THYMOCYTES WITH THE PREDICTED PROPERTIES OF PRE-T CELLS

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Peripheral T cells exhibit a large repertoire of specificities for foreign antigens that they recognize on cell surfaces in association with products of the MHC (1, 2). The structures responsible for this recognition are a set of surface receptor molecules clonally distributed among T cells. The clones that express the peripheral T cell repertoire of receptors are somehow selected from the universe of clones arising during T cell differentiation in the thymus (3–5), and it is logical to assume that the selection events are enacted through the surface receptor molecules expressed on thymocytes early in their differentiation. To understand this phenomenon it is necessary to determine the factors that control the surface expression of this receptor on thymocytes at this critical stage in their development.

The genes encoding the two chains (α and β) of these receptors are not rearranged until after the T cell precursors enter the thymus (6, 7). In mouse, the complete β chain gene rearrangements required for expression do not occur in significant numbers until day 16 of fetal life, although mRNA from unrearranged or incompletely rearranged genes is present earlier. α chain mRNA is barely detectable until day 17 (7, 8), and therefore it has been suggested that β chain protein expression precedes that of the α chain (9), a situation analogous to the initial synthesis of heavy chains in B cells before light chain expression (10).

Analysis of receptor expression at the protein level has been hampered by the lack of antisera to the constant regions of either α or β chains that can detect the native molecule in situ, although several anti–constant region antisera have been produced that will immunoprecipitate receptors from detergent lysates of thymocytes. Therefore, much of the information about expression of the receptor is extrapolated from work with two mAbs, KJ16-133.1 (KJ16) (11, 12) and F23.1 (13), which detect β chains from the frequently used $V_\beta 8$ family (~20% of β chains in most strains).

Cytofluorometric analysis of thymocytes with KJ16 has shown that surface expression of α/β receptors begins early in development, with the first receptor-positive cells detectable at day 17 of fetal life (14). These studies have also shown that from birth a high proportion (~50%) of cortical, immature thymocytes express receptor on their surface, but that the number of receptors per cell is 5–10-fold lower than on mature medullary thymocytes or on peripheral T cells.

KJ16 has also been used in electron micrographic studies to stain thymus thin sections (15). These studies revealed another frequent cortical thymocyte with high levels of cytoplasmic staining with KJ16.

In the current study we undertook to establish the relationship among these receptor-bearing populations. We found that cytoplasmic-free β chains were synthesized at a high rate primarily by cortical thymocytes with no detectable surface receptor. This species was synthesized at a much lower rate in cortical thymocytes with low-level surface receptor and was virtually undetectable in mature, high surface receptor–expressing medullary cells. These results indicate a frequent pre-T cell in the thymus cortex, which has rearranged and expressed the β-chain locus in the apparent absence of the α chain.

Materials and Methods

Mice. BALB/c By mice were bred at the National Jewish Center from breeding stock obtained from The Jackson Laboratory, Bar Harbor, ME. SWR mice were also purchased from The Jackson Laboratory. Fetal mice were obtained on appropriate days of gestation by counting from day 0, the day on which a vaginal plug was observed in the mother.

Reagents. All antisera and antibodies have been described elsewhere (11, 13, 16). Briefly, KJ16-133.1 (KJ16) is a rat IgG, and F23.1 a mouse IgG, both of which bind T cell receptors using β chains from the $V_\beta 8$ gene family. R3497 is a rabbit antiserum that was raised against the purified receptor of the T cell hybridoma DO-11.10, and precipitates all mouse T cell receptors yet tested.

Cell Preparations. Fetal, newborn, and adult thymocytes were obtained and used directly from thymocyte suspensions. Adult thymocytes were fractionated into peanut agglutinin–positive (PNA$^+$)[1] and PNA$^-$ populations by panning (17) on 10-cm petri dishes coated with PNA (20 μg/ml PNA in 0.15 M NaCl: 0.05 M Tris, pH 9.5, for 1 h at room temperature followed by 5% FCS/buffered saline solution (BSS) for 1 h at room temperature, and finally washed twice with BSS before panning). Cells were suspended in 5% FCS/BSS/1 mM sodium azide at 10^7 cells/ml and were panned for 90 min at 4°C. After gentle washing, PNA$^+$ (adherent) cells were harvested by incubating for 10 min with 0.2 M galactose/1% FCS/1 mM azide. PNA$^-$ cells were panned three times to assure purity, and PNA$^+$ cells were panned twice. PNA$^+$ F23.1$^+$ cells were obtained by panning PNA$^+$ cells on plates coated with F23.1 (100 μg/ml F23.1 in BSS for 1 h at room temperature followed by 5% FCS/BSS for 1 h). F23.1$^-$ cells were obtained by panning twice, and were found to be receptor-negative by flow cytometry and by surface iodination and receptor precipitation.

Cortisone-resistant thymocytes were harvested 48 h after peritoneal injection of 2.5 mg cortisone acetate.

Cell Labeling. Cells were surface-labeled with ^{125}I using iodogen as described previously (18). Metabolic labeling with [^{35}S]cysteine was performed at 37°C in cysteine$^-$ MEM at 10^7 cells/ml plus 10 μCi [^{35}S]cysteine/ml. In pulse-chase experiments, cells were metabolically labeled for 60 min, washed, resuspended in identical medium containing cold cysteine, and incubated at 37°C for varying times. PNA$^+$ F23.1$^+$ cells were metabolically labeled while adherent to the F23.1-coated plates after panning and washing.

Precipitations. Surface receptors were precipitated by adding 20 μl of F23.1 to a 15-ml suspension of labeled cells (in BSS/1 mM sodium azide), incubating 10 min at 37°C, washing once, lysing the cells in 3 ml 1% NP-40/PBS, spinning the lysate at 28,000 rpm for 1 h, and precipitating for 15 min at room temperature with protein A–Sepharose 4B beads. Internal receptor molecules were then harvested by reabsorbing the precleared lysate for 15 min at room temperature with beads preloaded with F23.1 or R3497. The beads were then washed well in 0.5% NP-40/50 mM NH$_4$HCO$_3$, and the bound material

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[1] *Abbreviations used in this paper:* BiP, immunoglobulin heavy chain binding protein; BSS, buffered saline solution; NEPHGE, nonequilibrium pH gradient electrophoresis; PNA, peanut agglutinin.

was eluted by brief vortexing with 50 mM diethylamine. The eluate was neutralized with 1.0 M acetic acid and taken to dryness in a Speed-Vac. Each sample was then dissolved in a sample buffer appropriate for the next desired procedure.

