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Human B-Cell Growth and Differentiation Factors, and Their Effects on Leukemic B-Cells

F. C. Rawle [1,2], R. J. Armitage [1], and P. C. L. Beverley [1]

A. Introduction

A wealth of data on B-cell growth and differentiation factors has been published recently, and so many different factors have been shown to act on B cells [1] that it is difficult to determine which, if any, of these factors are physiologically relevant. Initial models assumed that B-cell activation leads to an ordered expression of receptors for specific growth and then differentiation factors, but these now appear to be incorrect. Firstly, most of the factors with functional effects on B cells are not specific for cells of that lineage. IL2, IL1 and IFNγ have all been shown to have effects on B cells [1, 2]. Secondly, BSF-2 (B-cell differentiation factor BCDFγ), which has recently been cloned [3], has been shown to activate mast cells and basophils, and to cause T-cell proliferation [4], while BCGF II acts as a differentiation factor for eosinophils [5].

The same lymphokine may also have different effects at different stages of B-cell differentiation. BSF-1 acts on resting B cells to induce expression of class II antigens and prime cells for subsequent activation by anti-μ, but also acts on Staphylococcus A Cowan activated B cells to promote switching to production of the IgG1 subclass [3]. Since BSF-1 acts on resting B cells, it also appears that the assumption that antigen-specific activation is required before B cells can respond to non-specific lymphokines derived from T helper cells is not correct.

It is clearly of great importance to define at which stages of maturation and activation B cells express receptors for, and are capable of responding to, the various lymphokines. We have adopted two complementary approaches; first, the use of monoclonal antibodies to define molecules of functional importance on the B-cell surface, and second, the purification of cytokines for use in identifying receptors and in studies of their functional effects.

[1] ICRF Human Tumour Immunology Group, Faculty of Clinical Sciences, and [2] Department of Zoology and Comparative Anatomy, University College London, London, UK

B. Methods

BCDF activity was measured using the CESS assay [6]. Briefly, 5000 cells per well were cultured in flat-bottomed 96-well microtitre plates for 5 days, and IgG in the supernatant was measured by enzyme-linked immunosorbent assay (ELISA). Peripheral blood lymphocytes from prolymphocytic leukaemia (PLL) patients were cultured at 10^6 per well in 2 ml costar wells, with or without T24 supernatant at 10%. Cells were fixed with 70% ethanol and then subjected to ribonuclease digestion and propidium iodide staining, followed by analysis on the FACS IV (Becton Dickinson). For measurement of IgM secretion, PLL cells were cultured at 10^5 per well in flat-bottomed 96-well microtitre plates; supernatants from triplicate cultures were harvested at day 7, pooled and assayed for IgM by ELISA.

Table 1. BCDF activity in supernatants of bladder carcinoma cell lines in the CESS assay

	IgG secreted (ng/ml)	
	Experiment A	Experiment B
Medium control	24	76
MLR[a]	343	988
5637	340	613
T24	426	772
Supernatant concentration	20%	10%

[a] MLR is the supernatant from a 4-day mixed lymphocyte reaction with peripheral blood lymphocytes from three donors, cultured at a total concentration of 2×10^6/ml.

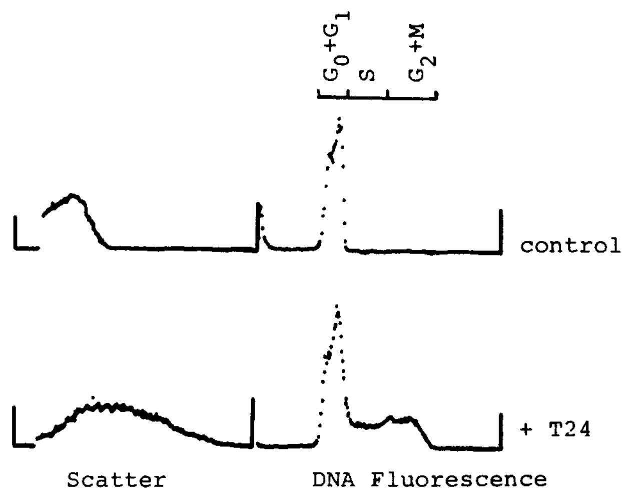

Fig. 1. Cell cycle analysis and cell size (measured by forward-angle light scatter) of PLL cells cultured for 5 days with T24 supernatant (10%) or medium only

