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In Vitro Maintenance of Differentiation Marker Synthesis by Subpopulations of Mouse Thymocytes

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Mouse thymocyte populations enriched in functionally incompetent, "immature" cells on the one hand, or in competent "mature" cells on the other hand, express different steady-state levels of certain surface antigens and marker enzymes. In the cases of the glycoproteins H-2 (K and D), Qa, and TL, and the DNA polymerase terminal deoxynucleotidyl transferase (TdT), these levels reflect different rates of de novo synthesis in the two populations. Thus each population appears to manifest a characteristic pattern of synthetic rates for the various products relative to total protein synthesis. To investigate the maintenance of these patterns, enriched pools of "immature" and "mature" thymocytes were incubated in vitro for 24 h, and the rates of product synthesis before and after culture were compared. H-2 synthesis, initially most rapid in the mature cells, continued to be made at the highest rate in this population. TdT synthesis, a characteristic activity of the immature cells, was not induced in the mature cells, but proceeded at an increased relative rate in the immature population. Therefore, the differences between the rates of H-2 and TdT synthesis were stable properties of the two thymocyte populations. Another marker of immature cells, TL, did not continue to be produced in parallel with TdT. Rather, its synthesis was selectively curtailed in relation to the continuing protein synthesis in the immature cultures. This non-coordinate regulation of TL and TdT production in immature thymocytes may be due to several mechanisms. These are discussed with regard to their implications for pathways of thymocyte maturation.

Key words: **thymocyte subpopulations, differentiation antigens, PNA fractionation, density gradient, biosynthetic labeling, short-term culture, non-coordinate regulation**

Mammalian T lymphocytes undergo crucial maturation processes in the thymus [1, 2]. Lymphoid cells isolated from the thymus comprise at least two subpopulations, which are distinguishable by their antigenic and physical properties and by their immunologic

Abbreviations: endo H, endo-β-N-acetylglucosaminidase H; HEPES, N-2-hydroxyethyl piperazine-N′-ethanesulfonic acid; PBS, Dulbecco's phosphate-buffered saline; PNA, peanut agglutinin; SDS, sodium dodecyl sulfate; TdT, terminal deoxynucleotidyl transferase; TL, thymus-leukemia.

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responsiveness [3]. These phenotypes probably correspond to different extents of maturation along one or more developmental pathways. Therefore, one may gain access to biochemical events involved in T cell maturation by defining specific molecular differences between the cells in various thymic subpopulations.

In contrast to those of B cells, the receptors and effector molecules involved in T cell responses are unknown. Consequently, the known T cell developmental markers simply correlate with immune functions; they do not necessarily participate in them. With this reservation, useful molecules to study are the glycoproteins bearing differentiation antigens, which are expressed to different extents in the various classes of thymocytes [3]. Examples of these are TL, displayed only by "immature" thymocytes, and H-2 K and D, expressed at highest levels on "mature," functionally competent cells. Another developmentally regulated marker is the template-independent DNA polymerase terminal deoxynucleotidyl transferase (TdT). This activity is found mainly or exclusively in immature thymocytes and in a small number of bone marrow cells [4–6]. If the expression of each marker is turned on and off at a different stage, unique patterns of combined marker display should characterize the cells at many points in their maturation.

Studying separated immature and mature populations of thymocytes, we recently found that the differences in their respective steady-state displays of H-2, TL, and TdT corresponded to differences in the rates at which these molecules were synthesized [7]. Thus the differentiation antigen phenotype seems to be regulated at a translational or pretranslational level. The purpose of the present study has been to investigate the stability of these biosynthetic phenotypes and to ask whether expression of these products is regulated coordinately or independently.

MATERIALS AND METHODS

Animals and Cells

C57BL/6-*Tla*^{a} (B6-TL$^+$) mice were bred in the animal colony at the Salk Institute from stock originally provided by Dr. E. A. Boyse (Memorial Sloan-Kettering Cancer Center, NY). They were used between 3 and 6 weeks of age. ASL1.1 lymphoma cells, adapted to culture from the spontaneous A strain leukemia ASL1, were kindly provided by Dr. Robert Hyman (Salk Institute).