Endoglycosidase Digestion.   Endoglycosidase F digestions were done at 40 U/ml for 20 h at 37°C in buffer containing 0.1 M sodium phosphate, pH 6.1, 0.05 M EDTA, 1% 2-ME (19). Endoglycosidase H digestions were done at 0.12 μg/ml for 20 h at 37°C in buffer containing 0.15 M sodium citrate, pH 5.25, and 1% NP-40 (20).

Electrophoresis.   One-dimensional (12.5%) and two-dimensional (10% nonreduced followed by 10% reduced) SDS-PAGE were performed on mini-slab gels (Idea Scientific, Corvallis, Oregon) in the buffer system of Laemmli (21). Nonequilibrium pH gradient electrophoresis (NEPHGE)/SDS-PAGE analyses were performed according to O'Farrell et al. (22), in which the samples were suspended in buffer containing 9M urea, 1% NP-40, 2% ampholytes (pH 3.5–10), and 1% 2-ME, and then electrophoresed for 1,200 V·h on mini-slabs containing 4% acrylamide, 9 M urea, 4% NP-40, and 4% ampholytes (pH 3.5–10) (22). Lanes from the NEPHGE slab gels were sliced out, gently rocked for 10 min in Laemmli sample buffer plus 5% 2-ME, and loaded sideways onto 10% polyacrylamide slab gels.

Results

Time-course Labeling and Precipitation of Receptor Molecules.   A previous ultrastructural study (15) detected a frequent cortical thymocyte with a high level of cytoplasmic material reactive with an anti-V_β reagent. We designed experiments to characterize this material biochemically. In our initial studies, cortical cells were enriched by using thymocytes from newborn mice, since at this age virtually no cells express surface receptor at the high level seen on mature medullary T cells (14). Cells were labeled with [^{35}S]cysteine and surface receptors were precleared by treating the labeled cells with the anti-V_β mAb, F23.1, before detergent lysis and precipitating with protein A–Sepharose after washing and lysis. Then the internal receptor was collected on beads preloaded with F23.1. Fig. 1 shows the two-dimensional (nonreduced vs. reduced) SDS-PAGE analysis of the precipitates at several time points over a 4-h labeling with [^{35}S]cysteine. The α and β chains of mature T cell receptors are disulfide-linked to form an 85-kD heterodimer. The disulfide bonds are broken before the running of the second dimension gels, and hence, the two chains migrate to their individual position, both around 43 kD. Therefore, these two-dimensional gels can discriminate between multimeric molecules, which appear as multiple species below the diagonal, and unassembled or monomeric species, which are found on the diagonal.

Even at 4 h very little labeled receptor was observed at the surface of the cells, indicating a long transit time for receptor through the cell to the surface. Internally, the vast preponderance of specifically precipitated material was seen as three rapidly labeling species on the diagonal, at 44, 41, and 38.5 kD. In additional experiments these molecules have been observed after only 2 min of labeling (data not shown). This was the first suggestion that incompletely assembled receptor molecules could be precipitated by any of our antireceptor antibodies or antisera. In addition, in the cytoplasm of these cells assembled α/β chains were labeled as well, but at a much lower level. This material was not always visible without extended exposure of the autoradiograms.

Specificity Control for Precipitation Reactions.   To ensure that the precipitated molecules were indeed receptor related and not merely artifactual, a similar
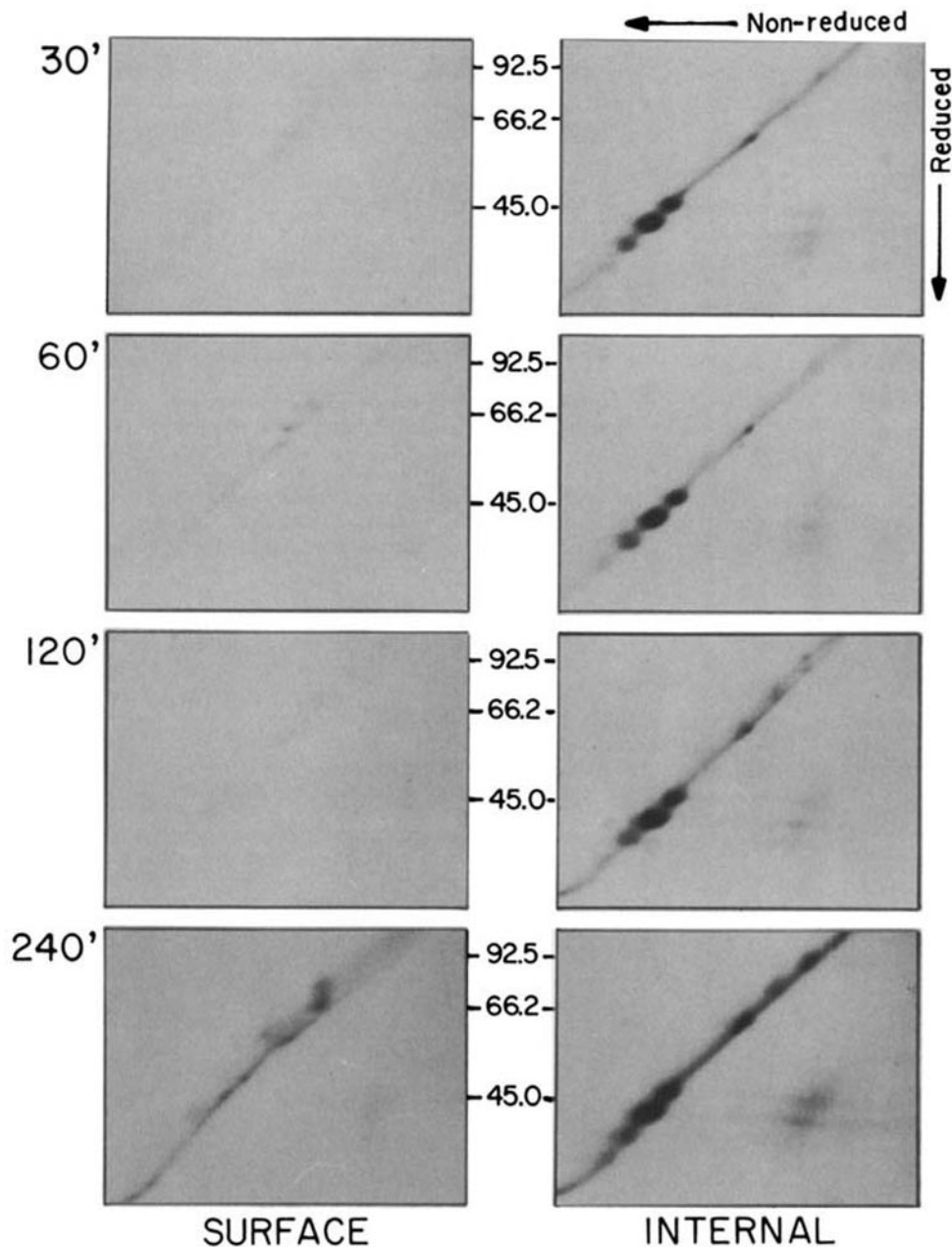
FIGURE 1. Two-dimensional SDS-PAGE (nonreduced followed by reduced) of F23.1 precipitates of newborn thymocytes metabolically labeled with [^{35}S]cysteine for the indicated length of time. Figures on the left are F23.1 preclears of surface material, while those on the right are the residual receptor molecules precipitated from the cytoplasm.

