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EVALUATION OF T AND B LYMPHOCYTE MEMBRANE MARKERS IN HUMAN NON-HODGKIN MALIGNANT LYMPHOMATA

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Summary.—Lymphoma cells from 25 patients were studied for the presence of B lymphocytes (membrane bound Ig and Fc receptor) and T lymphocytes (rosette formation with sheep erythrocytes) membrane markers. All cases of well differentiated lymphocytic lymphoma and of acute lymphosarcoma cell leukaemia and most cases of poorly differentiated lymphocytic lymphoma behaved as B cell monoclonal malignancies. However, the malignant cells of some patients were not definitely classified according to their B or T cell origin or lacked these membrane markers. The latter situation was encountered in 4 reticulum cell sarcomata. Polyclonal Ig were found on the surface of B cells in a case of hyperbasophilic undifferentiated lymphoma. The need for using several membrane markers to study the abnormal lymphoma cells is outlined. Such studies improve our understanding of these malignancies and may lead in the future to a satisfactory classification of non-Hodgkin lymphomata.

THE PRESENTLY usual classification of non-Hodgkin lymphomata (Rappaport, 1966) is based on purely morphological criteria. This classification is of great help for clinical and therapeutic evaluation of these patients. However, some lymphomata are difficult to define according to a rigid morphological basis and, in some patients, various well trained pathologists may disagree. Moreover, this terminology relies upon the appreciation of morphological similarities between normal and neoplastic cells, although the normal counterpart of some lymphoma cells is not easy to define (*i.e.* reticulum cell sarcoma).

It is therefore of great interest to attempt to characterize more precisely the nature and origin of the various types of malignant cells encountered in human lymphomata. The study of those cell membrane markers which enable us to distinguish between human B and T lymphocytes is a logical and reasonable approach since most of these malignancies seem to originate from lymphoid tissue and since the identification of these surface markers has been rewarding for our understanding of other human lymphoproliferative diseases (Seligmann, Preud'Homme and Brouet, 1973). We have, for instance, shown previously that Waldenström's macroglobulinaemia represents the proliferation of a clone of B cells with persistent maturation from the small lymphocyte to the IgM secreting plasma cell (Preud'Homme and Seligmann, 1972*a*), whereas chronic lymphocytic leukaemia (CLL) represents usually a monoclonal B cell malignancy with a maturation arrest of the proliferating lymphocytes (Preud' Homme *et al.*, 1971; Preud'Homme and Seligmann, 1972*b*). In a small minority of patients, CLL is identifiable as a T cell malignancy (Dickler *et al.*, 1973; Seligmann *et al.*, 1973). The membrane markers of the malignant cells of the Sezary syndrome indicate that the cells are related to thymus derived lymphocytes (Brouet, Flandrin and Seligmann, 1973) and the atypical mononuclear cells of infectious mononucleosis appear also to derive from T lymphocytes (Sheldon *et al.*, 1973). The purpose of this paper is to report our preliminary results in 25 patients with

non-Hodgkin malignant lymphomata using the 3 following membrane markers: for B lymphocytes, membrane bound Ig detectable by direct immunofluorescence (Pernis *et al.*, 1971) and the receptor for heat aggregated human IgG ("Fc receptor") (Dickler and Kunkel, 1972; Preud'Homme and Seligmann, 1972*c*); for T lymphocytes, the ability to form rosettes with sheep erythrocytes (Lay *et al.*, 1971; Jondal, Holm and Wigzell, 1972).

MATERIALS AND METHODS

Patients and cells.—The malignant lymphomata were classified according to the classification of Rappaport (1966). Controls consisted of subacute lymphadenitis, benign hyperplasia, CLL and Waldenström's macroglobulinaemia nodes.

Lymphoma cells were collected from surgically removed lymph nodes (13 cases), peripheral blood (6 cases) or bone marrow (6 cases). Morphological studies were performed on the same node as that used to extract the cells. The procedure used to isolate blood or marrow cells has already been described (Preud'Homme *et al.*, 1971). The nodes were obtained immediately after surgery, teased gently and filtered through a stainless steel mesh. The cell suspension was washed 3 times in Hank's solution made 5% in bovine serum albumin. Cytological examination was carried out on the cell suspensions.

Immunological methods.—The procedures used for the preparation of monospecific antisera to Ig chains and of heat aggregated IgG, for the conjugation with rhodamine or fluorescein and for the staining of living cells in suspension have been previously described (Preud'Homme *et al.*, 1971; Seligmann *et al.*, 1973). For these immunofluorescence experiments, microscopical examination was performed alternatively under ultraviolet vertical illumination and in phase contrast. In some cases trypsinization of the cells followed by short-term culture was performed in order to ascertain that the membrane bound Ig were a true cell product (Preud' Homme and Seligmann, 1972*c*).

