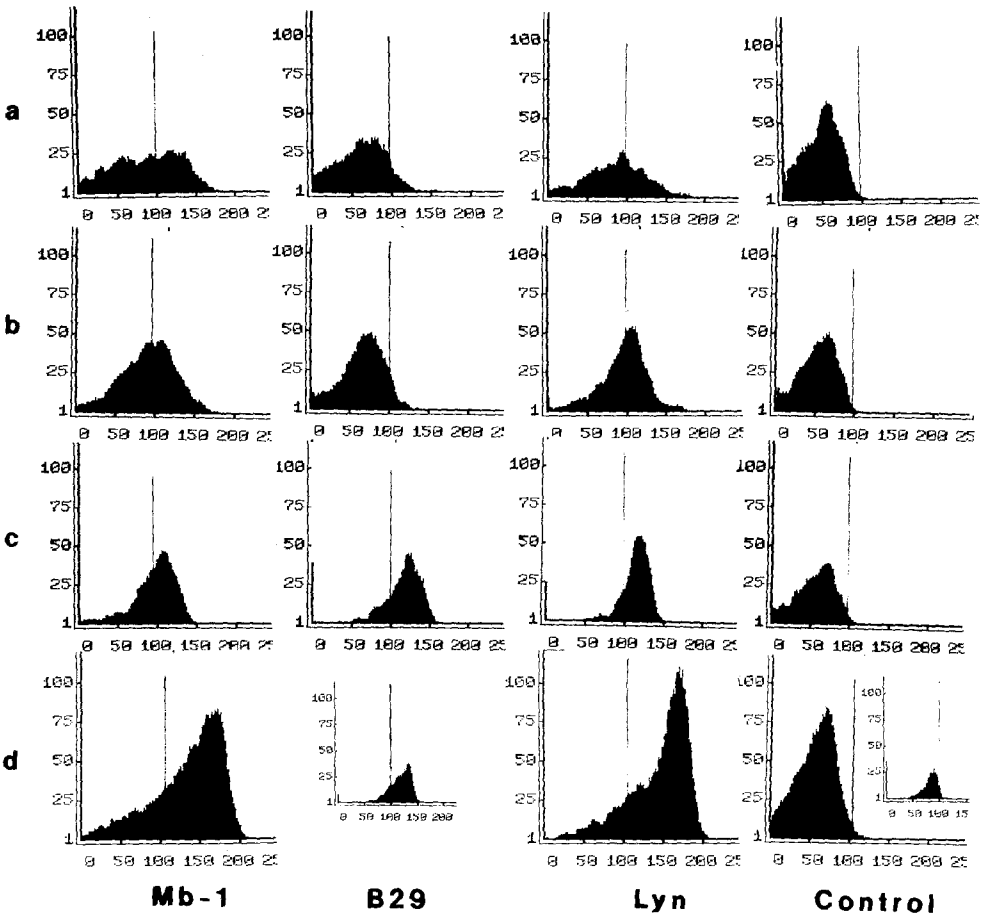


**Fig. 3** Flow-cytometric profiles of SCID-EBV-positive BML (*a, b*) and SCID-EBV-negative BML (*c, d*) in expression of Mb-1, B29 and Lyn. Cases *a–d* corresponding to Table 5. Also, *a* corresponds to B in Table 3 and Figure 2; *b* to D; *c* to E; *d* to F. B29 on case 4 was studied separately, and its negative control is listed on the left part of the control column

**Table 5** Correlation of immunohistology and flow cytometry in expression of membrane Ig-associated proteins Mb-1, B29 and Lyn, on EBV-bearing human BMLs maintained in SCID mice (*SCID-EBV-positive BMLs*), and EBV-negative BMLs maintained in SCID mice (*SCID-EBV-negative BMLs*)

| Case | Mb-1[a] | B29[a] | Lyn[a] |
|---|---|---|---|
| SCID EBV-positive BML | | | |
| a | ++,+ (30%)/+,– | –/– | ±/± |
| b | ±/± | –/– | +,±/± |
| SCID-EBV-negative BML | | | |
| c | +/+ | +/+ | ++/+ |
| d | ++/++ | ++/++ | ++/++ |

[a] The results on immunostaining are listed as the numerator; flow cytometry as the denominator
Case a corresponds to case number 1 on Table 3, b to case number 9

mIg was determined by flow cytometry in all five SCID-EBV-positive BMLs and all eight EBV-negative BMLs. Their classes matched fully with their cIgs (Table 3). Whereas Mb-1, B29 and Lyn were highly expressed in EBV-negative BMLs regardless of their Ig class (not listed in Table), EBV-positive BMLs exhibited a decrease of Mb-1 in three of five cases, B29 in four of five and Lyn in three of five (Table 3). In three of five cases of SCID-EBV-positive BMLs, the level of cIg was also decreased. In two of these, the mIg-associated proteins were decreased (cases 1 and 9 in Table 3), while Mb-1 and Lyn were well expressed in the remaining case (case 10). In the remaining two SCID-EBV-positive BMLs, the mIgs were expressed at the level of EBV-negative BMLs. Unexpectedly, in a EBV-positive ML where mIg (Iga) was well expressed, the mIg-associated proteins were heavily down-regulated. This was case 2 in Table 3, where mIgα was well expressed, whereas Mb-1, B29 and Lyn were unstained (Figs. 2, 4). In the control EBV-negative BMLs, on the other hand, all the mIgα- or mIgγ-bearing cases expressed these mIg-associated proteins intensely (Fig. 4). Further analysis of SCID-EBV-positive BMLs in terms of correlation between mIg and mIg-associated proteins could not be done because of the limited number of cases.

