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Immunologic Subsets in Human B-Cell Lymphomas in Relation to Normal B-Cell Development

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A. Introduction

Human non-Hodgkin lymphomas represent a complex group of diseases with extremely varied clinical courses and a large number of histopathologic subtypes. Immunologic approaches to this group of diseases have clearly shown that a great majority of these neoplasias are derived from B-cells (see Lennert 1978). Moreover, as with other neoplasias (Fialkow 1976) they appear, with very few exceptions, to be of monoclonal origin as shown by light chain isotype restriction (Levy et al. 1977).

Various morphological entities are included in B-cell lymphomas. Although the details and the nomenclature of the histopathologic classification remain an issue of controversy, there is general agreement that some B-cell lymphomas are derived from cells of germinal centers, whereas others, such as immunoblastic lymphomas and lymphomas with plasmacytoid features, appear to be at a maturation stage close to plasma cells with high intracellular concentrations of immunoglobulin as demonstrated by immunohistochemistry (Taylor 1978). These observations suggest that B-cell lymphomas are derived from different stages of B-cell differentiation and maturation pathways.

In our laboratory we are trying to obtain more detailed information on the relationship between human B-cell lymphomas and normal B-cell differentiation and maturation pathways. The problem is approached in two ways. First, a more detailed multiparameter analysis of surface markers on human lymphomas has been undertaken. Second, attempts are being made to trigger these lymphomas to proliferate and differentiate in vitro. Data from both these approaches will be summarized in the present paper. Our studies clearly demonstrate that human B-cell lymphomas can be divided into immunologically distinct subsets. Moreover, such lymphomas may be triggered to proliferate and differentiate as measured by immunoglobulin (Ig) synthesis by anti-Ig, usually in combination with tumor promotor (TPA).

B. Materials and methods

I. Lymphoma Biopsies

The studies to be reported have been carried out on cell suspensions from lymphoma biopsies containing more than 50% B-cells staining monoclonally for surface immunoglobulin (sIg). Histologic classification was performed according to the Kiel classification system (Lennert 1978).

II. Surface Markers and Capping

For identification of sIg, anti human Ig sera labeled with fluorescein isothiocyanate (FITC) were obtained from Dakopatts (Copenhagen, Denmark), with the exception of FITC-labelled anti-δ which was obtained from Behringwerke (Marburg, West Germany) to be described elsewhere (Godal et al., to be published). Complement receptors (CR) were identified by standard procedures with C_5-deficient mouse serum in a blind fashion (Godal et al. 1978). Capping was carried out as described by Elson et al. (1973).

III. Single Cell Flow Cytometry

Two parameter flow cytometric measurements of light scatter and fluorescence from lymphocytes stained with FITC-conjugated antisera were performed with a laboratory-built flow cytometer (FCM) (Lindmo and Steen 1977). The method will be described in detail elsewhere (Godal et al., to be published).

IV. Cell and Nuclear Volumetry

For determination of the cellular volume distributions, the cells were suspended in 10 ml counting solution (isotone to Coulter electronics) and immediately measured by means of a modified Coulter counter (Steen and Lindmo 1978). Nuclear volume distributions were obtained by the same procedure using a counting solution which removes cytoplasm and fixes the nuclei in acid formaldehyde (Stewart and Ingram 1967).

V. Lymphocyte Stimulation

For stimulation with anti-Ig and/or TPA (12-O-Tetra-decanoyl-phorbol-13-acetate) (Midland Corporation, United States), cells were cultured in microtitration plates with 2×10^5 cells per well with RPMI 1640 medium and 10% fetal calf serum final concentration (Henriksen et al. 1980).

VI. Ion Flux

Cellular uptake of ^{86}Rb, which is assumed to reflect membrane transport of potassium, was carried out essentially as described elsewhere (Godal et al. 1978; Iversen 1976).

C. Results

I. Immunological Subsets in Human B-Cell Lymphomas

Surface concentration of Ig was determined by FCM analysis of cells stained under saturating conditions. The data were expressed as mean intensity for the positive cells as expressed relative to the mean intensity of unstained cells.

As shown in Table 1, different histologic and immunologic groups showed large variations with regard to relative amounts of sIg. Thus, centroblastic/centrocytic lymphomas of the nodular type expressing IgG had only about one fifth of the sIg of lymphomas of the same histological type expressing IgM. Similarly, lymphocytic lymphomas or lymphoplasmacytoid lymphomas had clearly lower concentrations of sIgM. Similar findings were made with anti-light chains in a radioimmunoassay. Moreover, as shown in Fig. 1 the relative amounts of IgD to IgM on lymphoma cells expressing both these isotypes varied considerably from one lymphoma to the other, i.e., from a 1:1 ratio down to a 1:6 ratio. The variation was particularly striking within the nodular group.

