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Normal and certain leukaemic B cells express IL-2 receptors without *in vitro* activation

R. J. ARMITAGE & J. C. CAWLEY *Department of Haematology, University College, London, UK*

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SUMMARY

IL-2 receptor expression by B cells has previously been considered to be confined to activated normal B cells and, among the B cell leukaemias, to the hairy cells (HC) of hairy-cell leukaemia. In the present paper, using α-Tac monoclonal antibodies in a highly sensitive indirect rosette method, we show that both normal and certain leukaemic B cells other than HC express IL-2 receptors. The density of these receptors is low since they were not detectable by indirect immunofluorescence. Various controls excluded non-specific-reagent or exogenous receptor binding and blocking studies with recombinant IL-2 confirmed the presence of the IL-2 receptors. The significance of the findings is discussed and it is suggested that B cell IL-2 receptor expression without *in-vitro* activation may be a function of B cell maturity.

Keywords B cells B cell leukaemias IL-2 receptors anti-Tac monoclonal antibody

INTRODUCTION

The generation of IL-2 receptors is an important feature of T cell activation, and provides a means for the replication of T cells in response to IL-2. The expression of IL-2 receptors was initially thought to be confined to activated T cells. Recently, however, it was shown that first hairy cells (HC) with B-cell phenotype and later normal B cells after *in vitro* activation can also express receptors for IL-2 (Korsmeyer *et al.*, 1983; Tsudo, Uchiyama & Uchimo, 1984; Waldmann *et al.*, 1984). However, the precise role of IL-2 in B cell growth and differentiation remains unclear.

In this paper, using monoclonal antibodies against the IL-2 receptor in a highly sensitive indirect rosette assay, we show that normal and certain leukaemic B cells express IL-2 receptors when tested directly *ex vivo* without *in vitro* stimulation.

MATERIALS AND METHODS

Patients. The following leukaemias were studied, the number of patients being indicated either in Table 1 or in parentheses: chronic lymphocytic leukaemia (CLL), centroblastic-centrocytic cell leukaemia (CB-CCL), centrocytic-cell leukaemia (CCL), acute lymphoblastic leukaemia expressing the cALL antigen (cALL), hairy-cell leukaemia (HCL), acute myeloblastic leukaemia (AML) (two cases), myeloid blast crisis or chronic granulocytic leukaemia (CGL) (two cases), and T-ALL (one case).

Correspondence: R. J. Armitage, Department of Haematology, University College Hospital, Gower Street, London WC1, UK.

All cases of CLL had a peripheral lymphocyte count $> 15 \times 10^9$/l, and the cells possessed both receptors for mouse erythrocytes and the T65 antigen (detected with Leu-1) (Royston *et al.*, 1980). In both CB-CCL and CCL, the cells lacked mouse erythrocyte receptors and, in the case of CCL, were also T65 negative; many cleaved cells were present and in CB-CCL were accompanied by > 20% larger cells with abundant cytoplasm. The other leukaemias were diagnosed by conventional morphological and cytochemical methods.

Cell preparations. Normal B cells were obtained from Ficoll-Hypaque-separated tonsil or peripheral blood mononuclear cells (PBM) by depletion of sheep-erythrocyte-rosetting (E^+) and plastic-adherent cells. A routine preincubation in serum-free medium (2 h at 37°C) was performed to remove any extrinsic antigen.

In the various leukaemias, unfractionated PBM were usually employed, although in hairy-cell leukaemia an E-depletion step was employed since substantial number of reactive T cells are often present in this leukaemia.

Surface markers. AET-treated sheep erythrocytes were used for the separation of E^+ cells, and a direct rosette method was employed for the detection of κ and λ light-chain surface immunoglobulin (SIg).

Monoclonal antibodies. The following mouse monoclonal antibodies (MoAb) were employed: Leu-12, (anti-pan B, p95), J5 (anti-cALLA; IgG2a), Leu-1 (anti-T and some B, including CLL cells, p65), UCHT-1 (anti-T), anti-Tac (anti-IL-2 receptor, IgG-2a, ascitic fluid usually diluted 1:1000) (Uchiyama, Broder & Waldmann, 1981) and 4H3 (anti-IL-2 receptor, IgG-2a, ascitic fluid diluted 1/1000, a kind gift from Dr E. L. Reinherz) (Fox *et al.*, 1985).