## Discussion

The present study showed that down-regulation of mIg-associated proteins, Mb-1, B29 and Lyn, occurred frequently in AIDS-ML and its experimental model, SCID-EBV-positive BML. The down-regulation was first noted by immunostaining, and was further confirmed with flow cytometry. The down-regulation seems somehow specific to EBV-associated MLs, because none of the control (EBV-negative) BMLs showed such a decrease.

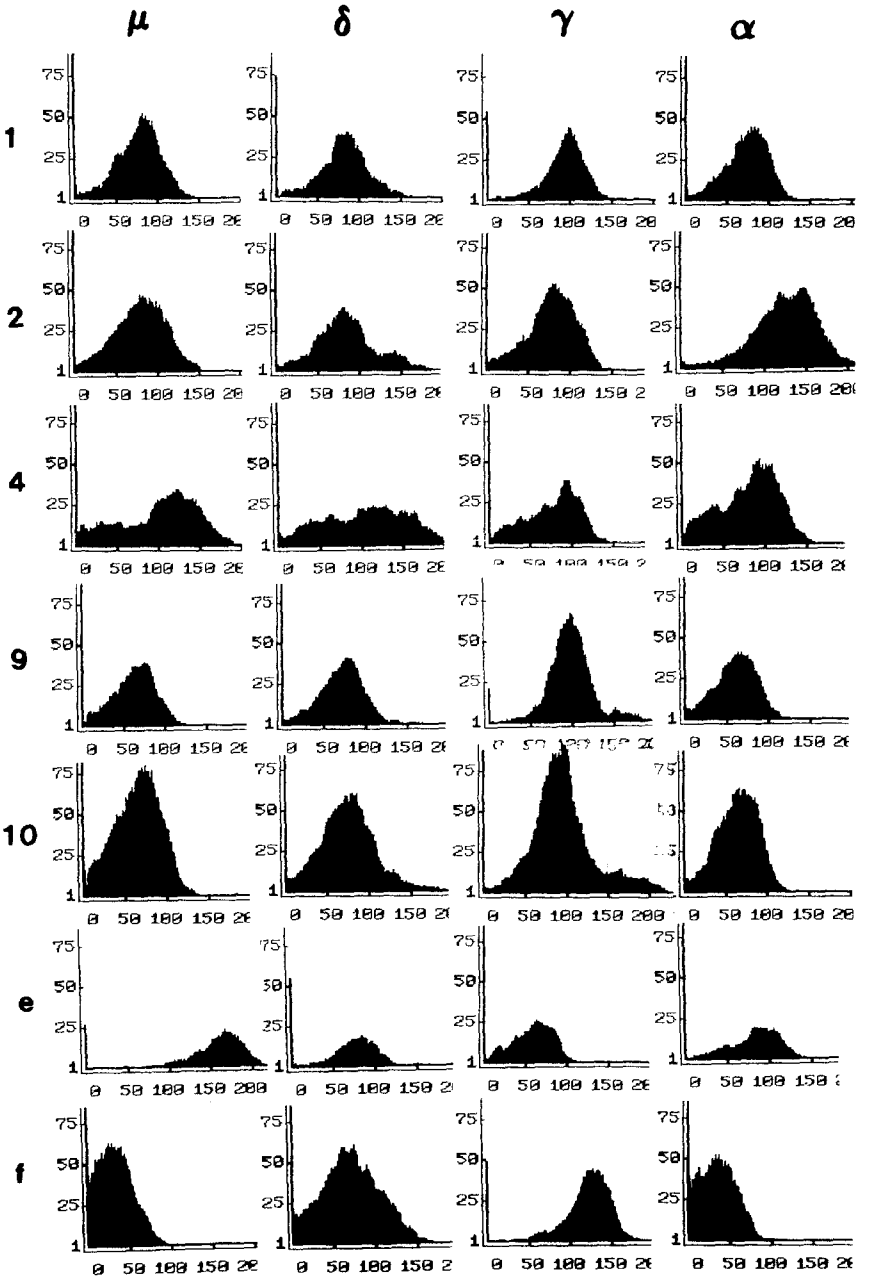


**Fig. 4** Demonstration of mIgs in SCID-EBV-positive BMLs (cases *1, 2, 4, 9, 10*,corresponding to Table 3) and EBV-negative BMLs (cases *e* and *f*)

