shown it to be far less effective in producing a disturbance in the venous flow. This may well explain their lack of success in preventing thrombosis in patients with malignant disease.

Conclusion

We have shown that our technique of peroperative intermittent calf compression, which produces the maximum haemodynamic disturbance in the leg veins, can reduce the incidence of post-operative thrombosis by more than 75% in patients aged over 40. More important, however, is the finding that in patients with malignant disease, hitherto regarded as "very high risk," the incidence can be reduced by almost 90%. We believe our technique to be the best currently available for preventing post-operative thrombosis.

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T and B Lymphocytes: Striking Differences in Surface Membranes

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Summary

Lymphocytes can be separated by electrophoresis into cells of high and of low electrophoretic mobility. Cells of high mobility are T cells and those of low mobility are B cells, with distinct immunological functions. Biophysical and biochemical studies on physically isolated and immunologically defined T and B cells in CBA mice showed striking differences in the chemical composition of the surface membranes.

Introduction

Cells of lymphoid origin can be separated on the basis of the net surface charge (negative) by free-flow electrophoresis (Hannig and Zeiller, 1969) into two distinct subpopulations of high and low electrophoretic mobilities without loss of viability and with very little overlap (Hannig and Zeiller, 1969; Zeiller *et al.*, 1972; Nordling *et al.*, 1972; Seiler *et al.*, 1974). Routine immunological methods show that the lymphocytes in the high mobility region are T cells whereas the cells in the low mobility region are B cells (Zeiller *et al.*, 1972; Nordling *et al.*, 1972; Seiler, *et al.*, 1974).

Studies (Mehrishi, 1972 a; Mehrishi, 1972 b) on normal human lymphocytes (isolated from the peripheral blood by a method such as the carbonyl iron-methyl cellulose method

(Coulson and Chalmers, 1964) but not separated further into subpopulations) showed the presence of positively charged groups and sulphhydryl groups and at least three types of negatively charged groups in the cell periphery.

Isolation of mouse T and B lymphocytes into high mobility regions and low mobility regions raised the obvious question: is the characteristic distribution of the electrophoretic mobilities of T and B cells, which perform distinct immunological functions, due to some striking differences in the chemical composition of their surface membranes? The different structures in the periphery of these cells probably give them their specific surface properties and govern the reactions taking place at the cell surface. Information on the surface chemistry of T and B cells would help us to understand the mechanisms of cellular interactions in molecular terms and would clearly be of much interest in immunobiological research.

We describe here the results of a study of the surface topography of physically and functionally defined T and B cells. The study showed striking differences in the chemical composition of the peripheral regions (within 8 Å from the surface) of T and B cells in CBA mice.

Methods

Lymph nodes, spleen, and thymus from several adult spf/CBA/J mice were removed, separately pooled, and the respective cell suspensions prepared for preparative free-flow electrophoresis (Hannig and Zeiller, 1969). The electrophoretic separation of the cells was carried out in a low ionic strength buffer at 6°C (210 mA 100 V/cm), using a refined model of the electrophoretic separator FF4 (Desaga, Heidelberg; Bender and Hobein, Munich) (Hannig and Zeiller, 1969). Cell fractions from the shaded portion of the curves shown in the graph contained homogeneous

populations of 90% viable cells of a single size. The electrophoretically isolated cells were washed in saline (0.145 molar NaCl/l. made 10^{-4} molar/l. with respect to NaHCO_3 ; pH 7.2 ± 0.2) and examined by cell electrophoresis ($25^\circ \pm 0.1^\circ\text{C}$). An electrophoresis cell of a circular cross-section equipped with reversible silver-silver chloride-potassium chloride (molar) electrodes was used (Mehrishi, 1972 b); the performance of the apparatus was monitored repeatedly with saline washed erythrocytes with a mobility of $-1.08 \pm 0.04 \mu\text{m}/\text{sec}/\text{V}/\text{cm}$. Within 15 minutes after treatment with mild specific reagents for the reversible blocking of amino groups (2, 3 dimethylmaleic anhydride; DMA) and sulphhydryl groups (6, 6'-dithiodinitrobenzoic acid; DTDNA) intact viable (90%) cells were also examined by cell electrophoresis to determine the numbers of amino groups and sulphhydryl groups on a per cell basis (Mehrishi, 1972 b).