Fractionation of Thymocyte Populations

Procedures were essentially as described before [7,8]. Briefly, thymuses were dissected free of lymph nodes and minced into phosphate-buffered saline (PBS) or Dulbecco's modified Eagle medium with HEPES buffer. The thymocytes thus released into single-cell suspension were washed and fractionated by flotation in discontinuous isopycnic gradients of bovine serum albumin (Path-o-cyte 5, Miles Research Products) as described elsewhere [7]. The A and B layers, constituting about 2% of the input cells (>90% viable) were pooled to give the "light" fraction. The D layer, with about 25% of the input cells (ca 80% viable), was taken as the "dense" fraction. Half of these dense cells were treated with peanut agglutinin [9] (PNA; Vector Laboratories, Burlingame, CA), at final concentrations of 0.5 mg lectin/ml and 2×10^8 cells/ml. The aggregates were isolated by settling through 20% or 50% heat-inactivated fetal calf serum at room temperature, and dispersed by washing in 0.2 M galactose in PBS. About 45% of the

dense thymocytes were recovered in this "dense, PNA^+" fraction with viabilities of >90%. During these fractionation procedures, a sample of the unfractionated thymocytes was kept on ice in HEPES-buffered Dulbecco's modified Eagle medium.

Cell Culture and Radiolabeling

One-third of each cell sample was labeled immediately with [^{35}S] methionine (500 μCi/ml; Amersham), harvested, and lysed as described previously [7,8]. The remainder was cultured overnight at 37°C in RPMI 1640 (Grand Island Biological Co.) with 10% heat-inactivated fetal bovine serum (Hy-clone lot number 100209, Sterile Systems, Logan, UT), 5×10^{-5} M 2-mercaptoethanol, and 2 mM L-glutamine, in a humidified atmosphere of 5% CO_2. Cell concentrations were $2–2.5 \times 10^7$/ml, or 1×10^7/ml for the light cells. After culture for 24 h, the cells were washed in PBS and labeled as before. Phenylmethylsulfonyl fluoride (Sigma) was added freshly to the lysis buffer to inhibit proteases.

Radioimmune Precipitation

Procedures and antisera were exactly as described before [7]. The monoclonal antibody against the TL.3 specificity, 18/20 [10], was kindly provided by Dr. Ulrich Hämmerling (Memorial Sloan-Kettering Cancer Center). Cell lysates were precleared by ultracentrifugation in the presence of fixed Staphylococcus aureus Cowan I strain (Pansorbin, Calbiochem-Behring), and the amount of acid-precipitable radioactivity in each supernatant was determined. Radioactive inputs for each immune precipitation sample are given in the figure legends. To distinguish TL from Qa, endo-β-N-acetylglucosaminidase H (endo H) treatment of the precipitated samples was carried out as described before [7,8], using enzyme generously donated by Phillips W. Robbins (Massachusetts Institute of Technology, Cambridge) at a concentration of 3 μg/ml.

Gel Electrophoresis

Samples were analyzed by electrophoresis on SDS-polyacrylamide slab gels as described elsewhere [7,8]. The gels were fixed and stained, processed for fluorography [11] and dried, and then exposed to presensitized X-ray film [12] (Kodak X-O-Mat R) at -70°C. The migration of the TL band, both before and after endo H digestion, appeared more diffuse in gels containing Matheson, Coleman, and Bell SDS than in gels with Bio-Rad SDS [cf 7,8].

RESULTS

The rate of synthesis of a specific product was estimated by monitoring the incorporation of [^{35}S]methionine into molecules that could be precipitated by the appropriate antibody and identified by electrophoresis in SDS-polyacrylamide slab gels. To minimize the influence of post-translational events on the yield of labeled product, and to approximate the instantaneous rate of synthesis, the labeling period was restricted to 30–40 min. Yields of product made in different cell samples were compared relative to constant amounts of incorporated radioactivity – ie, relative to constant amounts of newly-synthesized protein. Thus the analysis was weighted in favor of the contributions of the more biosynthetically active cells.

The TdT polypeptide, about 60,000 daltons, was detected with a rabbit antiserum against calf TdT, which crossreacts with the mouse enzyme [13]. H-2 K and D antigens, which are not considered separately here, were precipitated with an alloantiserum against the entire H-2^b haplotype [7]. Analysis of TL expression was complicated by the fact that the TL antiserum recognizes a second antigen, Qa-1, with a tissue distribution significantly different from that of TL [7,8,14,15]. Fortuitously, the TL and presumptive Qa-1 ("Qa") glycoproteins have similar apparent molecular weights. This similarity, however, results from the linkage of different-sized polypeptides to compensatingly different numbers of carbohydrate chains [8; E. Rothenberg and D. Triglia, manuscript in preparation]. The two glycoproteins were distinguished here by digestion with endoglycosidase H (endo H), which removes high-mannose carbohydrate chains, as described below.

