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Quantitative relationship between T3 and the T-cell antigen receptor on a human leukaemic line

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Summary. The T-cell antigen receptor complex consists of an idiotypic molecule, Ti, and T3. We have investigated the relationship between Ti and T3 on a leukaemic line HPB-ALL. This has shown that the two molecules are expressed on the cell surface in equal numbers, and that a similar stochiometric relationship is seen on sublines of HPB-ALL selected for diminished expression of Ti. Furthermore, the degrees of modulation and rates of resynthesis of Ti and T3 following modulation were very similar, regardless of whether anti-Ti or anti-T3 was used to produce the modulation. This work strongly suggests that the expression of Ti and T3 are under common control.

INTRODUCTION

The human T lymphocyte antigen receptor complex possesses at least two components (Meuer *et al.*, 1983a). These are an idiotypic structure (Ti) and T3 which is present on all immunocompetent T cells (Beverley, 1983). Tis have now been identified on the surfaces of a variety of T-cell subsets (Meuer *et al.*, 1983a, b; Hercend *et al.*, 1983) as well as leukaemic lines (Bigler *et al.*, 1983; Boylston, Goldin & Moore, 1984; Weiss & Stobo, 1984). They are disulphide-linked heterodimers with an overall molecular weight of 80,000–90,000, made up of two chains, α and β, with molecular weights of 37,000–52,000. T3 is made up of three non-covalently linked chains: γ, δ and ε with molecular weights of 25,000, 20,000 and 14,000, respectively (Borst *et al.*, 1984). While the components of T3 do not show polymorphism, gene sequencing has confirmed the existence of marked variability between the structures of different Tis (Yanagi *et al.*, 1984; Jones *et al.*, 1985).

The relationship between T3 and Ti has been demonstrated in a number of ways. Both structures are involved in antigen recognition. Monoclonal antibodies (McAbs) directed against either of them are able to inhibit a clonal response to antigen (Meuer *et al.*, 1983a). As a general rule, anti-T3 McAbs are able to inhibit the response of activated T-cell clones to the antigens for which they are specific, and are able to block the effector function of cytotoxic T lymphocytes (Reinherz, Hussey & Schlossman, 1980).

Ti and T3 that are non-covalently linked in the cell membrane comodulate (Meuer *et al.*, 1983c). A McAb directed against either antigen is able to produce modulation of the other. This suggests that these two structures are closely related in the cell membrane. Studies involving cytotoxic clones (Meuer *et al.*, 1983a) have suggested that there is a stochiometric relationship between the expression of T3 and Ti. One T-cell leukaemia line, Jurkat, had a series of mutants

Abbreviations: McAb, monoclonal antibody; PBSa, phosphate-buffered saline containing azide; Ti, T-cell idiotypic receptor.

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induced with ethyl methane sulphonate or radiation (Weiss & Stobo, 1984) and, using flow cytometry, those that did not express either Ti or T3 were selected. This study showed that mutants that were negative for one component of the receptor complex were negative for the other.

Under certain conditions, Ti and T3 can be made to coprecipitate although they are not covalently lined (Reinherz *et al.*, 1980). Finally, in the process of thymic ontogeny, the surface expression of Ti α and β chains appears to be linked to that of T3 (Royer *et al.*, 1984). We have raised a McAb against the Ti present on a T-cell leukaemia line HPB-ALL (Boylston *et al.*, 1984). In this study, we describe the results of a series of experiments in which the relationship between T3 and Ti has been investigated.

MATERIALS AND METHODS

Cell line HPB-ALL and the conditions for its culture have been described (Boylston, Anderson & Howarth, 1981).

McAb preparation H1-2D4, an anti-HPB-ALL Ti McAb, was prepared as described (Boylston *et al.*, 1984) and purified according to the method of Ey, Prowse & Jenkin (1978). Pure UCHT-1 was a gift from Dr P. Beverly, ICRF Tumour Immunology Unit, University College Hospital, London. MOPC-21 was used as a control mouse immunoglobulin. The antibodies were labelled by the choramine-T method using ^{125}I obtained from Amersham U.K., Amersham, Bucks (McConahey & Dixon, 1966).

