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Phaseolus vulgaris phytohaemagglutinin (PHA) binds to the human T lymphocyte antigen receptor

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The interaction of phytohaemagglutinin (PHA) with the human T lymphocyte antigen receptor (Ti) was explored. Nonidet-P40 lysates of surface-labelled HPB-ALL cells were immunoprecipitated with PHA, using a rabbit anti-(PHA)-serum, as well as clonotypic monoclonal antibodies (H1-2D4 and T40/25) and a rabbit antiserum (R-43) against Ti. One- and two-dimensional SDS-polyacrylamide electrophoresis under reducing and non-reducing conditions showed that both the clonotypic antibodies and PHA precipitated a disulphide cross-linked heterodimer having a mol. wt. of ~79 000 (unreduced) and a comprising subunits of mol. wts. ~50 000 and 39 000 (reduced). Further evidence that PHA binds Ti was obtained by (i) cross-immunodepletion with H1-2D4 and PHA; (ii) immunoprecipitation with H1-2D4 of a glycoprotein fraction specifically eluted from a PHA immunoprecipitate; (iii) immunoprecipitation with PHA of a solubilised H1-2D4 immunoprecipitate; (iv) 2-D (non-equilibrium pH gradient electrophoresis/SDS) analyses of H1-2D4 and PHA immunoprecipitates, indicated that H1-2D4 and PHA recognise coincident β polypeptides. PHA also binds a Ti-like disulphide cross-linked heterodimer on tonsil lymphocytes and two other T-cell leukaemias (HUT-78 and J6). The data further suggest that PHA and R-43 recognise a subpopulation of Ti molecules on HPB-ALL cells that are not bound by H1-2D4, suggesting that there may be at least two forms of Ti. Similar experiments indicate that Concanavalin A (Con A) and wheat germ agglutinin (WGA) also probably bind Ti, whereas *Helix pomatia* agglutinin (HPA) does not.

Key words: T cell antigen receptor/PHA/mitogenesis/lymphocyte surface

Introduction

T-lymphocyte activation by plant lectins, especially leucoagglutinating phytohaemagglutinin (PHA) and Concanavalin A (Con A), has been used as a model system for studying the biochemical pathways by which: (i) T-lymphocytes respond to (are activated by) antigen, and (ii) cells in general are initiated to grow. While the molecular basis of the initiating event is not known, it is generally assumed that lectin-induced mitogenesis involves interaction with one or more cell-surface molecules (Owen and Crumpton, 1981).

It was suggested almost a decade ago that in addition to an antigen-binding site, the T-cell antigen receptor is glycosylated and that potent mitogenic lectins, such as PHA and Con A, activate quiescent lymphocytes by binding to the carbohydrate moiety (Greaves, 1975). This model for lectin-induced T-cell activation is analogous to what is known about receptor-mediated activation of specific biochemical pathways in certain other systems. That is, antibodies directed against the cell-surface receptors for insulin (Kahn *et al.*, 1978), and thyroid stimulating hormone (Smith, 1981) can mimic the effects of the natural ligands. Testing of this hypothesis is complicated by the fact that both non-mitogenic and mitogenic lectins recognize multiple cell surface glycoproteins, the majority of which are completely uncharacterised, except at the level of one-dimensional SDS-polyacrylamide gel electrophoresis (1-D SDS-PAGE). Several of these glycoproteins are bound by both classes of lectins. Specificity with respect to mitogenicity is, however, indicated by the fact that some T-cell glycoproteins seem to be uniquely bound by mitogenic lectins (Dillner-Centerlind *et al.*, 1980) and the observation that not all lectins which bind to T-cells are mitogenic (Owen and Crumpton, 1981).

Several laboratories have described clonotypic monoclonal antibodies directed against human T-cell lines that bind to a cell surface molecule having the characteristics of the antigen receptor (Kappler *et al.*, 1983; Meuer *et al.*, 1983a; Boylston *et al.*, 1984). SDS-PAGE analysis, under non-reducing and reducing conditions, of immunoprecipitates prepared using these antibodies from lysates of cells either surface labelled with ^{125}I or biosynthetically labelled with [^{35}S]methionine and/or [^{35}S]cysteine have shown that the receptor is a disulphide cross-linked heterodimer of mol. wt. ~90 000, consisting of subunits of mol. wts. ~50 000 and 40 000 (designated α and β, respectively) (Kappler *et al.*, 1983; Meuer *et al.*, 1983a). The data further indicated that the antigen receptor on T-lymphocytes is glycosylated (Acuto *et al.*, 1983). Thus, one aspect of the model proposed by Greaves (1975) has been confirmed.

Inasmuch as monoclonal antibodies directed against the T3 (Van Wauwe *et al.*, 1980; Chang *et al.*, 1981; Burns *et al.*, 1982) and T11 antigens of human T cells (Meuer *et al.*, 1984), as well as, when linked to Sepharose, the T-cell antigen receptor (Ti) (Meuer *et al.*, 1983b) are mitogenic, the working hypothesis adopted for these studies is that these cell-surface antigens are likely to be amongst the molecules which interact with PHA, Con A, and other potent mitogenic lectins. A possible corollary to this hypothesis is that these antigens may not be recognised by non-mitogenic lectins. Although it has been proposed that PHA interacts with T3 (Palacios, 1982; Kammer *et al.*, 1984), a view which is supported by co-capping data, no biochemical evidence for direct binding of PHA to T3 has been reported. Suggestive, but inconclusive, evidence that PHA and Con A can bind the antigen receptor has, however, been obtained (Kanellopoulos *et al.*, in preparation). Thus, glycoproteins having mobilities corresponding to the approximate mol. wts. of the subunits of Ti were among the bands observed when PHA and Con A immunoprecipitates from HPB-ALL cell and peripheral blood

lymphocyte lysates were analysed by 1-D SDS-PAGE under reducing conditions. The studies described here provide strong evidence that PHA binds to the T-cell antigen receptor on HPB-ALL cells, tonsil lymphocytes, and probably HUT-78 and J6 cells. The results further suggest that there are at least two subpopulations of the antigen receptor on the surface of HPB-ALL cells. Evidence suggesting that Con A binds to the antigen receptor on HPB-ALL cells was also obtained. Although not explored in detail, it appears that wheat germ agglutinin (WGA) binds to Ti, while *Helix pomatia* agglutinin (HPA), which is not mitogenic for T-cells (Dillner-Centerlind *et al.*, 1980) does not.