analysis was carried out on a precipitate obtained using an antibody specific for a different T cell protein. Fig. 2a shows that an anti-Thy-1 monoclonal, T24/40, did not precipitate the species seen in Fig. 2b, when F23.1 was used. It can also
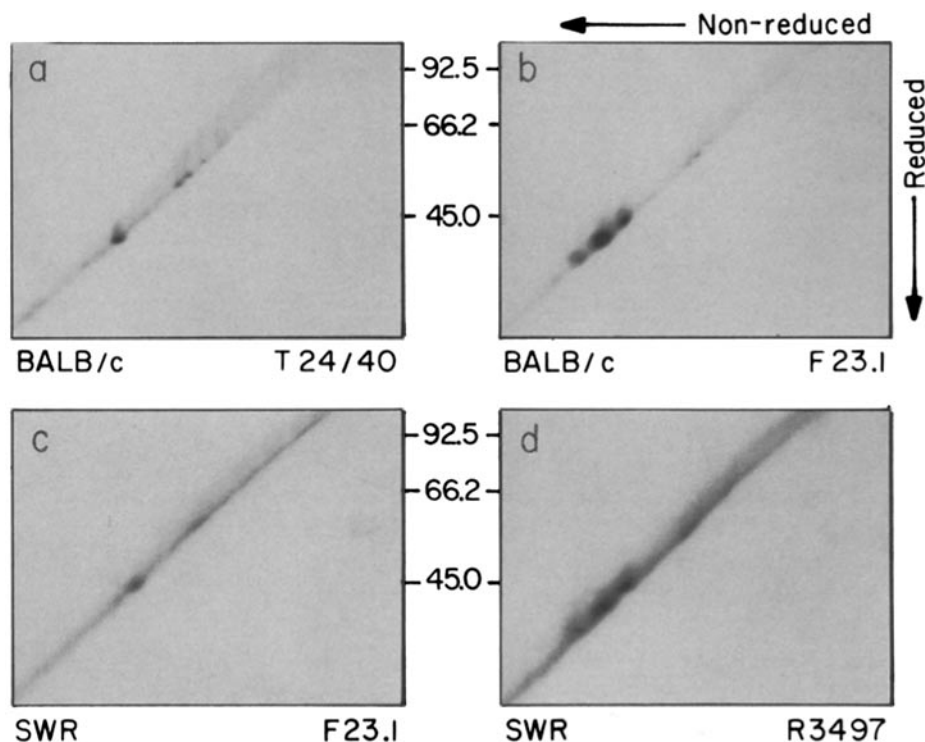
FIGURE 2.   Two-dimensional SDS-PAGE (nonreduced followed by reduced) of (a) T24/40 (anti-Thy-1) precipitate of [^{35}S]cysteine-labeled BALB/c thymocytes, (b) F23.1 precipitate of ^{35}S-BALB/c thymocytes, (c) F23.1 precipitate of ^{35}S-SWR thymocytes, and (d) R3497 precipitate of ^{35}S-SWR thymocytes.

be seen that the 60-min labeling of these BALB/c newborn thymocytes was insufficient time to label the Thy-1 molecule significantly. Since Thy-1 is a predominant surface protein on these cells it is apparent that the precipitated β chain–containing molecules were being synthesized at a very high rate. Fig. 2c shows that when thymocytes from SWR mice, which are missing the V$_β$8 gene family (12), were similarly labeled and precipitated with F23.1 these molecules were absent. R3497, however, which precipitates all receptors regardless of the V$_β$ used, continued to precipitate these species. These results showed that these molecules were receptor related and that at least one of them was a receptor β chain.