Electrophoretic distribution profiles of CBA mouse lymph node cells. ● = Total nucleated cells. ○ = Total lymphocytes. □ = α -positive lymphocytes. △ = Lymphocytes positive for mouse B lymphocyte specific antigen.

Vibrio cholerae neuraminidase (VCN; EC 3.2.1.18; Behringwerke, Germany) (Mehrishi, 1970) and ribonuclease (pancreatic and T_1 cells) (Mayhew and Weiss, 1968), which had been claimed by the manufacturers to have no proteolytic activity, were used for removing any susceptible groups from the surface membranes (at 37°C for 45 minutes). The material removed from the surface was stored for subsequent biochemical analyses. Assay for *N*-acetylneuraminic acid (NANA) in the material removed from the cell surface by VCN treatment confirmed the presence of sialic acid in the cell periphery of both T and B cells (see Appendix). The enzyme treated cells were washed twice in saline and examined by cell electrophoresis.

The processing of the electrophoretic data on normal and chemically-modified cells to derive the zeta potentials (mV), surface charge densities (e.s.u./ cm^2), and the numbers of groups or electron charges (from the increase or decrease of mobility) per cell surface area has recently been described (Mehrishi, 1969).

Results

The numbers of groups and charges on T and B cells are given in the table on a per-cell basis.

The difference in the chemical constitution of the periphery of T and B cells was striking. B cells, with a low mobility (median value $-0.90 \mu\text{m}/\text{sec}/\text{V}/\text{cm}$), had phosphate groups and sulphhydryl groups in the cell periphery and carried a large number of positively charged amino groups, and the α -carboxyl groups of VCN-susceptible NANA accounted for only 17% of the total corrected electron charges. T cells on the other hand exhibited a much higher mobility (median value $-1.33 \mu\text{m}/\text{sec}/\text{V}/\text{cm}$). In the T cell peri-

Surface Membrane Groups and Charges of Mouse T and B Lymphocytes

Type of Group or Charge	No. of Groups or Charges per Cell Surface Area ($\times 10^3$)		No. of Cells	No. of Experiments
	T Cells	B Cells		
Amino groups (positively charged) ..	6	11	357	26
Sulphydryl groups ..	Not detected	19.6	93	6
α -Carboxyl groups (neuraminidase-susceptible NANA) ..	24	12	278	15
Phosphate groups ribonuclease-susceptible) ..	Not detected	16	246	16
Unidentified anionogenic groups ..	76	43		
Apparent electron charges	100	60		
Corrected electron charges (negative charges + positive charges)	106	71		
Electrophoretic mobility	-1.33 ± 0.15	-0.90 ± 0.1		

Surface area of T and B cells was estimated as $100 \mu\text{m}^2$ (Zeiller *et al.*, 1974). Median values of mobilities are given because range of (s.d.) values is wide (see fig.).

phery phosphate and sulphhydryl groups were not detected, the number of the positively charged amino groups was much smaller, and the number of the α -carboxyl groups of NANA was much higher than on B cells (see table). Since the contributions of the negative charges attributable to NANA or phosphate groups did not account for all the corrected electron charges (the sum of the apparent electron charges and the positively charged groups) there must have been other negatively charged groups which have not yet been identified. Such acidic groups were present on both T and B cells. The number of the unidentified acidic groups on T cells was 76% higher than on B cells.

Discussion

Though we have confidence in our electrokinetic data and detailed biochemical analyses are being carried out the main purpose of this paper is to emphasize the differences which we have found in the surface topochemistry of T and B cells. This information is likely to be of interest to a number of research workers.

It is tempting to speculate on some of the biological implications of these differences. Though the general electrophoretic behaviour of lymphocytes from normal healthy individuals and from leukaemia patients seems to be similar (Mehrishi, 1972 b) evidence is accumulating that the cell surface of lymphocytes in chronic lymphocytic leukaemia differs from that of normal lymphocytes (Kornfeld, 1969). Also B lymphocytes in many cases of leukaemia, chronic lymphocytic leukaemia (Johansen and Klein, 1970; Wilson and Nossal, 1971; Grey *et al.*, 1971), and Burkitt's lymphoma (Klein *et al.*, 1968) have been reported as having surface immunoglobulins on the cell membrane and therefore representing a B lymphocyte malignancy.