For brevity, the terms 'PHA, Con A, WGA and HPA immunoprecipitates' are used to designate the molecules precipitated by these lectins with antibodies against the respective lectin.

Results

PHA and antibodies against Ti immunoprecipitate polypeptides having the same mol. wt.

In preliminary experiments PHA immunoprecipitates from tonsil lymphocytes and T-leukaemia cell lines (HPB-ALL, HUT-78, and J6) which had been labelled at the surface by lactoperoxidase-catalyzed iodination were analysed by 1-D SDS-PAGE under reducing conditions. All samples showed 10 or more labelled bands including several which migrated in the 35 000–55 000 mol. wt. range. As a result it is possible that PHA binds the T-cell antigen receptor, as well as other T-cell surface glycoproteins of similar mol. wts. such as T11 (51 000 mol. wt.; Meuer *et al.*, 1984), although the patterns were too complex for unambiguous interpretation. However, polypeptides corresponding to the subunits of the T3 were not observed (data not shown). Because of the availability of HPB-ALL cell-specific monoclonal antibodies (H1-2D4 and T40/25) as well as rabbit polyclonal antibodies (R-43) against the antigen receptor, we focused initially on the possible interaction between PHA and Ti on HPB-ALL cells. One-dimensional SDS-PAGE analyses under reducing conditions of immunoprecipitates prepared using the antibodies R-43, H1-2D4 and T40/25 as well as the lectins PHA, Con A and WGA, revealed in each case polypeptides which migrated at 49 000 (α) and 37 000 (β) mol. wts. The β polypeptide immunoprecipitated by H1-2D4 was more intense and compact than the α band, whereas the relative intensities of these polypeptides were reversed with R-43 and the lectins. Also, the band precipitated by PHA, Con A and WGA in the position of the α polypeptide was more diffuse than that with the monoclonal antibodies, suggesting that the lectins (and perhaps R-43) bind to a more variably glycosylated form. In contrast, no such polypeptides were detected with the lectin HPA. Although Ouchterlony analysis showed that the anti-(HPA) and anti-(WGA) sera were equally effective in precipitating their respective antigens, it is clear that the HPA immunoprecipitate contained insignificant amounts of ^{125}I-labelled polypeptides. Under non-reducing conditions, a band migrating at 79 000 mol. wt. was observed with T40/25, H1-2D4 and PHA immunoprecipitates. This polypeptide was not visualised under reducing conditions; on the other hand, the 49 000 and 37 000 mol. wt. species were observed on reduction only.

Since the T-cell antigen receptor is a disulphide cross-linked heterodimer, 2-D (non-reducing/reduced) SDS-PAGE allowed a greater degree of discrimination with respect to the presence of putative receptor subunits among the immunoprecipitated polypeptides (Figure 1). In each case heterodimers comprising subunits of 50 000 and 39 000 mol. wt., characteristic of the α and β subunits respectively, were observed. The putative α polypeptides precipitated by the lectins PHA, Con A and WGA (Figure 1B,C,D) were more intensely labelled than the β polypeptides, whilst the relative intensities of these spots were reversed in the H1-2D4 immunoprecipitate (Figure 1A). A third diffuse spot of mol. wt. 45 000 and of variable intensity was routinely detected with the lectin immunoprecipitates (Figure 1B,C,D); the origin of this polypeptide is not known.

PHA immunoprecipitates a disulphide cross-linked heterodimer from tonsil lymphocytes

In as much as HPB-ALL cells grow continuously in culture, it was important to demonstrate that Ti-like molecules are also immunoprecipitated by PHA from lysates of quiescent lymphocytes, for which PHA is mitogenic. Figure 2 shows that PHA precipitated a cross-linked heterodimer consisting of subunits of mol. wts. 51 000 (α) and 40 000 (β) from tonsil lymphocytes; the relative intensities of the α and β polypeptides were also similar to those noted previously for HPB-ALL cell lysates. A third polypeptide (γ), having a mol. wt. of ~35 000 was also detected. This may represent a non-receptor related dimer such as the T8 antigen which comprises subunits of mol. wts. 32 000 and 30 000 or degraded Ti.

H1-2D4 and PHA immunoprecipitate common polypeptides

The similarities between the polypeptides precipitated by PHA and H1-2D4 was explored further by cross-immunodepletion experiments. Treatment of aliquots of HPB-ALL cell lysate with a monoclonal antibody against class I antigens (W6.32) did not markedly reduce the amount of the 50 000 and 39 000 mol. wt. polypeptides precipitated by H1-2D4 and PHA. Although pre-absorption with H1-2D4 depleted most of the 39 000 mol. wt. polypeptide that was precipitated by PHA, a significant fraction of the 50 000 mol. wt. polypeptide remained. Despite the sharp reduction in the amount of radioactivity in the β polypeptide position, 2-D (non-reduced/reduced) SDS-PAGE analysis of a parallel PHA precipitate obtained after depletion with H1-2D4 showed that a cross-linked heterodimer was still precipitated by PHA. On the other hand, pre-absorption with PHA removed the majority of the 50 000 and 39 000 mol. wt. polypeptides that were precipitated by PHA, but Ti was still precipitated on subsequent addition of H1-2D4, although in markedly decreased amount.