Identification of the On-diagonal Molecules.   To determine which of the three on-diagonal species were receptor chains, and which, if any, were associated nonreceptor molecules, NEPHGE/SDS-PAGE two-dimensional analyses were performed on the R3497 precipitate from ^{35}S-labeled (60 min) newborn thymocytes. Fig. 3a shows that each of the three species was composed of a very charge-heterogeneous group of basic molecules, but that the sizes of the molecules within each group were very homogeneous. The receptor purified from ^{125}I surface-labeled newborn thymocytes was similarly analyzed (Fig. 3b) and showed size and charge heterogeneity among the basic β chains, as well as the less well-resolved acidic α chains, that was identical to that seen with surface
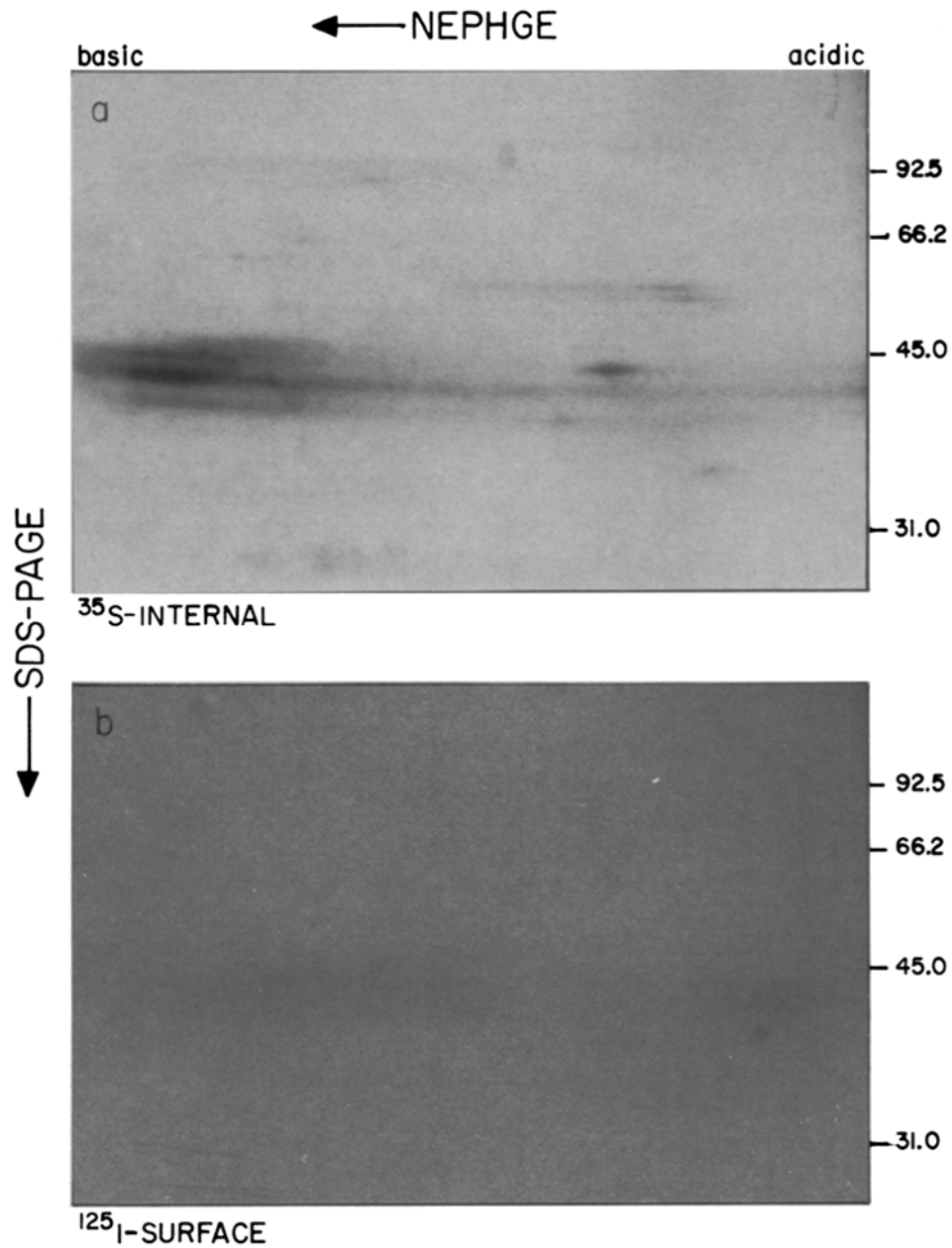
FIGURE 3. Two-dimensional gels (reduced NEPHGE followed by SDS-PAGE) of R3497 precipitates from newborn thymocytes: (a) metabolically labeled for 60 min with [^{35}S]cysteine or (b) surface labeled with ^{125}I. The pH gradient of the NEPHGE runs from 4.0 on the right to 8.8 on the left.

receptor on peripheral T cells (not shown). These results showed that the rapidly labeling receptor-related molecules in immature thymocytes were three forms of β chains without associated α chains, probably at distinct stages of glycosylation.
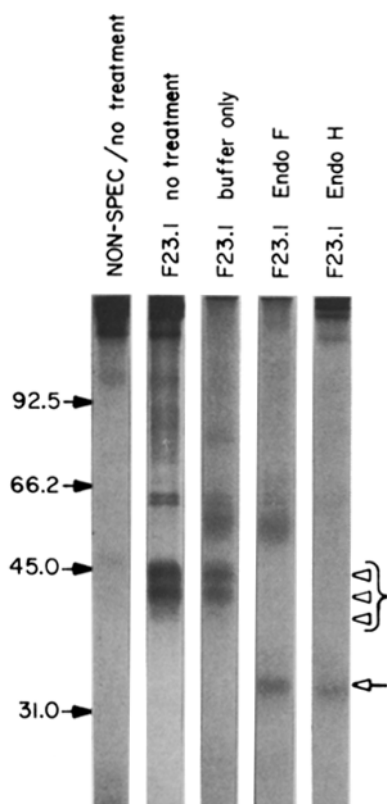
FIGURE 4. Nonreduced SDS-PAGE of material precipitated from [³⁵S]cysteine-labeled BALB/c newborn thymocytes. From the left these include material bound to and eluted from protein A beads alone, untreated F23.1-precipitated material, F23.1 precipitate incubated for 20 h at 37°C in Endo F buffer without enzyme, F23.1-precipitate digested with endoglycosidase F, and F23.1 precipitate digested with endoglycosidase H. The Endo F reaction buffer contained 2-ME while the Endo H buffer did not. (*Open arrowheads*) the positions of the internal β chains at 44, 41, and 38.5 kD; (*stemmed open arrow*) the reaction product at 33 kD.

Note also that the average charges of these early β chains were more basic than seen with surface β chains, suggesting that terminal sialylation had not yet occurred.

It is of interest that R3497, KJ16, and F23.1 all precipitated this material. The determinant recognized by KJ16 has been shown previously (11) to be stable to reduction of the β chain but not to its denaturation with either SDS or urea. This suggested that these thymocyte cytoplasmic β chains were in a somewhat native conformation, either as free β chains or in association with some unlabeled component. However, no molecule analogous to the 78-kD Ig binding protein (BiP) of pre-B cells (23, 24) was seen to be associated with these β chains.