All washing steps were carried out using phosphate-buffered saline (0·15 M NaCl, 0·02 M PO_4) containing 0·01 M sodium azide and 1% fetal calf serum (PBSa). Cell counts were carried out in a haemocytometer using trypan blue exclusion as an indicator of viability.

Ti-depleted clones

HPB-ALL cells, 20×10^6 in 2 ml PBSa, were incubated with 200 μl pure Hl-2D4 on ice for 1 hr. They were then washed three times in PBSa at 4°. The cells were then resuspended in 2 ml phosphate-buffered saline and 600 μl goat anti-mouse immunoglobulin (Seralab, Crawley Down, W. Sussex). After three washes, they were sorted into Ti-positive and -negative populations using a fluorescent activated cell sorter (Becton-Dickinson FACS IV). The faintest staining 8% of cells were grown up and then cloned by limited dilution, at half a cell per well on flat-bottomed polystyrene plates containing 5×10^4 feeder cells per well. Clones were selected for their ability to bind diminished quantities of ^{125}I Hl-2D4. Cells with this property were transferred to larger culture bottles.

Binding of radiolabelled McAbs

HPB-ALL cells, 1×10^6 in 100 μl PBS serum azide, were incubated with 1 μl of ^{125}I Hl-2D4 in 100 μl PBSa for 1 hr at 37°. They were then washed three times in PBSa and counted on a gamma counter.

Scatchard plots

Aliquots of 1×10^6 HPB-ALL were incubated with increasing quantities of radiolabelled McAb until saturation had been reached. The results were expressed in the form of Scatchard plots (Boeynaems & Dumont, 1975) to determine the number of bound sites and affinity of the McAbs.

Resynthesis following modulation

HPB-ALL cells were incubated with twice saturating quantities of either Hl-2D4 or UCHT-1 (determined using the binding curves obtained in the previous experiment) overnight at 37° in a CO_2 incubator. Control cells were incubated with equimolar amounts of MOPC-21. They were then washed twice with PBSa and resuspended in fresh medium at a concentration of 1×10^6 per ml. At various times, aliquots of 1×10^6 cells were incubated with saturating amounts of either radiolabelled Hl-2D4 or radiolabelled UCHT-1 as described before. The experiment continued until the binding of these McAbs to the modulated cells had returned to control levels.

Quantitive modulation

Cells were incubated at a concentration of 1×10^6 per ml with increasing amounts of either UCHT-1 or anti-Ti McAb (up to $2 \times$ saturating quantities) overnight. The next day, they were washed twice and aliquots incubated with either HL-2D4 or UCHT-1 in saturating doses to assess the degree of modulation.

RESULTS

Number of UCHT-1 and Hl-2D4 binding sites on HPB-ALL- and Ti-depleted sublines

Cells were incubated with increasing quantities of either ^{125}I-labelled anti-Ti (Hl-2D4) or anti-T3 (UCHT-1) McAbs until saturation had been reached. The results for the parent HPB-ALL line showed that

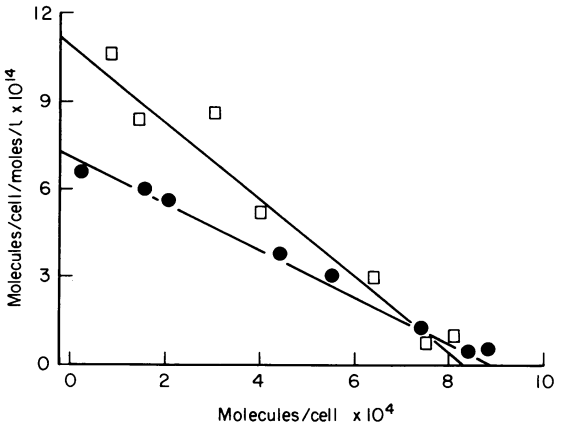

Figure 1. Scatchard plot of the binding of Hl-2D4 (●) and UCHT-1 (□) to HPB-ALL.

