The EMBO Journal Vol.2 No.10 pp.1807 – 1814, 1983

Biosynthesis and molecular nature of the T3 antigen of human T lymphocytes

Jean M. Kanellopoulos[1], Noel M. Wigglesworth, Michael J. Owen[2] and Michael J. Crumpton*

Imperial Cancer Research Fund Laboratories, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, UK

Communicated by M.J. Crumpton
Received on 5 July 1983

Immunoprecipitates of the T3 antigen prepared from HPB-ALL cells by using the monoclonal antibody UCH-T1 were analysed by SDS-polyacrylamide gel electrophoresis. Cells which had been biosynthetically labelled for up to 4 h gave a major polypeptide of mol. wt. 19 000 plus two weaker, more diffuse bands of mol. wts. 21 000 and 23 000, whereas surface labelled cells gave a prominent band of mol. wt. 19 000, a major band of 21 000 and a weaker diffuse band of ~26 000. As judged from their sensitivity to proteinase-K digestion, all the above polypeptides possess a transmembrane orientation. Digestion with endoglycosidases H and F (endo-H and endo-F), and tunicamycin treatment indicate that all the polypeptides, except that of 19 000 mol. wt. are N-glycosylated. The 21 000 and 23 000 mol. wt. chains possess both immature and mature oligosaccharide units, whereas the 26 000 mol. wt. band apparently has mature units only. Pulse chase experiments combined with digestion by endo-F and endo-H suggest that the N-glycosylated polypeptides are derived from two polypeptides of mol. wts. 14 000 and 16 000. It is concluded that the T3 antigen is derived from three different non-glycosylated polypeptides two of which are subsequently N-glycosylated to give the 21 000, 23 000 and 26 000 forms. The cell surface T3 antigen most probably comprises at least two distinct, non-covalently associated polypeptides, but the number and types of polypeptides giving rise to the whole molecule and whether different complexes exist is at present unclear.

Key words: T3 antigen/biosynthesis/lymphocyte surface/membrane glycoprotein

Introduction

Monoclonal antibodies directed against human T-lymphocyte surface antigens have been extensively used to identify and characterise subpopulations of human T-cells. Some of these monoclonal reagents have been shown to interfere with immune responses when added to functional assays. Several monoclonal antibodies, namely OKT3, Leu-4 and UCH-T1, that were described independently in different laboratories (Kung *et al.*, 1979; Ledbetter *et al.*, 1981; Beverley and Callard, 1981), apparently recognise the same surface antigen. Thus, these antibodies share the common properties of reacting with human mature thymocytes and all peripheral blood T-lymphocytes as well as of inducing T-lymphocytes to grow and divide (Van Wauwe *et al.*, 1980; Chang *et al.*, 1981; Burns *et al.*, 1982). Furthermore, the OKT3 antibody inhibits target cell lysis by cytolytic T-lymphocytes including allogeneic T-cells (Chang *et al.*, 1981) and T-cell clones directed against either class I or class II major histocompatibility antigens (Biddison *et al.*, 1982; Meuer *et al.*, 1982). This inhibitory effect has been correlated with a lack of recognition of the target cells rather than with a defect in the lytic components (Meuer *et al.*, 1982). In addition to inhibiting T-killer (i.e., effector) cells, the OKT3 antibody blocks T-cell proliferative responses to cell surface antigens (Reinherz *et al.*, 1980; Chang *et al.*, 1981). These biological properties have led to the suggestion that the molecule recognised by the OKT3-like antibodies corresponds, or is closely related, to the antigen recognition structure of T-cells (Reinherz *et al.*, 1980; Chang *et al.*, 1981). Recently, in support of this view Meuer *et al.* (1983) described a monoclonal antibody which recognises an idiotypic structure on a cytotoxic T-cell clone; in particular, this antibody inhibited cell-mediated lysis and antigen-specific proliferation of this cell clone. In addition, the T3 antigen on the surface of this T-cell clone redistributes with the clonotypic molecules (Meuer *et al.*, 1983). A model for the antigen receptor on human T-lymphocytes was subsequently formulated on the basis of the above data (Reinherz *et al.*, 1983).

Few biochemical studies have been performed on the T3 antigen. Initially, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analyses of OKT3 immunoprecipitates from labelled cells showed one band only of ~19 000 mol. wt. The structure of the antigen was, however, shown subsequently to be much more complex than was suggested by these initial results (Ledbetter *et al.*, 1981; Borst *et al.*, 1982; Kanellopoulos *et al.*, 1982).

Here, we show that the UCH-T1 monoclonal antibody immunoprecipitates predominantly three major polypeptides from Nonidet-P40 (NP-40) lysates of T-lymphocytes. Analyses of pulse-chase experiments, using biosynthetic labelling with [^{35}S]methionine, combined with digestion of immunoprecipitates by endoglycosidases-H and F (endo-H and endo-F, respectively) have indicated that two of the major polypeptides are N-glycosylated. These polypeptides have mol. wts. of 16 000 and 14 000 in the non-glycosylated states. The third major polypeptide of mol. wt. 19 000 is not N-glycosylated. Although only one of these polypeptides is labelled by reagents specific for integral membrane proteins (Borst *et al.*, 1982; Kanellopoulos *et al.*, in preparation), proteinase-K digestion of microsomes showed that all three polypeptides have a transmembrane orientation. At present, however, it is unclear whether the UCH-T1 (i.e., T3) antigen exists at the cell surface as a hetero-dimeric or -trimeric structure.

The data presented here partly agree with those of Borst *et al.* (1982). The latter workers, however, failed to identify the 19 000 mol. wt., non-glycosylated polypeptide. The reason for this discrepancy is not clear, but may reflect the relative resolutions of the respective SDS-polyacrylamide gel systems.

[1]Present address: INSERM U-23, 184 Rue du Faubourg, St Antoine, Paris, Cedex 12, France.

[2]Present address: ICRF Tumour Immunology Unit, Department of Zoology, University College London, Gower Street, London WC1E 6BT, UK.

*To whom reprint requests should be sent.