Endoglycosidase Digestion of Unassembled β Chains. To assess the maturity of the carbohydrates linked to these β chains, whole immunoprecipitates of the ³⁵S-labeled newborn thymocyte receptor were digested with either endoglycosidase F, which cleaves both immature high mannose and mature complex N-linked oligosaccharides, or endoglycosidase H, which cleaves only N-linked sugars of the high mannose forms. Fig. 4 shows that both enzymes digested the receptor chains in nearly identical fashion, with almost all the receptor material moving to 33 kD, the approximate size of the β chain polypeptide. A weak reaction product was also present with both enzymes at 35 kD. The fact that the contaminating nonreceptor bands around 62 kD were not altered indicated that proteolytic degradation had not occurred. These results showed that virtually all
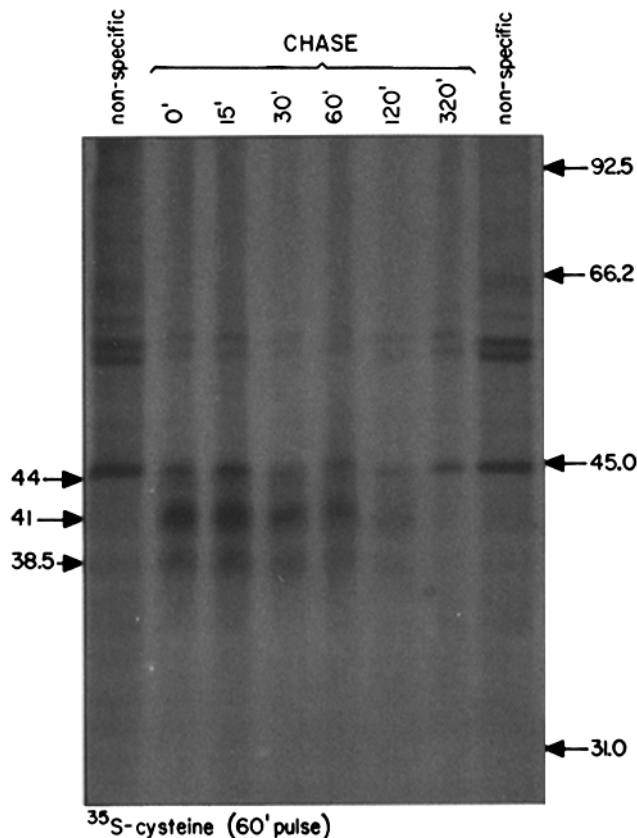
FIGURE 5. Nonreduced SDS-PAGE of F23.1 precipitates of BALB/c newborn thymocytes pulsed for 60 min with [^{35}S]cysteine and chased with cold cysteine for the indicated lengths of time. The sizes of the three internal β species are indicated on the left. Nonspecific precipitates are material bound to and eluted from protein A beads alone.

of the unassembled β chains in immature thymocytes were polypeptides with immature oligosaccharides attached, and suggested that transport through the Golgi apparatus to the surface, with accompanying oligosaccharide modification to complex forms, had not occurred in these cells, perhaps due to the absence of an accompanying α chain. Therefore, transport of the β chains may be blocked in what, according to the ultrastructural pictures, appears to be the endoplasmic reticulum.

Fate of Internal Receptor Material. The fact that 4 h of metabolic labeling of immature thymocytes resulted in very little detectable surface expression of newly synthesized receptor molecules made the fate of the rapidly labeling internal β chains unclear. Newborn thymocytes were labeled with [^{35}S]cysteine for 60 min and then chased in an identical medium containing a 10^4-fold excess of cold cysteine. Fig. 5 shows that over several hours of cold chase the three species all virtually disappeared, and at the same relative rate. None of the species were chased into either of the other two, and no detectable counts are chased to the position of assembled dimeric receptor molecules at 85 kD. Therefore, although newly synthesized receptor molecules are capable of transport to the surface of tumor cells in 20 min (25), immature thymocytes appear to make and degrade β chains very rapidly. This, along with the electron micrographic evidence of Farr et al. (15), suggested that the majority of the receptor observed

with KJ16 inside thymocytes was a rapidly turning-over pool of immature β chains in the endoplasmic reticulum.

Purification and Analysis of Thymocyte Subpopulations. Our experiments established that some proportion of thymocytes synthesized a great deal of internal unassembled β chains. Previous work had shown that about half of all adult thymocytes do bear surface receptors (14), and also that a very significant fraction of cortical thymocytes contained internal receptor protein (15). We then designed experiments to determine whether this unassembled β chain was in cortical cells with no surface receptor (apparent pre-T cells) or accumulated as excess free β chain in those cortical cells synthesizing low levels of surface α/β receptor.

To obtain thymocyte subpopulations of varying states of maturity and surface receptor expression, adult BALB/c thymocytes were fractionated by panning on lectin and antibody-coated plates. Mature thymocytes (PNA⁻) were obtained by exhaustive adsorption of immature cells on plates coated with PNA. The adherent immature cells were then further fractionated into surface receptor–positive and –negative populations by panning on plates coated with F23.1. Each population was then metabolically labeled for 60 min with [^{35}S]cysteine and its internal receptor forms were precipitated and analyzed on two-dimensional gels. The cell equivalents in each precipitation were identical except with the PNA⁺ F23⁺ population, where a group with only one-fifth as many cell equivalents was included. This was because in a normal population, ~20% of cells are F23.1⁺, whereas in this set of selected cells, 100% were F23.1⁺. Also included were analyses of the receptors inside cortisone-resistant cells, the most mature cells in the thymus.

Unfractionated adult thymocytes (Fig. 6a) showed high levels of internal unassembled β chains, consistent with the fact that 90% of the cells in an adult thymus are immature. The PNA⁺ F23⁻ cells (Fig. 6b), the most immature cells of the fractionated subpopulations, bore no detectable F23.1⁺ surface receptor yet contained high levels of unassembled β. The PNA⁺ F23⁺ cells (Fig. 6, c and d), which are cortical cells with low levels of surface receptor, had much less of the unassembled β pool. The purified medullary thymocytes, PNA⁻ (Fig. 6f) or cortisone-resistant (Fig. 6e) cells, had very low levels of unassembled β chains.