More definitive evidence that PHA and H1-2D4 bind a common molecule was obtained by re-precipitating solubilised (dissociated) H1-2D4 and PHA immunoprecipitates with PHA and H1-2D4 respectively. PHA immunoprecipitates were incubated with lysis buffer containing either N-acetylgalactosamine, or glucose as a non-specific eluant, and samples of the eluants were immunoprecipitated twice with H1-2D4. Approximately 70% of the radioactivity was eluted by N-acetylgalactosamine compared with 37% by glucose or lysis buffer alone. As shown in Figure 3, the putative Ti subunits were still strongly represented in the *Staphylococcus aureus* pellet after extraction with glucose (lane 7) whereas they were significantly depleted by elution with N-acetylgalactosamine (lane 1). Furthermore, H1-2D4 precipitated α and β polypeptides from the N-acetylgalactosamine eluate (lane 3), but not from the glucose eluate (lane 6), whereas a control an-

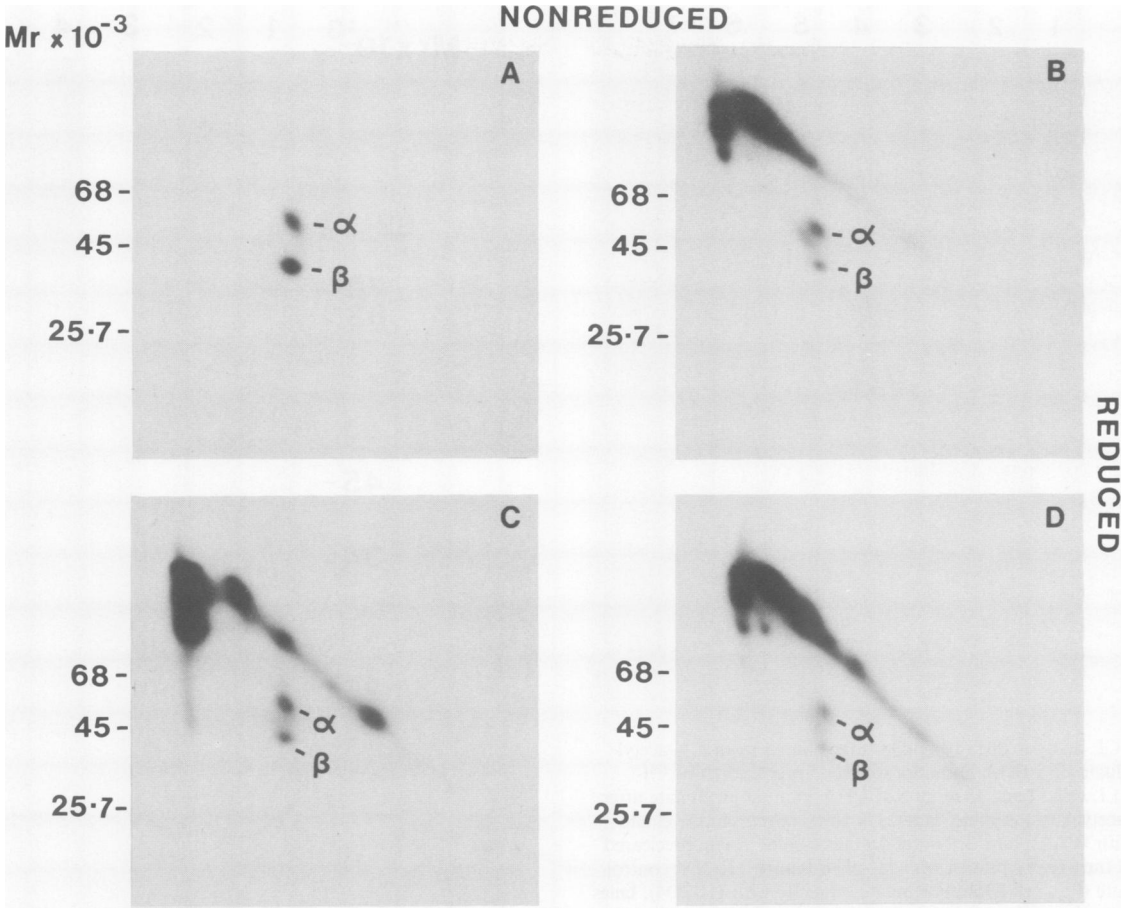

Fig. 1. 2-D (non-reduced/reduced) SDS-PAGE analysis of polypeptides precipitated by Ti antibodies and various lectins from HPB-ALL cells. Lysate of surface-labelled cells was precipitated with monoclonal Ti antibody H1-2D4 **(panel A)**, PHA **(panel B)**, Con A **(panel C)** and WGA **(panel D)**. The precipitates were analysed under reducing conditions in the first dimension and non-reducing conditions in the second dimension.