Results

Tunicamycin treatment of HPB-ALL cells

The T3 antigen of human peripheral blood T-lymphocytes has been shown to be glycosylated (as judged by specific binding to Concanavalin-A) and to comprise three major polypeptides (Van Agathoven *et al.*, 1981; Borst *et al.*, 1982; Kanellopoulos *et al.*, 1982). To determine which of these polypeptides possess N-linked carbohydrate moieties and whether N-glycosylation is necessary for reactivity with the UCH-T1 antibody, immunoprecipitates were prepared from NP-40 lysates of tunicamycin-treated and untreated, biosynthetically-labelled HPB-ALL cells. As shown in Figure 1A, SDS-PAGE analysis of UCH-T1 immunoprecipitates from untreated cells revealed a strongly labelled band of apparent mol. wt. 19 000 and two weaker bands of 21 000 and 23 000 mol. wt. In contrast, immunoprecipitates from tunicamycin-treated cells gave a strong band of 19 000 and a weaker band of 16 000 mol. wt. (Figure 1B). Control experiments were carried out using a monomorphic monoclonal antibody, namely W6.32, against HLA-A,B,C antigens. With untreated cells, the HLA-A,B,C heavy chain of apparent mol. wt. 43 000 was immunoprecipitated together with β_2-microglobulin of mol. wt. 11 500; the band of 38 000 mol. wt. most probably represents a protease-cleaved fragment of the heavy chain (Figure 1C). With tunicamycin-treated cells, the anticipated four bands were observed corresponding to the intact heavy chain, its non-glycosylated counterpart (mol. wt. 40 000), the non-glycosylated proteolytic fragment (mol. wt. 35 000) and β_2-microglobulin (Figure 1D).

The results of these experiments indicated that the two polypeptides of 23 000 and 21 000 mol. wt. precipitated by the UCH-T1 antibody possess N-linked glycan units. In contrast, the 19 000 mol. wt. polypeptide is apparently not N-glycosylated. Although, on the basis of the relative sizes and intensities of the respective bands, the results clearly imply that both the 23 000 and 21 000 mol. wt. glycosylated polypeptides are derived from the 16 000 mol. wt. band detected after tunicamycin treatment, other explanations are also possible. If, as seems likely from the results of the control experiments, tunicamycin suppressed N-glycosylation of the T3 antigen, then the UCH-T1 antibody does not recognise an N-linked carbohydrate moiety.

Pulse-chase analysis and endo-H sensitivity of the T3 antigen

HPB-ALL cells were pulsed for 5 min with [^{35}S]methionine and were then chased with unlabelled methionine for 0, 5, 15, 35, 70 and 140 min. At the latter two time periods, cells which had been treated in an identical manner with the pulse-chased cells except that they had not been biosynthetically labelled, were labelled at the surface by lactoperoxidase-catalysed iodination. Immunoprecipitates, prepared from NP-40 lysates of the labelled cells, were divided into two; one aliquot was digested with endo-H and the other incubated without the enzyme.

SDS-PAGE analyses of the control W6.32 immunoprecipitates (Figure 2, lower panel) revealed the anticipated pattern of increased resistance to endo-H digestion of the HLA-A,B,C antigen heavy chains with increased time of chase (Owen *et al.*, 1980). As noted in Figure 1, the immunoprecipitates apparently also contained a lower mol. wt. form of the heavy chain. Since this band was of similar intensity in the endo-H-treated and untreated samples, it is unlikely to have been produced by contaminating proteases in the

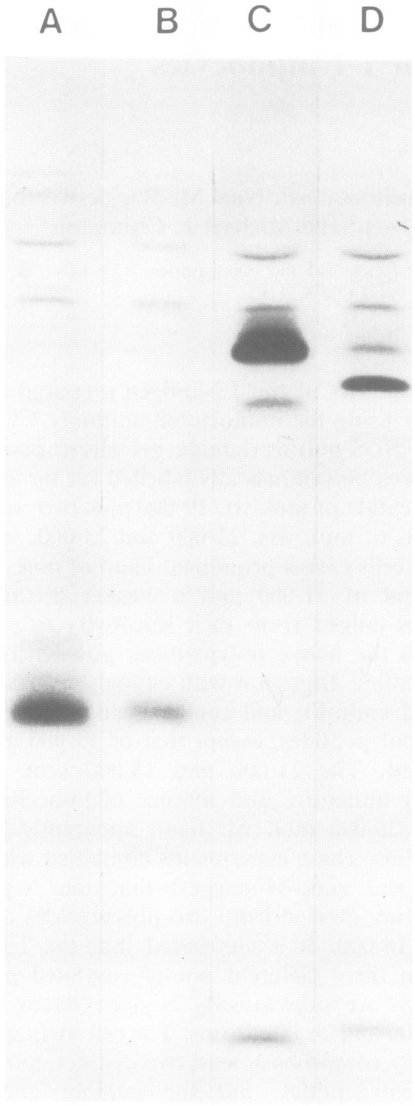

Fig. 1. Effect of tunicamycin treatment on the size of the T3 antigen. HPB-ALL cells (10^6/ml) were preincubated in either medium alone (**tracks A** and **C**) or medium supplemented with 3 μg tunicamycin/ml (**tracks B** and **D**) for 2 h at 37°C before labelling with [^{35}S]methionine (10 μCi/ml) for 4 h. The cells were washed, counted for viability and dispersed in lysis buffer at 2 x 10^7 viable cells/ml. Samples (250 μl) of the lysate were precipitated with either the UCH-T1 (**tracks A** and **B**) or the W6.32 (**tracks C** and **D**) monoclonal antibodies, as described in Materials and methods. Immunoprecipitates were analysed on 12.5% polyacrylamide gels in SDS under reducing conditions, and the bands were visualised by autoradiography.

endo-H preparations. Figure 3A and B shows that the HLA-A,B,C antigen heavy chain separated from the surface-labelled cells was resistant to digestion with endo-H.

The polypeptide compositions of the UCH-T1 immunoprecipitates were much more complex than those of the HLA-A,B,C antigens (Figure 2, upper panel). A series of biosynthetically-labelled polypeptides with apparent mol. wts. of 23 000, 21 000, 19 000 and 18 000 were detected at early chase times. The bands of 23 000, 21 000 and 18 000 mol. wt. decreased in intensity and those of 23 000 and 21 000 mol. wt. became more diffuse with increasing time of chase. In contrast, the 19 000 mol. wt. band increased in intensity with time of chase. Since, at each time point, the intensities of this band were similar in the endo-H-treated and untreated