Rosette formation with sheep red blood cells (E. rosette) was performed according to Jondal *et al.* (1972) with a few modifications (Brouet *et al.*, 1973).

RESULTS

Control patients

In patients with subacute lymphadenitis, 20–40% of the cells bore membrane bound Ig, as shown by the sum of cells positive with antisera to μ, γ and α chains or to κ and λ chains. Mu positive cells were predominant and the prevalent light chain was κ. Forty to 70% of the cells formed E. rosettes. Cytological examination of the cell suspensions revealed 60–80% of small lymphocytes and 15–20% of large lymphoid cells. Both small lymphocytes and large cells showed either membrane Ig or rosette formation although most rosetting cells appeared to be small lymphocytes.

In patients with benign lymph node hyperplasia, most cells obtained from the nodes were small lymphocytes and a majority of rosette forming cells was apparent (80–95%), even in a patient affected with Waldenström's hyperimmunoglobulinaemic purpura in whom only 15% of the lymphocytes bore membrane bound Ig with predominance of IgG.

In lymph nodes from patients with documented B derived CLL or with Waldenström's macroglobulinaemia, 80% of the lymphocytes bore a monoclonal Ig which was identical to that detected on blood lymphocytes. However, 10–20% of the lymphocytes appeared to be thymus-derived since they exhibited rosette formation.

Malignant lymphomata

The results are summarized in the Table.

Poorly differentiated lymphocytic lymphoma.—In 9 of 12 cases, the lymphoblasts bore monoclonal membrane bound Ig. The cells were obtained from the blood (4 patients), the marrow (2 patients) or the nodes (3 patients). All cases were of the diffuse type. The staining of the cells was usually very bright, with a spotty appearance similar to that of normal lymphocytes. A $\mu\lambda$ monoclonal spike was detected in the serum of a patient with a

TABLE.—*Membrane Markers of Lymphoma Cells*

	No. of cases	Membrane bound Ig		Fc receptor	E. rosettes
Poorly differentiated lymphocytic lymphoma (diffuse)	12	$\mu\kappa$	3	3/3	0
		$\mu\lambda$	5	3/5	1/5
		$\gamma\kappa$	2	ND	0
		$\mu\gamma\ \kappa\lambda$	1	1/1	0
		None	1	0	0
Well differentiated lymphocytic lymphoma	3	$\mu\kappa$	3	3/3	0*
Poorly differentiated reticulum cell sarcoma (diffuse)	4	0		0	0
Mixed lymphoma (reticulolymphosarcoma)	1	$\gamma\lambda$ (all types of cells)		+	0
Undifferentiated lymphoma (acute lymphosarcoma cell leukaemia)	4	$\mu\kappa$	1	ND	0
		$\mu\lambda$	2	2	0
		$\gamma\kappa$	1	ND	0
Unclassified hyperbasophilic lymphoma (see text)	1	Polyclonal 60% of cells		+ 60% of cells	5%

* In one of these patients, 40% of E rosette forming cells were found besides the $\mu\kappa$ B cells. (ND: not done.)

$\mu\lambda$ lymphoblastic proliferation. Seven of these cases were studied for the presence of the Fc receptor on the lymphoblasts. This marker was detected in 6 patients and was absent in one case. The abnormal cells of these patients did not form E. rosettes. The vast majority of the small lymphocytes which were found in the cell suspension besides the abnormal cells formed E. rosettes and did not bear membrane bound Ig.

In another case the malignant cells had a polyclonal membrane Ig pattern since all cells were stained simultaneously by antisera to γ, μ, κ and λ chains. The Fc receptor was detected on these cells. No further studies could be performed in this patient.

In one patient, nearly all malignant cells bore a monoclonal membrane bound IgM but were devoid of Fc receptor and formed rosettes with sheep erythrocytes.

In the last patient, who was the only child in this series, none of the surface markers were detectable on the abnormal cells extracted from a lymph node.

Well differentiated lymphocytic lymphoma (*WDLL*).—The lymph nodes from 3 patients were studied. The cells appeared on Giemsa staining to be mainly small lymphocytes. In all 3 cases, a monoclonal $\mu\kappa$ B cell proliferation was readily detectable. However, in one case (which was the only one of the nodular type), 40% of lymphocytes forming E. rosettes were also found. Double labelling experiments showed clearly that we were dealing with 2 distinct populations of cells bearing either the B or the T cell marker.

