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Total and Surface-located Sialic Acid Levels in Normal and Leukaemic Lymphocytes: Relationship to T and B Cell Nature and to Location *In Vivo**

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Abstract—*Unfractionated normal blood lymphocytes (predominantly T lymphocytes) showed significantly elevated total and neuraminidase-susceptible sialic acid compared with CLL lymphocytes (predominantly B lymphocytes). T-enriched fractions from normal blood contained about three-fold the sialic acid content of the corresponding T-depleted fractions, and similarly low values were also obtained for T-depleted fractions from tonsils. Thus the reduced sialic acid levels in CLL lymphocytes appear to be due to their B cell nature. In contrast to the situation in normal peripheral venous blood, tonsillar T and B cells did not differ markedly in sialic acid content, suggesting that for T cells maturity and/or location* in vivo *are important determinants of this parameter. Results with calf thymocytes were in agreement with this. Sialic acid was estimated in malignant B and T lymphoblasts. Shedding of sialic acid-containing moieties from lymphocytes was also examined.*

INTRODUCTION

SIALIC acid, terminally located on surface glycoconjugates, is an important determinant of the net negative charge on cells and is considered to be involved in many behavioural characteristics such as cell contact, adhesion, antigenicity, immunogenicity and receptor function [1, 2].

The question of whether CLL lymphocytes possess abnormal overall amounts of surface sialic acid is pertinent to these problems. Assessment of electrophoretic mobility, which can give an indication of surface sialic acid, yielded conflicting results when CLL lymphocytes were compared with those from normal blood [3–6]. Moreover, sialic acid is not the sole determinant of surface charge and thus not invariably correlated with electrophoretic mobility [1, 7], so that biochemical analyses are needed to resolve the question. Due to difficulty in obtaining sufficient lymphocytes from normal human blood, few authors have directly analysed normal and CLL lymphocytes for sialic acid and, once again, the results are conflicting [4, 8, 9]. More recent work [10] using isolated membrane preparations indicates lower amounts of sialic acid in those from CLL than from normal blood lymphocytes.

Direct comparison of unfractionated normal blood with CLL samples is, however, of limited value, since CLL is almost invariably a B lymphocyte proliferation [11, 12], while normal blood lymphocytes consist of 60–70% T, 10–30% B and 10–30% null cells [12]. Thus, any differences found may relate to lymphocyte type rather than to malignant origin. More recently, advances in lymphocyte separation techniques have enabled certain comparisons to be made between lymphocyte sub-populations from normal human blood, and electrophoretic studies have shown a mainly bimodal distribution, with T cells having a higher mobility than B cells [13], in confirmation of earlier findings for rat and mouse lymphocytes [7]. The mobility of human null cells was found [14] to be lower than that of most of the T cell population and close to the value for B cells. These data suggest that B and null lymphocytes from normal human blood may have less surface sialic acid than their T cell counterparts.

The main purpose of the present study was to measure the sialic acid content (total and

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surface-located) of normal and CLL lymphocytes and to evaluate the relative contributions of malignant origin, B cell nature and functional immaturity of CLL cells to any differences found. Because of the low B cell content of normal blood, human tonsils were also used: like CLL blood these comprise mainly B lymphocytes [15, 16]. Calf cortical thymocytes and cultured Namalva cells [17] were analysed as examples of immature T cells and malignantly transformed human lymphoblasts of B cell type respectively.

In order to obtain a measure of the surface-located sialic acid the lymphocytes were treated with *Vibrio cholerae* neuraminidase (VCN). The enzyme releases sialic acid in free form and gives a reasonable estimate of surface sialic acid [1]. An additional method of exploring membrane glycoprotein differences was measurement of sialic acid released from lymphocytes during incubation in phosphate-buffered saline alone or in the presence of the proteolytic enzymes trypsin or protease 1 of *A. oryzae* (brinase). Trypsin has been widely used to liberate protein-bound sialic acid complexes from cell surfaces. The use of brinase was prompted by the fact that it has been shown to increase T lymphocyte (E) rosetting *in vitro* [18], in contrast to the action of trypsin but similar to that of papain [19]. Brinase also restores depressed cellular immunity *in vivo* and causes the removal of leukaemic cells from the circulation [20].