Further evidence for immunological heterogeneity among human B-cell lymphomas was uncovered by CR and capping studies. Only 26 of 51 lymphomas expressed CR, and a very close association between the presence of CR and capping with anti-μ was found. Out of 45 lymphomas tested by both parameters, 42 could be allocated into groups positive or negative in both test systems, whereas only two were clearly positive in one test system and negative in the other. This relationship between expression of complement receptors and capping was specific to sIgM, as cappinq with anti-δ has been found positive irrespective of whether IgM was capped or not. Moreover, sIgG positive lymphomas revealed capping with anti-γ, whereas all these lymphomas tested so far lack CR.

Based on these combined data we can at present distinguish between five immunological subsets in B-cell lymphomas. The relationship between these groups and the histopathologic classification is shown in Table 2. All five immunologic groups were found among the

Table 1. The relative amounts of μ and γ heavy chains on lymphocytes from various histologic types of B-cell lymphomas by flow cytometric analysis

Histologic type	Mean intensity, positive subpopulation	
	μ	γ
Centroblastic/centrocytic (nodular type) (n=4)		4.8±0.5[a]
Centroblastic/centrocytic (nodular type) (n=4)	25 ±7	
Centroblastic/centrocytic (diffuse type) (n=3)	24 ±5	
Lymphoplasmacytoid (n=3)	10 ±4	
Lymphocytic (n=3)	6.4±0.8	

[a] ±s.e. (mean)

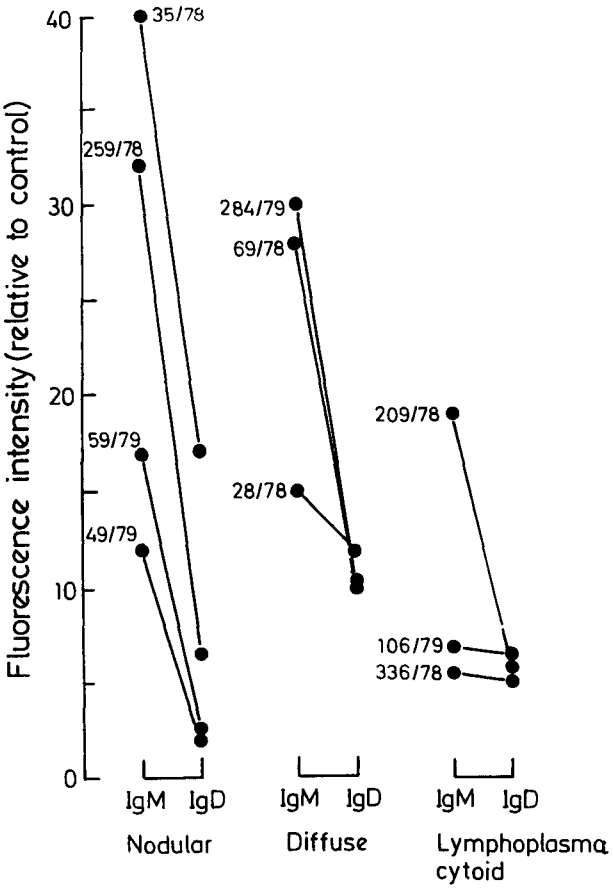

Fig. 1. The relative amounts of μ and δ heavy chains on lymphocytes from nodular and diffuse centroblastic/centrocytic lymphomas and lymphoplasmacytoid lymphomas as assessed by flow cytometric analysis. The *lines* connect the results with anti-μ and anti-δ on each lymphoma tested. Cell suspension (biopsy) number is shown

nodular lymphomas, whereas in contrast all six lymphomas examined in the diffuse lymphocytic group belonged to one immunologic group. The other histopathologic groups fall between these two, indicating a more limited heterogeneity with regard to immunologic types, but the numbers are here too small to allow definite conclusions to be drawn.

II. Responses of Lymphoma Cells to TPA and Anti-Ig

Among various substances tested which are known to induce differentiation in erythroleukemia cells, such as di-methyl-sulphoxide, we have found that TPA (10^{-7} M) induced striking changes in a majority of lymphomas. These alterations include a rapid enlargement in cellular volumes which can be measured within hours after exposure and the induction of cytoplasmic protrutions). In about 50% of the lymphomas nuclear volume also increases after addition of TPA. When anti-Igs are added to TPA, mitogenic effects can be observed as measured by thymidine incorporation and FCM. The mitogenic effects have a clear dose-response relationship to anti-Ig, with peak responses ranging from 2.5 to 250 µg per ml final concentration. The response shows a sharp peak on day 2 or 3. Among anti-Igs anti-μ most commonly gave a positive proliferative response (8 out of 14 cases), whereas 4 out of 11 responded to anti-δ and only one out of five to anti-γ.

