The T3 Complex on Human T Lymphocytes Involves Four Structurally Distinct Glycoproteins*

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Monoclonal antibodies allow for the detection of antigens which are specific for human thymus-derived lymphocytes. Among these antigens, the T3 complex is of particular interest since it is involved in several T cell functions. The main target antigen of the anti-T3 reagents is borne by a 20-kDa glycoprotein. In addition, glycoproteins of 25-28, 37, and 44 kDa are found in anti-T3 immunoprecipitates derived from surface-labeled cells. The four antigens appeared to be strongly associated with each other in detergent-containing solutions. Comparative studies of the four proteins, facilitated by the use of endo-β-N-acetylglycosaminidase F, revealed that their polypeptide backbones have different molecular weights and PI values. Moreover, peptide maps of the 20-kDa T3 and the 25-28-kDa T3 were quite different. Metabolic labeling experiments suggested that the 25-28-kDa protein might become associated with the 20-kDa T3 antigen during biosynthesis. The 37-kDa and 44-kDa proteins could not, however, be detected and, therefore, might become associated with the 20-kDa T3 on the cell surface. Evidence has been found for the existence of a fifth member of the T3 complex, namely an unglycosylated 20-kDa T3 species.

Human thymus-derived lymphocytes express several cell surface structures which have some degree of specificity for this subset of lymphoid cells (1-3). Most T lineage-associated surface markers can now be detected by monoclonal antibody reagents. One of these cell surface markers, T3, which is recognized by the monoclonal antibodies OKT3 (1), anti-Leu-4 (4), and UCHT-1 (5), is present on most mature thymocytes and virtually all peripheral blood T lymphocytes, but on no other lymphoid or myeloid cells. Recently, UCHT-1 and anti-Leu-4, but not OKT3, were also found to react with cells of a completely different origin, namely Purkinje cells in the cerebellum (6). However, the target structure of UCHT-1 and anti-Leu-4 on Purkinje cells has yet to be compared to that on T cells.

The T3 antigen(s) seem to be involved in several T cell-specific functions. Addition of anti-T3 monoclonal antibody in very low concentrations to human peripheral blood lymphocytes induces DNA synthesis (7). In higher concentrations, the antibody blocks the proliferative response to cell surface and soluble antigens and hence inhibits the generation of cytotoxic T cells in a mixed lymphocyte culture and abrogates the ability of T cells to provide help to B cells in antibody production (9). In addition to an involvement in T cell proliferation, the T3 antigen appears to play some role in target cell lysis by allogeneic cytotoxic T cells (9). Anti-T3 monoclonal reagents inhibit cytotoxicity of cytotoxic T lymphocyte clones directed at either class I or class II major histocompatibility complex antigens (10, 11). On the basis of these data, T3 is suspected to be involved either in T lymphocyte specific cell-cell interactions, or most likely indirectly in antigen recognition structures. A study of the structural aspects of T3 should, therefore, aid in our understanding of these T cell functions.

We have previously identified the target antigen for OKT3 and anti-Leu-4 as a 20-kDa glycoprotein which is heterogeneous in charge due to sialic acids (12, 13). In addition to the major glycoprotein of 20 kDa, glycoproteins of 25-28, 37, and 44 kDa were detected in smaller amounts in immunoprecipitates (13).

The data presented in this paper support the notion that all four members of the T3 complex are indeed glycoproteins. Using radiiodination, biosynthetic labeling techniques, and endoglycosidases, we determined that the protein backbones of the 20-, 25-28-, 37-, and 44-kDa molecules have molecular mass of 14, 16, 32, and 34 kDa, respectively. The 20-kDa protein appeared to be structurally distinct from the 25-28-, 37-, and 44-kDa species. In addition, our data suggest that a nonglycosylated 20-kDa T3 may be a part of the complex. MATERIALS AND METHODS

Cells—For all studies described in this paper, we used the human T leukemia cell line HPB-ALL (14), cultured in RPMI-1640, supplemented with 5% fetal calf serum, at 37 °C in a 5% CO2 atmosphere.