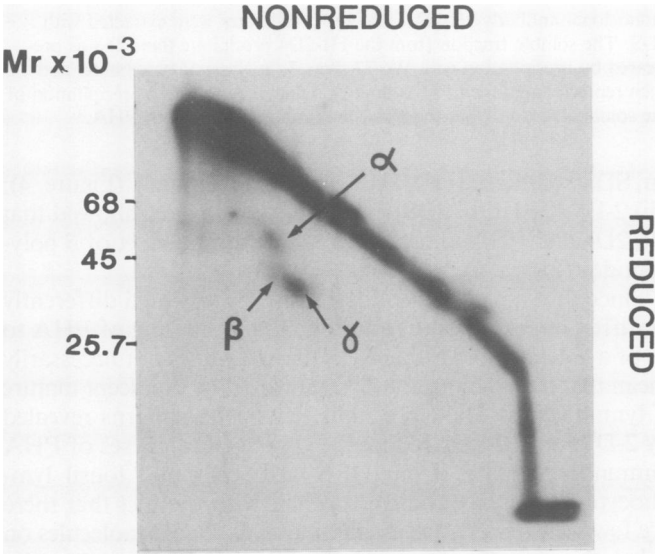

Fig. 2. 2-D (non-reduced/reduced) SDS-PAGE analysis of polypeptides precipitated by PHA from human tonsil lymphocytes. Microsomes prepared from tonsils were labelled by lactoperoxidase-catalysed iodination, solubilised in lysis buffer, and the pre-cleared lysate was immunoprecipitated with PHA. The precipitate was analysed under non-reducing conditions in the first dimension (10% acrylamide) and under reducing conditions in the second dimension (12.5% acrylamide).

tibody (W6.32) added prior to H1-2D4 did not immunoprecipitate Ti from the glucose eluate (lane 5), although faint bands were observed with the N-acetylgalactosamine eluate (lane 2).

In the reciprocal experiment, an H1-2D4 immunoprecipitate which had been solubilised in SDS was re-precipitated with PHA (Figure 4). SDS-PAGE analysis of the resulting PHA immunoprecipitate (lane 2) revealed bands of similar mobilities and intensities to those of the α and β polypeptides of the original H1-2D4 immunoprecipitate (lane 1). A control antibody (W6.32), which was added to the solubilised H1-2D4 immunoprecipitate prior to PHA, gave faint bands only in the positions of the α and β polypeptides (lane 3). The effect of solubilisation on reprecipitation is represented in lanes 5 and 4 which show the compositions of PHA immunoprecipitates before and after solubilisation and subsequent reprecipitation; in the latter instance, the 50 000 mol. wt. polypeptide was more strongly represented.

2-D(NEPHGE/SDS) PAGE analysis of immunoprecipitates

The subunits of Ti differ significantly in their isoelectric point; thus, the α polypeptide is acidic whereas the β polypeptide has a pI near neutrality (Kappler *et al.*, 1983). Additional evidence that H1-2D4 and PHA bind common molecules was obtained by 2-D(NEPHGE/SDS) PAGE analyses of their respective immunoprecipitates. As shown in Figure 5, both H1-2D4 and PHA clearly immunoprecipitate a coincident

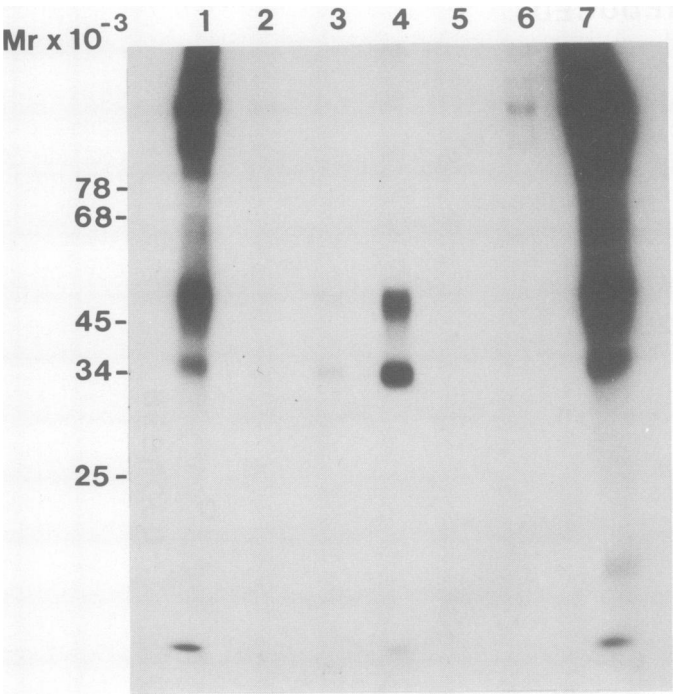

Fig. 3. SDS-PAGE analysis of polypeptides precipitated from a N-acetylgalactosamine eluate of a PHA immunoprecipitate prepared from ^{125}I-labelled HPB-ALL cells. **Lane 1:** residue of PHA immunoprecipitate after elution with N-acetylgalactosamine; **lane 2,** N-acetylgalactosamine eluate reprecipitated with W6.32; **lane 3,** N-acetylgalactosamine eluate precleared with W6.32 and then reprecipitated with H1-2D4; **lane 4,** H1-2D4 control immunoprecipitate (i.e., cell lysate precipitated directly with H1-2D4); **lanes 5** and **6** were the same as those of **lanes 2** and **3**, respectively, except that the eluate of the PHA immunoprecipitate was prepared using glucose instead of N-acetylgalactosamine; **lane 7,** residue of PHA immunoprecipitate after elution with glucose.