By FCM analyses simultaneously measuring DNA and Ig contents in cells it can be clearly shown that lymphomas also often start to synthesize Ig. Moderate increases were found with TPA alone, whereas this was greatly enhanced by anti-Ig. Cells staining most strongly for Ig are found both with a G_1 and G_2

Table 2. Relationship between immunologic subtypes and the histopathologic classification in human B-cell lymphomas

	Lymphocytic (diffuse)	Centroblastic/centrocytic (diffuse)	Centroblastic/centrocytic (nodular)	Centroblastic (diffuse)	Immunoblastic (diffuse)	Lymphoplasma cytoid (diffuse)	Total
IgM, IgD, CR[a]		4[c]	4			3	11 (22%)
IgM, IgD[b]			3				3 (6%)
IgM, CR[a]	6	1	4	1	1	2	15 (30%)
IgM[b]		2	4	3	2	4	15 (30%)
IgG		1[d]	5				6 (12%)
Total	6 (12%)	8 (16%)	20 (40%)	4 (8%)	3 (6%)	9 (8%)	50

[a] The great majority also positive for capping with anti-μ
[b] The great majority negative for capping with anti-μ
[c] One in addition expressing sIgG
[d] Also expressing sIgM

content of DNA, showing that at least in many cells DNA synthesis and Ig synthesis may occur simultaneously.

By studying ion flux with anti-Igs we have found that early ^{86}Rb uptake is closely associated with a proliferative response to anti-Ig plus TPA. This uptake can be measured within minutes after addition of anti-Ig and takes place without TPA, whereas there was no correlation between ^{86}Rb-uptake and anti-Ig induced capping.

D. Discussion

The present study demonstrates that human B-cell lymphomas can be subdivided into a number of distinct immunologic subsets. These subsets correspond only partially to the histopathologic classification. Some histologic groups comprise several distinct immunologic subsets. The prognostic significance of immunologic subclassification in B-cell lymphomas remains to be determined. The material is enlarged and will be followed up to answer this question.

Our findings raise a number of questions with regard to the B-cell maturation and differentiation processes. Of particular interest in this regard is sIgD. Studies based on cell sorting (Black et al. 1978; Zan-Bar et al. 1979) and parental administration of anti-IgD serum in mice (Dresser and Parkhouse 1978) have shown that IgD positive cells are involved in primary responses but that IgD becomes lost and is not present on mature memory cells. In our study IgD was found only on a proportion of nodular lymphomas derived from germinal center cells. Moreover, the mean amounts of IgD on those lymphomas which were positive were shown by FCM to vary from relatively strongly positive to almost negative. These findings suggest that IgD becomes lost during the B-cell maturation processes taking place in germinal centers. It is interesting to note that a dissociation between capping of IgM and IgD was also found on a distinct subset of nodular lymphomas, raising the possibility that IgD plays a distinct role in germinal centers.

The lymphomas were also found to be heterogenous with regard to CR. Thus, all lymphomas expressing sIgG only were CR negative, whereas CR positive and CR negative lymphomas were found both in the IgM+ IgD and IgM groups. The most likely explanation for these findings would be that CR may also be lost during B-cell maturation. This view is supported by experimental data from studies of thoracic duct lymphocytes, where Mason (1976) found 19S AFC precursors exclusively in the CR positive fraction, whereas 7S AFC precursors were found both in the CR positive and CR negative population. Since nodular lymphomas of germinal center cell origin showed this CR heterogeneity, our findings would indicate that CR may also be lost during B-cell maturation taking place in germinal centers. Thus, our data would be compatible with a differentiation (bifurcation) process taking place in germinal centers, by which B-cells loose IgD but retain CR for the generation of recirculating sIgM and CR positive memory cells, whereas the loss of CR may be an event along another maturation pathway involving a switch to sIgG.

Lymphocytic lymphomas would be candidates a neoplastic counterparts to recirculating sIgM and CR positive memory cells. These lymphoma cells are different from chronic lymphocytic leukemia (CLL) cells (Godal et al. 1978) because they cap with anti-IgM.

The role of CR negative B-cells remains unclear, but since CR negative cells are found in immunoblastic lymphomas which have high intracellular concentrations of Ig (Landaas et al., in press; Stein 1978; Taylor 1978) and also plasma cells are known to lack CR (Burns et al. 1979), these cells may represent precursors to antibody-producing cells. The lack of capping of sIgM in these cells is interesting. This would make them suitable as antigen-presenting cells to T-cells, which are essential for maturation to antibody secretion (Rohrer and Lynch 1979) and possibly to other B-cells for producing antibodies of higher affinity.