Pulse-labeled TL and Qa migrate slightly more slowly than actin, with apparent M_r of 44,000–45,000 daltons (Fig. 1, lane 3; cf lane 1). Treatment of these samples with endo H destroyed the 44,000–45,000 dalton band and revealed two digestion products in the TL antiserum precipitate, with M_r of about 40,000 and about 35,000 daltons (Fig. 1, lane 4). Only one of these represented the TL glycoprotein backbone, as shown when a monoclonal antibody against TL was used instead of the oligospecific TL antiserum (Fig. 1, lanes 5 and 6). This defined the 40,000 dalton molecule as the digestion product of TL glycoprotein; the 35,000 dalton molecule, which is also made in peripheral lymphoid tissues and cortisone-resistant thymocytes [7,8], came from the Qa glycoprotein.

Because of its specificity for high-mannose chains, endo H could not be used to analyze cell-surface forms of these glycoproteins. Before transport to the cell surface, TL and Qa glycoproteins undergo carbohydrate processing, which leaves them with exclusively endo H-resistant, complex-type oligosaccharide chains [8; manuscript in preparation]. Thus TL glycoprotein labeled with ^{125}I on the cell surface was not susceptible to endo H digestion (Fig. 1, lanes 7–10). Surface-labeled Qa was similarly resistant (not shown). In addition, the carbohydrate-free protein actin, nonspecifically precipitated by normal mouse serum, was insensitive to endo H, as expected (Fig. 1, lanes 1 and 2).

Fractionation of Cell Populations

The immunologically competent subpopulation of thymocytes can be separated from the immature majority by several methods. In vivo cortisone treatment eliminates the majority of thymocytes, sparing the mature cells [16]. The mature cells tend to be lower in buoyant density than the small, dense immature cells; furthermore, the lectin peanut agglutinin (PNA) agglutinates immature thymocytes but fails to bind to the functionally competent cells [9,17,18]. The majority and minority populations isolated by different methods may not correspond exactly, but they are very similar. In each case [3,5,6,17, 18], the majority of cells express unusually high levels of Thy-1 and low levels of H-2, easily detectable surface TL in appropriate mouse strains, and appreciable intracellular activity of TdT. The minority show about tenfold higher levels of H-2 K and D, with TdT and TL correspondingly reduced to the threshold of detection and Thy-1 reduced to the levels typical of peripheral T cells. The rates of synthesis of TL, TdT, and H-2 differ sufficiently in the majority and minority populations to account for these phenotypic differences [7] (see also Fig. 2, lanes 1 and 5; Fig. 3, lanes 1 and 5; and Fig. 4, lanes 2 and 5). Thus, not only the steady-state levels of these molecules but also their instantaneous rates of production serve to distinguish populations of cells in different developmental stages. Tentatively, then, we can regard the combined labeling rates of this panel of marker molecules as defining a biosynthetic phenotype for a given cell population.

