Brief Definitive Report

THE EXPORT OF IMMUNOGLOBULIN D BY HUMAN NEOPLASTIC B LYMPHOCYTES*

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Immunoglobulin D (IgD) shows an unusual distribution in being found commonly on the cell surface of circulating normal human B lymphocytes, but only in very low amounts in the serum (1). Similarly, the incidence of IgD-producing myeloma is rare, perhaps reflecting the low numbers of normal plasma cells which secrete IgD (2). Another unusual feature of IgD is that although cell surface IgD displays the usual (two-thirds) predominance of light chains of the κ type, the myeloma proteins described have been very largely (90%) of the λ light chain type (3). A similar predominance of IgD λ has also been demonstrated for normal human serum IgD (4).

In the B lymphocytic neoplasm chronic lymphocytic leukemia (CLL), IgD is frequently found on the cell surface together with IgM of the same idiotype (5) and the predominance of tumors bearing the κ light chain type reflects that of the normal B cell population. Some cases of non-Hodgkin's lymphoma (NHL) involving B lymphocytes, usually of the well-differentiated lymphocytic group, also display IgD and IgM (6).

In our studies on the ability of cells from patients with CLL to export Ig in culture (7), it was demonstrated that 9/9 exported IgM and that 3/9 were also able to export IgD: in two of the cases where anti-idiotypic antibody was available, the IgD was shown to be of the same idiotype as the IgM. The three IgD-exporting cell populations were all of the λ light chain type.

The present investigation has been concerned with the patterns of Ig secretion of 23 patients with CLL or NHL and has demonstrated export of IgD by 10/12 neoplastic cell populations which displayed surface Ig of the λ type and no export of IgD by 11/11 cell populations displaying surface Ig of the κ type.

Materials and Methods

Preparation of Cells. Peripheral blood was obtained from patients with CLL, all of which had elevated numbers of lymphocytes ($>3 \times 10^{10}$/liter). Lymphocytes were isolated by gradient centrifugation at 37°C as described previously (7). The lymph nodes and spleen from the patients with NHL were chopped and passed through a sterile sieve to prepare cell suspensions before being separated by gradient centrifugation as for peripheral blood. Lymphocytes were finally suspended at 2×10^7/ml in Eagle's minimal essential medium (MEM) containing 1% nonessential amino acids (Flow Laboratories, Inc., Rockville, MD), 2 mM L-glutamine, 100 IU/ml of both penicillin and streptomycin, and 10% fetal calf serum. Cells were swirled gently at 37°C, and samples were taken at intervals for assessment of Ig production in the supernatants. The culture period was usually 5–6 h and cell viability was monitored by Trypan blue

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exclusion. Surface Ig isotypes of cell preparations were examined by immunofluorescence as described previously (7).

Radioimmunoassay (RIA). This was used to measure levels of IgM, IgD, κ, and λ light chains in cell supernatants: the assays have been described previously (7), and it should be noted that the light chain assays are relatively insensitive to light chain in combination with μ or δ chain and that they are largely detecting free light chain. The RIA for IgD has also been described (7): the solid phase was Sephadex G-25 coupled to sheep antibody to normal human Fd δ and radiolabeled normal human Fab δ as antigen. Normal human IgD obtained from serum by immunosorption was used as a standard, and soybean trypsin inhibitor (20 μg/ml) was present in the RIA buffer (7). The assay was sensitive to IgD in the range of 1–200 μg/ml, and specificity was demonstrated by <0.1% interference by IgM, κ, or λ light chains.

Results

The results of investigations of lymphocyte populations from 23 patients are shown in Table I (cells of the λ chain type) and Table II (cells of the κ chain type). The surface Ig isotypes were monotypic with >95% of the cells displaying only one light chain type; the two exceptions to this being patient Sto (Table I) who displayed both κ and λ light chains but who exported only λ, suggesting the presence of extrinsic IgG, and patient Far (Table II), whose cells displayed no detectable Ig but did demonstrate Fcγ receptors and failed to form rosettes with sheep erythrocytes, thus strongly suggesting their B cell nature. Plasma cells were not detectable in any of the populations studied, and the neoplastic lymphocytes were histologically in the category of the well-differentiated lymphocytic group. The intensity of surface fluorescence among the different neoplasms was variable with some tendency for the λ-positive group to show stronger reaction.