MATERIALS AND METHODS

Blood lymphocytes

Heparinised blood samples were obtained from normal healthy volunteers: plasmapheresis by IBM cell separator was used to obtain lymphocyte-rich blood fractions yielding sufficient normal lymphocytes for biochemical analyses. Chronic lymphocytic leukaemic (CLL) blood was (except where stated) obtained from patients with high lymphocyte counts who had been receiving chemotherapy for varying lengths of time. The patients were ambulatory and in partial remission, as judged by lack of evidence of tissue infiltration. Blood was collected by venipuncture in heparinised tubes. Comparison of samples obtained by cell separator and by venipuncture from normal and CLL subjects showed that the method of blood collection did not affect the results for total lymphocyte sialic acid.

Tissue lymphocytes

Human tonsils, obtained at tonsillectomy, were processed as soon as possible by teasing out in phosphate-buffered saline and filtering through cotton gauze before lymphocyte isolation. Calf thymus glands were processed as soon as possible after slaughter: pieces of cortical tissue were teased out in PBS and filtered through gauze. Since staining showed these preparations to consist of 95% thymocytes, no further separative procedures were used.

Lymphocyte isolation

Lymphocytes were isolated from human blood, normal and leukaemic, and from tonsils by centrifugation on Ficoll-metrizoate [21]. They were suspended in PBS (Dulbecco A, Oxoid Ltd.) and washed twice in this medium with centrifugation at 500 ***g*** for 7 min. Platelets were removed from blood lymphocyte preparations by a final slow wash (100 ***g*** for 1 min). All preparations comprised >90% mononuclear cells. Smears were stained for non-specific esterase and chloroacetate esterase [22]. Blood lymphocyte preparations were found to contain 2% monocytes (mean of 5 experiments, range 0–7%) and 3% granulocytes (mean of 7 experiments, range 0–8%). Contamination of tonsil preparations was even lower, being never greater than 0.5% for either cell type. Previous authors have shown lymphocyte preparations from tonsils to be almost exclusively composed of T and B cells, with B cells predominating [15, 16]. The T cell content of our preparations was $27 \pm 3\%$ (mean ± S.E.M. of 7 experiments), in agreement with these authors, indicating a B cell content of *ca.* 70% for unfractionated tonsil samples.

Sub-fractionation of lymphocytes

This was carried out on lymphocyte preparations obtained from normal human blood and tonsils. Initially the nylon wool column method [23] was used, but this procedure was found to cause gross depletion of lymphocyte membrane material, including sialic acid [24]. An additional problem was that components of fetal calf serum used in this method tended to adhere to lymphocytes, resulting in excessively high sialic acid levels unless the cells were subjected to extra washing. Results obtained with the nylon wool method could not, therefore, be used in the present study and rosette depletion methods were adopted instead. An unfortunate outcome of the changeover was that few volunteers for plasmaphoresis remained at this stage, so that the numbers of experiments on separated lymphocyte poulations from normal blood were less than originally planned.

Lymphocyte preparations from normal blood or from tonsils were depleted of T cells by

sheep red blood cell rosetting and removal of rosettes by centrifugation through Ficoll-metrizoate [25]. Interface cells were washed twice with PBS before use. Such T-depleted preparations were virtually free (<2%) of E rosetting cells and showed negligible esterase staining for monocytes or granulocytes. Normal blood lymphocytes were depleted of B cells by mouse erythrocyte rosetting [26] and removal of rosettes by centrifugation on Ficoll-metrizoate as described above.