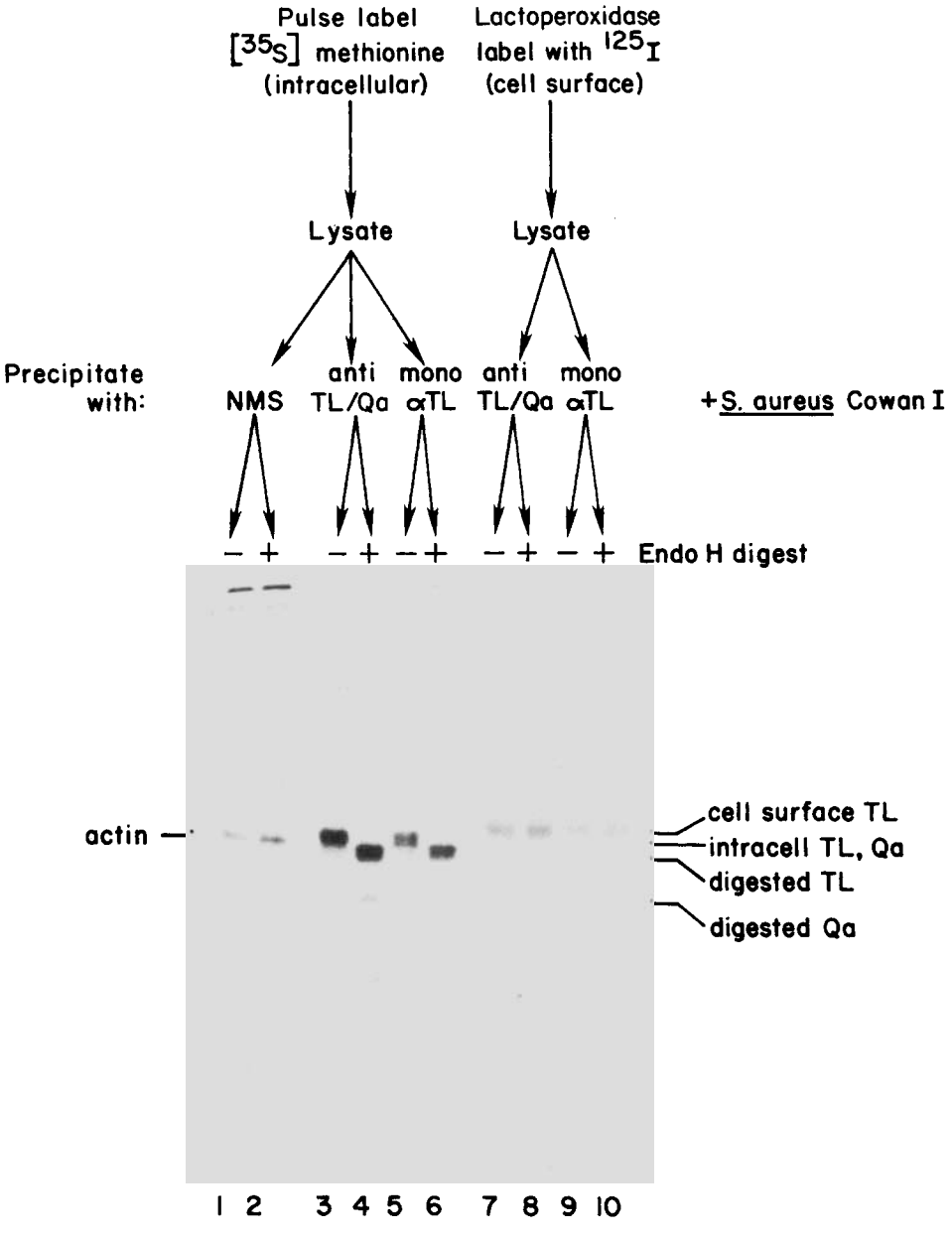

Fig. 1. Resolution of TL and Qa glycoproteins by endoglycosidase H digestion. Lanes 1–6: B6-TL$^+$ thymocytes were metabolically labeled with [^{35}S] methionine for 40 min and lysed. Aliquots of lysate were incubated with normal mouse serum (lanes 1, 2), conventional TL alloantiserum (αTL/Q_a) (lanes 3, 4), and monoclonal antibody against the TL.3 specificity (lanes 5, 6). The samples were split after precipitation and incubated with or without 3 μg/ml of endo H for 19.5 h at 37°C. Each lane contains the antigen recovered from 3 × 10^6 cpm of acid-precipitable radioactivity.

Lanes 7–10: ASL1.1 tumor cells were radioiodinated with lactoperoxidase and lysed as described previously [8]. Portions of the lysate were immune-precipitated and treated or not treated with endo H, as indicated. Each sample represents the precipitate from 7.8 × 10^4 protein-bound cpm. Fluorography of all lanes was for 5 days.