These results indicated that receptor synthesis in very immature thymocytes begins with rapid production of β chains alone. To check this we adopted an alternate method of examining receptor expression in immature cells, using fetal thymocytes. Day 16 fetal thymocytes have no surface receptor and virtually no detectable α chain message, yet a long exposure of the two-dimensional electrophoresis of the F23.1 precipitate of ^{35}S-labeled day 16 cells revealed a small pool of unassembled β chains (Fig. 6g). By day 17, the first day that traces of surface receptor can be found, this β chain pool had greatly increased in size and was in fact similar in magnitude to that seen in neonatal or adult thymocytes (Fig. 6h). This burst of β chains was consistent with the rapid accumulation of potentially functional β chain rearrangements occurring at this time (6). Since the rate of surface expression of mature receptors is very slow over the course of fetal development (14), this burst of unassembled β chain between days 16 and 17 was perhaps the best evidence for the existence of pre-T cells, i.e., cells containing one chain (β) of the T cell receptor but not the other.
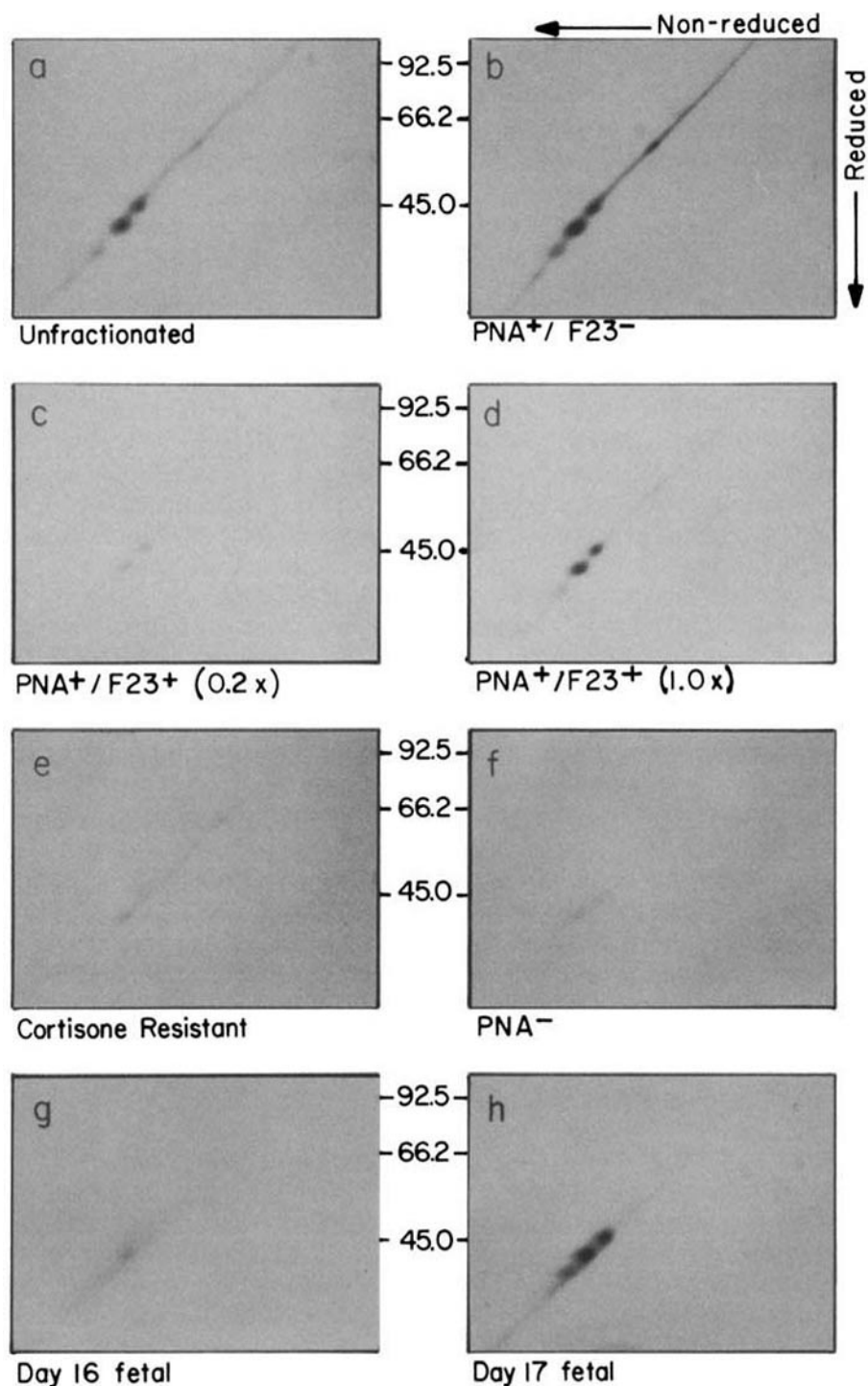
FIGURE 6. Two-dimensional SDS-PAGE (nonreduced followed by reduced) of F23.1 precipitates of [³⁵S]cysteine-labeled (a) unfractionated adult BALB/c thymocytes, (b) adult thymocytes that bound to PNA-coated plates but not to F23.1 plates, (c and d) adult thymocytes that bound to PNA plates and F23.1 plates, (e) cortisone-resistant adult thymocytes, (f) adult thymocytes that did not bind to PNA plates, (g) day 16 fetal BALB/c thymocytes, and (h) day 17 fetal thymocytes. a–c, e, and f are directly comparable, while d contains five times as much material as c and is included to show that PNA⁺/F23⁺ cells do contain small amounts of internal unassembled β chains. g is a comparatively much longer exposure to show that day 16 fetal thymocytes also contain unassembled β chains.

Receptor Synthesis in a T Cell Hybridoma. To obtain a picture of the comparable pools of receptor forms in cells that bear large amounts of surface receptor, a time-course label identical to that done with newborn thymocytes and shown in Fig. 1 was performed with the T cell hybridoma DO-11.10/S4.4, a mouse hybridoma with specificity for chicken OVA plus I-A^d. Fig. 7 shows that in this hybridoma, made from a lymph node T cell, the internal β pool was observable at 30 min of labeling but that the internal dimeric receptor pool was also very well represented. At 60 min of labeling both the free β and internal dimer pools were even more heavily represented, while the preclear of surface receptor showed only the slightest hint of typically diffuse surface receptor. By 120 min of labeling, the internal pools of free β and immature dimers plateaued, and the surface preclear revealed a strong surface receptor spot of the typically diffuse nature due to glycosylation heterogeneity. At 240 min the internal picture remained basically unchanged while the precleared surface receptor spot was much stronger.

