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Cell surface expression of β_2-microglobulin (β_2m) correlates with stages of differentiation in B cell tumours

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SUMMARY Cell surface β_2-microglobulin (β_2m) densities of malignant B cells were determined by enzyme immunoassay in 97 cases of immunologically defined lymphoproliferative disease. Absolute β_2m densities were found to depend on disease category with the lowest levels found on cells from chronic lymphocytic leukaemia (mean = 5·6 ng/10^6 cells, n = 27); atypical chronic lymphocytic leukaemia (mean = 5·9 ng/10^6 cells, n = 8); and prolymphocytoid chronic lymphocytic leukaemia variant (mean = 6·0 ng/10^6 cells, n = 16). β_2m densities for B non-Hodgkin's lymphoma (n = 14) and B prolymphocytic leukaemia (n = 17) cases were 8·1 and 10·0 ng/10^6 cells, respectively, and the highest densities were found on cells from "late-B cell" tumours (mean = 14·3 ng/10^6 cells). Plasma cells from cases of Ig secreting tumours expressed unexpectedly low β_2m densities (mean = 9·3 ng/10^6 cells; n = 6).

β_2-microglobulin (β_2m) forms the invariant light chain of the HLA class I molecule[1] and is expressed, in non-covalent association with the 43000 dalton heavy chain,[2] on the surface of cells from many tissues.[3 4] HLA antigens seem to play a part in regulating the immune response by major histocompatibility complex restriction of T cell mediated cytotoxicity,[5] and β_2m is required for the structural integrity of the HLA molecule.[6–8] Defective expression of HLA has been reported in a variety of tumours,[4 9] and it is possible that this impairs T cell mediated responses to tumour cells.[10 11] β_2m is shed into plasma as a result of normal membrane turnover[12] and is raised in many pathological conditions.[13] Correlations between serum β_2m and estimated tumour mass have been noted in chronic lymphocytic leukaemia[14] and myeloma,[15] and serum β_2m is also closely correlated with prognosis in myeloma.[16] The underlying importance of these observations has yet to be determined. Further insights may be gained by examining relations between serum concentration and cell surface expression. We present the findings of a vertical study into surface β_2m expression in malignant lymphoid cells from patients with morphologically and immunologically classified B cell disorders.

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Material and methods

Mononuclear cell fractions were obtained from 97 cases of lymphoproliferative disease by density centrifugation (Lymphoprep: Nyegaard) of edetic acid and anticoagulated peripheral blood or bone marrow. Cell viabilities exceeded 90% in all cases.

All patients had B cell disorders as defined by monoclonal antibodies to B cell determinants (Leu12: CD19; and B1: CD20) and surface or cytoplasmic immunoglobulin light chain restriction. Cases were classified as chronic lymphocytic leukaemia (CLL: n = 27), prolymphocytic leukaemia (PLL: n = 17), non-Hodgkin's lymphoma (NHL: n = 14), or leukaemic reticuloendotheliosis (LRE: n = 7) by conventional morphological and previously described immunological criteria.[17] A further 24 cases were investigated, comprising two groups defined as (*a*) cases with morphology suggestive of typical chronic lymphocytic leukaemia which showed increased (>20%) FMC7-positive components or surface immunoglobulin (SIg) densities (CLL-atyp; n = 8); and (*b*) cases which were morphologically and immunologically consistent with chronic lymphocytic leukaemia in "prolymphocytoid transformation"[18] (CLL-Pro; n = 16). In addition, plasmacytoid and plasma cells from eight cases of immunoglobulin-secreting tumours were also examined: Walden-

strom's macroglobulinaemia (WM; n = 2) and myeloma (n = 6).

Surface β_2m determinations were performed by immunoenzyme assay[19] of fractionated lymphoid cells from cases with leu12 or SIg/CIg light chain restriction, or both, in at least 80% of cells, or a peripheral white cell count > 100 × 10^9/l (two cases).

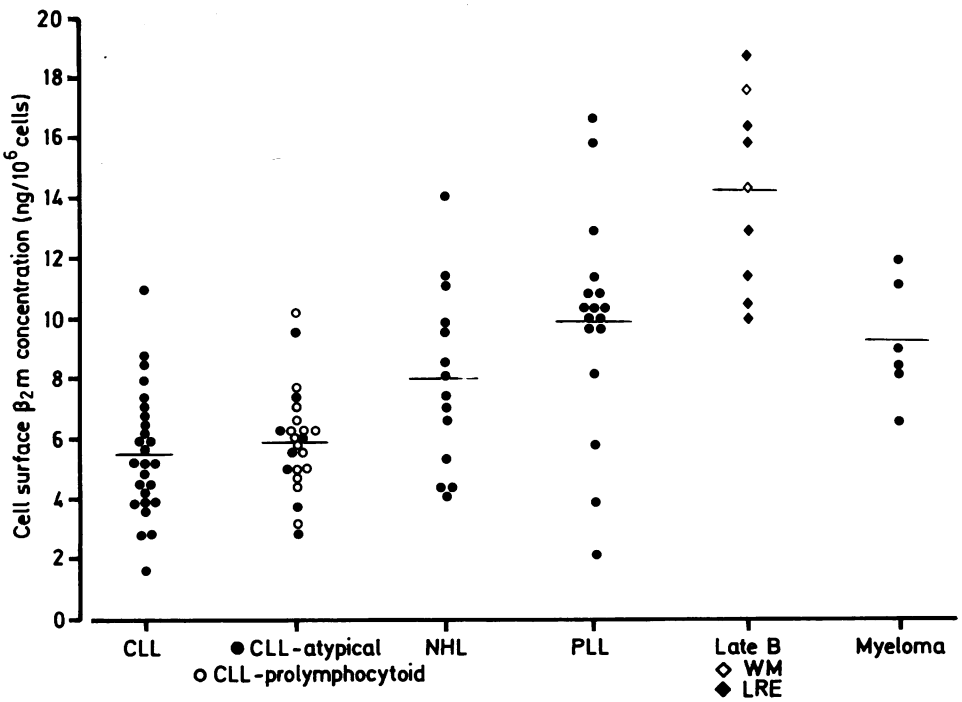
Statistical comparisons of surface β_2m expression by malignant B cells from the various diagnostic categories were made using the non-parametric Mann-Whitney U test.