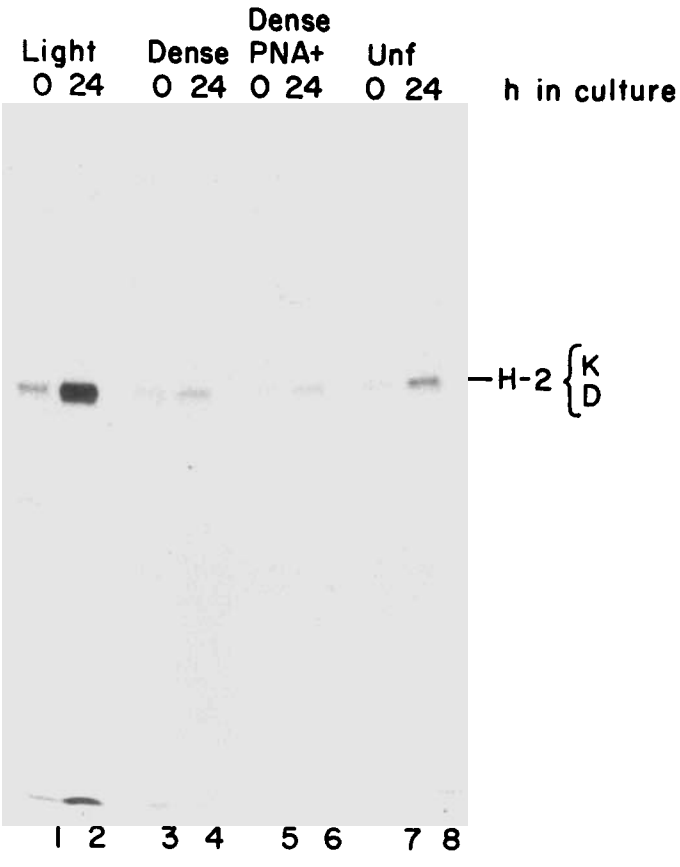

Fig. 2. H-2 synthesis in separated populations of thymocytes before and after culture. B6-TL$^+$ thymocytes were separated into light ("mature") and dense fractions as described in Materials and Methods. Part of the dense fraction was further enriched in "immature" cells by agglutination with PNA (lanes 5, 6). The enriched populations and a sample of unfractionated thymocytes ("unf") were labeled for 30 min with [^{35}S]methionine either immediately (lanes 1, 3, 5, and 7) or after 24 h in culture (lanes 2, 4, 6, and 8). In each case H-2 glycoproteins were immune-precipitated from 250,000 cpm of newly synthesized protein. Fluorographic exposure was for 3 days.

STABILITY OF MARKER SYNTHESIS IN CULTURE

To explore whether this biosynthetic phenotype was a stable attribute of a cell population, immature and mature thymocytes were physically separated and then incubated in short-term culture. Because these were primary cells and not continuously proliferating tissue culture lines, some trypan blue-excluding cells were usually lost from the cultures. These would range from 15% to 60% of the input cell number in the first day and a smaller percentage thereafter. In addition, the rate of protein synthesis per culture routinely decreased 2- to 5-fold, as measured by methionine incorporation during a 30-min pulse. Under these circumstances, "stability" needs special definition.

By measuring synthesis of specific products in relation to the rate of total protein synthesis in a culture, we necessarily confined our analysis to those cells in each population that continue to be biosynthetically active. Relative to overall synthesis, then, a

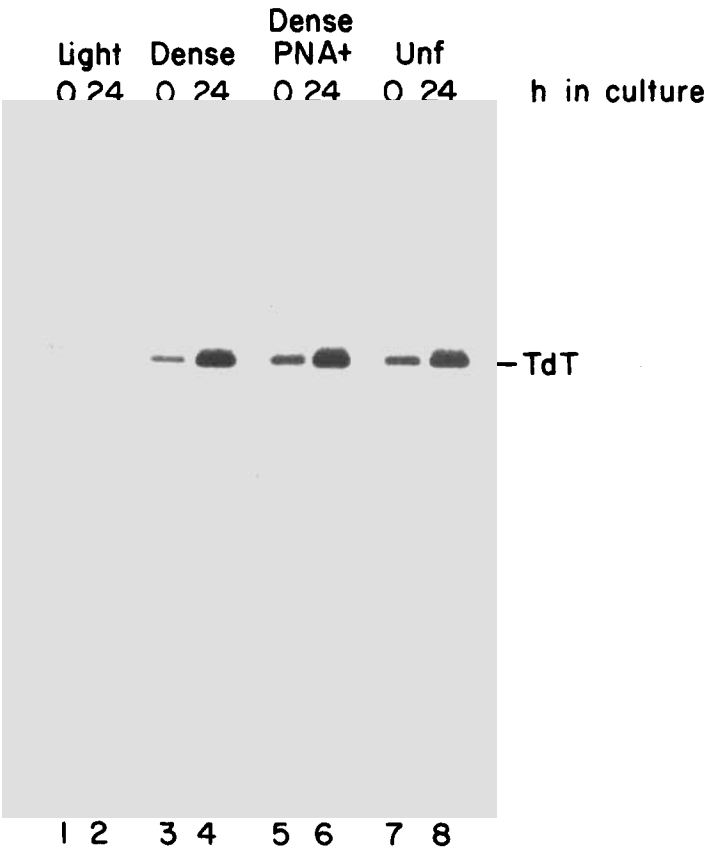

Fig. 3. TdT synthesis in separated populations of thymocytes before and after culture. Samples of the same lysates shown in Figure 2 were precipitated with antiserum against TdT. Each sample represents the precipitate from 250,000 cpm of acid-precipitable radioactivity and fluorography was for 3 days.

stable rate of product synthesis for the 24 h culture period would have the following implications. First, continuing production of that molecule must be compatible with cell viability for at least 24 h. Second, most likely the cells making the product stay active in protein synthesis at least as frequently as the average cell in the culture. If this is not so, some cells must actually be making the product at an accelerated rate.

Using this approach, it was of special interest to ask whether the cells surviving in immature thymocyte cultures were representative of the input cells. Maintenance of TL or TdT synthesis would indicate that the active cells in these cultures were not simply contaminating mature cells, which apparently survive better in vitro [19,20]. The specific questions we wished to answer were the following:

1) Is synthesis of each product maintained at its initial level?