Monoclonal Antibodies—The monoclonal reagent OKT3 was purchased from Ortho Pharmaceuticals, Raritan, NJ. Anti-Leu-4 was a generous gift of Dr. Robert Evans, Sloan Kettering Memorial Institute, New York. Anti-β2m monoclonal antibody was kindly supplied by Dr. Lee Nadler, Sidney Farber Cancer Institute, Harvard Medical School, Boston.

Radiolabeling—Cell surface iodination with Na125I (ICN Radiochemicals, Irvine, CA) was catalyzed either by lactoperoxidase (15), or by 1,3,4,5-tetrachloro-3a,4a-diphenylglycouril (Bodogen, Pierce Chemical Co., Rockford, IL) (16). HPB-ALL cells were labeled metabolically with [35S]methionine or a mixture of [35S]methionine and [35S]cysteine using two methods. First, in pulse-chase experiments, cells were washed once in RPMI-1640 without [35S]methionine and [35S]cysteine (15). Second, they were incubated in this medium, which contained 10% dialyzed fetal calf serum, for 1 h at 10 × 10⁶ cells/ml. Subsequently, they were spun down, resuspended at 50 × 10⁶ cells/ml and pulsed for 5 min with 250 μCi/ml [35S]methionine and [35S]cysteine (American Merck, Rockland, NY). They were precultured in this medium, which contained 10% dialyzed fetal calf serum, for 1 h at 10 × 10⁶ cells/ml. Subsequently, they were spun down, resuspended at 50 × 10⁶ cells/ml and pulsed for 5 min with 250 μCi/ml [35S]methionine and [35S]cysteine (American Merck, Rockland, NY). They were cultured with 5 volumes of RPMI-1640, containing 0.5 mm nonradioactive methionine and cysteine. At various time points, 6-ml samples (50 × 10⁶ cells) were taken and diluted 10 times in ice-cold phosphate-buffered saline. The cells were spun down immediately and lysed in immunoprecipi-
P-N-acetylglycosaminidase H; Endo-F, endo-/3-N-acetylglycosaminidase focusing was performed on vertical slab gels according to Van AgtSDS, 5% 2-mercaptoethanol, 0.2% Ampholytes. After 45 min, an equal
5-8 as 101:1 were used. Samples were taken up in 8.8
anode. Two-dimensional electrophoresis was done in accordance with
electrophoresis was carried out on discontinuous vertical slab gels
according to a modification of the Laemmli procedure (19). Gradient
immunoprecipitate. The protein was resuspended in 0.1 M sodium
phosphate buffer, pH 6.1, containing 50 mM EDTA, 1% 2-mercapto-
complex or on the T3 antigen which had been purified from an
immunocomplex was resuspended with OKT3 or anti-Leu-4 and rabbit
anti-mouse IgG at 4°C (13), and immunoprecipitates were removed
from the lysate by centrifugation at 13,000 X g. Precipitates were
resuspended in 0.2 ml Tris-NaCl buffer with 0.5% deoxycholate
and boiled for 3 min. Endo-H was added at 3 pg/ml and incubation
for 15 min at 37°C. This technique, developed by Helenius and Simons
(22), determines whether a protein is amphiphilic. It has been shown
that amphiphilic proteins bind relatively large amounts of
interactions with the hydrophobic domains of these proteins. Amphiphilic proteins show,
differences in mobility when subjected to electrophoresis in neutral, cationic, or anionic detergents because
their net charge depends upon the charge contributed by the
detergent which is bound to them. Hydrophilic proteins show
no differences in mobility, but migrate solely according to their own net charge.

The T3 Complex Consists of a 20-kDa Glycoprotein That
Is Associated with 25-28-, 37-, and 44-kDa Glycoproteins—

The T3 Complex of Human T Lymphocytes

The human T cell-specific cell surface antigen T3 is recognized by the monoclonal reagents OKT3, anti-Leu-4, and UCHT-1. Earlier, we showed that OKT3 and anti-Leu-4 precipitated a 20-kDa glycoprotein from resting and phytohemagglutinin-activated peripheral blood T lymphocytes and the T leukemic cell line HPB-ALL. In addition three glycoproteins of apparent
molecular mass of 25-28, 37, and 44 kDa were present (in smaller quantities than the 20-kDa T3) in the anti-T3 immunoprecipitates (13). Only the 20-kDa T3 could be labeled with the hydrophobic reagent iodonaphtylazide (13).