there were approximately 90×10^3 Hl-2D4 binding sites and approximately 80×10^3 UCHT-1 binding sites (Fig. 1). Assuming that each McAb molecule bound to a single receptor, this gives the number of Ti and T3 molecules present on each cell and gives a molar ratio of 1:0·9. The Scatchard plots for the Ti-depleted sublines confirmed that there had been a marked reduction in anti-Ti binding sites. For the subline showing the largest decrease, 2C3, this was to 36% of that of the parent line. When the number of UCHT-1 binding sites was determined, there was a striking parallel decrease in T3 expression (Fig. 2). For the subline 2C3, this was to 30% of normal. There was

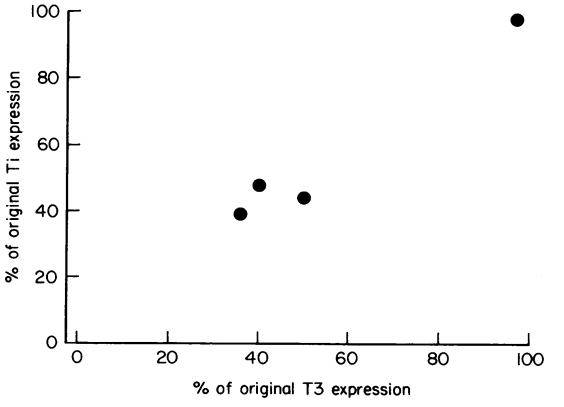

Figure 2. Comparison of Hl-2D4 and UCHT-1 binding to the four sublines of HPB-ALL selected for decreased Ti expression with that to the parent line.

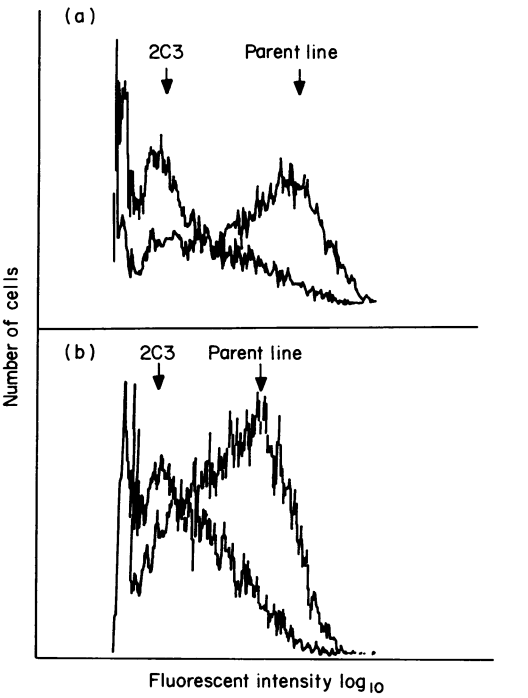

Figure 3.(a) Fluorescent activated cell sorter profiles of Hl-2D4 binding to the HPB-ALL subline, 2C3, showing the greatest decrease in Ti expression and that of the parent line. (b) Fluorescent activated cell sorter profiles of UCHT-1 binding to the HPB-ALL subline, 2C3, showing the greatest decrease in Ti expression and that of the parent line.

a very strong correlation between the reduction in Ti and the reduction in T3 (correlation coefficient = 0·9). It should be noted that this reduction in Ti and T3 expression was a stable property of the sublines, even when they had been grown for several weeks. The binding of both Hl-2D4 and UCHT-1 were further studied in a flow cytometer, and the results for one clone, 2C3, are shown in Fig. 3. This confirms the reduction in Ti and T3 sites. It should be noted that there was no difference in cell size between the parent line and the Ti-depleted clones.