MoAb staining. The MoAb were normally used in an indirect rosette assay employing ox erythrocytes (E_{ox}) coupled with chromic chloride to affinity-purified goat anti-mouse Ig (E_{ox}-α-MIg) (Mills, Armitage & Worman, 1983). In selected experiments, the anti-Tac was employed in an indirect immunofluorescent method using FITC-conjugated goat anti-mouse Ig; staining was then assessed by fluorescence microscopy or by FACS analysis.

To control for reagent Fc binding, another antibody of the same subclass as α-Tac (J5; IgG-2a) was included in all experiments; this (except in cALL) and second-layer-only controls was always negative.

To control for passive binding of any Tac antigen in the serum, Tac was capped with anti-Tac, or 4H3 and unbound second layer. After ensuring, by indirect rosette testing, that all anti-Tac or 4H3 positivity had disappeared from the surface of the test cells, they were subsequently re-tested for Tac re-expression after up to 72 h culture.

Double labelling experiments for coexpression of Leu-12 and Tac on normal and CLL B cells were performed as follows: Cells were first stained directly with FITC-conjugated Leu-12 and then with unconjugated goat anti-mouse Ig to block any subsequent binding of anti-mouse Ig to the Leu-12 present on the cells. Indirect staining with anti-Tac and J5 and E_{ox}-α-MIg second layer was then

Table 1. Tac and other antigen expression by normal and leukaemic B cells

Cell type	No. of Cases	Positive cells ± 1 s.d. (%)					
		SIg	Leu-12	J5	Leu-1	UCHT-1	α-Tac
Normal PB	6	82±4	81±5	5±2	2±1	4±2	37±10
Normal tonsil	2	86	84	2	2	2	39
CLL	14	88±7	90±5	2±2	64±16	6±5	71±16
CB-CCL	2	93	92	5	61	5	66
CCL	2	92	90	3	9	6	10
cALL	2	6	87	77	2	4	10
HCL	3	72	60	5	—	3	66

carried out. The J5 staining was invariably low, therefore excluding any non-specific binding of α-Tac to the Leu 12/anti-mouse Ig already present.

Blocking experiments. Test cells were incubated with a range of concentrations of recombinant IL-2 (Biogen, Geneva, Switzerland, 5-500 ng/ml) for 1h at 4°C. After washing, the cells were then tested for α-Tac positivity (α-Tac or 4H3 1/1000) by indirect rosetting. As a control, cells were incubated with recombinant γ-Interferon (γ-IFN) (Biogen, Geneva, Switzerland) in the place of IL-2.

RESULTS

Normal B cells express Tac

A proportion of normal B cells from both tonsil (32–45%) and peripheral blood (29–48%) expressed Tac antigen when tested directly *ex-vivo* by the indirect rosette method (Table 1). When tested by indirect immunofluorescence, very low levels of staining (< 3%) were observed by both fluorescence microscopy and FACS analysis (three samples tested).

Certain leukaemic B cells express Tac

In all CLL and HCL patients a high proportion of cells expressed Tac antigen (44–94% & 60–70% respectively) (Table 1). When tested by indirect fluorescence microscopy and FACS, CLL cells were unreactive (< 5%; five cases), while moderate staining was demonstrated on the same proportion (60–70%) of hairy cells that displayed α-Tac positivity in rosette assays (three cases). α-Tac positivity was also shown in both cases of CB-CCL tested, while centrocytic, acute lymphoblastic (Table 1) and myeloblastic leukaemia cells lacked α-Tac reactivity.

The specificity of anti-Tac staining

In all instances where Tac positivity was high, reactivity with another IgG-2a antibody (J5) was low, this, together with the uniformly low reactivity observed after second-layer-only staining, excludes non-specific reagent binding as an explanation of the α-Tac positivity observed. In addition, the low reactivity observed on myeloblasts (two cases of AML and two of blast crisis CGL), in certain lymphoproliferative disorders (Table 1), and on freshly isolated normal (normal E^+ cells < 10% α-Tac positive, ten cases) or ALL T cells (1% α-Tac positive) argues against non-specific staining.

Furthermore, identical Tac expression was detected on CLL and normal B cells with an alternative α-Tac antibody (4H3) (for four patients with CLL, α-Tac 75 ± 13, 4H3 72 ± 12; for normal blood B cells from three patients α-Tac 37 ± 5, 4H3 41 ± 4).