C. Results and Discussion

We have found that the human bladder carcinoma cell lines T24 and 5637 secrete BCDF detectable in the CESS assay (Table 1). The factor from T24 is a soluble molecule which elutes from an ACA54 gel filtration column as a single peak with a molecular weight of approximately 25 kD, and has a pI in the range 5.5–6.0 on isoelectric focusing (data not shown). We are in the process of purifying this molecule in order to determine its relation to the T-cell-derived BCDF described by other workers [7].

We have also found that supernatants from these two cell lines cause PLL cells to undergo a significant increase in cell size, as measured by forward-angle light scatter, and to enter cell cycle (Fig. 1). In contrast, chronic lymphocytic leukaemia (CLL) cells do not respond to these supernatants, and preliminary data indicate that normal non-T cells can respond with an increase in size but do not enter cycle. Normal T cells show no response.

PLL cells stimulated with 5637 or T24 supernatant are also induced to secrete IgM (Table 2), and this is accompanied by a decrease in expression of surface IgM detected by staining with monoclonal anti-μ and FACS analysis (data not shown). Thus, the PLL cells appear to differentiate into antibody-producing cells. It is not clear whether this is due to the BCDF or whether proliferation and differentiation are both due to a single signal, as in the response of murine BCL1 cells to BCGF II [1].

Table 2. IgM secretion by PLL cells stimulated with bladder carcinoma supernatants

	IgM secreted (ng/ml)
Control	5
T24 20%	121
T24 2%	97
5637 20%	78
5637 2%	167

These cell lines had been repeatedly tested for mycoplasma contamination by orcein acetate staining and culture, with negative results. However, recent data suggest that the growth-promoting activity for PLL cells may be associated with a mycoplasma or ureaplasma. It is not yet clear whether production of BCDF is associated with mycoplasm infection.

Regardless of their origin, these factors/activities have clearly defined effects on B cells and are thus likely to be binding to receptors of functional importance. They can therefore be used to investigate the normal function of these receptors and of the signals transmitted through them.

Identification of the receptors should be possible either by receptor-ligand cross-linking or by blocking studies with monoclonal antibodies. We have already used the latter strategy to search for antibodies which interfere with differentiation induced by mixed lymphocyte reaction (MLR) supernatant, and have identified several B-cell surface molecules with functional roles [8, 9].

It is of interest that cells at different stages of differentiation (PLL and CLL cells) differ in responsiveness to bladder carcinoma factors. Clearly, future strategies for treatment of leukaemia, based on manipulation of growth and differentiation, need to define which receptors and factors are important in regulation of each stage of B-cell development. Cytokines and monoclonal antibodies provide powerful tools for approaching these questions.

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References

1. Kishimoto T (1985) Annu Rev Immunol 3:135–159
2. Melchers F, Anderson J (1984) Cell 37:715–720
3. Noma Y, Sideras P, Naito T, Bergstedt-Lindquist S, Azuma C, Severinson E, Tanabe T et al. (1986) Nature 319:640–646
4. Vitetta E (in press) Proceedings of the International Conference on Lymphocyte Activation and Immune Regulation
5. Sanderson CJ, O'Garra A, Warren DJ, Klaus GGB (1986) Proc Natl Acad Sci USA 83:437–440
6. Muraguchi A, Kishimoto T, Miki Y, Kuritani T, Kaieda T, Yoshizaki K, Yamamura Y (1981) J Immunol 127:412–416
7. Hirano T, Taga T, Nakano N, Yasukawa K, Kashiwamura S, Shimizu K, Nakajima K, Pyun H, Kishimoto T (1985) Proc Natl Acad Sci USA 82:5490–5494
8. Golay JT, Rawle FC, Beverley PCL (1985) In: Reinherz EL, Nadler LM (eds) Leucocyte typing II. Springer, Berlin Heidelberg New York Tokyo, pp 463–472
9. Golay JT, Clark EA, Beverley PCL (1985) J Immunol 135:3795–3801