2) If not, do the biosynthetic phenotypes of the subpopulations remain distinct or tend to converge?

3) Are TL and TdT – ostensibly, markers for the same subpopulation – maintained or lost coordinately or independently?

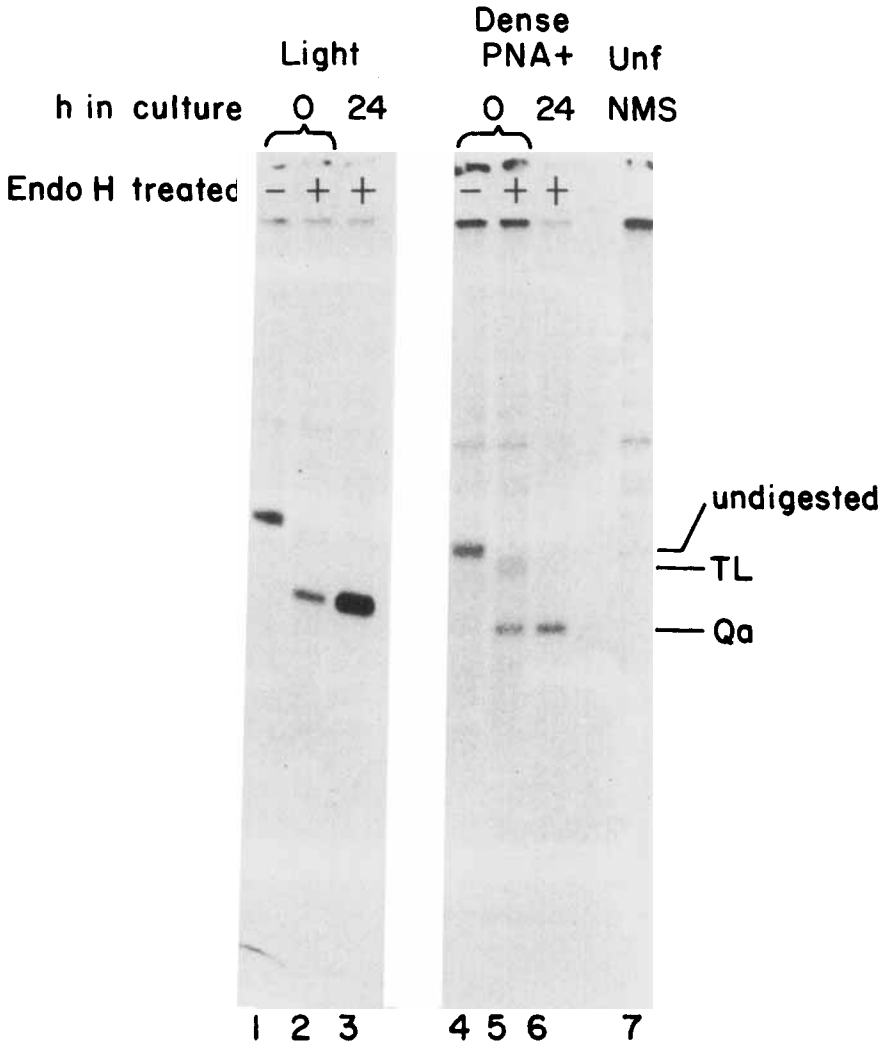

Fig. 4. Stability of TL and Qa synthesis in mature and immature thymocyte populations. Samples of the same lysates described in the legend to Figure 2 were precipitated with alloantiserum against TL and Qa, and digested with endo H. Half of the precipitate from each preculture sample was incubated in parallel without enzyme (lanes 1 and 4). A control sample from unfractionated thymocytes was precipitated with normal mouse serum (NMS) (lane 7). Each lane represents the precipitate from 10^6 cpm of newly synthesized protein, and fluorography was for 23 days. Results with unfractionated thymocytes and dense-fraction thymocytes not subjected to PNA agglutination were the same as those shown for dense, PNA^+ thymocytes (lanes 5 and 6).

Mature cells were taken from the two lowest-density fractions resolved on bovine serum albumin density gradients (A and B layers [7]); immature cells were harvested from the high-density layer (D layer) and then further enriched by agglutination with PNA at limiting cell concentration. The cells were then pulse-labeled with [^{35}S] methionine, either immediately after the separation or after 24 h in culture, and the fractions of

label incorporated into H-2, TdT, TL, and Qa were determined by immune precipitation and gel electrophoresis. The extent to which these patterns of synthesis were subpopulation-specific was estimated by comparison with those of unfractionated thymocytes at both time points.