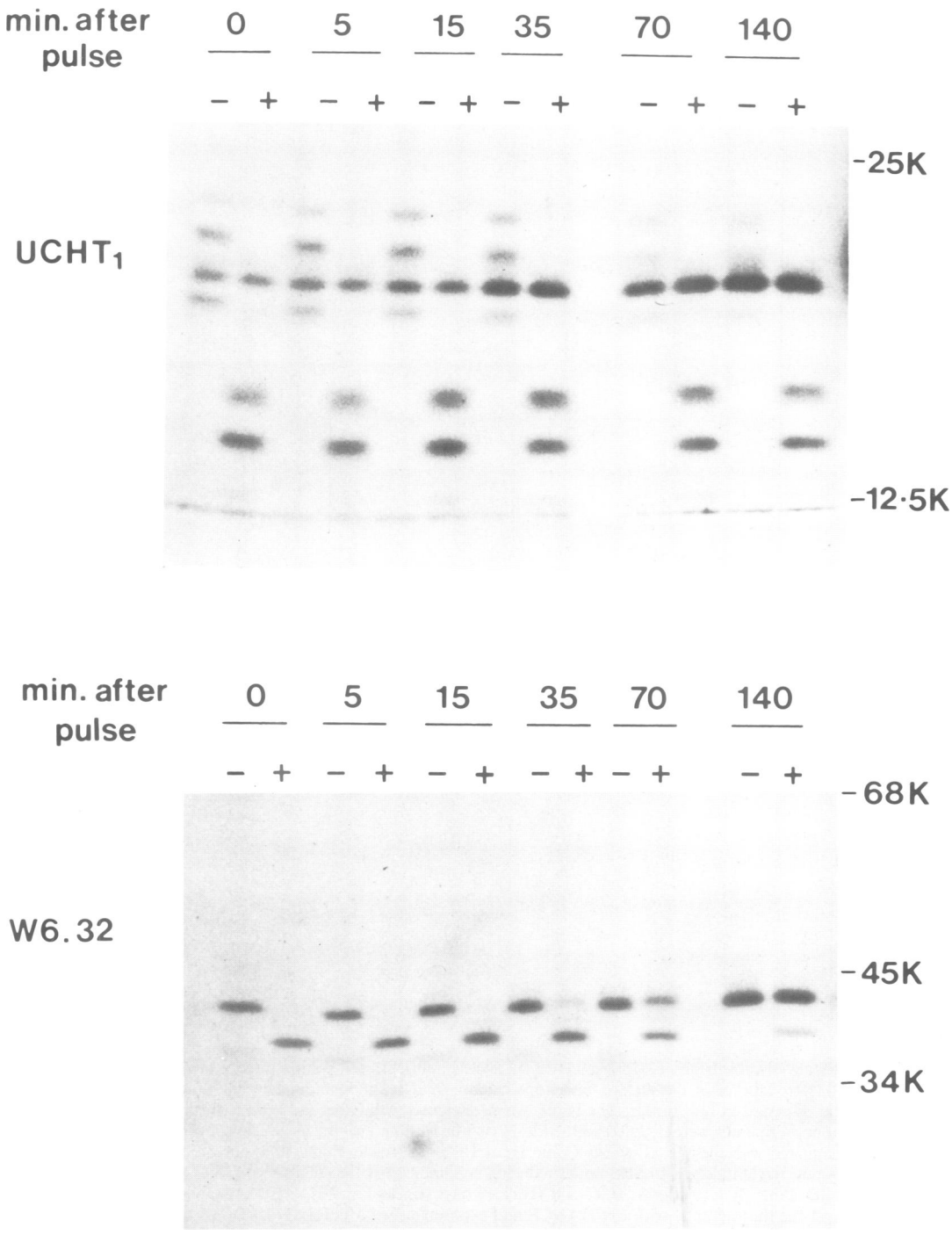

Fig. 2. Pulse-chase analysis and endo-H sensitivity of the T3 antigen. HPB-ALL cells were labelled at 37°C for 5 min with 75 μCi/ml of [^{35}S]methionine. After adding RPMI 1640 medium containing an excess of unlabelled methionine, a sample (2 x 10^7 cells) was diluted immediately in ice-cold PBS (0 min after pulse). Further samples were removed at the times indicated. Cells were pelleted at 4°C, lysed in 1 ml of lysis buffer and immunoprecipitated with either the UCH-T1 **(upper panel)** or the W6.32 **(lower panel)** monoclonal antibodies. Immunoprecipitates were digested with endo-H (+) or without enzyme (−). After precipitation with acetone, samples were analysed on 12.5% polyacrylamide gels in SDS under reducing conditions.

samples, it appears likely that this polypeptide is not N-glycosylated. Endo-H digestion resulted in the production, at each time point, of two prominent bands of 16 000 and 14 000 together with a very faint component of 12 500. The analyses also revealed a faint band of ~39 000 mol. wt. that was endo-H sensitive as well as another band of ~51 000 that was endo-H insensitive (Figure 3E, F). Neither of these higher mol. wt. bands were detected in the control immunoprecipitates (Figure 3C, D).

UCH-T1 immunoprecipitates prepared from surface-labelled HPB-ALL cells revealed the expected profile (Kanellopoulos *et al.*, 1982) of an intense compact band of 19 000, an intense more diffuse band of ~21 000 and a diffuse weak band of ~26 000 mol. wt. (Figure 3G). Endo-H treatment resulted in no apparent change in the position or intensity of the 26 000 and 19 000 bands but the middle band was deleted (Figure 3H). Two new intense bands of ~18 000 and 17 000 were generated by endo-H digestion (Figure 3H) plus two much weaker bands of ~16 000 and 14 000 that were revealed only after exposing the gel for longer periods (Figure 3J).