Namalva lymphoblasts

This lymphoblastoid cell line, originating from a Burkitt's lymphoma [17], was grown in RPMI 1640 medium containing 20 mM HEPES (4 - (2 - hydroxyethyl) - 1 - piperazine - ethanesulphonic acid) buffer, 10% foetal calf serum, penicillin (100 IU/ml) and streptomycin (10 μg/ml). The line was maintained by reseeding every three days at 0.5×10^6 viable cells/ml. For experimental use cells were harvested on day three by centrifugation and washed twice in PBS.

E rosetting (T cells)

One-tenth of a ml of lymphocytes (4×10^6/ml) were incubated with 0.1 ml sheep erythrocytes (4×10^7/ml) for 20 min at room temperature, pelleted and maintained overnight at 4°C. After gentle resuspension the percentage lymphocytes forming rosettes was determined in triplicate, three or more adherent erythrocytes being taken as positive.

Cell counts and viability

Counts were carried out in a Burker cytometer under phase-contrast microscopy and concentrations adjusted with phosphate-buffered saline (PBS). Viability, as assessed by trypan blue (0.25 g/l final concentration) was always greater than 90% both before and after incubation. All experiments were commenced immediately after preparation of the suspensions.

Incubation conditions

All incubations were in a shaking water bath at 37°C for 45 min in PBS alone or containing the enzymes neuraminidase, brinase or trypsin. *Vibrio cholerae* neuraminidase (VCN), obtained from Beringwerke AG, Marburg, was used in final concentration of 25 U/10^7 cells/ml. A unit is defined as the amount required to release 1 μg of *N*-acetylneuraminic acid from a standard glycoprotein substrate in 15 min at 37°C. After incubation the cells were removed by centrifugation for 7 min at 500 ***g*** and the supernatants dried down and retained for sialic acid analysis. Brinase (protease 1 of *Aspergillus oryzae*), supplied by Astra AB, Sweden, was used in a final concentration of 6 μg/5×10^7 cells/ml. The characteristics of this enzyme have been described by Bergkvist [27]. Trypsin (bovine pancreas, Type III, twice crystallised), obtained from Sigma, was used in final concentration of 6 μg/5×10^7 cells/ml. After removal of cells by centrifugation (500 ***g*** for 7 min), supernatants from proteolytic treatment together with PBS controls were treated with trichloroacetic acid (TCA) at a final concentration of 5% (w/v) at 4°C overnight to precipitate protein. Precipitates were spun down and aliquots of supernatants analysed directly for free sialic acid and, after acid hydrolysis, for bound sialic acid in non-protein form. The latter was calculated by subtraction of free from hydrolysed values. TCA precipitates were acid-hydrolysed for analysis of protein-bound sialic acid. Hydrolysis of cell samples for estimation of total cellular sialic acid was as previously described [28]. All acid hydrolyses were for 1 hr at 80°C, using 0.1 NHCl. Sialic acid was determined by a modification by O'Kennedy [28] of the Warren thiobarbituric acid method [29].

Sephadex chromatography

Supernatants from brinase-treated CLL lymphocytes were fractionated by gel filtration on Sephadex G-50. Dialysed supernatants were concentrated and applied to a 100×0.9 cm Sephadex G-50 (fine) column. The eluent was 0.01 M phosphate buffer, pH 7.4, at a rate of 15 ml/hr. The void volume was determined with Blue Dextran. Absorbance at 280 nm was measured on a Beckman quartz spectrophotometer. Column fractions were also analysed for hexose [30] and for sialic acid by the fluorimetric method of Hammond and Papermaster [31].