We examined the migration of the four proteins in the
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T3 antigens were isolated using the OKT3 antibody, disso-
ciated from immunoglobulin with 1 M MgCl2, and subjected
to charge shift electrophoresis. This experiment demonstrated that the T3 antigens are indeed amphiphilic (Fig. 1). Bands 1 cm long, in the direction of electrophoresis, were excised from the agarose gels after charge shift electrophoresis and run on a second dimension consisting of an SDS-polyacrylamide gel (Fig. 2). This experiment pointed out that the 20-kDa T3 and
the 25-, 28-, 37-, and 44-kDa molecules migrated together in
charge shift electrophoresis. This was interpreted as an indica-
tion that the four proteins are associated in the presence
of various detergents. An alternative interpretation, which cannot
be excluded, is that all four T3 proteins bind equal amounts of detergents and thus migrate over the same distance upon electrophoresis. It should be noted that the 37-
and 44-kDa molecules are present in anti-T3 immunoprecri-

1The abbreviations used are: NP-40, Nonidet P-40; Endo-H, endo-
β-N-acetylgalactosaminidase; Endo-F, endo-/3-N-acetylgalactosami-
dase F; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel elec-
trophoresis.
Triton performed according to Helenius and Simons cells by means of immunoprecipitation with antigens were purified from a NP-40 lysate of "I-labeled HI'B-ALL MgCl2

Niobium bromide long in the direction of electrophoresis and run on a 10-15% polytive of the labeling technique (Fig. 2). This was particularly in charge due to the presence of variable amounts of acrylamide gradient gel, made according to Laemmli in the presence of deoxycholate tates in much smaller amounts than the 20-kDa T3 irrespective of the labeling technique (Fig. 2). This was particularly the case when anti-Leu-4 antibody was used. In fact, if an immunoprecipitate, made with anti-Leu-4, was washed with a buffer containing 1 M MgCl2, only the 20-kDa protein could be detected (Fig. 3, lanes C and D).

The charge shift and immunoprecipitation studies suggest that the main target antigen for anti-Leu-4 and OKT3 is the 20-kDa glycoprotein, which is associated in solution with the 20-kDa T3 and the 16kDa band from the 25-28-kDa protein (data not shown). This result was corroborated in another experiment in which the 20-kDa T3 was isolated using the monoclonal reagent anti-Leu-4. Upon washing the immunoprecipitate with 1 M MgCl2 in 0.01 M Tris-HCl, 0.5% NP-40, the only antigen that remained bound to the antibody was the 20-kDa T3 (Fig. 3, lane C). After treatment with Endo-F, only a 14-kDa protein was found (Fig. 3, lane D). It was established that the set of 37-kDa and 44-kDa molecules gave the set of 32-kDa and 34-kDa Endo-F breakdown products.

Two-dimensional electrophoresis showed that the 14-kDa and 16-kDa Endo-F breakdown products had little, if any, charge heterogeneity as would be expected after the removal of the N-linked, complex-type carbohydrate carrying sialic acids (Fig. 4, A and B). The isoelectric points of the 14-kDa and 16-kDa proteins were 4.5 and 7-8, respectively, corre-

Previous studies of iodinated T3 antigens had already given us some information about the nature of their carbohydrate moieties. The 20-kDa T3 antigen was found to be heterogeneous in charge due to the presence of variable amounts of sialic acids. Endo-H treatment yielded a number of digestion products of 14, 16, and 18-19 kDa in molecular mass and left part of the 20-kDa molecule unaffected. This suggested that the 20-kDa T3 molecule, as it is present on the cell surface, carries both high mannose and complex-type, N-linked sugars in variable amounts. The charge heterogeneity of the 25-28-kDa protein was also caused by sialic acids, but this protein was not sensitive to Endo-H. The 37-kDa and 44-kDa molecules were possibly also heterogeneous in charge, but did not resolve well in two-dimensional electrophoresis. The 37-kDa protein was susceptible to Endo-H; the 44-kDa protein was not (13).