Poorly differentiated reticulum cell sarcoma (*diffuse*).—In all 4 cases which were studied, the large neoplastic cells did not bind aggregated IgG and did not form E. rosettes. In 3 cases, no membrane bound Ig was detectable on the lymphoma cells. In another case, a small percentage of the large cells (10–15%) were stained by the 5 antisera used. The grossly spotted and patchy appearance of the fluorescence was unusual.*

Mixed lymphoma (*reticulolymphosarcoma*).—The lymph node cells from one such patient were studied. All the proliferating cells ranging from lymphocytic cells to large "reticulum cells" bore a monoclonal surface IgG λ and the Fc receptor, and they did not form E. rosettes. A small homogeneous IgG λ component was detectable at immunoelectrophoretic analysis of the serum of this patient.

* More cases have been studied since this manuscript was submitted. In 2 cases supervening on other B cell neoplasia (1 CLL and 1 macroglobulinaemia), the large malignant cells were shown to be of B cell origin and produced the same Ig chains as the neoplastic cells of the primary proliferation. In another case, the malignant cells bore a receptor with strong affinity for IgG, suggesting their origin from histiocytes.

Undifferentiated lymphosarcoma.—We have studied 4 patients (3 children and 1 adult) with a tumoral disease involving the lymph nodes, spleen and liver and with similar unusual cytological features. Large lymphoblastic cells of varying size, with a strongly basophilic and vacuolated cytoplasm were found in the nodes, invaded completely the marrow and were also present in small percentages in the blood. The precise classification of these cases is difficult and they could possibly be labelled as acute lymphosarcoma cell leukaemia (Flandrin *et al.*, 1974).

In all 4 patients, the malignant cells bore a monoclonal surface IgM (3 cases) or IgG (one case) which were proved to be actual cell products in the 2 cases where trypsinization and *in vitro* synthesis experiments were performed. The cells were able to bind aggregated IgG in both cases which were studied for this marker. Rosette formation was not observed.

Unclassified lymphoma.—We have studied a patient whose tumoral disease was characterized by a neoplastic lymph node proliferation involving mainly large basophilic cells with a tendency towards plasma cell differentiation, by the presence of similar abnormal cells in the peripheral blood, a strikingly high serum level for all Ig classes without any monoclonal component, and by the presence of anti-smooth muscle antibodies and a positive Coombs test. This peculiar syndrome (Flandrin *et al.*, 1972) has now been observed in more than 20 patients in our Institute and could perhaps be classified as " hyperbasophilic malignant lymphoma ".

Sixty per cent of the cells obtained from the lymph node biopsy and almost all the circulating cells isolated from the blood bore membrane bound Ig and possessed the Fc receptor. The pattern of the surface Ig was of the polyclonal type, the percentage of positive cells bearing μ, γ and α chains being 45%, 35% and 20% respectively with roughly equal percentages for κ and λ chains. Ten per cent of the lymph node cells and 65% of the circulating cells contained detectable Ig by intracytoplasmic staining, with a roughly similar distribution for the various Ig classes.

DISCUSSION

To our knowledge, very few studies have as yet been devoted to the detection of the membrane markers of B and T lymphocytes on the malignant cells from non-Hodgkin lymphomata. One should probably be very cautious in the interpretation of the results obtained in such studies. The validity of the various membrane markers at present available has indeed been established for the distinction between B and T *normal* lymphocytes but it may be dangerous to extrapolate these data to *abnormal* lymphoma cells. The absence of any marker of B or T lymphocytes on lymphoma cells does not definitely argue against their possible lymphoid origin since we are dealing with neoplastic and often poorly differentiated cells. Conversely, the detection of some membrane properties similar to those of B or T lymphocytes does not necessarily imply that these lymphoma cells are lymphoid in nature. The phenomenon of rosette formation, the precise signification of which is unknown, could apply to non-lymphoid malignant cells. The presence of a receptor for IgG on cells of the monocytic series is a well established fact. For all these reasons the simultaneous use of several membrane markers for B and T cells, whenever possible, is obviously of great interest.

The first reported demonstration of surface Ig on human lymphoma cells concerned a Burkitt tumour (Klein *et al.*, 1968). Most studied Burkitt tumours and derived lines have now been shown to be B cells carrying monoclonal bound Ig, mainly of the IgM class.

In the present preliminary report, we have found that all studied cases of well differentiated lymphocytic lymphoma and of a peculiar undifferentiated lymphoma

(" acute lymphosarcoma cell leukaemia ") and most cases of poorly differentiated lymphocytic lymphoma were featured by a monoclonal B cell proliferation.

In all our 3 cases of well differentiated lymphocytic lymphoma and in the single other case studied by Aisenberg and Bloch (1972), the proliferating cells in the node bore a monoclonal IgM. This finding is not astonishing in view of the close relationship between this condition and CLL, a disease featured in most cases by the proliferation of IgM bearing B lymphocytes (Preud'Homme and Seligmann, 1972*b*).