Results

The fig shows the results of β_2m expression by malignant B cells in the different diagnostic categories. Cases of LRE and WM were combined to form the "late-B" group for statistical analysis. The lowest β_2m densities were found in the chronic lymphocytic leukaemia (mean 5·6 ng/10^6 cells), CLL-pro (mean 6·0), and CLL-atyp (mean 5·9) groups. No significant differences in the expression of β_2m were found between the three CLL groups, and for the purpose of subsequent statistical analysis, the CLL-pro and CLL-atyp groups were combined. Absolute β_2m values exceeding 9·0 ng/10^6 cells were found in one of 27 of CLL, two of 24 of atyp-CLL/CLL-pro, five of 14 of non-Hodgkin's lymphoma, 13 of 17 of prolymphocytic leukaemia, and nine of nine of late-B cases. When compared with the CLL groups, significantly increased surface β_2m densities were found in non-Hodgkin's lymphoma (mean 8·1) at $p < 0.05$ and in prolymphocytic leukaemia (mean 10·0) and late-B (mean 14·3) at $p < 0.001$. Surface expression was further increased in late-B compared with that in prolymphocytic leukaemia ($p < 0.01$) and non-Hodgkin's lymphoma ($p < 0.001$). Plasma cells, however, expressed significantly less ($p < 0.01$) β_2m than late B cells (mean 9·3), with mean expression not significantly different from non-Hodgkin's lymphoma, indicating decreasing expression with terminal B cell differentiation.

Discussion

The use of immunological markers provided a means whereby the various stages of B cell maturation could be defined. By examining the immunological profile of B cell neoplasms, it was possible tentatively to establish their relative location in order of differentiation as CLL, CLL-Pro, PLL, LRE, WM, and myeloma. It is evident from the results in this



Expression of cell surface β_2m by lymphoid cells in B cell malignancies. (____) indicates mean value for each category.

study that the increasing expression of surface β_2m closely parallels this sequence, with the notable exception of myeloma. In this respect β_2m is similar to the membrane densities of FMC7, B1/B4, SIg and HLADr (Ia) determinants, which increase with maturation but are lost at the terminal plasma cell stage.[20-23] Interestingly, the prolymphocytoid variants of CLL (CLL-Pro), in which PLL-like morphology is seen despite retention of many of the CLL phenotypic characteristics,[18 23 24] showed surface β_2m expression similar to that of typical CLL.

The position of non-Hodgkin's lymphoma in the differentiation pathway is less clear, as development from centroblasts to centrocytes within active lymph nodes is not solely related to the generation of antibody secreting cells.[25 26] Considerable variation in surface β_2m expression was found in our cases of non-Hodgkin's lymphoma, and it is considered that this reflects the immunological heterogeneity of this group.

The function of surface β_2m with respect to B cell differentiation is unknown. Cytotoxic T cells recognise antigen in combination with the class I HLA molecule on the target cell surface.[27] β_2m has also been found in association with non-HLA molecules, such as H-Y and T1 antigens, and a structure cross reacting serologically with β_2m is an integral part of the Qa antigen.[28-30] In vitro experimental evidence suggests that non-HLA β_2m may be associated with trigger molecules of B cell function,[31 32] and it has been suggested that β_2m may have a role in regulating the expression of certain cell membrane antigens during cellular differentiation.[28] If this is the case then it is not unreasonable to speculate that membrane β_2m density will vary as a function of cellular maturity and that its decline at the terminal plasma cell stage is consistent with loss of requirement for surface components entailed in control of differentiation. A common feature of disease progression in various malignancies is raised serum β_2m. Its measurement is considered to be of value as an indicator of disease activity in CLL and is a powerful prognostic indicator in myeloma.[33] The origin of increasing β_2m production in malignant disease progression has not been established, but an inverse relation has been shown between surface β_2m expression and rate of secretion in carcinoma cell lines.[34] Non-haemopoietic tumours often exhibit reduced β_2m expression, compared with their normal counterparts,[3 4 9] and it has been proposed that this may provide a means of evading T cell cytotoxic mechanisms,[35] since HLA expression on target cells is required for T cell cytotoxicity.[36] Whether B cell neoplasms behave in similar fashion has not been determined, but it is tempting to speculate that the rise in serum β_2m seen in the progression of some B cell tumours to more clinically aggressive forms[33] may be accompanied by a reduction in surface β_2m expression. Studies are currently in progress to examine β_2m surface expression and secretion rates in relation to serum concentrations in B cell malignancies.

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