Rate of Hl-2D4 and UCHT-1 modulation

Cells were incubated with twice saturating quantities of either ^{125}I Hl-2D4 or UCHT-1 (determined using the binding curves described above). They were then washed and the rate of modulation observed by counting the residual binding of radiolabelled McAb

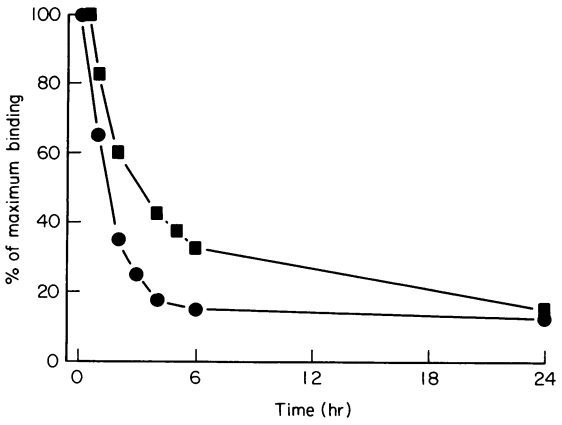

Figure 4. The rates of modulation of T3 (■) by UCHT-1 and Ti (●) by Hl-2D4.

to aliquots of 1×10^6 cells at a number of intervals (Fig. 4). Although the rate of modulation for up to 24 hr of T3 was somewhat lower than that and Ti ($t\frac{1}{2} = 1\frac{1}{2}$ and $2\frac{1}{2}$ hr, respectively) the overall shape of the curves was similar as was the maximum degree of modulation (14% and 11%, respectively).

Quantitative modulation

Cells were modulated overnight with increasing quantities of pure McAb up to twice saturating. The residual Ti and T3 expression were determined the following day using ^{125}I Hl-2D4 and UCHT-1. The results show (Fig. 5) that equimolar amounts of UCHT-1 and Hl-2D4 produced very similar reductions in Ti and T3 expression. Therefore, regardless of the McAb used to produce the modulation, there were very similar decreases in both Ti and T3.

Ti and T3 resynthesis following modulation

Following maximal modulation of T3 and Ti with either Hl-2D4 or UCHT-1, the rates of re-expression of the two cell surface components were determined using radiolabelled McAbs. Very similar results were obtained for T3 and Ti, regardless of the McAb used to produce the modulation (Fig. 6). The $t_{\frac{1}{2}}$ were 9 hr for Ti and 10 hr for T3.

DISCUSSION

This study strongly supports the intimate relationship between T3 and Ti on the human T-cell leukaemia HPB-ALL. Firstly, the quantification of Ti and T3 binding sites using Scatchard plots indicated that the numbers were very similar, 90×10^3 and 80×10^3, respectively. These figures are similar to, but some-

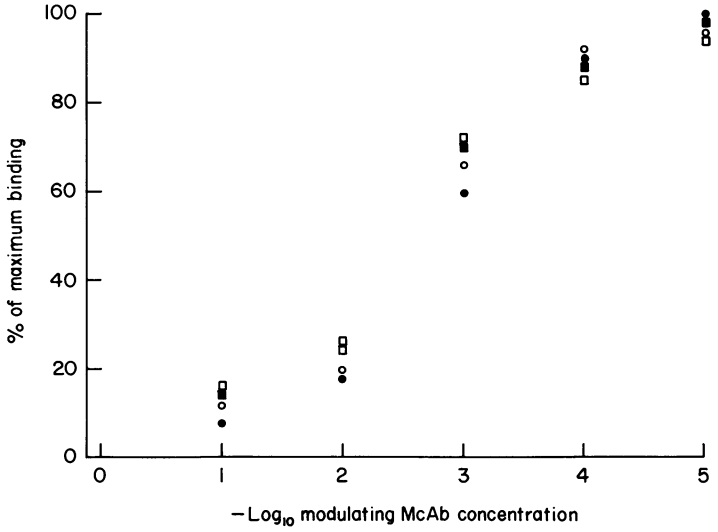

Figure 5. Quantitative comodulation of T3 and Ti by UCHT-1 and Hl-2D4: (●) modulated by UCHT-1 and subsequent UCHT-1 binding; (○) modulated by UCHT-1 and subsequent Hl-2D4 binding; (■) modulated by Hl-2D4 and subsequent UCHT-1 binding; (□) modulated by Hl-2D4 and subsequent Hl-2D4 binding.