RESULTS

Total and VCN-susceptible sialic acid

Table 1 shows the values obtained for lymphocytes from the various sources. It is notable that the total sialic acid content of B-depleted, T-rich preparations from normal blood was found to be 348 nmol/10^9 cells, while that of T-depleted fractions was only 95 nmol, a difference which is more than three-fold. It can also be seen that total sialic acid in CLL lymphocytes is almost identical to that of T-depleted fractions from normal blood and from tonsils. While only two values are available for sub-populations from normal blood

Table 1. Total and neuraminidase (VCN)-susceptible sialic acid in lymphocytes from various sources

Lymphocyte source	Total	*n*	VCN-susceptible	*n*
Normal blood				
Whole preparations	256 ± 43	9	191 ± 17	9
B-depleted fractions	348 (346, 350)	2	n.d.	
T-depleted fractions	95 (103, 87)	2	54	1
CLL blood				
Whole preparations	88 ± 5*	9	64 ± 5*	9
Tonsils				
Whole preparations	99 ± 7*	10	80 ± 11*	11
T-depleted fractions	96 ± 9*	6	65 ± 12*	5
T cells	[107]†			
Cultured Namalva				
Lymphoblasts	247 (222, 272)	2	143 (142, 144)	2

Sialic acid in nmol/10^9 cells: mean values ± S.E.M.
n: Number of experiments.
*Significant difference from normal blood whole preparations ($P < 0.01$).
†[ ] Approximate: calculated from whole and T-depleted preparations on the basis of 27% T and 73% B cells in whole preparations.
n.d.: Not determined.

depleted for T or B cells (see Materials and Methods), it will be noted that individual values agree closely. Furthermore, if one disregards null cells as common to each fraction (and we have observed extremely low total sialic acid in a sample of lymphocytes from a patient with null cell CLL), the results are consistent with the mean total sialic acid (256 ± 43 nmol/10^9 cells) found for 9 experiments with unfractionated normal blood lymphocytes containing on average [32] 63% T and 19% B cells ($0.63 \times 348 + 0.19 \times 95 = 237$).

The significantly lower ($P < 0.01$) sialic acid content of CLL lymphocyte samples as compared with whole preparations from normal blood cannot be attributed to null cells since the null cell content of CLL samples is at most 19% and generally lower [33], and this is of the same order as in normal blood samples. Thus, the difference in sialic acid is due to the B cell nature of CLL and the predominance of T lymphocytes in normal blood.

The amount of VCN-susceptible sialic acid in CLL lymphocytes was similar to that of T-depleted preparations from normal blood and tonsils and significantly lower ($P < 0.01$) than the value for unfractionated lymphocytes from normal blood. The single value available for VCN-susceptible sialic acid in T-depleted lymphocyte preparations from normal blood (54 nmol/10^9 cells) is in keeping with the low total sialic acid content (95 nmol) in these cells. These findings strongly suggest that the lower level of VCN-susceptible sialic acid in CLL lymphocytes is related to their B cell nature.

Low values for total and VCN-susceptible sialic acid were a feature of all individuals in the CLL group in Table 1, which consisted of patients who had received treatment and were in temporary remission. A single, untreated case (not included in Table 1) tested for VCN-susceptible sialic acid gave a value of 56 nmol/10^9 cells, which is similar to the results for CLL in remission and for normal B lymphocytes.

Since the whole preparations from normal blood gave a value of 191 nmol/10^9 cells for VCN-susceptible sialic acid compared with 54 nmol/10^9 cells for the T-depleted fraction, it is evident that a marked difference exists in this respect between the T and non-T cell populations in normal blood. On the basis of 63% T and 19% B cells in normal blood lymphocyte preparations, it can be calculated that the T cell VCN-susceptible sialic acid is in the region of 270 nmol/10^9 cells, or five times that of the non-T fraction.

Whole lymphocyte preparations from human

tonsils (27% T cells, 73% B cells) had a mean total sialic acid content of 99 nmol/10^9 cells. This is significantly lower than that for corresponding preparations from normal blood ($P < 0.01$). Tonsillar preparations which had been depleted of T cells showed results for total sialic acid (96 nmol) almost identical to whole preparations, and it is calculated that the sialic acid content of tonsillar T cells is of the order of 107 nmol/10^9 cells. Thus T lymphocytes from tonsils apparently contain only about one-third of the amount of sialic acid in normal peripheral human T cells.