As shown in Fig. 3, lane B, the "I-labeled T3 antigens were all susceptible to digestion with Endo-F. After Endo-F treatment, a series of digestion products was found of 14, 16, 32, and 34 kDa in molecular mass. Treatment of the 20-kDa and 25-28-kDa proteins with Endo-F after separation by SDS-PAGE and elution from the gel revealed that the 14-kDa band was derived from the 20-kDa T3 and the 16kDa band from the 25-28-kDa protein (data not shown). This result was corroborated in another experiment in which the 20-kDa T3 was isolated using the monoclonal reagent anti-Leu-4. Upon washing the immunoprecipitate with 1 M MgCl2 in 0.01 M Tris-HCl, 0.5% NP-40, the only antigen that remained bound to the antibody was the 20-kDa T3 (Fig. 3, lane C). After treatment with Endo-F, only a 14-kDa protein was found (Fig. 3, lane D). It was established that the set of 37-kDa and 44-kDa molecules gave the set of 32-kDa and 34-kDa Endo-F breakdown products.

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The charge shift and immunoprecipitation studies suggest that the main target antigen for anti-Leu-4 and OKT3 is the 20-kDa glycoprotein, which is associated in solution with the 25-28-, 37-, and 44-kDa proteins. Whether this means that these proteins are also associated with each other on the cell surface remains to be determined.

The 20-, 25-28-, 37-, and 44-kDa Glycoproteins Have Distinct Protein Backbones—Co-precipitation of the four glycoproteins could also be explained by a sharing of epitopes recognized by anti-T3 monoclonal antibodies. We therefore analyzed and compared the structures of the 20-, 25-28-, 37-, and 44-kDa proteins. Since all four proteins are glycosylated, we made use of a recently described enzyme, Endo-F, to study their protein moiety (18). This enzyme was found to cleave high mannose (23) as well as complex-type (23) N-linked carbohydrate groups from various well defined molecules, such as Rauscher murine leukemia virus glycoprotein 70 and HLA-A and -B antigens. Like Endo-H (24), Endo-F is thought to hydrolyze the glycosidic bond between the first two N-acetylglucosamines through which N-linked carbohydrate groups are attached to asparagine residues in the protein backbone.

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sponding with those found for the 20-kDa and 25–28-kDa proteins after neuraminidase treatment (13) (data not shown). The Endo-F digestion products of the 37-kDa and 44-kDa proteins gave clearer patterns upon two-dimensional electrophoresis than their glycosylated counterparts and displayed no distinct charge heterogeneity (Fig. 4B).

These results indicate that the 20-kDa T3 molecule, as it is present on the cell surface, consists of a protein backbone of 14 kDa in molecular mass and carries a variable number of N-linked, high mannose and/or complex sugars. The 25–28-kDa protein has a 16-kDa protein backbone and carries only N-linked, complex-type carbohydrate. The 37-kDa and 44-kDa proteins also have asparagine-linked oligosaccharides and have protein backbones of 32 and 34 kDa, but we did not determine which respective digestion product corresponded to which protein. The 25–28-kDa protein was apparently not simply a differently glycosylated form of 20-kDa T3 protein. Peptide maps of Endo-F digestion products of these two proteins were very similar to those of their glycosylated forms simply a differently glycosylated form of 20-kDa T3 protein to which protein. The 25–28-kDa protein was apparently not simply a differently glycosylated form of 20-kDa T3 protein. The peptide maps of the 37-kDa and 44-kDa proteins also have asparagine-linked oligosaccharides and have protein backbones of 32 and 34 kDa, but we did not determine which respective digestion product corresponded to which protein. The 25–28-kDa protein was apparently not simply a differently glycosylated form of 20-kDa T3 protein.