The results obtained by analysis of culture fluids obtained from cells are shown as ng of Ig product/2 × 10^7 cells per h, but all cultures were monitored hourly over 5–6 h, and production was only considered positive where amounts in the supernatant increased with time. The patterns of Ig export were variable, but all the neoplastic populations appeared to export light chain of either κ or λ type, which corresponded to the surface Ig light chain. The light chains exported were presumably free rather than combined in Ig molecules, as they are in excess over the IgM and IgD, and have been demonstrated previously (7) by separation of culture fluids of patients Wat and War (Table I).

The amounts of IgM found in culture fluids were also variable among different neoplastic populations. Levels of <2 ng IgM/2 × 10^7 cells per h were considered negative due to the limitations of the assay. Thus 8/12 of cells of the κ type (Table I) and 8/11 of the λ type (Table II) exported significant amounts of IgM.

The major difference between the κ-positive and the λ-positive groups was in the export of IgD. The assay for IgD is more sensitive and levels of <1 ng IgD/2 × 10^7 cells/h were considered negative. Thus 10/12 neoplastic populations of the λ type showed significant levels of exported IgD (160–620 molecules of monomer/cell per h), whereas 0/12 of the κ type exported IgD. There was no apparent association between export of IgM and IgD, and some populations (Sur and Mai in Table I) exported only IgD.

Discussion

The majority of normal B lymphocytes in human peripheral blood display both IgM and IgD, and there is predominance of the κ light chain type (1). Similarly, in

TABLE I

Surface Expression and Export of Ig by Lymphocytes from Patients with B cell Neoplasms of the λ Light Chain Type

Patient	Disease*	Source of lymphocytes	Surface Ig‡ isotypes	Exported Ig§			
				IgM	IgD	κ	λ
				ng/2 × 10^7 cells/h			
Bee	NHL	Lymph node	IgM(D)Gλ ++	7.4	1.0	0	46
Bun	CLL	Blood	IgMDGλ ++	1.4	3.8	0	22
Phi	CLL	Blood	IgMDGλ +	6.8	1.7	0	24
Sto	CLL	Blood	IgMDGκλ ++	12	3.3	0	19
Sur	NHL	Blood	Ig(M)Dλ ++	0	2.3	0	55
Mai	CLL	Blood	IgMGλ (+)	0	1.8	0	47
Wat	CLL	Blood	IgMDGλ ++	14	3.3	0	14
War	CLL	Blood	IgMDGλ +	9.5	1.3	0	31
Tay	CLL	Blood	IgM(D)λ ++	9.4	2.0	0	31
Twe	CLL	Blood	IgMDλ (+)	20	2.8	0	62
Wet	CLL	Blood	IgM(D)λ (+)	2.2	0.4	0	43
Hol	CLL	Blood	IgMλ (+)	0.6	0.2	0	20

* The NHL cases were both of the well-differentiated lymphocytic lymphoma (WDLL) group.

‡ IgG was detectable on the cell surface of seven patients' cells but is probably extrinsic (8). Levels of fluorescence are indicated by plus marks, and where an individual heavy chain class is of low fluorescence it is in parentheses.

§ Exported Ig was measured in culture fluids by RIA. The κ and λ light chain assays are relatively insensitive to light chain in whole Ig molecules and are detecting mainly free light chain (7).

TABLE II

Surface Expression and Export of Ig by Lymphocytes from Patients with B Cell Neoplasms of the κ Light Chain Type

Patient	Disease*	Source of lymphocytes	Surface Ig‡ isotypes	Exported Ig§			
				IgM	IgD	κ	λ
				ng/2 × 10^7 cells/h			
Far	CLL	Blood	ND‖	6.5	0	20	0
Hal	CLL	Blood	IgMDGκ (+)	12	0	48	0
Han	CLL	Blood	IgMDGκ (+)	1.3	0.4	19	1.6
Hut	NHL	Spleen	IgMDκ (+)	3.7	0.5	16	0
Owe	CLL	Blood	IgMDκ ++	6.6	0.4	5.6	0
Smi	CLL	Blood	IgM(D)Gκ (+)	0	0.4	12	0
Wil	CLL	Blood	IgM(D)Gκ +	2.9	0	20	0
Win	CLL	Blood	IgMDκ +	0.9	0.2	28	0.5
Woo	CLL	Blood	IgMGκ (+)	20	0.2	32	0
Ant	CLL	Blood	IgMDGκ ++	11	0.3	16	0
Mat	NHL	Lymph node	IgMDκ (+)	4.0	0	2.5	0

* The NHL cases were both of the WDLL group.