It was of interest to examine a sample which became available from a patient with T cell acute lymphocytic leukaemia, consisting of 80–90% blast cells. VCN-susceptible sialic acid was found to be 132 nmol/10^9 cells, a value notably less than the calculated level for normal peripheral T cells. Results for Namalva lymphoblasts, a malignant line of B cell origin, are also given in Table 1, which shows mean values for two experiments in triplicate. Total and VCN-susceptible sialic acid were of the same order as found with whole preparations from normal blood and, therefore, markedly higher than those for non-malignant, T-depleted preparations from blood or tonsils or for CLL cells. This relatively high value may be due to the larger size of lymphoblasts [12] as compared with normal peripheral lymphocytes. VCN-susceptible sialic acid in Namalva cells comprised 58% of the total content, a value much lower than the mean of 75% obtained with whole lymphocyte preparations from normal blood. A similarly low value (57%) was found in a single experiment using the T-depleted fraction from normal blood, but further work is necessary before any significance can be attached to this finding.

Release of sialic acid during incubation in saline with and without addition of proteolytic enzymes

Incubation in PBS alone. Whole lymphocyte preparations from normal blood, CLL blood, calf thymus, and human tonsils and T-depleted fractions from tonsils were incubated in PBS. Supernatants were analysed for free sialic acid and for sialic acid bound to protein and 'non-protein'. The latter term refers to bound sialic acid in TCA supernatants, and evidence for the peptide nature of this fraction will be presented below. As shown in Table 2, whole lymphocyte preparations from normal blood incubated in PBS released a total of 43 nmol sialic acid/10^9 cells. Approximately two-thirds of this was protein-bound and one-third was bound to non-protein moieties, a negligible amount being in free form. Lymphocyte preparations from the other sources tested were similar to those from normal blood in their release of free and non-protein bound sialic acid. As seen in Table 2, however, the amount of protein-bound sialic acid released from CLL lymphocytes (12 nmol/10^9 cells) was significantly lower than that from whole preparations from normal blood. The mean value for T-depleted fractions from tonsils was even lower (6 nmol). Comparison of the latter with the value of 19 nmol/10^9 cells obtained for unfractionated tonsillar lymphocytes (27% T, 73% B cells) indicates a specific deficiency in protein-bound sialic acid release in the T-depleted fraction and a calculated value for tonsillar T cells of approximately 54 nmol/10^9 cells. Thus, B-rich lymphocyte preparations, whether from tonsils

Table 2. Release of sialic acid in free and bound form from lymphocytes during incubation in PBS alone and PBS containing brinase

Lymphocyte source	Incubation conditions					
	PBS alone			PBS + brinase		
	Free	Non-protein bound	Protein bound	Free	Non-protein bound	Protein bound
Normal blood (4)	3 ± 3.0	13 ± 8.0	27 ± 3.0	23 ± 7.0†	40 ± 4.0††	23 ± 8.0
CLL blood (8)	5 ± 1.0	10 ± 4.0	12 ± 4.0*	4 ± 1.0	17 ± 4.0	11 ± 3.0
Tonsils						
Whole (7)	8 ± 3.0	5 ± 2.0	19 ± 3.0	11 ± 3.0	17 ± 3.0†††	18 ± 2.0
T-depleted fractions (2)	6	0	6	10	0	8
Calf thymus (3)	3 ± 0.4	3 ± 0.5	3 ± 0.4**	2 ± 0.3	6 ± 0.5††	9 ± 1.8†

Sialic acid in nmol/10^9 cells: mean ± S.E.M. Number of matched analyses in parentheses.
Significant difference from normal blood lymphocytes in PBS alone denoted by *($0.02 > P > 0.05$) and **($P < 0.01$).
Significant effect of brinase denoted by †($0.05 > P > 0.02$), ††($0.02 > P > 0.01$) and †††($P < 0.01$).

or from CLL blood, differ from T-rich preparations from tonsils or normal blood in having less protein-bound sialic acid available for spontaneous release during incubation in saline. The finding of a similarly low value for calf thymocytes (3 nmol/10^9 cells), taken in conjunction with other results (see Discussion), suggests that this parameter may be related to immaturity, irrespective of T or B nature.