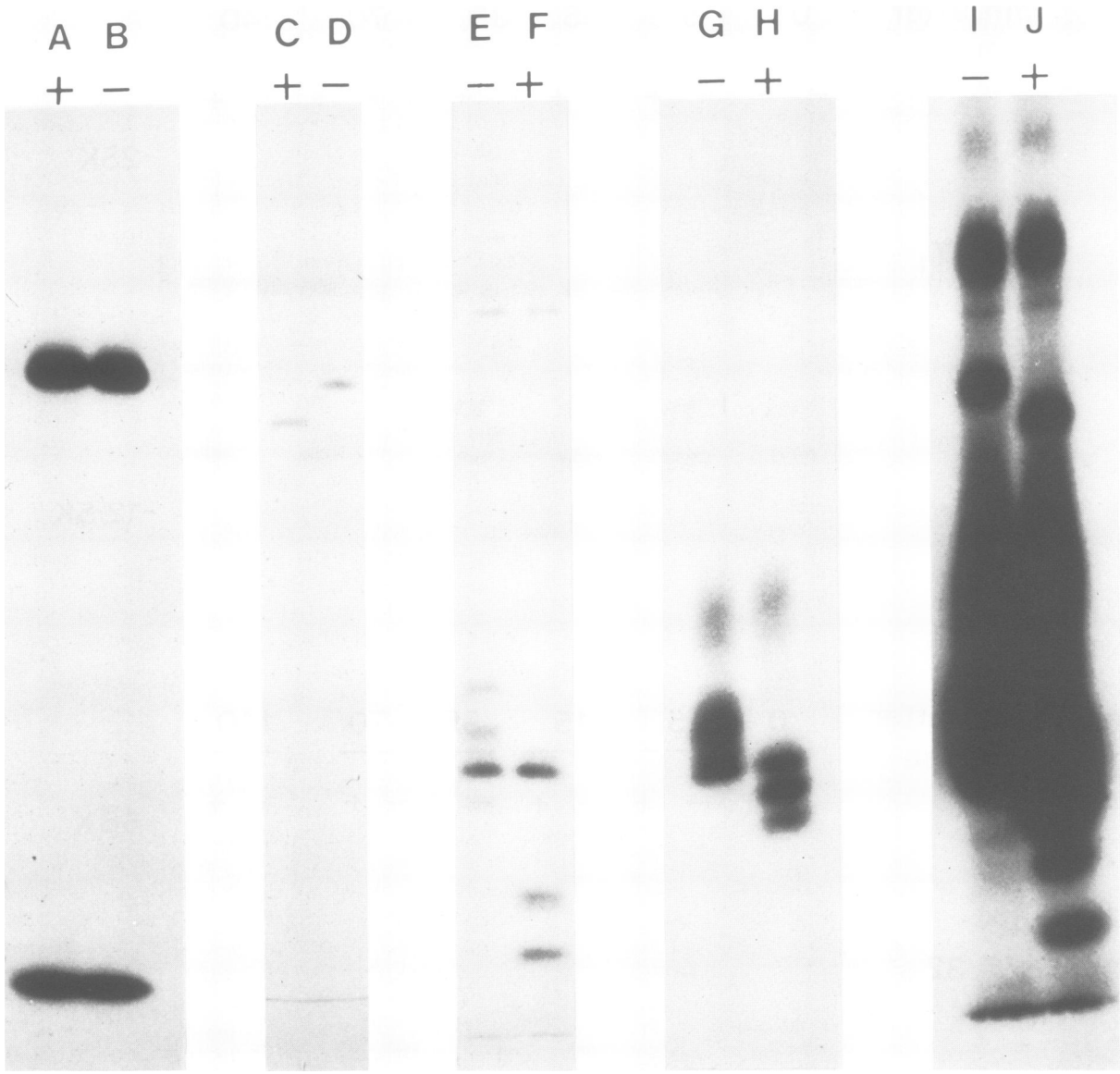

Fig. 3. Sensitivity to endo-H digestion of cell surface T3 antigen. HPB-ALL cells that had been cultured under the same conditions as those used for the pulse-chase experiment (Figure 2), were labelled at the surface by lactoperoxidase-catalysed iodination using the procedure described by Walsh and Crumpton (1977). The cells were washed and dispersed in lysis buffer. The lysate was precipitated with either the W6.32 (**tracks A** and **B**) or the UCH-T1 (**tracks G, H, I** and **J**) antibodies and the immunoprecipitates were digested with endo-H (+) or without enzyme (−). The 35 min pulse-chase samples from the experiment shown in Figure 2 were also precipitated with the W6.32 (**tracks C** and **D**) or UCH-T1 (**tracks E** and **F**) antibodies. After precipitation with acetone, the samples were analysed on 12.5% polyacrylamide gels in SDS under reducing conditions. ^{125}I-Labelled polypeptides were revealed by autoradiography at −70°C with intensifying screens for either 48 h (**tracks A, B, G** and **H**) or 19 days (**tracks I** and **J**). [^{35}S]Methionine-labelled polypeptides (**tracks C, D, E** and **F**) were revealed by treating the gel for fluorography with EN^{3}HANCE and exposing at −70°C for 19 days.

This longer exposure also revealed two minor bands of higher mol. wt.; one of ~39 000 that was endo-H sensitive and another of ~51 000 that was endo-H resistant (Figure 3I, J).

The resistance of the ^{125}I-labelled HLA-A,B,C antigen heavy chain to endo-H digestion argues strongly that the lactoperoxidase-catalysed iodination was restricted to the cell surface. In consequence, the above results indicate that the T3 antigen expressed on the surface of HPB-ALL cells comprises some polypeptides with immature (i.e., incompletely processed) N-glycan units.

Endo-F sensitivity of the cell surface T3 antigen

Endo-F cleaves N-linked glycan units of both the high mannose (i.e., immature) and complex (i.e., mature) type compared with endo-H which cleaves immature units only (Elder and Alexander, 1982). Immunoprecipitates prepared from surface-labelled HPB-ALL cells were digested with endo-F prior to SDS-PAGE analysis. Digestion of the HLA-A,B,C antigen control immunoprecipitates revealed the expected decrease of ~3000 mol. wt. in the size of the heavy chain (Figure 4C, D). It is however, apparent that the experimental conditions employed resulted in partial cleavage only. In the case of the T3 antigen, the 26 000 and 21 000 mol. wt. bands were completely digested with the appearance of two new bands of 16 000 and 14 000, whereas the 19 000 mol. wt. band appeared to be resistant to digestion (Figure 4A, B).

Collectively the results of the endo-H and endo-F digestions suggest: (i) the 19 000 mol. wt. band is not N-glycosylated; (ii) the 21 000 mol. wt. band has both high mannose

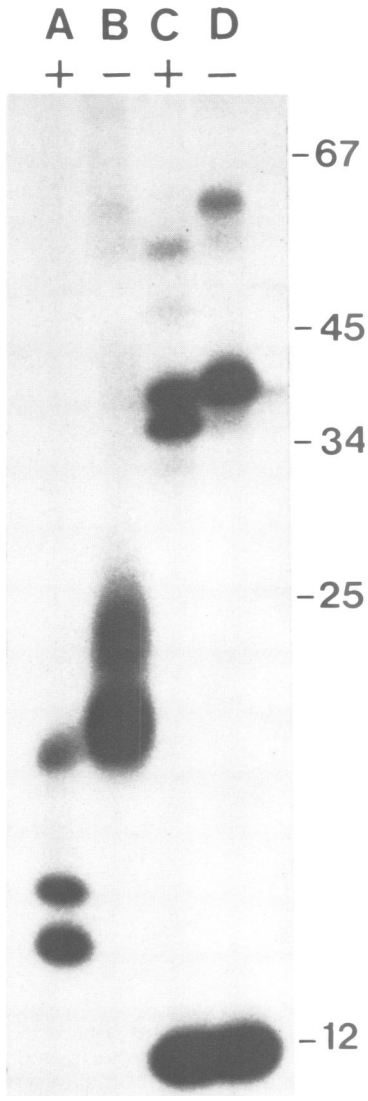

Fig. 4. Sensitivity to endo-F digestion of cell surface T3 antigen. HPB-ALL cells were labelled at the surface by lactoperoxidase-catalysed iodination and lysed in lysis buffer. W6.32 (**tracks C** and **D**) and UCH-T1 (**tracks A** and **B**) immunoprecipitates were digested with either endo-F (+) or without enzyme (−). Samples were analysed on 12.5% polyacrylamide gels run in SDS under reducing conditions.