‡ IgG was detectable on the cell surface of six patients' cells but is probably extrinsic (8). Levels of fluorescence are indicated by plus marks, and where an individual heavy chain class is of low fluorescence it is bracketed.

§ Exported Ig was measured in culture fluids by RIA. The κ and λ light chain assays are relatively insensitive to light chain in whole Ig and are detecting mainly free light chain (7).

‖ Not detected.

the B cell neoplasms such as CLL, where cells bear IgM and IgD of the same idiotype (5), the greater incidence of tumors of the κ type reflects the normal pattern. However, 90% of myelomas secreting IgD are of the λ type (3), and normal serum IgD also appears to be mainly of the λ type (4). These findings have led to the suggestion that surface and exported IgD differ in some fundamental way, such as belonging to different subclasses (9), although this has not been demonstrated (10).

This investigation bears on this question at the level of the B lymphocyte. It has been shown previously (7) that apparently homogeneous neoplastic B lymphocytes

that display IgM and IgD can export small amounts of IgM (9/9 patients) and in some cases IgD (3/9 patients) with neither Ig arising from the cell surface (7). In our experience, cell surface material is found in culture fluids only as a large molecular weight, possibly vesicular form which is not detected by our techniques (11), although it would be included if the commonly used procedures of immune precipitation and electrophoresis in denaturant were applied. In two of the IgD-exporting neoplasms for which anti-idiotype was available, the IgD was shown to be of the same size as normal IgD and of the same idiotype as the IgM (7). These three IgD-exporting neoplasms were all of the λ type and are included in Table I (Phi, Wat, and War).

The association of IgD export with expression of the λ light chain in the three patients studied led to the present analysis of a further 20 patients, and such an association has been confirmed. Thus, the dissociation between ability to express and ability to export IgD is present already at the stage of the B lymphocyte, in that those cells synthesizing λ light chain are able to and frequently do export IgD, whereas those synthesizing κ light chains are able only to express IgD. The mechanism of such a dissociation presumably could involve either lack of biosynthetic machinery or degradation of the IgDκ destined for export.

The ability of human lymphoma-derived B cell lines to both express and secrete IgM has been shown to arise from two independent mRNA molecules, presumably derived from a primary nuclear transcript by differential splicing (12). A similar procedure appears to operate for synthesis of membrane and secreted IgD (13). It is of interest that investigation of a human lymphoblastoid cell line expressing surface IgM and IgD of κ light chain type, but secreting only IgM, revealed the presence of two mRNA molecules encoding δ chains (14). One appeared to encode surface δ, the other a δ chain of lower molecular weight consistent with that of secreted IgD. If such mRNA is generally present in B cells that express the κ light chain type, lack of secretion of IgDκ might be due to a block subsequent to translation. An alternative possibility for the failure of cells expressing IgDκ to export IgD is that the IgDκ destined for export may be degraded inside the cell. A case of CLL with intracellular IgDκ that was not secreted has been described (15), but no intracellular IgD has been detected in normal B lymphocytes or other cases of CLL. Therefore, if such degradation occurs, it must be rapid.

Summary

An investigation has been made into the ability of human neoplastic B lymphocytes expressing surface IgM and IgD to export IgD in culture. Cells that expressed surface Ig of the λ light chain type frequently exported IgD (10/12 patients), whereas cells expressing surface Ig of the κ light chain type exported no IgD, although most (8/11 patients) were able to export IgM. It appears, therefore, that in most of the 23 cases studied, cells synthesizing IgD with λ light chains can both express and export IgD, whereas those synthesizing IgDκ can only insert it into the surface membrane. This finding and the known preponderance of λ in plasma IgD imply that the possession of a λ chain facilitates the IgD secretory pathway, a conclusion that implicates a control mechanism subsequent to the surface/secretory dichotomy arising from different splicings of heavy chain messenger RNA.

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