In summation, this experiment defined three pools of receptor forms detected with our antireceptor antibodies. First, were the unassembled, immature β chains, apparently confined to the cytoplasm in the absence of α chain partners. Second, were internal α/β dimers that also appeared to be immature due to their distinct sizes, indicating that very little sugar modification had yet occurred. Third, was the surface receptor which had had its high mannose sugars modified to complex forms during passage to the cell surface. In experiments with ^{125}I surface-labeled receptor from DO-11.10/S4.4 (data not shown), it was found that all of the surface receptor was resistant to endoglycosidase H, showing that all its sugars had been converted to complex forms.

Discussion

In attempting to understand the expression of T cell receptor proteins in the immature cells of the thymus one must struggle with the complicating fact that a thymus contains a spectrum of cells in various states of differentiation and, more importantly, that the window to these events is opened only as wide as is permitted by the set of specificities in the antireceptor antibodies available. Fortunately, the thymus is in large part populated by immature cells (26). Specifically, in an adult thymus only ~15% of thymocytes are medullary in phenotype, that is PNA$^-$, cortisone-resistant, L3T4$^+$/Ly-2$^-$ or L3T4$^-$/Ly-2$^+$, and with surface receptor at levels equivalent to those seen on peripheral T cells. The remaining 85% of thymocytes fall into the broad category of cortical cells, that is PNA$^+$, cortisone-sensitive, L3T4$^+$/Ly-2$^+$ or L3T4$^-$/Ly-2$^-$, and expressing somewhere between 0 and 10% of the level of surface receptor seen on peripheral T cells. In a newborn thymus there are virtually no cells with the medullary phenotype, so analysis of immature cells is greatly simplified by looking at newborn thymocytes, which allow a fairly clear observation of receptor synthesis in immature cells without the inclusion of a cell fractionation step.

The approach used in this study was to metabolically label immature and mature thymocytes and to analyze all surface and internal receptor forms precipitable with the antireceptor antibodies available to us. Our results lead us to two major conclusions. First, cytoplasmic β chains are rapidly synthesized and
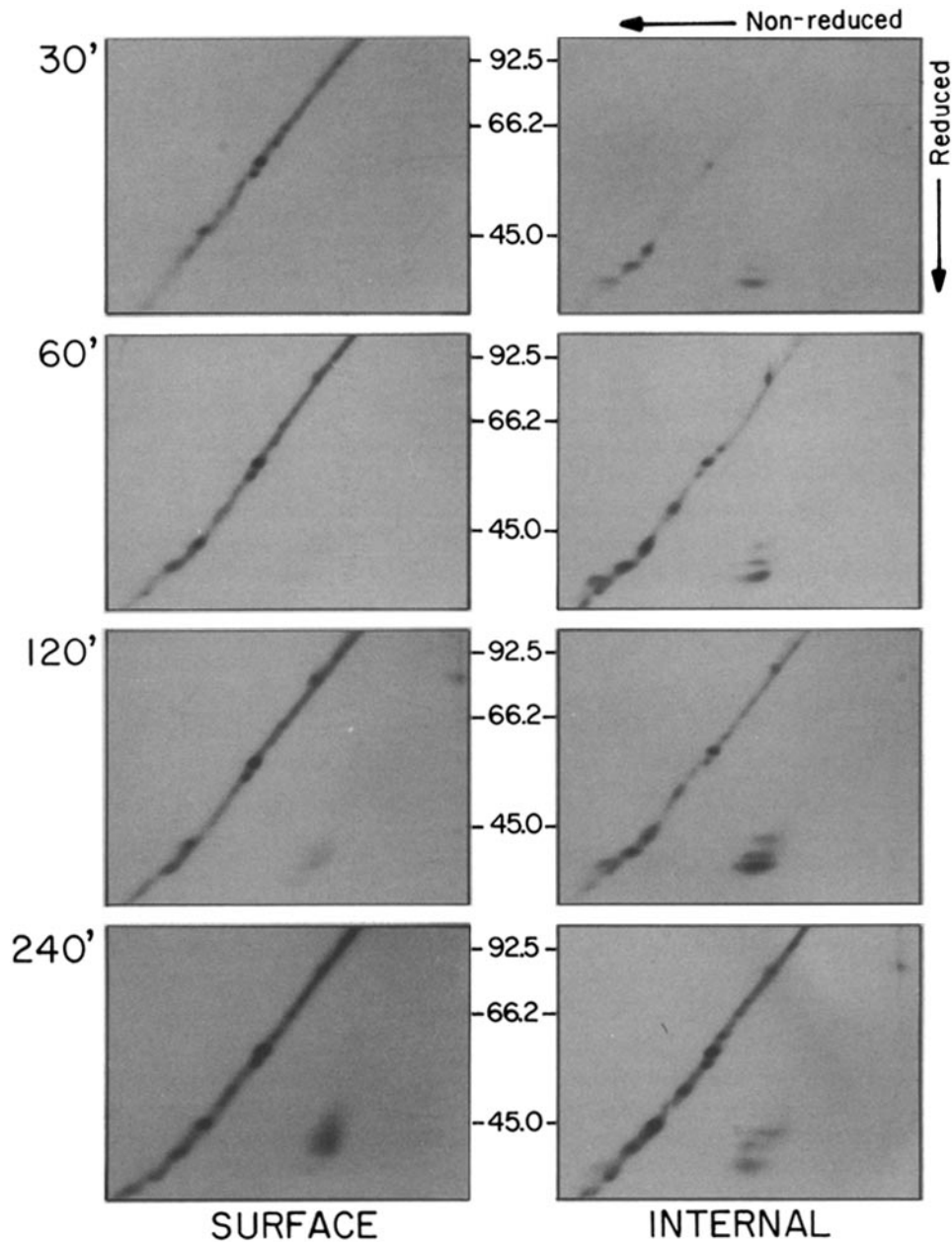
FIGURE 7.   Two-dimensional SDS-PAGE (nonreduced followed by reduced) of F23.1 precipitates of the T cell hybridoma DO-11.10/S4.4 that was metabolically labeled with [^{35}S]cysteine for the indicated lengths of time. Figures on the left are F23.1 preclears of surface material, while those on the right are the residual receptor molecules precipitated from the cytoplasm.