In order to confirm "specificity", many more control EBV-negative BML cases would have to be studied. Mason et al., using the same anti-Mb-1 antibody, showed that 63 of 64 BML cases and 24 of 25 acute lymphoblastic leukaemias (ALLs) were well stained [16]. Neither they, nor others, reported the results of staining for B29 in ML biopsy samples, although Mason et al. did report data for ALL (13 of 25). We also have our own unpublised data on EBV-negative BMLs: Mb-1 was clearly observed in 23 of 24 cases, B29 in 20 of 24 and Lyn in 24 of 24. We did not include these data in the main body of the present paper because in a few of these biopsy samples, the immunohistological findings were difficult to evaluate because of a mixture of non-neoplastic B cells bearing the mIg-associated molecules. MLs maintained in SCID mice, and those originating in extranodal non-lymphoid organs such as brain or liver, were much more easy to evaluate because the degree of infiltration of non-neoplastic B cells remained very low. The previous reports and our unpublished data, together with the present control cases, undoubtedly support the assumption that mIg-associated proteins are down-regulated somewhat specifically in AIDS-ML and its experimental model, the SCID-EBV-positive BML.

Meanwhile, down-regulation was not evident in some SCID-EBV-positive BMLs. This apparently implies that down-regulation does not occur evenly in all SCID-EBV-positive BMLs. This lack of down-regulation was most frequent in Lyn (four of ten), followed by Mb-1 (three of ten) and least common in B29 (one of ten). The present study was unable to show any evidence to explain this difference. Several assumptions may be possible in this

context. The first is that these differences might be related to the difference in the amount of EBV-encoded proteins in neoplastic cells, as will be discussed later. The second possibility is that it is the reflection of the difference in progression of SCID-EBV-positive BMLs: that is to say, a change in cell characteristics after initial transformation of the B cell. The third is that the difference is reflected in the differentiation level of neoplastic B cells, although this is least likely because all the control EBV-negative BMLs, regardless of their immunophenotype or morphology, expressed the mIg-associated proteins intensely.

mIgμ and mIgδ were recently found to be expressed on the cell membrane through non-covalent coupling of Igs produced in cytoplasm with Mb-1 and B29 [6, 19]. This implies that Mb-1 and/or B29 act as a lift to pull up cIgs onto the cell membrane. The role of Mb-1 and B29 in the membrane expression of cIgγ or cIgα has not been elucidated, although the presence of a similar mechanism is speculated. In fact, in mice, Mb-1 and B29 are shown to appear on the surface of B cells bearing any class of Ig, including Igμ, γ, δ, and α [25]. In relation to this, Nakamura et al. [20] showed that the surface expression of mIgs of all isotypes coincides strictly with the expression of Mb-1 and B29. Similar results were reported for Igμ by Ishihara et al. [11]. Thus, we considered it of interest to investigate the correlation in the expression of mIg and mIg-associated proteins in SCID-EBV-positive BMLs. We found that the mIg level was also depressed in three of five cases, and that in two of these, the decrease of mIg-associated proteins occurred together. However, we also found a case in which Mb-1, B29 or Lyn was down-regulated while mIg (mIgα) was well expressed. Also, there was another case in which Mb-1 and Lyn were well expressed in spite of very low expression of mIg (case 10). These cases are the first of their kind reported that show dissociation of mIg and mIg-associated proteins. However, the limited number of cases prevented us carrying out much more detailed analysis, because the SCID-EBV-positive BMLs were easily lost during passage due to difficulties with engraftment of the tumour cells. Thus, in the present study, fresh cells capable of withstanding flow cytometry could be prepared in five of ten SCID-EBV-positive BMLs. Further studies based on many more cases should be done in future.

What, then, is the biological significance of this down-regulation. Generally, down-regulation of certain molecules in the signal transduction cascade can occur when other constituents situated downstream are activated. Thus, it is highly probable that some molecules downstream from the mIg-associated proteins are activated constitutively in these SCID-EBV-positive BMLs, presumably by EBV-encoded proteins such as EBNA2 or LMP, and that this activation will lead to a decrease of mIg-associated proteins located upstream. EBNA2 and LMP are known to play crucial roles in transformation of infected cells, while their function as activators of host cell signal transduction is becoming much clearer [3, 15, 26, 27]. Thus, detailed analysis of these proteins may give further clues for clarification of the mechanism of this down-regulation.

In summary, this study has shown the frequent and specific down-regulation of mIg-associated proteins in AIDS-ML and its experimental model, SCID-EBV-positive BML. It can be speculated that this down-regulation is caused by EBV-encoded proteins that constitutively activates some host proteins located downstream from the mIg-associated proteins. Elucidation of the abnormal expression of these proteins at the molecular level will add further information on the tumorigenesis of AIDS-MLs, or MLs found in immunocompromized hosts.

**Acknowledgements** We wish to thank Dr. David Mason for supplying us with anti-Mb-1 and -B29 antibodies. Drs. Tadashi Yamamoto and Kumao Toyoshima for Lyn-9, and to Ms. Reiko Akino for editing the manuscript. This work was supported by Grants-in-Aid for Scientic Research on Priority Areas from the Japanese Ministry of Education, Science and Culture.

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