Plasma cell development must also take place by events not involving germinal center formation, because the appearance of antibody-producing plasma cells occurs before germinal center formation (White et al. 1975). In fact, the appearance of germinal centers coincides with the occurrence of circulating immune complexes (White et al. 1975), and immune complexes are highly effective in generating memory cells (Klaus 1979). The lymphoplasmacytoid group is interesting in this regard. Histopathologically, these lymphomas show morphologic evidence of maturation towards plasma cells. This can also be demonstrated

with immunohistochemical methods (Landaas et al., in press; Stein 1978) by which cytoplasmic immunoglobulin can easily be detected. This group comprised 3 immunologic subsets (sIgM, sIgD, and CR positive; IgM and CR positive; only IgM positive), demonstrating that plasma cells may develop from different subsets of B-cells. The sIgM, sIgD, and CR positive cells have the surface characteristics of B-cells involved in a primary response and could reflect a plasma cell maturation pathway branching off prior to germinal center formation.

The present study revealed definite differences with regard to the concentration of sIg. The highest concentrations were found in positive lymphomas of follicular center cell origin, whereas positive lymphomas within the same category had a substantially lower sIg concentration. Similarly, low concentrations were also present on lymphocytic lymphomas. As has been discussed above, both these types of lymphomas may correspond to B-cells at a higher maturation stage. Their lower concentrations of sIg may therefore represent a maturation-associated loss of sIg. The pathway leading to sIgG positive cells may be associated with affinity maturation processes (Herzenberg et al. 1980).

A hypothetical picture of the relationship between the different lymphomas and normal B-cell maturation and differentiation as discussed above is outlined in Fig. 2.

Our findings that B-cell lymphomas can be triggered to proliferate and synthesize Ig in vitro by anti-Ig, usually in combination with TPA, represent a new approach which may help in delineating these relationships and put them on a firmer basis. It has already been shown that lymphocytic lymphomas may differentiate towards plasma cells.

Such studies may also provide important information with regard to mechanisms of B-cell triggering. Our studies so far have clearly shown that sIg of either the μ, γ, or δ type may deliver transmembrane signals giving rise to an increased influx of K^+ and in concert with other agents, especially TPA, induce proliferation and differentiation in neoplastic B-cell arrested at different stages of differentiation.

References

Black SJ, van der Loo W, Loken MR, Herzenberg LA (1978) Expression of IgD by murine lymphocytes. Loss of surface IgD indicates maturation of memory B-cells. J Exp Med 147:984–996 – Burns GF, Worman CP, Roberts BE, Raper CGL, Barker CR, Cawleys JC (1979) Terminal B cell development as seen in different human myelomas and related disorders. Clin Exp Immunol 35:180–189 Dresser DW, Parkhouse RME (1978) The effect of the parental administration of rabbit anti-(mouse)-IgD serum on the immune response of mice to sheep erythrocytes. Immunology 35:1027–1036 – Elson