**Biochemical Analysis of the T3 Antigens**—To investigate in more detail if any structural similarities between these two proteins exist, we subjected 125I-labeled 20-kDa and 25–28-kDa proteins to two-dimensional peptide mapping (Fig. 5, A–C). The peptide maps of the two proteins were clearly different, as emphasized by a mixing experiment (Fig. 5C). This indicated that the 20-kDa T3 protein differs greatly in structure from the 25–28-kDa protein. Peptide maps of Endo-F digestion products of these two proteins were very similar to those of their glycosylated forms (data not shown). Due to a shortage of iodinated material, peptide maps of the 37-kDa and 44-kDa proteins could not be compared to those of the other proteins. Further structural comparisons will have to await large scale purifications of the different glycoproteins.

**Biosynthetic Labeling of the T3 Antigens**—The biosynthesis of the T3 antigens in the human T leukemic cell line HPB-ALL was studied and compared with the results obtained after cell surface labeling. After labeling with [3H]methionine and [35S]cysteine for 16 h, only the major 20-kDa T3 form was found, and not the 25–28-, 37-, or 44-kDa proteins.

The 20-kDa T3 was also the only species found in immunoprecipitates from HPB-ALL cells labeled with [3H]mannose and [3H]glucosamine (data not shown). In a control precipitate made with an anti-β-m monoclonal antibody, the only detectable label was present in the HLA heavy chain, and not in the associated unglycosylated β-m. This indicated that none of the [3H]mannose or [3H]glucosamine had been converted metabolically into amino acids. (These findings support the view that the monoclonal antibodies OKT3 and anti-Leu-4 react only with the mature 20-kDa form of T3.)

Analysis of the 20-kDa T3 species labeled with [35S]methionine and [35S]cysteine by isoelectric focusing (Fig. 6, lane E) revealed that the majority of the radioactivity was present in three bands, namely 20-kDa T3 (a, b, and c). After labeling with 1H-carbohydrate, most of the label was found in the 20-kDa T3 c band (Fig. 6, lanes B, C, and D). After metabolic labeling for 16 h, only a relatively low amount of radioactivity had been incorporated in the most acidic T3 forms, whereas these forms are strongly labeled by [125I] (Fig. 6, lane A).

Having shown previously that the more acidic bands of T3 antigens—The biosynthesis of the T3 antigens in the human T leukemic cell line HPB-ALL was studied and compared with the results obtained after cell surface labeling. After labeling with [3H]methionine and [35S]cysteine for 16 h, only the major 20-kDa T3 form was found, and not the 25–28-, 37-, or 44-kDa proteins.

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surface-iodinated 20-kDa T3 could be converted into 20-kDa T3 \((a, b, \text{ and } c)\) by treatment with neuraminidase \((13)\), we concluded that the most acidic forms were generated by sialic acids. From these results, it would appear that even upon overnight labeling with \(^{14}S\)-amino acids or \(^3H\)-carbohydrates, the terminal phase of glycosylation involving sialyltransferases had not yet been completed. Another possibility is that there is a higher turnover in T3 biosynthesis and mature T3 forms are rapidly lost from the cell surface.

Glycosylation of the Biosynthetically Labeled T3 Antigen(s)—In order to study the glycosylation of the 20-kDa T3 in more detail, and to allow a comparison of Endo-H and Endo-F digestions, pulse-chase experiments were carried out. HPB-ALL cells were labeled for various time periods in the presence or absence of tunicamycin \((23)\). In addition, pulse-chase samples were treated with Endo-H and Endo-F. As shown in Fig. 7, lane A, a band of 20 kDa was observed after labeling cells for 5 min with a mixture of \([^{35}S]\)methionine and \([^{35}S]\)cysteine. Within 15 min, a 23-kDa molecule became visible, which was most clearly present after 1 h (Fig. 7, lane B).