The behaviour of chronic lymphocytic leukaemia lymphocytes in vitro after treatment with phytohaemagglutinin (PHA) was explained initially on the basis of deficiency of intracellular organelles alone, and subsequently by the suggestion that PHA acted predominantly on T cells whereas chronic lymphocytic leukaemic lymphocytes belonged chiefly to the B cell line. Since the biochemical sequences leading to PHA-induced RNA, DNA, and protein synthesis and eventually to mitosis are initiated at the cell membrane, defects in the structures of the cell membrane are clearly important. The decrease in the PHA receptors of the leukaemic lymphocyte membrane may possibly be a consequence of predominant B cell involvement in leukaemia but it may also reflect the changes in membrane structures associated with neoplastic and potentially malignant cells.

We believe that the present study of the surface membranes of T and B cells will lead to a better understanding

of the molecular mechanism of the interactions in which these cells participate. A combination of the methods described for obtaining data on T and B cells may be developed for clinical use, especially for leukaemias.

Phillips and Roitt (1973) reported that B cells in man can be transformed by PHA, strongly suggesting that PHA transformation is thus an unreliable criterion of T cell numbers in the human species. Also, quite recently, Dickler *et al.* (1974) reported that when the B and T cell markers were evaluated simultaneously on individual (human) lymphocytes a small, real subpopulation reproducibly bore both markers.

Clearly care must be taken in using the present immunological methods to attribute the cell surface markers of either T or B cells as absolute. The extrapolation between species in relation to the distinguishing characteristics of the two cell types is also hazardous.

Our approach should be useful in establishing a quantitative chemical basis at the cellular level and characterizing cell types by physico-chemical methods (Mehrishi, 1972 b). There is a pressing need for such studies on cell types in disease.

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Appendix

Marked intramembraneous differences between T and B cells, not directly seen in ultra-thin sections, have also been shown by electron microscopy of replicas of membranes of mouse lymphocytes prepared by freeze-fracturing (Mandel, 1972).

The initial and critical event in lymphocyte stimulation by both specific (antigen) and non-specific agents—for example, phyto-mitogens—is the combination of the agents with binding sites on or in the lymphocyte. Evidence is now available that VCN-susceptible NANA molecules of the lymphocyte surface are the binding sites for PHA (Lindahl-Kiesling and Peterson, 1969; Kornfeld, 1969; Johanson and Klein, 1970; Zatz *et al.*, 1972) and that VCN treatment completely blocks the PHA-absorptive capacity of lymphoid cells and the mitogenic effects of PHA (Adler *et al.*, 1972). The proposal that “the α -carboxyl groups of VCN-susceptible sialic acid residues in the cell periphery are the sites for the binding of PHA” now seems plausible in view of recent finding that sialic acid is present in the periphery of both T and B cells, though in much smaller amounts in B cells (table I), and that B cells under certain conditions do respond to PHA (Phillips and Roitt, 1973). The response of B cells to PHA, especially in three-day cultures, was not observed *before*, perhaps because the number of carboxyl groups of sialic acid is too few for the PHA binding to be effective (Mehrishi 1972 b).

In the above preliminary paper the results of biochemical analyses of the material removed from the cell surface, which is still being investigated thoroughly, were not included. As stated earlier, the presence of NANA in the cell periphery was confirmed from the analyses. During the past 10 years, however, various workers have found no close relation between the loss of the α -carboxyl groups of cell-surface NANA by VCN treatment and a decrease in the electron charges per cell surface area as estimated from electrophoretic data. The seeming futility of trying to correlate the loss of NANA molecules released by enzyme treatment with a decrease in the electron charges per cell surface area calculated from the electrophoretic mobility of treated cells has often been emphasized (Mehrishi, 1972 b). Invariably *more* NANA is released than would be expected from the electrophoretic data—assuming that the loss of one molecule of NANA corresponds to the loss of one electron charge (1:1 ratio). This assumption may seem reasonable but its validity remains to be established.

The evidence for the sulphhydryl groups is, however, most satisfactory. Analyses of the supernatant after treatment of B cells with DTDNA (ultraviolet absorption peak 290 nm) for 20 minutes (at 18°C) to estimate the amount of the 6-mercapto-nicotinic acid (6MNA) (ultraviolet absorption peak 344 nm) indicated the presence of sulphhydryl groups blocked in the cell periphery (Mehrishi, 1972 b). There was good agreement between the number of sulphhydryl groups per cell surface area calculated from the electrophoretic data on DTDNA-treated cells and the ultraviolet spectrophotometric data on the released 6MNA. It was not the prime purpose of the above paper to discuss the biochemical data in detail.

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