Incubation in PBS containing proteolytic enzymes. The effects of brinase on sialic acid release can be seen in Table 2. Comparison with PBS controls shows that the enzyme caused the release from normal blood cells of significant amounts of free ($0.05 > P > 0.02$) and non-protein bound ($0.05 > P > 0.02$) sialic acid, but had no effect on the protein-bound fraction. Total net release from normal blood lymphocytes due to the enzyme was 43 nmol/10^9 cells, which represents a 100% increase over that using PBS alone. In contrast, CLL lymphocytes were not significantly affected by exposure to brinase, nor were T-depleted fractions from tonsils. Significant increases in the non-protein fraction of bound sialic acid were noted with whole tonsillar populations and calf thymocytes, and in the latter case protein-bound sialic acid also showed an increase. These findings indicate that release of sialic acid from lymphocytes by brinase occurs mainly from T cells, whether of solid tissue origin or from circulating blood.

Another feature of the results in Table 2 was the finding that brinase released sialic acid in free form from normal blood lymphocytes but not from any of the other lymphocyte populations tested. The effect of trypsin on whole lymphocyte preparations from normal blood, CLL blood and tonsils was also examined. The enzyme-specific release of sialic acid was of the same order as that found using brinase, with the exception that it only concerned the non-protein-bound moiety and no free sialic acid was released.

CLL cells appeared to have little protease-sensitive sialic acid, since no significant release was observed either with trypsin or with brinase. Tonsillar lymphocytes, which were sensitive to brinase in this respect, released no measureable sialic acid due to exposure to trypsin.

Supernatants from unfractionated normal and CLL blood lymphocytes incubated with PBS containing brinase were examined by Sephadex G-50 chromatography. Similar profiles were found in each case, and a typical result is shown in Fig. 1. Two main peaks are

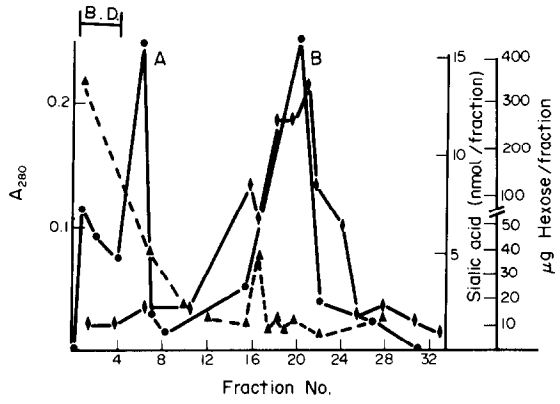

Fig. 1. Gel filtration of supernatants from brinase-treated CLL lymphocytes. Dialysed supernatants were concentrated and applied to a Sephadex G-50 column. The eluent was 0.01 M phosphate buffer (pH 7.4) and fractions of 2 ml were collected. Void volume was determined using blue dextran (BD). Fractions collected before the elution of BD were discarded. ◆——◆ A_{280}; ●——● sialic acid; ▲----▲ hexose.

found; peak A, consisting of high molecular weight material (> 30,000), and peak B, having lower molecular weight (3000–5000). Since both peaks contained hexose and sialic acid but only peak A was precipitatible with TCA (5%, w/v), it is likely that peak B corresponds to the 'non-protein' fraction of bound sialic acid already described: this fraction would therefore appear to be mainly glycopeptide in nature, as previously found in this laboratory for ascites tumour cells [34]. As already noted (Table 2), brinase caused increased release of non-protein-bound sialic acid (peak B) in whole lymphocyte preparations from normal blood, tonsils and calf thymus but not from CLL lymphocytes.