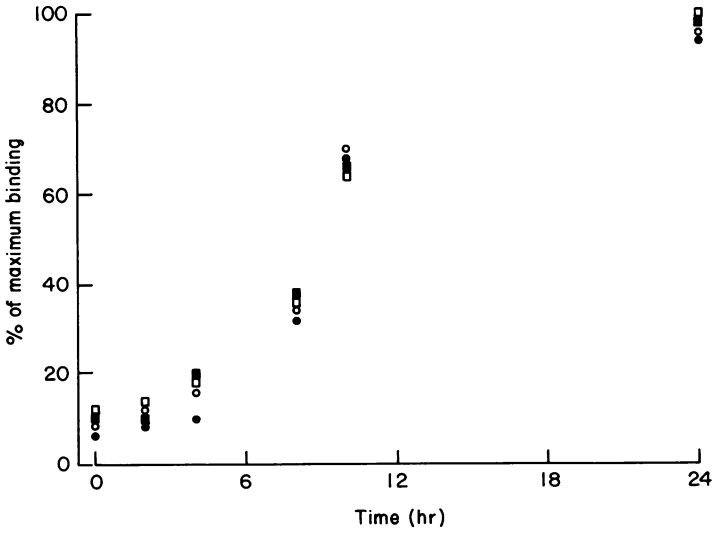

Figure 6. Rates of resynthesis of T3 and Ti after comodulation by UCHT-1 and Hl-2D4: (●) modulated by UCHT-1 and subsequent UCHT-1 binding; (○) modulated by UCHT-1 and subsequent Hl-2D4 binding; (■) modulated by Hl-2D4 and subsequent UCHT-1 binding; (□) modulated by Hl-2D4 and subsequent Hl-2D4 binding.

what greater than, the corresponding ones for T4+ and T8+ cytotoxic T-cell clones: 29,000–42,000 Ti sites and 30,000–48,000 T3 sites (Meuer *et al.*, 1983a). The increased numbers of these molecules in HPB-ALL may be partially explained by the relatively large size of the cells. This confirms that a stochiometric relationship exists.

HPB-ALL cells selected for their reduced expression of Ti were cloned to see whether there was a parallel reduction in T3. This was shown to be the case. This striking observation strongly suggests that the surface expression of these two molecules is under some form of common control. This observation strengthens that of Weiss & Stobo (1984) whose Ti and T3 negative mutants of Jurkat, a T-cell leukaemia line, had completely lost both components. The fact that the magnitude of the reductions in the Ti-depleted clones remained constant, even though the cells had been cultured for some time, shows that it was not a cell cycle-dependent phenomenon.

Modulation refers to the decreased expression of an antigen following exposure to the corresponding antibody. The fact that Ti and T3 comodulate means that the two must be physically associated in the cell membrane, although they are not covalently linked. The relationship between the modulation of these two cell surface components was quantitated in a series of experiments. Firstly, it was shown that, while the rates of modulation produced by the two McAbs (UCHT-1 and Hl-2D4) were different, the maximum degrees of modulation were similar. Studies into the modulation of various antigens using different antibodies measured using several techniques have shown that the maximum level of modulation varied considerably (Chatenad & Bach, 1984). However, the degrees of modulation and either Ti or T3 produced by equimolar amounts of Hl-2D4 or UCHT-1 were very similar over a wide range of McAb concentrations. Therefore, not only is there a stochiometric relationship between Ti and T3 expression, but also modulation. Finally, the rates of re-expression of T3 and Ti following modulation were investigated. These were very similar, regardless of the McAb used to produce the modulation. This further argues for a common mechanism co-ordinating the expression of T3 and Ti in the cell membrane.

Therefore, Ti and T3, the two components of the T lymphocyte antigen receptor complex, can be seen to behave in a parallel manner under a wide range of conditions. While this does not explain the significance of the relationship, its stochiometric nature is consistent with T3 being necessary for stabilizing Ti within the cell membrane, as suggested by sequencing studies which indicate the possible existence of an

ionic bond between the two molecules in this region (van den Elsen *et al.*, 1984). If stabilization of the complex in the cell membrane is a property of only one component, then it should be possible to dissociate it from the others so that the non-essential components disappear. For example, the heavy chain of the class I MHC antigen can be lost without losing β_2 microglobulin expression, and the heavy chain of immunoglobulin molecule can be lost without losing light chain expression.

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