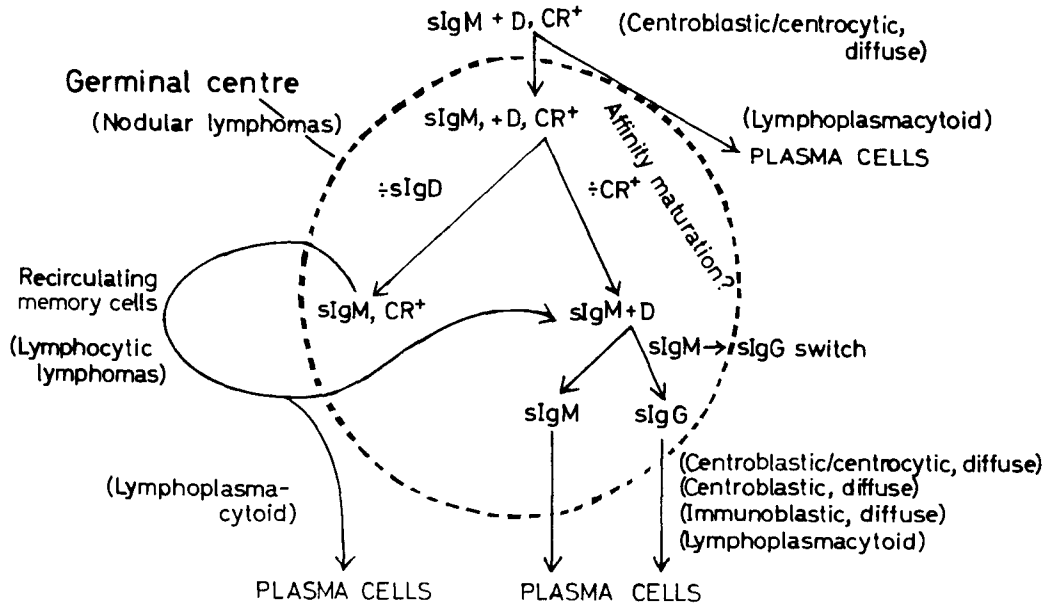

Fig. 2. Simplified and hypothetical scheme of human B-cell lymphomas in relation to normal B-cell development. The scheme suggests that plasma cell maturation may take place from different levels of B-cell differentiation. The position of different histologic types of lymphomas is indicated in brackets

CJ, Singh J, Taylor RB (1973) The effect of capping by antiimmunoglobulin antibody on the expression of cell surface immunoglobulin and on lymphocytic leukemic lymphocytes. Scand J Immunol 2:143–149 – Fialkow PJ (1976) Clonal origin of human tumors. Biochim Biophys Acta 458:283–321 – Godal T, Henriksen A, Iversen J-G, Landaas TØ, Lindmo T (1978) Altered membrane-associated functions in chronic lymphocytic leukemia cells. Int J Cancer 21:561–569 – Godal T, Lindmo T, Marton PF, Landaas TØ, Langholm R, Høie J, Abrahamsen AF (to be published) Immunological subsets in human B-cell lymphomas – Henriksen A, Godal T, Landaas TØ (1980) Mitogenic effect on human lymphocytes of insolubilized anti-immunoglobulins. I. Specificity of the stimulating agent. J Immunol 124:921–925 – Herzenberg LA, Black SJ, Tokuhisa T, Herzenberg LA (1980) Memory B cells at successive stages of differentiation. J Exp Med 151:1071–1087 – Iversen J-G (1976) Unidirectional K^+ fluxes in rat thymocytes stimulated by concanavalin A. J Cell Physiol 89:267–276 – Klaus GGB (1979) Generation of memory cells. III. Antibody class requirements for the generation of B-memory cells by antigen-antibody complexes. Immunology 37:345–351 – Landaas TØ, Godal T, Marton PF, Kvaløy S, Langholm R, Lindmo T, Jørgensen OG, Høst H (1981) Cell-associated immunoglobulin in human non-Hodgkin lymphomas. A comparative study of surface immunoglobulin on cells in suspension and cytoplasmic immunoglobulin by immunohistochemistry. Acta path microbiol scand, sect A (in press) – Lennert K (ed) (1978) Malignant lymphomas other than Hodgkin's disease. Springer, Berlin Heidelberg New York – Levy R, Warnke R, Dorfman RF, Haimovich J (1977) The monoclonality of human B-cell lymphomas. J Exp Med 145:1014–1028 – Lindmo T, Steen HB (1977) Flow cytometric measurement of the polarization of fluorescence from intracellular fluorescein in mammalian cells. Biophys J 18:173–187 – Mason DW (1976) The requirement for C3 receptors on the precursors of 19S and 7S antibody-forming cells. J Exp Med 143:1111–1121 – Rohrer JW, Lynch RG (1979) Immunoregulation of localized and disseminated murine myeloma: Antigen-specific regulation of MOPC-315 stem cell proliferation and secretory cell differentiation. J Immunol 123:1083–1087 – Steen HB, Lindmo T (1978) Cellular and nuclear volume during the cell cycle of NHIK 3025 cells. Cell Tissue Kinet 11:69–81 – Stein H (1978) The immunologic and immunochemical basis for the Kiel classification. In: Lennert K (ed) Malignant lymphomas other than Hodgkin's disease, part 6. Springer, Berlin Heidelberg New York – Stewart CC, Ingram M (1967) A method for counting phytohemagglutininstimulated lymphocytes. Blood 29:628–639 – Taylor CR (1978) Immunocytochemical methods in the study of lymphoma and related conditions. J Histochem Cytochem 26:496–512 – White RG, Henderson DC, Eslami MB, Nielsen KH (1975) Localization of a protein antigen in the chicken spleen. Effect of various manipulative procedures on the morphogenesis of the germinal centre. Immunology 28:1–21 – Zan-Bar I, Strober S, Vitetta ES (1979) The relationship between surface immunoglobulin isotype and immune function of murine B lymphocytes. IV. Role of IgD-bearing cells in the propagation of immunologic memory. J Immunol 123:925–930