and complex N-glycan units; (iii) the 26 000 mol. wt. band possesses complex N-glycan units only; (iv) the 21 000 as well as probably the 26 000 mol. wt. bands give rise independently to both the 14 000 and 16 000 mol. wt. bands; (v) the 14 000 and 16 000 mol. wt. bands possess neither high mannose nor complex glycan units. The 17 000 and 18 000 mol. wt. bands detected after endo-H digestion are probably related to the 14 000 and 16 000 bands by the presence of complex glycan units. This interpretation implies that the endo-H digestion was not limiting, a view that is consistent with the apparently complete digestion of the biosynthetically-labelled T3 and HLA-A,B,C antigens (Figure 2).

Transmembrane orientation of the T3 antigen

Transmembrane polypeptides can be designated on the basis that proteinase-K cleaves the cytoplasmic domains of polypeptides inserted in endoplasmic reticulum vesicles (Owen *et al.*, 1980). HPB-ALL cells were pulse labelled for 5 min with [^{35}S]methionine prior to cell breakage and separation of a microsomal fraction. Immunoprecipitates were subsequently prepared before and after digesting the microsomes with proteinase-K. A comparison of the polypeptide compositions of the control immunoprecipitates (Figure 5D, E) revealed that proteinase-K induced the expected decrease of ~4000 mol. wt. in the HLA-A,B,C heavy chains. In contrast, no decrease in size of the β_2-microglobulin was detected, a result which agrees with the ascribed location of this entire polypeptide within the lumen of the endoplasmic reticulum (Owen *et al.*, 1980).

In the case of the T3 antigen, microsomes incubated without proteinase-K gave four bands of 18 000, 19 000, 21 000 and 23 000 mol. wt. (Figure 5A; compare Figure 2, upper panel, left-hand track). Each of these four bands was apparently reduced in size by proteinase-K digestion (Figure 5B), and on this basis appears to have a transmembrane orientation. Although it is not possible to designate precursor-product relationships, it appears likely that the most intense bands are related, in which case the 19 000 mol. wt. band gives rise to one of 14 000 mol. wt. If the microsomes were permeabilised by treatment with NP-40 prior to proteinase-K digestion, then the majority of the HLA-A,B,C antigens were degraded (Figure 5F), whereas three bands of 14 000, 13 000 and 12 000 mol. wt. were still detected in the UCH-T1 immunoprecipitates (Figure 5C).

Discussion

The characteristics of the specific polypeptides detected in UCH-T1 immunoprecipitates from HPB-ALL cells are summarised in Table I. The overall results argue strongly in support of the T3 antigen comprising at least two distinct, non-covalently associated polypeptides. The primary questions concern the number of distinct polypeptides included in the UCH-T1 immunoprecipitates, whether they differ one from another in their glycosylation status and/or their amino acid sequence, whether they associate to form more than one type of molecular complex and the composition(s) of the complex(es) with respect to the number and types of chains? An important parameter is the molecular size of the T3 antigen. Preliminary results (Kanellopoulos *et al.*, in preparation) suggest that the deoxycholate-solubilised, cell surface T3 antigen is polydisperse with a mol. wt. within the range 50 000−75 000, including bound detergent.

UCH-T1 immunoprecipitates from both biosynthetically- and surface-labelled HPB-ALL cells contain a prominent polypeptide of ~19 000 mol. wt. (designated α). No decrease in size of this polypeptide (as judged by SDS-PAGE analysis) was detected by endo-H or endo-F digestion (Figures 3 and 4), by tunicamycin treatment (Figure 1) or during pulse-chase experiments (Figure 2), although the polypeptide when incorporated in microsomal vesicles was sensitive to digestion with proteinase-K (Figure 5). These results collectively suggest that this polypeptide is not N-glycosylated and that it spans the plasma membrane with a cytoplamsic domain of ~5000 daltons in size. Since transmembrane polypeptides are invariably glycosylated (Bretscher and Raff, 1975), it seems likely that this polypeptide is O-glycosylated. However, it is apparent that transmembrane insertion of a polypeptide has no obligatory requirement for glycosylation (Owen *et al.*, 1980).

The apparent increase in intensity of the ^{35}S-labelled α polypeptide during the chase period of the pulse-chase experiment (Figure 2) is not inconsistent with the above assignment.