DISCUSSION

Our results indicate that the total sialic acid content of CLL cells is approximately 60% lower than that of whole lymphocyte preparations from normal blood. This is in agreement with the results of Kornfeld [8] and in contrast to the report by Lichtman and Weed [4] that no such difference existed, a statement based on analysis of only one normal sample. McClelland and Bridges [9] reported a remarkably high total sialic acid value for normal blood lymphocytes (1037 nmol/10^9 cells), but their value for CLL (129 nmol/10^9 cells) is of the same order as that found in the present study.

Our values for VCN-susceptible sialic acid confirm those of other authors using intact cells [8, 35] or isolated membranes [10] in indicating a diminution in surface-located sialic acid in CLL cells when compared with whole lymphocyte populations from normal blood.

The present study extends the investigations

of the various authors quoted by showing that the low sialic acid levels in CLL lymphocytes are a property of their B cell nature, similarly low values also being found in T-depleted preparations from normal blood and from tonsils.

An important outcome of the present study was the marked difference in total sialic acid content found between normal peripheral blood preparations enriched or depleted for T cells: the results indicated that the level in peripheral T cells is more than three-fold that of the non-T population.

Our results with VCN also suggest that a difference of at least the same magnitude, if not more, exists for surface sialic acid in these lymphocyte fractions from peripheral blood. As far as we are aware, the present report represents the first direct analyses of sialic acid in human blood lymphocyte sub-populations.

Electrophoretic mobility measurements have shown normal human peripheral T cells to have higher surface electronegativity than their B cell counterparts [13, 14]. While this provided an indication that T lymphocytes may be richer in surface sialic acid, it is also recognised that the contribution of sialic acid to total cell surface charge is only 45% in the case of lymphocytes, as compared to 60% in other cell types [7]. The present results, therefore, support the electrophoretic mobility studies and suggest that the higher mobility of human peripheral T lymphocytes may, indeed, be associated with an increased amount of sialic acid on the surface of these cells. Since total sialic acid levels were virtually identical in tonsillar whole preparations and T-depleted fractions, it appears that no such enrichment of T as compared to B cell sialic acid exists in tonsil lymphocytes. Thus, circulating T lymphocytes possess almost three-fold the sialic acid content of T lymphocytes from tonsils. Häyry *et al.* [36] analysed T and B cell fractions from mouse lymph nodes for sialic acid. While scarcity of cells necessitated their using an indirect method, the fact that they detected no difference in sialic acid between T and B cells from this source suggests that this situation may hold for tissue-derived lymphocytes in general and that T cells may undergo a specific enrichment of sialic acid on passing into the peripheral blood. In agreement with this proposal, an increase in sialylation of thy-1 antigen and other glycoproteins has been found to coincide with functional maturation of mouse T lymphocytes [37].

The present results would appear to conflict with a recent report [38] that T lymphocytes from tonsils are of higher electrophoretic mobility (negativity) than either peripheral blood T cells or B cells from tonsils. It seems possible that these mobility measurements may have been influenced by the fact that the cells were incubated overnight in 20% fetal calf serum before electrophoresis. We have found that even a short exposure to fetal calf serum results in inordinately high surface sialic acid levels in T lymphocytes and that the relative increase is greater in tonsil T cells than in peripheral blood T cells [24].

Namalva lymphoblasts were found to have levels of total and VCN-susceptible sialic acid more than double those in the other B-enriched preparations analysed, whether of malignant (CLL) or non-malignant origin. While this may be a reflection of the larger size of blast cells, it appears that malignant lymphoblasts may not invariably possess more sialic acid than non-malignant lymphocytes of the same type. Reduced VCN-susceptible sialic acid in human acute lymphocytic leukaemic lymphoblasts as compared with normal blood (T-rich) lymphocytes has been reported previously [35, 39], and in the present study a single sample tested from a patient with T cell acute leukaemia showed a VCN-susceptible sialic acid level considerably lower than the estimated value for normal peripheral T cells.