H-2 Synthesis

After 24 h in culture, recoveries of viable cells were 40% of the unfractionated thymocytes and 45% of the small, dense thymocytes with or without PNA selection. In agreement with previous reports [19,20], the net survival of the buoyant mature cells was better, with 64% of the input cells recovered as viable. This provided an explanation for the significant enhancement in H-2 synthesis relative to total protein synthesis in unfractionated thymocytes after culture overnight (Fig. 2, lanes 7 and 8). Much of this increase could be attributed to the selective survival or proliferation of buoyant mature cells synthesizing high levels of H-2 (Fig. 2, lanes 1 and 2). Small, dense cells, especially when further purified by PNA selection, made very low levels of H-2 after 24 h of culture as well as before (Fig. 2, lanes 3–6). The slight enhancement of H-2 synthesis in the immature population after culture could well be due to a few contaminating mature cells. Thus rapid H-2 synthesis appears to be a specific property of the mature thymocytes that survive well in vitro.

TdT Synthesis

TdT synthesis was found to be highest in the immature, cortisone-sensitive thymocytes [7, 13], (Fig. 3, lanes 1 and 5). Although these cells are less viable in vitro, as a class, than the mature subpopulation, TdT synthesis relative to total protein synthesis was maintained and even enhanced in unfractionated thymocytes after culture (Fig. 3, lanes 7 and 8). This was due to cells in the dense, PNA-binding population (Fig. 3, lanes 3–6), and not to the buoyant cells; the latter population actually exhibited a lower rate of TdT synthesis after incubation in vitro than it did initially (Fig. 3, lanes 1 and 2). Thus, a high rate of TdT synthesis is a stable characteristic of cells in the immature population, which are relatively viable. It cannot be attributed to the same cells that make the highest levels of H-2, either before or after culture.

Synthesis of TL and Qa

The stability of TdT synthesis in vitro made it especially interesting to study TL synthesis. Clearly some immature thymocytes could survive and maintain their expression of a unique marker polypeptide. Would this be equally true for the other immature thymocyte product?

As shown in Figure 4, in the same population that showed augmented TdT synthesis, TL synthesis was not maintained (lanes 5 and 6). It was shut off more rapidly than any decline in cell viability or overall biosynthetic activity, since the pre- and post-culture samples were compared relative to the same amount of newly synthesized protein. Furthermore, in the same lanes, the label incorporated into Qa was remarkably stable; this is consistent with the widespread synthesis of this gene product in thymocytes of various classes [7]. The same responses of TL and Qa synthesis to culture were found in the dense thymocytes without PNA selection and in unfractionated thymocytes (not shown). TL synthesis was arrested in thymocytes cultured in 4 different lots of fetal bovine serum from different sources, irrespective of the addition of supplemental amino acids and pyruvate.

Cultures that ceased to make TL were not grossly depleted of cells bearing surface TL. The percentage of unfractionated thymocytes that were detectably stained by monoclonal anti-TL and fluorescein-labeled goat anti-mouse Ig was the same (ca 60%) both before and after 24 h in vitro (data not shown). Thus, if most of the cells with surface TL were initially active in TL synthesis, they stopped or underwent general metabolic arrest without actually lysing. TL synthesis might have been restricted to a minority of these cells, however. Selective loss of a fraction of the TL-positive cells might not have been detected.

The buoyant population of thymocytes synthesized little if any TL, with no hint of TL induction after culture. In these cells, however, Qa synthesis did appear to be augmented, to about the same extent as that of H-2. It is possible that the parallel production of both Qa and H-2 at enhanced rates is due to the selective survival or activation of the same subpopulation of the mature cells.

Whatever the mechanisms regulating Qa synthesis, it clearly contrasts with the synthesis of TL, which is restricted to the dense, PNA^+ thymocyte population. Furthermore, the cells that make TL must either be especially poorly viable – notably less viable than those making TdT – or else programmed to shut off TL synthesis selectively under the stress of in vitro culture.

DISCUSSION

Thymocytes in different developmental states have been resolved into discrete populations according to steady-state phenotype. The object of these studies is eventually to redefine the stages of their differentiation in terms of the ongoing biosynthetic activity of each class of cells. As a tool for studying differentiation in vitro, this approach has the drawback of requiring pooled cell samples. It can give only a weighted average "phenotype" over a population, not the single-cell resolution of fluorescent or cytotoxic antibody assays. However, it has a number of important compensating advantages. It measures active properties of the cells, and therefore, in a sense, constitutes a set of functional assays. It also offers superior kinetic resolution in two ways. When a new cell-surface glycoprotein is induced or an old one is shut off, metabolic pulse-labeling indicates the changes earlier than the total surface display. It can also define the timing of such a change within an interval of minutes, in contrast to standard T cell functional assays, which involve incubations of days.