It is of interest that all 4 cases grouped under the heading of acute lymphosarcoma cell leukaemia were characterized by a monoclonal B cell proliferation. The patient labelled similarily in whom Aisenberg *et al.* (1973) found a surface IgG lambda may belong to the same group. These findings differ clearly from those obtained in acute lymphocytic leukaemia since a single case of our series has been identified as a B cell malignancy (Seligmann *et al.*, 1973).

Ten of our 12 cases of poorly differentiated lymphocytic lymphoma appear to represent a B cell proliferation, with proven monoclonality in all these patients but one. Similar findings were reported in a few other cases (Aisenberg and Bloch, 1972; Piessens *et al.*, 1973; Wilson and Hurdle, 1973). In one of our cases we have observed a mixed staining pattern characterized by the simultaneous presence of μ, γ, κ and λ chain determinants on all the malignant cells. Although further experiments could not be performed in this patient, it seems likely, in view of our previous experience in CLL (Preud' Homme and Seligmann, 1972*b*), that we were dealing with a false polyclonal appearance. In all CLL patients with a similar mixed staining of freshly drawn cells, we have shown indeed by trypsinization followed by *in vitro* culture that the actual cell product was a monoclonal Ig. The apparently polyclonal pattern of the surface Ig was due either to an anti-IgG antibody activity of a monoclonal IgM (Preud'Homme and Seligmann, 1972*c*) or to the attachment of immune complexes to the B lymphocyte surface, or to the simultaneous presence at the surface of the leukaemic lymphocytes of a synthesized monoclonal Ig and of an antibody directed against surface antigenic determinants. Conversely, the findings in the patient with an unclassified hyperbasophilic lymphoma reflect presumably a true polyclonal B cell proliferation since the various Ig chain classes were detected on different cells. Whether these Ig producing cells were malignant or " reactive " is open to question.

In most of these patients in whom we conclude to be a B cell malignancy, the lymphoma cells bound aggregated IgG and thus bore the receptor for Fc in addition to membrane bound Ig. Furthermore, in all those cases where trypsinization experiments were performed, we were able to show that the surface Ig were actual cell products. It should be emphasized that, in most cases of poorly differentiated or undifferentiated lymphosarcoma with membrane bound Ig, the fluorescence staining was very bright and the density of the Ig at the cell surface seemed much higher than in CLL patients. In CLL patients, the homogeneous and faint staining reflects presumably a block in the maturation process of the proliferating B lymphocytes (Preud'Homme *et al.*, 1971; Pernis *et al.*, 1971; Preud'Homme and Seligmann, 1972*b*). It seems possible therefore that the brightly stained lymphosarcoma cells could represent activated B lymphocytes rather than undifferentiated B cells. The same question has been put forward for the Ig bearing blast cells found in acute crisis supervening on CLL (Brouet *et al.*, 1973*b*).

The malignant cells of one of our patients with poorly differentiated lymphocytic lymphoma showed both E. rosette formation and membrane bound IgM λ. One may recall that both B and T membrane markers have been found in a mouse thymoma cell line (Harris *et al.*,

1973). However, since trypsinization experiments could not be performed in our patient, we cannot assume that the surface Ig were true cell products. Since these lymphoma cells did not bear the receptor for Fc, the possibility should be raised that we were dealing with a T cell malignancy. We are aware of 2 cases of poorly differentiated lymphocytic lymphoma (Smith *et al.*, 1973; Bryland and Rappaport, personal communication, 1973) which were considered to represent a T cell proliferation on the basis of E. rosette formation by the malignant cells and lack of surface Ig.

In 5 patients of this series, none of the studied membrane markers were detected on the malignant cells: one case of poorly differentiated lymphocytic lymphoma and 4 cases of reticulum cell sarcoma. These negative findings do not necessarily mean, in our opinion, that reticulum sarcoma cells do not derive from the lymphoid tissue and are of histiocytic origin. It is worth noting that Stein, Lennert and Parwaresch (1972), using a different approach, have claimed that reticulum sarcoma cells produced Ig molecules, that conversely Aisenberg and Bloch (1972) were unable to detect surface Ig in the single case of reticulum cell sarcoma studied by them and that Bryland and Rappaport (1973) found some B cell characters in one case and T cell properties in another case. In view of these discrepancies and of the small number of reported cases, further studies on the membrane markers of reticulum sarcoma cells are clearly required. They should include the use of specific antisera to T and possibly B cells since we have found that blast cells from acute lymphocytic leukaemia patients may be killed by specific antisera to T cells although they showed little or no tendency to E. rosette formation.

In conclusion, the identification of B and T cell membrane markers provides a new tool for the study of non-Hodgkin lymphomata and probably represents a fruitful conceptual approach. There is much hope that such studies will lead to a better understanding and to a scientifically accurate and operational classification of these diseases. However, it would be premature to establish presently such a classification.

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