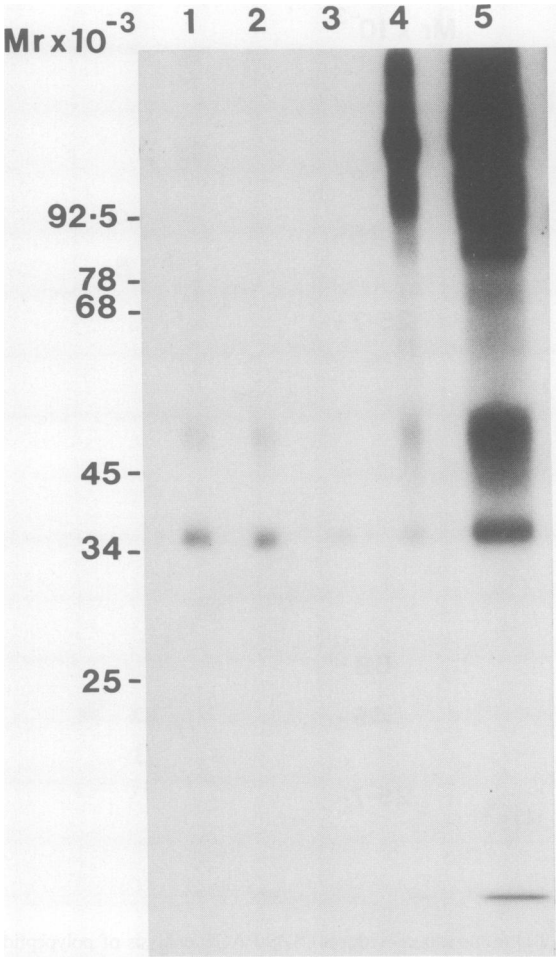

Fig. 4. SDS-PAGE analysis of polypeptides precipitated by PHA from the SDS soluble fraction of a H1-2D4 immunoprecipitate. Lysate of ^{125}I-labelled HPB-ALL cells was precipitated with PHA **(lane 5)** or the monoclonal antibody H1-2D4, and the precipitates were extracted with SDS. The soluble fraction from the H1-2D4 precipitate **(lane 1)** was precleared by precipitation with W6.32 **(lane 3,** analysis of precipitate) and then reprecipitated with PHA **(lane 2). Lane 4** shows the reprecipitation of the soluble fraction from the PHA immunoprecipitate with PHA.

family of β polypeptides having an average pI of ~6.5. The α polypeptide precipitated by H1-2D4 was located at the acidic edge of the pH gradient; in this respect it behaves similarly to that observed by Acuto *et al.* (1983). A corresponding polypeptide was not delineated in PHA immunoprecipitates; conceivably it has a more acidic pI and was lost. Similar results were obtained with R-43 and with Con A, although in the latter case the patterns were somewhat more complex due to the presence of class I antigens and higher mol. wt. polypeptides. However, the band corresponding to the β subunit of Ti was clearly present in all instances.

Discussion

The results presented here provide strong evidence that PHA binds to the antigen receptor on HPB-ALL cells. In summary, monoclonal antibodies against Ti of these cells (T40/25, Kappler *et al.*, 1983; H1-2D4, Boylston *et al.*, 1984) and PHA both immunoprecipitated a disulphide cross-linked heterodimer having an apparent mol. wt. of ~80 000 when unreduced, and comprising subunits of ~50 000 and 39 000 mol. wt. (Figure 1A,B). Polypeptides of the same apparent mol. wt. were also observed by using a polyclonal rabbit antiserum against Ti of HPB-ALL cells. Cross-immunodepletion experiments showed that PHA and H1-2D4 recognise at least a portion of the same polypeptides. Further support for this hypothesis was obtained by: (i) immunoprecipitation of Ti by H1-2D4 from the glycoprotein fraction eluted from PHA immunoprecipitates with N-acetylgalactosamine (Figure 3); (ii) immunoprecipitation of a Ti-like molecule by PHA from an SDS-solubilised H1-2D4 immunoprecipitate (Figure 4); (iii) 2-D(NEPHGE/SDS) PAGE analyses which showed that H1-2D4 and PHA immunoprecipitate a similar set of β polypeptides (Figure 5).

Since the same polypeptide can be glycosylated differently in different cell types (Crumpton, 1982), binding of PHA to Ti of a T-leukaemia cell line (HPB-ALL) does not necessarily mean that it also binds the Ti's expressed by quiescent mature T lymphocytes. The close similarity in the patterns revealed by 2-D (non-reduced/reduced) SDS-PAGE analyses of PHA immunoprecipitates from HPB-ALL cells and tonsil lymphocytes (Figures 1 and 2), together with the fact that there are few well-represented disulphide cross-linked molecules on T-lymphocytes (Goding and Harris, 1981) argues strongly, however, that PHA also binds to the antigen receptor(s) on circulating T cells.

Demonstration that PHA binds to the T cell antigen receptor is consistent with the hypothesis that activation of quiescent lymphocytes by PHA is mediated by direct binding to, and perhaps cross-linking of, the antigen receptor. It does not, of course, indicate that PHA necessarily stimulates growth *via* Ti, or exclusively *via* Ti. Obviously PHA binds a