The experiments described here examined the synthesis of a panel of gene products in physically separated populations of thymocytes, which were then subjected to tissue culture. First, we wanted to determine whether differential patterns of synthesis between the two populations were maintained. This was necessary to justify using these patterns to define "biosynthetic phenotypes" characteristic of each class of cells. Second, we asked whether two products characteristic of one population were regulated coordinately or not, under the stress of culture.

For at least 24 h in this culture system, the biosynthetic activities of mature and immature thymocytes remained distinct. H-2 synthesis continued preferentially in the low-density cells, while TdT synthesis continued preferentially in the dense, PNA^+ cells. There was no interconversion or randomization of synthesis. In this regard, therefore, the differential production of these markers was a stable property of each of the two cell populations.

The actual rates of H-2 and TdT synthesis, relative to overall synthesis, did change during culture; ie, each increased. This might be the result of selective cell death within each population, or the result of specific induction processes. Thymocytes displaying high levels of surface H-2 are known to enjoy a survival advantage in culture [20], and these are likely to be the cells that make H-2 K and D at the highest rate [7]. Even as a minor contaminant in the immature population, these cells would account for an increased share of overall protein synthesis in the culture after 24 h, leading to a relative enhancement of H-2 production.

In this context, however, the persistent synthesis of TdT in dense, PNA^+ cells after culture is even more striking. In vivo, this is a specific marker for immature thymocytes [5,6,7,13] which, as a class, are poorly viable in culture [20]. Still, relative to the overall residual protein synthesis in these cultures, TdT synthesis actually accelerates. This is unlikely to represent TdT induction (or re-induction) in contaminating cells from the mature population, since relative TdT synthesis declines after culture in the buoyant cell class. Rather, it suggests that some immature cells, at least, do survive while actively maintaining this aspect of their phenotype.

Other components of their initial phenotype are less stable. Although TL and TdT appear to mark very similar populations of thymocytes [5,7], TL synthesis does not continue in culture, either in unfractionated thymocytes or in specifically enriched immature cells.

The non-coordinate expression of TL and TdT may have several explanations. First, within the dense, PNA^+ thymocyte population, some cells may make TdT but not TL, while others are responsible for all the TL synthesis, with or without concurrent synthesis of TdT. If this were the case, selective death of the second subclass would eliminate TL synthesis as observed. The phenotype of the TdT-synthesizing cells would depend on the relationship between the two subpopulations. Although they might derive from separate lineages, so that the surviving TdT producers would lack surface TL, these cells might alternatively display profuse surface TL as a result of prior synthesis. Second, most or all cells may synthesize both gene products initially, but the trauma of culture could have differential effects on them. Components of the culture medium or lack of thymic factors could selectively repress transcription or speed degradation of TL mRNA. Furthermore, the two polypeptides – an integral membrane glycoprotein and a nonglycosylated, soluble nuclear enzyme – are likely to be made in separate subcellular compartments. Thus, even less specific physiological changes, for example in the local concentrations of initiation factors or oligosaccharide donors, could affect one product more than the other. Finally, although all cells may synthesize both TdT and TL initially, the shutoff of TL may be preprogrammed within the thymus. One way such preprogramming might operate would be via translation of TL from an mRNA with a short half-life. If synthesis of this mRNA were not continuous within the thymus, but restricted to a short period in differentiation, TL glycoprotein synthesis from this limited message pool could not persist under any culture conditions. In the same cells, TdT synthesis could be maintained either by continued production of TdT mRNA or simply because the mRNA half-life was longer.

Each of these explanations has interesting implications for our understanding of T cell maturation. The first entails a further subdivision of the immature thymocyte population. This implies either a new developmental branch point resulting in separate lineages or an additional phenotypic transition, and possible regulatory point, within one lineage. The second explanation involves product-specific biosynthetic changes in the cells in re-

sponse to manipulation in vitro. This predicts that distinct environmental factors can separately regulate expression of the individual constituents of the immature phenotype. Within the organization of the thymus, these signals may be delivered simultaneously, but independent mechanisms could be delivered simultaneously, but independent mechanisms could be involved in each phenotypic and functional change. The third explanation requires that a limiting pretranslational step of TL synthesis occur only transiently in the thymus. This event would then narrowly define one phase of thymocyte maturation, and might serve as a highly specific indicator of response to an early developmental stimulus – eg interaction with cells of the thymic stroma.

To choose among these explanations will require a more detailed characterization of those immature cells with the best survival rates, isolated both before and after culture. Of course, the various explanations may ultimately converge in any of several ways. Meanwhile, the process of exploring their corollaries provides a wealth of specific challenges.

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