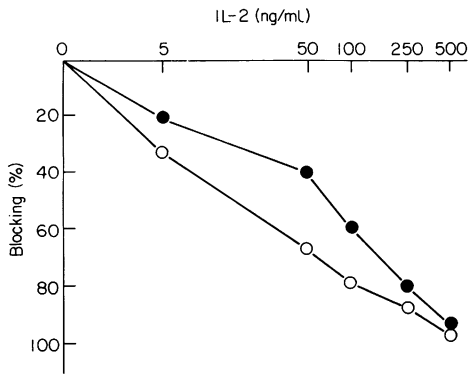

Fig. 1. IL-2 blocks α-Tac reactivity. Results represent the means of two experiments. α-Tac staining performed at 1/1,000 dilution by indirect rosetting. (○) Normal B cells; (●) CLL B cells.

Tac is an intrinsic product of B cells

After capping and shedding, Tac was re-expressed to pre-stripping levels in up to 72 h by normal (four experiments) and CLL (five experiments) B cells.

Furthermore, in the case of both normal and CLL B cells, capping with α-Tac or 4H3 abrogated reactivity with both these reagents. After stripping with α-Tac, both α-Tac and 4H3 positivity reappeared after 72 h culture, as it did after capping and shedding with 4H3.

In double-labelling experiments involving both normal and CLL B cells, almost all cells expressing Tac coexpressed Leu-12 (normal B cells – 88% of Tac-positive cells expressed Leu-12 (two experiments), CLL B cells – 94% of Tac-positive cells expressed Leu-12 (two experiments)).

α-Tac reactivity is blocked by IL-2

The α-Tac and 4H3 reactivity of both normal and CLL B cells was blocked by IL-2 in a linear fashion (Fig. 1); indicating the presence on these cell types of the IL-2 receptor. Incubation with γ-IFN had no effect on Tac positivity.

DISCUSSION

The present study shows that normal and certain leukaemic B cells express IL-2 receptors without *in vitro* activation. However, the density of receptor expression is clearly low since the more sensitive indirect rosette method (Mills *et al.*, 1983) was necessary to detect Tac on all cell types except HCL cells. The sensitivity of our rosette method, as compared with immunofluorescence probably explains previous failures to detect Tac on CLL (Uchiyama *et al.*, 1981) or non-activated normal B cells (Tsudo *et al.*, 1984; Waldmann *et al.*, 1984).

The specificity of the α-Tac staining on normal and CLL B cells was confirmed with a second MoAb against IL-2 receptors and by the inclusion of first-layer controls of the same Ig subclass as α-Tac. The low α-Tac reactivity of non-activated T, myeloid and certain cells of B lineage further argued against non-specific binding.

Capping and re-expression experiments showed that the Tac antigen detected on normal and CLL B cells is an endogenous product, and double labelling directly confirmed that the antigen is present on Leu-12 positive B cells. The blocking of α-Tac and 4H3 staining with recombinant IL-2 indicates that the epitopes detected constitute at least part of the IL-2 receptor.

Our demonstration of low levels of IL-2 receptor on normal and certain leukaemic B, but not T, cells without *in vitro* activation supports previous suggestions that the function of the receptor on B cells differs from that on T cells in not being directly concerned with proliferation (Waldmann *et al.*, 1984). Several laboratories have shown the presence of Tac on B cells after *in vitro* activation (Waldmann *et al.*, 1984; Tsudo *et al.*, 1984), and it is therefore possible that our observations are a consequence of low levels of *in vivo* activation. However, the distribution of Tac positivity among the B cell leukaemias raises the possibility that Tac expression *in vivo* reflects B cell maturation. Thus, very early B cells as represented by cALL and the late B cells of CCL lack IL-2 receptor, while the intermediate CLL and the related CB-CC cells express Tac positivity. The presence of IL-2 receptors on HC and not CC cells may reflect the fact that HC have no potential for plasmacytoid differentiation (Cawley, Burns & Hayhoe, 1980) and therefore are not on the main lymphoplasmacytoid differentiation pathway. The intermediate Tac expression of normal peripheral blood and tonsil B cells may be due to the presence of subpopulations of B cells at different stages of maturation. The concept that IL-2 receptor expression by B cells *in vivo* is a maturation phenomenon is compatible with suggestions that IL-2 is concerned with B cell differentiation rather than proliferation (Waldmann *et al.*, 1984).

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