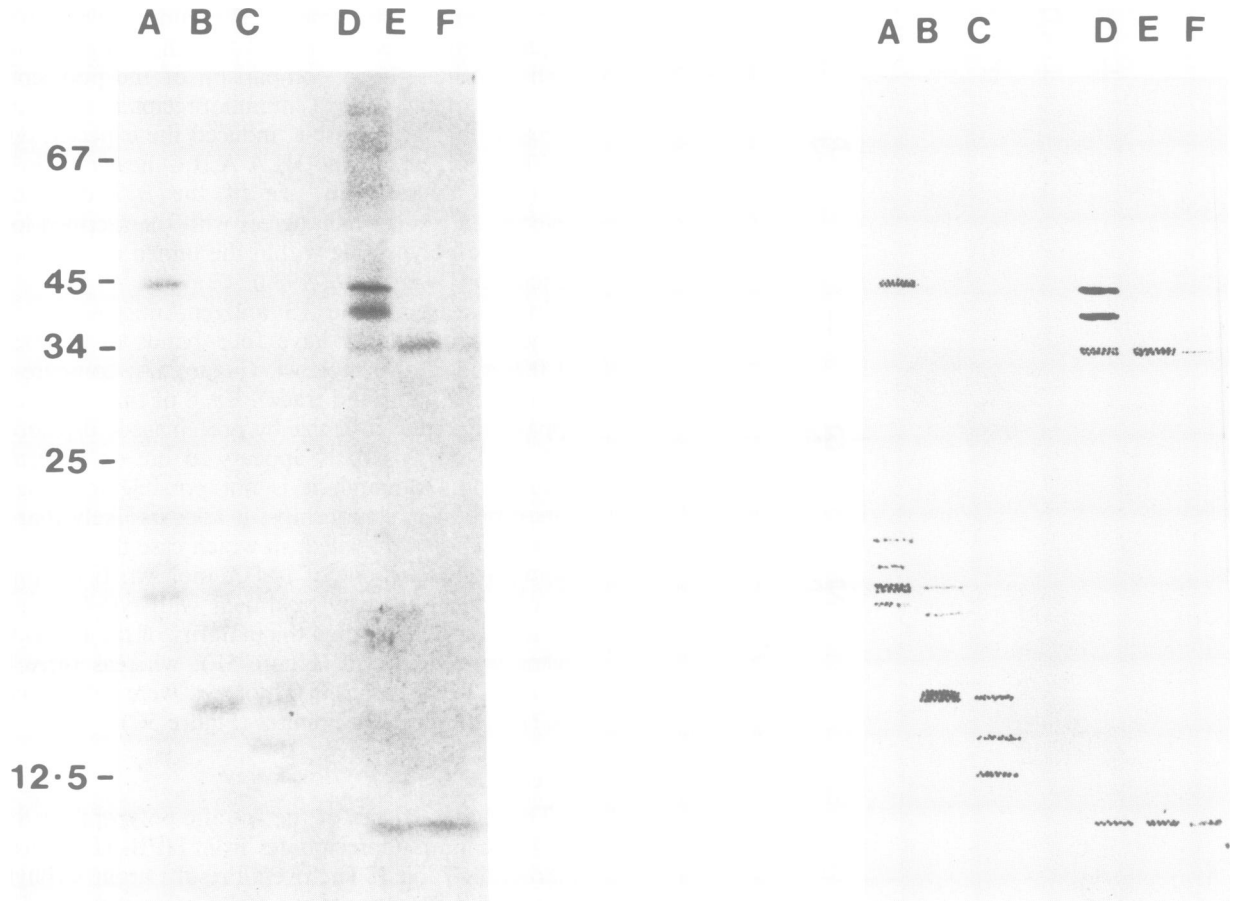

Fig. 5. Sensitivity to proteinase-K digestion of T3 antigen incorporated in microsomal vesicles. Proteinase-K digestion of microsomes prepared from [^{35}S]methionine, pulse-labelled (5 min) HPB-ALL cells, was performed as described in Materials and methods. **Tracks A** and **D** represent the control digest (i.e., no added enzyme), **tracks B** and **E** represent microsomes digested with proteinase-K and **tracks C** and **F** represent microsomes digested wiht proteinase-K in the presence of NP-40. Samples in lysis buffer were immunoprecipitated with either the W6.32 (**tracks D, E** and **F**) or the UCH-T1 (**tracks A, B** and **C**) antibodies. The immunoprecipitates were analysed on 15% polyacrylmide gels run in SDS under reducing conditions. The left-hand panel shows an autoradiograph of the gel and the right-hand panel a drawing of the autoradiograph.

Thus, this increase can be accounted for if the UCH-T1 antibody recognises the α chain only when it is associated with another polypeptide and if an excess of labelled α chains is synthesised during the pulse. Under these circumstances, labelled α chains would combine with newly synthesised, unlabelled polypeptide during the chase and, at late time points, increasing amounts of the labelled α chain would be immunoprecipitated. A similar situation has been described previously for the β_2-microglobulin component of HLA-A,B,C antigens (Krangel *et al.*, 1979; Owen *et al.*, 1980).

The 21 000 and 23 000 mol. wt. polypeptides (designated β γ, respectively) are N-glycosylated and possess a transmembrane orientation, as judged by their sensitivity to digestion by proteinase-K, and by endo-H and/or endo-F, as well as by treatment with tunicamycin. As each of these polypeptides was detected at 0 min in pulse-chase experiments (Figure 2), it appears that neither was generated from a precursor form. The γ polypeptide was not apparent in immunoprecipitates from surface-labelled cells whereas a polypeptide of ~26 000 mol. wt. was observed only using surface-labelled cells (compare Figure 3E with 3G). As the 26 000 mol. wt. polypeptide was resistant to endo-H digestion (Figure 3H) and as both biosynthetically- and surface-labelled cells gave the same pattern of polypeptides after digestion with endo-H and endo-F (Figures 3F and 4A), it appears likely that the 26 000 mol. wt. polypeptide represents a more mature glycosylated form of the β and/or γ polypeptides. In contrast to the 26 000 mol. wt. polypeptide, the cell surface form of the β polypeptide possesses both simple (i.e., high mannose) and complex oligosaccharide units (Figure 3H and 4A).

There is some confusion over the number of different non-glycosylated polypeptides that give rise to the β, γ and 26 000 mol. wt. chains. The results of the endo-H and endo-F digestions argue strongly that the latter polypeptides are derived from one or other or both of a 14 000 and a 16 000 mol. wt. polypeptide (Figures 2, 3 and 4). Further, it appears most likely that the 14 000 and 16 000 polypeptides differ in their amino acid compositions, since O-glycosylation would not be expected to have occurred as early as 0 min after a 5 min pulse (Johnson and Spear, 1983). On the other hand, after treatment with tunicamycin (an inhibitor of N-glycosylation; Tkacz and Lampen, 1975), only one non-glycosylated candidate of 16 000 mol. wt. was observed (Figure 1). The absence of the 14 000 polypeptide after tunicamycin is puzzling, although one possible explanation is that this polypeptide

Table I. Specific polypeptides comprising UCH-T1 immunoprecipitates from HPB-ALL cells

Properties	Polypeptides						
Mol. wt.[a]	51 (51–53)	39 (39–41)	26 (26–28) γ(mature?)	23 (23–24) γ	21 (21–23) β	19 (19–20) α	18[b] (18–19)
^{125}I surface labelling	±	±	++	?	+++	++	–
[^{35}S]Methionine biosynthetic labelling (4 h)	±	±	?	+	+	+++	+
Endo-H sensitive	no	yes	no	yes	yes	no	yes
Endo-F sensitive	?	?	yes	yes	yes	no	n.d.[c]
Proteinase-K sensitive	?	?	n.d.	yes	yes	yes	yes

[a]Mol. wt. x 10^{-3}. The numbers in parentheses indicate the limits of variation in mol. wt. for each band. The lower figures have been used in the text for the sake of clarification.
[b]Detected in pulse-chase experiments only (Figure 2).
[c]Not directly testable under experimental conditions used.

associates with the other chain(s) only after glycosylation. This explanation implies that the UCH-T1 antibody recognises the molecular complex, a suggestion which is consistent with the results of 'Western blot' analysis.