degraded in cells that express no surface α/β receptor and precede cells with surface α/β receptor during ontogeny. Second, the accumulation of this free β chain pool drops precipitously in both cortical and medullary cells that express

Selection
and
Tolerance

β   α   αβ low   Survivors   αβ high
Rearrangement   Rearrangement

○   βhigh   βlow   βlow

Earliest   Pre-T cell   Immature   Mature
Thymocyte   T cell   >90%   T cell
                     PNA⁺ F23⁻   PNA⁺ F23⁺   death   PNA⁻ F23⁺

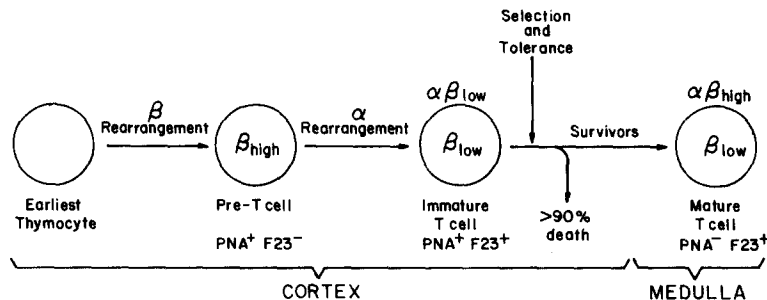   CORTEX   MEDULLA

FIGURE 8. Model for T cell Ag/MHC receptor synthesis, assembly, and surface expression during thymocyte differentiation that is consistent with our experimental results.

surface α/β receptor. Taken together with previous results on the distribution and kinetics of α/β surface expression, these results are consistent with the pathway of expression shown in Fig. 8 in which the precursor of thymocytes with surface α/β receptor is a pre-T cell that has rearranged and expressed β chain. We should stress, however, that none of our experiments establish directly the precursor/product relationship among the subpopulations shown in Fig. 8.

Similar analyses of the receptor forms in the T cell hybridoma DO-11.10/S4.4 delineated three pools of receptor-related molecules, the unassembled immature β chains, the immature internal α/β dimers, and the mature surface α/β receptors. The assembled forms appeared very quickly during the time-course labeling at levels much greater than seen in newborn thymocytes, supporting the view of receptor synthesis, assembly, and surface expression in mature cells obtained from pulse-chase experiments with the mouse T lymphoma C6VL (25). In these cells both the α and β chains are synthesized within 2 min of labeling and are immediately disulfide linked, glycosylated, and rapidly processed, with the idiotype appearing within 10 min.

There are positively charged amino acids within the transmembrane region of the α and β chain (27, 28). Corresponding negatively charged amino acids occur in several of the components of T3 (29). It has been suggested (29) that pairing of these amino acids is essentially the stable assembly and transport of the $\alpha/\beta/T3$ complex to the cell surface. The failure of β chain to be transported to the surface in pre-T cells may, therefore, be due both to the absence of α-chain and to its failure to associate with a stabilizing T3 component.

Our results paint a picture of receptor expression in surface receptor–negative thymocytes similar to that observed with Ig synthesis in pre-B cells, where heavy chain synthesis precedes that of light chain (10). In pre-B cells, the heavy chain is synthesized on the rough endoplasmic reticulum where it is cotranslationally glycosylated during movement into the lumen. In the absence of light chain, the heavy chain is noncovalently bound by a 78-kD BiP and does not move out of the endoplasmic reticulum, through the Golgi with concomitant oligosaccharide modification, and to the surface until light chain is synthesized and displaces BiP. It is thought that movement of Ig through the various compartments involves specific receptors that recognize the heavy chain/light chain complex (30). This is probably the same scenario that is being followed by these pre-T cells, where β chain synthesis precedes that of the α chain. The newly synthesized β chains,

with their immature high-mannose oligosaccharides, are not recognized by the receptors that allow movement out of the endoplasmic reticulum, through the Golgi, and to the surface. Consequently these immature β chains accumulate either inside the endoplasmic reticulum or between the endoplasmic reticulum and the Golgi in anticipation of the synthesis and translation of α message. Once α chains are made, α/β dimerization takes place very rapidly, and movement through the Golgi occurs with accompanying modification of the N-linked oligosaccharides to produce complex sugars with sialic acids intact.

One apparent difference between Ig and T cell receptor synthesis is the absence in the pre-T cell of the analogue of BiP, though it is possible that the conditions of lysis and immunoprecipitation used in these studies may dissociate this noncovalent interaction. It is also possible that a component of T3 may fulfill the task of BiP in thymocytes, though it is not known that such a requirement exists.

Finally, these experiments confirm that the thymic cortex is the site where receptor expression begins (6, 14), thus supporting a theory of repertoire development in which the selective processes act on a subpopulation of receptor-positive immature thymocytes (31, 32). It is known that there is a great deal of cell death in the cortex and that even most of the receptor-positive cortical thymocytes do not fully mature (33). Whether this is due to a failure to be selected (poor affinity for self) or tolerance (high affinity for self) remains unknown. We can now say, however, that α/β receptors of apparently identical biochemical structure to those found on peripheral T cells are present on the surfaces of these immature thymocytes, though at much lower densities.

Summary

T cell receptor synthesis in thymocytes was examined by the differential immunoprecipitation of receptors from the surfaces and interiors of metabolically labeled newborn and adult thymocytes. Precipitated molecules were then analyzed for size, charge, and state of glycosylation.

Our experiments identified cells within the thymic cortex that contained a large pool of cytoplasmic-free receptor β chain. The β chain in this pool was synthesized and degraded rapidly and bore only high-mannose N-linked oligosaccharides. This pool was found predominantly in cells that lacked surface α/β receptors and appeared in ontogeny before cells expressing surface α/β. These results are consistent with a model in which the progenitor of cells with surface α/β expression is the T cell equivalent of the pre-B cell, which has rearranged and expressed β chain, but not α chain.

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