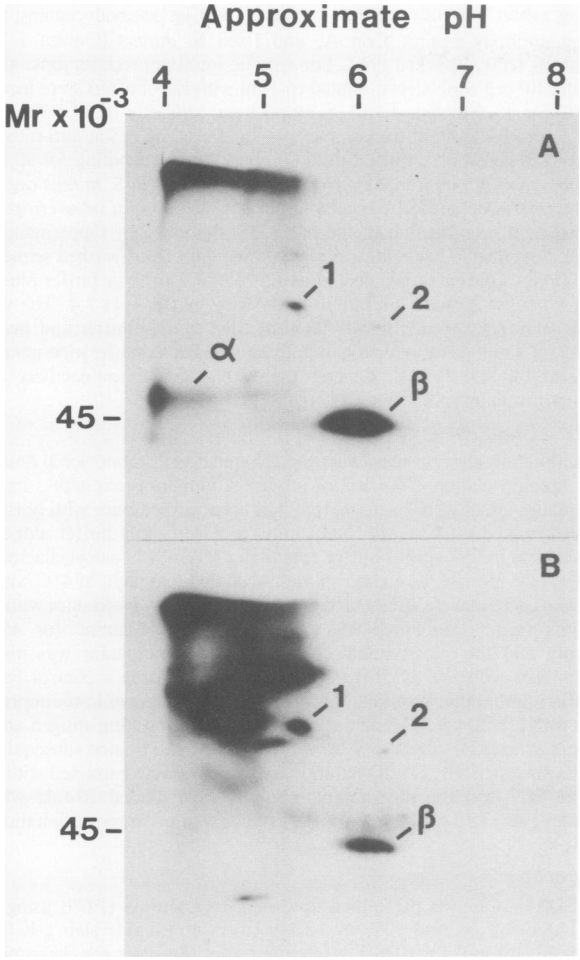

Fig. 5. 2-D (NEPHGE/SDS) PAGE analysis of H1-2D4 and PHA immunoprecipitates from HPB-ALL cells. Lysate of surface-labelled cells was precipitated with monoclonal Ti antibody H1-2D4 **(panel A)** and PHA **(panel B)**. Precipitates were analysed by NEPHGE in the first dimension and SDS-PAGE (10% acrylamide) in the second dimension. Spots 1 and 2 were routinely observed and proved useful in aligning the autoradiographs.

variety of other surface molecules (Figure 1B), one or more of which may conceivably also act as initiators of cell growth.

In addition to the above implications, the interaction of PHA with Ti provides a rational explanation for the disparate observations that, whereas PHA induced cell surface redistribution of the T3 antigen, no evidence for direct PHA-T3 interaction was obtained by immunoprecipitation (Kanellopoulos *et al.*, 1983). Thus, since Ti and T3 are associated on the cell surface (Meuer *et al.*, 1983a), redistribution of T3 by PHA is analogous to the redistribution induced by Ti antibodies. The reason for the failure of PHA to immunoprecipitate T3 despite binding to the cell surface T3/Ti complex is not known, although various explanations are possible including induced dissociation (Simonsen, 1984). Interestingly, T3 was not precipitated by Con A unless saturating levels were used, whereas Ti was precipitated at lower levels of Con A. It appears that Con A has a higher affinity for Ti than T3 in which case Con A conceivably stimulates quiescent T cells by interacting with Ti rather than T3.

Taken as a whole, the above results suggest that the antigen receptor of HPB-ALL cells exists in at least two subpopulations that differ in their glycosylation and/or sialylation; also, the α and β polypeptides of these subpopulations are apparently not equally susceptible to lactoperoxidase-catalysed iodination. This suggestion is based on the following observations: (i) H1-2D4 antibodies (and T40/25) precipitate a cross-linked heterodimer from surface-labelled HPB-ALL cells whose β subunit is always more heavily labelled than the α polypeptide (Figure 1A). In contrast, the α polypeptide of PHA immunoprecipitates was consistently more intensely labelled and diffuse than the β polypeptide (Figure 1B); the rabbit polyclonal antiserum (R-43) against Ti of HPB-ALL cells gave a similar result. (ii) The results of the immunodepletion experiments indicated that whilst H1-2D4 and PHA recognise at least some of the same Ti molecules, the cross-absorption was incomplete. In particular, SDS-PAGE analyses of the respective immunoprecipitates suggest that PHA preferentially binds a subpopulation of Ti molecules for which H1-2D4 has a lower affinity and which is more extensively glycosylated/sialylated especially on the α polypeptide. Inasmuch as HPB-ALL is an established cell line, it is unlikely that the proposed multiple forms of Ti reflect differences in primary structure. Rather it seems more probable that as the cultures are asynchronous, the multiple forms represent differences in Ti glycosylation associated with different stages of the cell cycle, or that differently glycosylated forms are expressed on the cell surface irrespective of the cell cycle.

Evidence was also obtained that PHA binds a molecule on the surface of two additional T leukaemia cell lines (HUT-78 and J6) with similar structural features to those of the Ti molecule of HPB-ALL cells. Thus, analyses of PHA immunoprecipitates from surface-labelled HUT-78 and J6 cells revealed an unreduced molecule of ~80 000 mol. wt. which on reduction gave one recognisable spot only of ~43 000 mol. wt. The distinct difference between this result and that obtained for HPB-ALL cells could reflect polymorphism of the antigen receptor, differential iodination of the subunits (see above), or different patterns of glycosylation. In contrast to the above data, direct evidence for the presence of distinct subunits was obtained from 2-D analyses of PHA immunoprecipitates from biosynthetically labelled ([^{35}S]cysteine) HUT-78 and J6 cells (data not shown).

Although not examined in detail, the marked similarity in the immunoprecipitation patterns obtained with Con A and WGA compared with those given by PHA (Figure 1) indicate that Con A and WGA also bind the antigen receptor on HPB-ALL cells. This interpretation is endorsed by the results of 2-D (NEPHGE/SDS) PAGE analyses of Con A immunoprecipitates (data not shown). The result obtained with WGA is of especial interest in view of the variable conclusions which have been drawn concerning the capacity of WGA to stimulate T cell growth and to initiate biochemical responses (Brown *et al.*, 1976; Greene *et al.*, 1976; Parker *et al.*, 1976; Dillner-Centerlind *et al.*, 1980). Our results do not resolve these discrepancies; also, it is essential to demonstrate that WGA binds to the receptor on peripheral blood T-lymphocytes. However, the present results do suggest that failure to stimulate T-cell growth by WGA is not due to inability of this lectin to bind to Ti. The similarities in the relative intensities of the putative α and β polypeptides of Con A and WGA immunoprecipitates with those of PHA immunorecipitates, particularly the diffuse nature of the α polypeptide (Figure 1B,C,D), suggest that Con A and WGA, like PHA, have a higher affinity for a more extensively glycosylated form of Ti than the clonotypic antibody.