An 18 000 mol. wt. polypeptide was also revealed by the pulse-chase experiments (Figure 2). As this polypeptide was not observed after chase periods of >3 h nor by surface labelling, it seems likely that it was processed to a higher mol. wt. form.

Apart from the above polypeptides, UCH-T1 immunoprecipitates often, but not invariably, contained two minor polypeptides of ~39 000 and 51 000 mol. wt. The characteristics of these polypeptides are summarised in Table I. These polypeptides were never detected in the control W6.32 immunoprecipitates and, on this basis, are judged to be specific to the UCH-T1 immunoprecipitates. Their presence may reflect an intermolecular association of the T3 antigen with another cell surface glycoprotein, although the possibility of a fortuitous cross-reaction has not been ruled out. Recently, Meuer *et al.* (1983) described a clonotypic monoclonal antibody against a clone of cytotoxic T cells that induces co-modulation of the cell surface T3 antigen with the clonotypic molecules. SDS-PAGE analyses of immunoprecipitates formed with this antibody revealed two major polypeptides of similar mol. wt. to those of the minor components detected in the UCH-T1 immunoprecipitates. Thus, these results suggest that the above minor components correspond to the polypeptides comprising the clonotypic molecules (i.e., the putative T-cell antigen receptor) described by Meuer *et al.* (1983).

Materials and methods

Cells

The thymus acute lymphoblastic cell line HPB-ALL was cultured in RPMI 1640 medium containing penicillin (100 units/ml), streptomycin (50 μg/ml) and 2% (v/v) fetal calf serum (Flow Laboratories, Irvine, Ayrshire, UK). The cells were labelled either biosynthetically with [^{35}S]methionine (25–50 μCi/mol) (Owen *et al.*, 1981) or at the surface by lactoperoxidase-catalysed iodination using the procedure described by Walsh and Crumpton (1977).

Monoclonal antibodies

The mouse monoclonal antibody UCH-T1 (an IgG_1) was described by Beverley and Callard (1981). The hybridoma was grown in mice and the ascitic fluid was precipitated with 45% saturated ammonium sulphate. The precipitated antibody was further purified using Protein A-Sepharose (Pharmacia Fine Chemicals) as described by Ey *et al.* (1978).

W6.32 (an IgG_{2a}) is a mouse monoclonal antibody which recognises a common epitope expressed by all HLA-A,B,C antigens, provided the heavy (i.e., 43 000 mol. wt.) chains are associated with β_2-microglobulin (Barnstable *et al.*, 1978). It was used either as ascitic fluid or as purified immunoglobulin prepared using Protein A-Sepharose.

Immunoprecipitation

Immunoprecipitation was carried out using NP-40 (BDH Chemicals Ltd., Poole, UK) lysates of labelled HPB-ALL cells as previously described (Owen *et al.*, 1980). The lysate (250 μl) was incubated at 4°C for 1–3 h with the monoclonal antibody (10 μg of purified IgG or 4 μl of ascitic fluid) prior to precipitation of the immune complexes by adding 100 μg of affinity purified rabbit anti-(mouse immunoglobulin) antibodies and incubating overnight at 4°C. Immunoprecipitates were centrifuged for 5 min at 4°C in an Eppendorf centrifuge and were washed sequentially as previously described (Owen *et al.*, 1980).

Tunicamycin treatment

HPB-ALL cells were washed twice in phosphate buffered saline (PBS; 0.15 M sodium chloride, 10 mM sodium phosphate buffer, pH 7.4) and were resuspended at 1 x 10^6 cells/ml in methionine-free RPMI 1640 medium containing 2% dialysed fetal calf serum. The cell culture was divided into two samples, one of which acted as the control whereas the other was treated with 3 μg of tunicamycin/ml (a kind gift from R. Hamill of Eli Lilly Co.). After 2 h at 37°C, 10 μCi/ml of [^{35}S]methionine was added to both samples and incorporation was allowed to proceed for 4 h. After two washes with medium, the cells were counted and were then lysed at 2 x 10^7 cells/ml in lysis buffer (10 mM Tris/HCl buffer, pH 7.4, containing 1% (w/v) NP-40, 0.15 M NaCl, 1 mM EDTA, 1 mg/ml bovine serum albumin (BSA), 2 mM phenylmethylsulphonyl fluoride (PMSF), 20 mM iodoacetamide, and 1 international unit of aprotinin/ml).

Pulse-chase labelling experiments

These were performed as previously described (Owen *et al.*, 1980). HPB-ALL cells were washed twice in PBS and resuspended at 5 x 10^6 cells/ml in methionine-free RPMI 1640 medium containing 2% dialysed fetal calf serum. They were incubated at 37°C for 1 h before adding [^{35}S]methionine (75 μCi/ml). After 5 min at 37°C, the cell suspension was diluted with 4 volumes of a prewarmed RPMI 1640 medium containing 10% (v/v) fetal calf serum and 2.5 mM methionine. Samples (~2 x 10^7 cells) were removed at various times and diluted into 4 volumes of ice-cold phosphate buffered saline (PBS). At each time point, the cells were centrifuged at 4°C and dispersed immediately in lysis buffer. Each lysate was then divided into two equal parts (~1 x 10^7 cell equivalents) which were used for immunoprecipitations with the UCH-T1 or W6.32 monoclonal antibodies.

Enzyme digestions

Digestion of immunoprecipitates by endo-β-N-acetylglucosaminidase-H (endo-H; purchased from F. Maley, State of New York Department of Health, Albany, NY) was performed as described by Owen *et al.* (1980).

Immunoprecipitates from HPB-ALL cells that had been labelled at the surface by lactoperoxidase-catalysed iodination were also digested with endo-β-N-acetylglucosaminidase-F (endo-F; Elder and Alexander, 1982). A solution of endo-F was kindly donated by S. Alexander, Department of Immuno-

pathology and the Committee for the Study of Molecular Genetics, Research Institute of Scripps Clinic, La Jolla, CA. The immunoprecipitates were solubilised by boiling for 4 min in 10 μl of 100 mM Tris-HCl buffer, pH 7.4, containing 1% SDS and 1% β-mercaptoethanol, and diluted by adding 90 μl of 100 mM sodium phosphate buffer, pH 6.1, containing 50 mM EDTA, 1% NP-40 and 1% β-mercaptoethanol prior to adding 5 μl of endo-F solution. The mixture was incubated at 37°C overnight, was reincubated for a further 2 h with a second dose (5 μl) of enzyme and was subsequently precipitated with 10% trichloroacetic acid and washed with acetone as described for the endo-H digestion.