![Isoelectric focusing of the 20-kDa T3 antigen, labeled by various techniques.](http://www.jbc.org/) HPB-ALL cells were labeled either chemically with \(^{125}I\), according to the iodogen method \((16)\) (lane A); or biosynthetically with \([^{14}C]\)glucosamine (lanes B and D); \([^{3}H]\)mannose (lane C); or \([^{35}S]\)methionine and \([^{35}S]\)cysteine (lane E). Immunoprecipitation was performed in all cases with anti-Leu-4. The immunoprecipitate made from \(^{125}I\)-labeled cells was washed with 1 M MgCl\(_2\) and analyzed by SDS-PAGE (Fig. 9). This washing procedure removed the 23-kDa molecule completely. Upon digestion with Endo-F, only the 14-kDa product was obtained (Fig. 9, lane B), which was similar to the result found with \(^{125}I\)-labeled T3 antigens (Fig. 3, lanes C and D). In addition, the 23-kDa protein had a basic pI, which was most clearly present after 1 h (Fig. 7, lane B).

![Pulse-chase labeling of T3 antigens with \(^{35}S\)-amino acids in the presence or absence of tunicamycin.](http://www.jbc.org/) HPB-ALL cells were pulsed for 5 min with a mixture of \([^{35}S]\)methionine and \([^{35}S]\)cysteine and chased with unlabeled methionine and cysteine for 8 h. Samples were taken at various time points, cells were lysed in NP-40, and immunoprecipitation was carried out with OKT3. Lanes A-D show T3 antigens synthesized in the absence of tunicamycin during a period of, respectively, 5 min (lane A), 1 h (lane B), 4 h (lane C), and 8 h (lane D) after the pulse. Lanes E-H show T3 antigens synthesized during those respective time periods in the presence of tunicamycin (2 \(\mu g/ml\)). Samples were analyzed on a 14-20% SDS-polyacrylamide gradient gel.

![Endo-F and Endo-H treatment of \(^{35}S\)-labeled T3 antigens.](http://www.jbc.org/) HPB-ALL cells were either chemically labeled with \(^{125}I\) (lanes A and B), or pulse labeled with \([^{35}S]\)methionine-\([^{35}S]\)cysteine for 1 h as described in Fig. 8, lanes c-f. T3 antigens were isolated with OKT3. Samples in lanes a and b are \(^{125}I\)-labeled T3 antigens, not treated and treated with Endo-F, respectively. Lanes c and d show \(^{35}S\)-labeled T3 antigens not treated and treated with Endo-H, respectively. Lanes e and f show identically labeled T3 antigens not treated and treated with Endo-F, respectively. Lane g, control immunoprecipitate.
cells were labeled with a mixture of [%]methionine and [%]cysteine with anti-Leu-4. The anti-Leu-4 immunocomplex was washed with a buffer containing MgCl₂ and was subsequently incubated without Endo-F (lane A), or with Endo-F (lane B). Lane C shows a control immunoprecipitate.

much like the 25-28-kDa protein after neuraminidase and Endo-F treatment (data not shown). Taken together, these observations suggest that the 23-kDa molecule is a precursor form of the 25-28-kDa T3 which was found on the cell surface.

The Endo-H and Endo-F treatments also revealed that a substantial portion of the 20-kDa T3 labeled with [%]amino acids was resistant to either enzyme. Along with the finding of a tunicamycin-resistant 20-kDa T3 form, these data suggest that, in addition to a glycosylated 20-kDa T3 form (derived from a 14-kDa precursor), another 29-kDa T3 form, which is not glycosylated, may exist.

DISCUSSION

The results in this paper describe the complexity of the human T cell surface antigen T3. The major component of the T3 complex is a glycoprotein of 20 kDa in molecular mass, which is associated with three other glycoproteins of 25-28, 37, and 44 kDa in detergent-containing solutions. Whether these proteins are also associated on the cell surface remains to be determined. Using the monoclonal antibody anti-Leu-4 in immunoprecipitation studies, we could demonstrate that, after washing with 1 M MgCl₂, only the 20-kDa T3 was retained on the immunocomplex. As the peptide maps prepared from [%]-labeled 20-kDa T3 and the 25-28-kDa protein appeared to differ greatly, and since the molecular masses and isoelectric points of the four polypeptides remained different after removal of all N-linked oligosaccharides, it is plausible that they differ in primary structure. However, these experiments do not exclude that all four glycoproteins are recognized by the monoclonal antibodies OKT3 and anti-Leu-4.