In certain instances, monoclonal antibodies directed against the T11 antigen are also mitogenic for human T

lymphocytes (Meuer *et al.*, 1984). Since immunodepletion studies with T11 antibodies were not carried out in the present studies, the close correspondence in molecular sizes of the α subunit of Ti (50 000) and the T11 antigen (51 000) do not allow conclusions to be drawn in respect of whether PHA binds T11. However, as T11 does not contain interchain disulphide bonds, it would remain on the diagonal in 2-D (non-reduced/reduced) SDS-PAGE. As a result, the T11 molecule, if present in PHA immunoprecipitates, does not confuse the Ti patterns.

Materials and methods

Cells

Lymphocytes were prepared from tonsils obtained from individual donors. Tissue (5 – 10g) was dispersed in 40 ml of RPMI 1640 medium and after centrifuging (1 min at 100 *g*) to remove connective tissue, the cell suspension was layered onto an equal volume of Ficoll-Paque and centrifuged at 2000 *g* for 15 min. The cells at the interface were collected and washed twice with medium. In those cases when cells were held overnight at 4°C, the RPMI 1640 medium was supplemented with 10% fetal calf serum (FCS). Microsomes were prepared from tonsil tissue as described by Allan and Crumpton (1970). T-leukaemia cell-lines (HPB-ALL, J6 and HUT-78) were cultured in RPMI 1640 medium containing penicillin (100 units/ml), streptomycin (50 μg/ml) and 10% FCS (or 5% FCS plus 20 mM glutamine). Cell viability was determined by trypan blue staining and was generally >90%.

Antibodies

UCHT1 is a mouse monoclonal antibody (IgG_1) directed against the pan T-cell T3 antigen (Beverley and Callard, 1981). The hybridoma was grown in mice and the antibody was precipitated from ascitic fluid with 45% saturated ammonium sulfate and further purified using protein A-Sepharose as described by Ey *et al.* (1978). W6.32 is a mouse monoclonal antibody (IgG_1) which recognizes a common epitope expressed by all HLA-A,B,C antigens, providing the heavy chains are associated with β_2 microglobulin (Barnstable *et al.*, 1978). T40/25 is a protein A-binding mouse monoclonal antibody which recognises the T-cell antigen receptor on HPB-ALL and HPB-MLT cells (Kappler *et al.*, 1983); ascitic fluid was kindly donated by Dr. Ian Trowbridge (The Salk Institute, La Jolla, CA) and was used without further purification. H1-2D4 is a mouse monoclonal antibody (IgG_1) which is clonotypic for HPB-ALL cells; it recognises a cross-linked heterodimer having the molecular characteristics of the T-cell antigen receptor (Boylston *et al.*, 1984). Purified antibody was obtained as a gift from Dr. Arthur Boylston (St. Mary's Hospital Medical School, London). R-43 is a rabbit antiserum directed against Ti on HPB-ALL cells. The antiserum, which had been absorbed with B-lymphoblastoid cells, was obtained as a gift from Dr. Boylston. Rabbit antibodies against PHA were used in the form of an IgG fraction separated from anti-(PHA)serum by chromatography on DEAE-Sepharose (Dr. R. Lyall, ICRF) and protein A-Sepharose. Rabbit antibodies against Con A that had been purified by affinity chromatography on Con A agarose were provided by Dr. J. Kanellopoulos. Rabbit antibodies against WGA and HPA were used in the form of an IgG fraction separated from anti-(WGA) and anti-(HPA)sera by chromatography on protein A-Sepharose. Antisera against these lectins were prepared by immunising rabbits according to conventional protocols. Rabbit anti-(mouse Ig) and anti-(human Ig) sera and mouse anti-(human Ig) serum (DAKOPATTS, Mercia Brocades Ltd., Weybridge, Surrey, UK) were used without further purification.

PHA, Con A, WGA and HPA, Sepharose, Ficoll-Paque and protein A-Sepharose were from Pharmacia Fine Chemicals.

Radiolabelling

Cells were washed twice with phosphate-buffered saline (PBS) immediately prior to being surface labelled by lactoperoxidase catalysed iodination, as described by Walsh and Crumpton (1977).

Immunoprecipitation

Labelled cells were washed once with ice-cold PBS containing 10% FCS and twice with PBS alone. Lysates were prepared, essentially as described by Owen *et al.* (1980), by incubating cells on ice for 30 min in lysis buffer [1% Nonidet-P40 (BDH Chemical Ltd., Poole, UK) in 0.15 M NaCl, 10 mM Hepes buffer, pH 7.4] containing bovine serum albumin (1 mg/ml), 2 mM phenylmethylsulphonyl fluoride, 20 mM iodoacetamide and aprotinin, (1 international unit/ml; Sigma Chemical Co.). The lysates were cleared by high speed centrifugation and stored at −70°C until used.