Proteinase-K digestion of labelled microsomes was performed as described by Owen *et al.* (1980). HPB-ALL cells were washed twice in methionine-free medium RPMI 1640 and resuspended at 5 x 10^6 cells/ml in the same medium containing 2% dialysed fetal calf serum. They were incubated for 1 h at 37°C, before pulsing for 5 min with 100 μCi/ml of [^{35}S]methionine. After adding 5 volumes of ice-cold RPMI 1640 medium, the cells were sedimented by centrifugation, resuspended in cold PBS and broken using a disrupting pump. The microsomal fraction, prepared as previously described (Crumpton and Snary, 1974), was resuspended in 60 μl of 10 mM Hepes buffer, pH 7.4, and divided into three samples. The first sample was incubated with 3 μl of proteinase-K (10 mg/ml), the second was incubated with 3 μl of proteinase-K in the presence of NP-40 which permeabilises (disrupts) the microsomal vesicles and the third sample was incubated without enzyme. Digestion was stopped by adding 5 μl of BSA (10 mg/ml) and 4 μl of PMSF (1 mg/ml) and the samples were dispersed in lysis buffer prior to immunoprecipitation.

SDS-PAGE analysis

Polyacrylamide gel electrophoresis was performed using 12% and 15% (w/v) acrylamide slab gels (overall length, 20 cm), a 5% (w/v) stacking gel and the SDS buffer of Maizel and Laemmli as described by Ames (1974). The gels were run, fixed and dried as previously described (Owen *et al.*, 1981). Mol. wts. were calculated by reference to the mobilities of the following standard proteins (subunit mol. wts. in parenthesis): BSA (67 000), ovalbumin (45 000), glyceraldehyde-3-phosphate dehydrogenase (34 000), immunoglobulin light chain (25 000), cytochrome c (12 500). ^{125}I-Labelled protein bands were detected by autoradiography using Kodak X-Omat H-X ray film with Dupont intensifying screens (Cronex Lighting Plus). Gels containing ^{35}S were treated with EN^{3}HANCE (New England Nuclear) before autoradiography using X-Omat H or SB5 film.

Acknowledgements

We wish to express our gratitude to Drs. P.C.L. Beverley and E. Zanders for stimulating discussions and for the UCH-T1 monoclonal antibody.

References

Ames,G.F.L. (1974) *J. Biol. Chem.*, **249**, 634-644.
Barnstable,C.J., Bodmer,W.F., Brown,G., Galfre,G., Milstein,C., Williams,A.F. and Ziegler,A. (1978) *Cell*, **14**, 9-20.
Beverley,P.C.L. and Callard,R.E. (1981) *Eur. J. Immunol.*, **11**, 329-334.
Biddison,W.E., Rao,P.E., Talle,M.A., Goldstone,G. and Shaw,S. (1982) *J. Exp. Med.*, **156**, 1065-1076.
Borst,J., Prendiville,M.A. and Terhorst,C. (1982) *J. Immunol.*, **128**, 1560-1565.
Bretscher,M.S. and Raff,M.C. (1975) *Nature*, **258**, 43-49.
Burns,G.F., Boyd,A.W. and Beverley,P.C.L. (1982) *J. Immunol.*, **129**, 1451-1457.
Chang,T.W., Kung,P.C., Gingras,S.P. and Goldstein,G. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 1805-1808.
Crumpton,M.J. and Snary,D. (1974) *Contemp. Top. Mol. Immunol.*, **3**, 27-56.
Elder,J.H. and Alexander,S. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4540-4544.
Ey,P.L., Prowse,S.J. and Jenkin,C.R. (1978) *Immunochemistry*, **15**, 429-436.
Johnson,D.C. and Spear,P.G. (1983) *Cell*, **32**, 987-997.
Kanellopoulos,J.M., Beverley,P.C.L., Zanders,E. and Crumpton,M.J. (1982) *Biochem. Soc. Trans.*, **10**, 101-102.
Krangel,M.S., Orr,H.T. and Strominger,J.L. (1979) *Cell*, **18**, 979-991.
Kung,P.C., Goldstein,G., Reinherz,E.L. and Schlossman,S.F. (1979) *Science (Wash.)*, **206**, 347-349.
Ledbetter,J.A., Evans,R.L., Lipinski,M., Cunningham-Rundles,C., Good, R.A. and Herzenberg,L.A. (1981) *J. Exp. Med.*, **153**, 310-323.
Meuer,S.C., Hussey,R.E., Hodgdon,J.C., Hercend,T., Schlossman,S.F. and Reinherz,E.L. (1982) *Science (Wash.)*, **218**, 417-473.
Meuer,S.C., Fitzgerald,K.A., Hussey,R.E., Hodgdon,J.C., Schlossman,S.F. and Reinherz,E.L. (1983) *J. Exp. Med.*, **157**, 705-719.
Owen,M.J., Kissonerghis,A.M. and Lodish,H.F. (1980) *J. Biol. Chem.*, **255**, 9678-9684.
Owen,M.J., Kissonerghis,M.A., Lodish,H.F. and Crumpton,M.J. (1981) *J. Biol. Chem.*, **256**, 8987-8993.
Reinherz,E.L., Hussey,R.E. and Schlossman,S.F. (1980) *Eur. J. Immunol.*, **10**, 758-762.
Reinherz,E.L., Meuer,S.C. and Schlossman,S.F. (1983) *Immunol. Today*, **4**, 5-8.
Tkacz,J.S. and Lampen,J.O. (1975) *Biochem. Biophys. Res. Commun.*, **65**, 248-257.
Van Agathoven,A., Terhorst,C., Reinherz,E. and Schlossman,S. (1981) *Eur. J. Immunol.*, **11**, 18-21.
Van Wauwe,J.P.V., De Mey,J.R. and Goossens,J.G. (1980) *J. Immunol.*, **124**, 2708-2713.
Walsh,F.S. and Crumpton,M.J. (1977) *Nature*, **269**, 307-311.

Note added in proof

Since the work described in this paper was completed and submitted for publication, similar results have been published.

Borst,J., Alexander,S., Elder,J. and Terhorst,C. (1983) *J.Biol. Chem.*, **258**, 5135-5141.
Borst,J., Prendiville,M.A. and Terhorst,C. (1983) *Eur. J. Immunol.*, **13**, 576-581.