The mature cell surface T3 glycoprotein of 20 kDa contains both complex and high mannose-type N-linked oligosaccharides. In a previous report (13), we have shown that radioiodinated 20-kDa T3 and the 37-kDa molecules, but not the 25-28-kDa and 44-kDa molecules, were susceptible to Endo-H digestion. This indicated that the mature 20-kDa and 37-kDa glycoproteins contain high mannose-type carbohydrate. In contrast, all four glycoproteins were susceptible to digestion with Endo-F, which removes both complex and high mannose-type N-linked sugars. Endo-F treatment reduced the molecular mass of the 20-kDa T3 to 14 kDa. The 25-28-kDa protein was digested to 16 kDa; the 37-kDa and 44-kDa proteins were digested to 32 and 34 kDa, respectively. The Endo-F breakdown products showed very little charge heterogeneity. This suggested that most of the complexity in the isoelectric focusing pattern was caused by N-linked, as opposed to O-linked, carbohydrate.

Treatment of the 20-kDa and 25-28-kDa T3 antigens with trifluoromethane sulfonic acid, which hydrolyzes intrachain carbohydrate bonds and O-linked protein-carbohydrate bonds (26), gave hydrolysis products with molecular weights identical with those of the Endo-F digestion products. This indicates that no substantial amount of O-linked carbohydrate can be present on these molecules (data not shown).

The results obtained with Endo-H and Endo-F treatment of surface-radioiodinated 20-kDa T3 are largely consistent with the observations made after biosynthetic labeling. However, after overnight labeling with [%]-amino acids, only part of the 20-kDa T3 was susceptible to Endo-F and Endo-H treatment. In contrast to the [%]-labeled 20-kDa T3, the [%]-labeled T3 could never be completely converted into the 14-kDa species. This indicated that a 20-kDa T3 molecule exists which does not carry any N-linked carbohydrate. The results of experiments using the drug tunicamycin are consistent with the existence of such a form, since even at very high tunicamycin concentrations (5 μg/ml) a 20-kDa T3 molecule was synthesized (data not shown). It is possible that this 20-kDa T3 form is not susceptible to iodination.

During the pulse-chase experiments, a 23-kDa polypeptide was most distinctly present after a 1-h chase, but disappeared after 4 h. This 23-kDa protein was converted into a 16-kDa form by Endo-F and Endo-H and was not found if biosynthetic labeling took place in the presence of tunicamycin. When an anti-Leu-4 precipitate was washed with 1 M MgCl₂-containing buffer, the 23-kDa molecule was not found. The 23-kDa protein had an isoelectric point similar to that of the 25-28-kDa protein after neuraminidase or Endo-F treatment (13). Taken together, these data suggest that the 23-kDa form is a precursor of the 25-28-kDa T3 which had been found on the cell surface. Why it disappears after 4 h and why we did not find its mature form in biosynthetic labeling experiments remains unclear. There are a number of possibilities. First, after the final steps of glycosylation have taken place and the mature 25-28-kDa has been formed, this molecule could be immediately transported to the cell surface and be lost there. Second, the interaction with the 20-kDa T3 protein might be different for intermediates of the 25-28-kDa T3 biosynthesis. Third, the monoclonal antibodies could bind some, but not all, precursor forms of the 25-28-kDa molecule. With the available monoclonal antibodies, it is not possible to elucidate this problem. Antibody reagents which react with the various members of the T3 complex and their precursors will need to be generated for a more detailed study of their biosynthesis.

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Structural Analysis of the T3 Complex of Human T Lymphocytes

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REFERENCES
The T3 complex on human T lymphocytes involves four structurally distinct glycoproteins.

J Borst, S Alexander, J Elder and C Terhorst


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