In a typical experiment, lysate from ^{125}I labelled HPB-ALL cells (3 x 10^8 cell equivalents in 8 ml) was pre-cleared by incubating at 4°C overnight with 100 μg rabbit antibody against mouse Ig, 111 μg antibody against PHA, 120 μg antibody against Con A, and fixed *S. aureus* (Cowan 1 strain) organisms (100 μl packed vol.). For specific immunoprecipitations, aliquots (usually 10^7 cell equivalents, diluted to 1 ml with lysis buffer) were incubated at 4°C for 1 – 5 h with 4 – 30 μg monoclonal antibody or 15 – 30 μg lectin prior to precipitation of the complexes with 20 – 60 μg rabbit anti-(mouse Ig) or 50 – 120 μg rabbit anti-(lectin) antibodies. After incubating for 90 min or overnight at 4°C, immune complexes were adsorbed to *S. aureus* organisms or protein A-Sepharose by incubating at 4°C for 90 min or overnight, and washed by a modification of the procedure described by Oppermann *et al.* (1979). Sedimented bacteria or protein A-Sepharose was washed sequentially with: (a) lysis buffer supplemented to 0.5 M NaCl; (b) lysis buffer plus 0.1% SDS; (c) 0.1% Nonidet-P40 in 10 mM Hepes buffer, pH 7.4. The washed pellets were resuspended in 60 – 90 μl of SDS sample buffer and heated at 100°C for 4 min; after centrifugation to remove bacteria, the supernatant was analysed by SDS-PAGE. Greater than 80% of the radioactivity of the immunoprecipitate was routinely solubilised.

Dissociation of immunoprecipitates

Lectin-binding glycoproteins were reprecipitated with monoclonal antibodies after specific elution from lectin/anti-lectin immunoprecipitates. Immunoprecipitates attached to bacteria, that had been washed once with lysis buffer supplemented to 0.5 M with NaCl and twice with lysis buffer alone, were resuspended in 250 μl lysis buffer containing 450 mM N-acetylgalactosamine (or 450 mM glucose, as a control) and incubated overnight at 4°C with mixing. The bacteria were sedimented by centrifugation, washed once with 100 μl of lysis buffer containing 450 mM N-acetylgalactosamine (or 450 mM glucose) and the supernatants combined. The supernatant was immunoprecipitated with W6.32 plus rabbit anti-(mouse Ig) as a control for non-specific immunoprecipitation, followed by two successive immunoprecipitations with H1-2D4 plus rabbit anti-(mouse Ig). The resulting antigen-antibody complexes were adsorbed to *S. aureus* organisms and treated subsequently as previously described. H1-2D4 immunoprecipitates were extracted with 100 μl of 1% SDS and the superantant fractions were diluted 10-fold with 2% Nonidet-P40 in 10 mM Hepes buffer, pH 7.4, prior to re-precipitation with PHA.

Electrophoretic analyses

1-D SDS-PAGE was performed according to Laemmli (1979) using a 5% (w/v) stacking gel and 10% or 12.5% (w/v) acrylamide slab gels (overall length 20 cm) in 0.1% SDS-Tris/glycine buffer. Samples for electrophoresis were suspended either in sample buffer A [2% (w/v) SDS, 10% (v/v) glycerol, 80 mM Tris-HCl buffer, pH 6.8, and 0.02% bromphenol blue] or B [4% (w/v) SDS, 20% (v/v) glycerol, 250 mM Tris HCl buffer, pH 6.8, and 0.02% bromphenol blue] with or without 5 – 10% (v/v) 2-mercaptoethanol, and heated to 100°C for 4 min. The gels were run overnight at 80 – 90 V, stained with Coomassie blue, then destained and dried as described by Owen *et al.* (1980). For ^{125}I-labelled samples, autoradiography was performed at −70°C using Kodak X-Omat RP X-ray film with an intensifying screen. Mol. wts. of bands were routinely determined by reference to the mobilities of standard proteins (subunit mol. wts. in parenthesis): prestained myosin heavy chain (200 000), phosphorylase B (92 500), bovine serum albumin (68 000), ovalbumin (45 000), α-chymostrypsinogen (25 700), β-lactoglobulin (18 400), and cytochrome C (12 300), from Bethesda Research Laboratories or as described by Owen *et al.* (1980).

2-D (non-reduced/reduced) SDS-PAGE was performed by a modification of the procedure described by Goding and Harris (1981). The first dimension (non-reduced) was run in tubes (3 mm x 85 mm) containing 10% (w/v) acrylamide with a 3% stacking gel (1.5 cm) at 40 V for 20 h using the Tris/glycine buffer of Laemmli (1979). Mol. wts. in the first dimension were estimated relative to a tube gel containing prestained standards (*vida supra*). The tube gels were then incubated for 2 h in 10 ml sample buffer B with 10% 2-mercaptoethanol, prior to electrophoresis on 10 or 12.5% (w/v) acrylamide slab gels (Laemmli, 1979), using a 5% (w/v) acrylamide stacking gel (overall length, 11 cm). Gels were stained, destained, dried and autoradiographed as described above.

The procedure employed for 2-D (NEPHGE/SDS) PAGE was similar to that of O'Farrell *et al.* (1977), as described by de Kretser *et al.* (1982). Briefly immunoprecipitates adsorbed to *S. aureus* were extracted with NEPHGE sample buffer [9.2 M urea, 2% Nonidet-P40, 5% ampholines (pH range 3.5 – 10; LKB Instruments Ltd., Croydon, UK) 5% 2-mercaptoethanol] at 50°C for 30 min. The first dimension was carried out in 11 cm cylindrical polyacrylamide gels with an essentially linear pH gradient from 4 to 9. These samples were equilibrated for 2 h in sample buffer A prior to separation in the second dimension on 10% polyacrylamide slab gels, using the Tris/glycine buffer of Laemmli (1979). Staining, destaining and autoradiography was as described above.

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