Incubation in PBS alone showed that CLL cells released significantly less protein-bound sialic acid than normal blood lymphocytes. Values of a similarly low order were noted with T-depleted fractions from tonsils, so that a general deficiency in membrane sialoglycoprotein (available for spontaneous release during saline incubation) appears to exist in B lymphocytes from these two sources. This may be contrasted with the abnormally elevated spontaneous release observed by us [40] with peripheral blood lymphocytes from certain patients with advanced cancer and anergy.

Calman [41], using cationic staining and electron microscopy, found the glycocalyx layer in CLL cells to be thinner than that of normal blood lymphocytes, but did not distinguish between normal T and B cells in this regard. The present results suggest that this depletion may be a property of B cells, whether from normal or from CLL blood: membrane material in general would appear to be lower in B than in T lymphocytes, as evidenced by our previous observations that cholesterol and phospholipid levels are *ca.* 60% lower in CLL cells and in B-enriched preparations from tonsils [24, 42] than in whole lymphocyte preparations from normal blood. This depletion is unlikely to be

merely due to differences in cell size. While CLL lymphocytes are smaller in volume by 10–14% than unfractionated normal peripheral (T-rich) lymphocytes [43, 44] and normal T cells [45], size differences of this order would not account for either the reduction in membrane lipid components observed previously [24, 42] or the 3 to 4-fold difference in sialic acid found in the present study. Attempts at estimation of lymphocyte size are, however, complicated by the existence of microvilli in variable numbers on most human lymphocytes, normal and CLL [33], and by reports that B cells tend to be more villous than T cells [46, 47].

When brinase or trypsin was included in the PBS medium during incubation it was noted that significant enzyme-specific release of sialic acid occurred with whole preparations from normal blood and tonsils and also with calf thymocytes, but not with CLL cells or T-depleted tonsillar lymphocytes. Thus protease-specific sialic acid release, as measurable by biochemical methods, appeared to be confined to T lymphocytes in general, whether mature or immature (thymocytes). Brinase released free sialic acid from normal blood lymphocytes but not from any other preparations tested (Table 2). This effect, also found in peripheral lymphocytes from patients with advanced cancer and anergy [40], appears to be a feature of mature peripheral T cells and may depend on activation of a fully developed lysosomal system. CLL cells, for example, are known to be deficient in lysosomes [11].

The lack of any marked effect of brinase or trypsin on sialic acid release from CLL lymphocytes and the evidence presented which indicates an overall reduction in surface sialic acid in B lymphocytes, both normal and CLL, does not preclude the existence in CLL cells of certain glycoproteins whose abnormally high molecular weight appears to be due, at least in part, to increased sialylation. Van Beek *et al.* [48] detected these components by the use of fucose labelling, exposure of the cells to trypsin and gel filtration of supernatants. It has been found in this laboratory, however, that fucose-labelled sialic acid-enriched moieties comprise only a small proportion of the sialic acid released during incubation [49]. In agreement, Speckhart *et al.* [50], while noting increased sialylation of certain components, observed an overall deficiency in sialylation of CLL surface glycoprotein in general. Thus it seems unlikely that these specific sialic acid-enriched moieties on CLL cells exert any marked influence on net cell surface charge or associated behaviour patterns.

In conclusion, the main outcome of the present study is the demonstration that while CLL cells have less total and VCN-susceptible sialic acid than normal peripheral lymphocytes, this is probably due to their B cell nature. It also appears that normal peripheral T and B lymphocytes differ markedly in sialic acid content. Since our results also indicate considerable sialic acid enrichment in circulating T cells as compared with T cells from tonsils, it is evident that location *in vivo* must be taken into account in comparative studies on surface properties of T and B lymphocytes.

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