Regulation of T-cell receptor gene expression in human T-cell development

(antigen receptor/thymus)

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ABSTRACT A cDNA clone encoding the α chain of the human T-cell antigen receptor was isolated by screening a library from the human T-cell line Jurkat with a mouse α -chain cDNA clone. This human α -chain clone, together with a human antigen receptor β -chain cDNA clone, was used to determine the stage of T-cell development at which antigen receptor mRNAs first appear. Blot-hybridization analysis of mRNA isolated from a panel of human thymic tumor lines clearly demonstrated that β -chain transcripts could be detected in all T-lineage cells. However, α -chain transcripts were only found in the most phenotypically mature lines, which express the antigen receptor-associated molecule T3. Furthermore, β -chain transcripts were abundant in RNA prepared from purified T3-negative thymocytes, whereas α -chain transcripts were virtually absent. From these results we conclude that α -chain expression occurs later in thymic ontogeny than that of the β chain and propose that it controls surface expression of the antigen receptor–T3 complex.

Biochemical characterization of the T-cell antigen receptor has defined it as a disulfide-linked heterodimer (designated Ti) comprising an α and a β chain (1–3). Although the $\alpha\beta$ heterodimer is at least partially responsible for conferring antigenic specificity to a T cell, it is clear that several molecules are involved in T-cell activation in response to antigen. Most notable amongst these is the T3 glycoprotein, which apparently is associated physically with the Ti structure at the T-cell surface (4, 5); indeed, it has been suggested that coexpression of T3 and Ti at the cell surface is obligatory (5). Knowledge of the stage of thymocyte ontogeny at which α and β chains (and T3) are expressed is important for any understanding of the acquisition of the T-cell repertoire and thymic education. It appears that β -chain transcripts are expressed at an early stage in thymic ontogeny because rearrangement of β -chain gene segments and high levels of β -chain mRNA have been detected in T-lineage tumors derived from immature [as defined on the basis of surface phenotype (6, 7)] thymocytes (8, 9). Recently, cDNA clones encoding mouse (10, 11) and human (12) α chains have been described. In this paper we use a human α -chain cDNA clone to investigate α -chain mRNA expression during thymic ontogeny. We show that expression of β -chain mRNA precedes that of α -chain mRNA, which appears to be restricted to more mature thymocytes and T cells.

MATERIALS AND METHODS

Isolation and Sequencing of an α -Chain Clone. An amplified cDNA library (13) constructed in the vector λ gt 10, of 500,000

recombinant phage, was screened by standard procedures (14) with a constant region fragment from pHDS58 (10). Five positive hybridizing clones were identified. The clone with the largest insert was subcloned into the *Eco*RI site of pBR322. This plasmid pJ6a2, was mapped and sequenced by the method of Maxam and Gilbert (15).

Blot-Hybridization Analysis. Inserts from α - and β -chain plasmids were oligo-labeled (16) to specific activities of $2\text{--}6 \times 10^8$ cpm/ μ g and hybridized to blots containing poly(A)⁺ RNA or total RNA denatured with formaldehyde prior to electrophoresing on 1.1% agarose gels (14). After hybridization for 24 hr, blots were washed in 0.015 M NaCl/0.0015 M sodium citrate, pH 7, at 50°C (twice for 15 min each), and three different exposures were taken to ensure linearity of response during scanning densitometry.

Surface Phenotype Analysis. The level of T3 expression in each batch of cells used to make RNA was determined by flow cytometric analysis using the antibody UCHT1 (kindly provided by P. Beverley).

RESULTS

The α -chain cDNA clone used in this study was isolated by screening a phage λ gt10 library constructed by using RNA from the human leukemic T-cell line Jurkat (13) with a labeled insert from a mouse α -chain cDNA clone (10). The insert from one positive clone (about 900 base pairs) was subcloned into pBR322 to yield the human α -chain clone pJ6a2. The sequence of this insert is shown in Fig. 1 *Lower*, and the sequencing strategy used is indicated in Fig. 1 *Upper*. Comparison of this sequence with that of an homologous clone isolated by Sim *et al.* (12) from a HPB-MLT cDNA library reveals that the Jurkat cDNA clone commences at a position corresponding to amino acid 147 of the HPB-MLT clone and, thus, comprises about three-quarters of the constant region coding sequence plus almost the entire 3' untranslated region of the α -chain mRNA. The sequences of the two human α -chain clones are identical over the entire coding region but differ in several positions in the 3' untranslated region.

The Jurkat α -chain clone together with a β -chain cDNA clone isolated from the same cDNA library (13) were used to determine the pattern of expression of the corresponding mRNAs by blot-hybridization analysis of mRNA isolated from various T-lineage cells. T3 expression was assessed on each batch of cells by using flow cytometry. When Jurkat mRNA was hybridized to the insert from pJ6a2, a transcript of about 1.4 kilobases (kb) was detected (Fig. 2 *Upper*, Jurkat lanes). This transcript, which has been described previously by Sim *et al.* (12), is specific to T-cell mRNA since it was not found in mRNA from two human lymphoblastoid B-cell lines

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Abbreviation: kb, kilobase(s)

FIG. 1. (Upper) Restriction map of pJ6a2. The plasmid pJ6a2 was isolated as described. The sequencing strategy is diagrammed along with the restriction sites that were 5'-end-labeled. Vector, C_α and 3' untranslated regions are indicated by —, □, and □, respectively. (Lower) Nucleotide sequence of pJ6a2. The predicted amino acid sequence was determined by an examination of open reading frames and in comparison with pHDS58.

(Fig. 2 Upper, BRI8 and Maja lanes). The level of α -chain mRNA expression varied somewhat in independent preparations of Jurkat mRNA, although the ratio of α - and β -chain transcripts remained constant (Fig. 2). We believe that differences in the level of T-cell receptor mRNAs in various Jurkat cell cultures reflect a difference in the growth state of the cells (unpublished results). α -Chain transcripts also were detected in peripheral blood T lymphocytes (Fig. 2 Upper, lane HPBL) and in a human helper T-cell clone (HA1.7) that recognizes influenza hemagglutinin (Fig. 3, lane A) (17). Blot-hybridization analysis of mRNA from the immature thymic tumors MOLT 4, HSB2, and RPMI 8402 failed, however, to detect expression of the 1.4-kb α -chain mRNA species at the exposure shown (Fig. 2 Upper). At longer exposures, a faint α -chain transcript was detected in MOLT 4 but not in HSB2 or RPMI 8402. Interestingly, an α -chain transcript also was detected only at very low levels (<1% of β -chain expression) in a Sezary cell tumor HUT 78, which is most probably derived from a peripheral blood T cell of helper lineage (Fig. 2 Upper, lane HUT 78) (18).

When the same blot hybridization was stripped and hybridized with a β -chain probe, 1.3-kb and 1.1-kb β -chain transcripts were readily detected, demonstrating a high level of expression in all of the lines examined (Fig. 2 Lower). This result indicates that β -chain expression may be a characteristic of the majority of thymocytes and is in agreement with previously published results (9). In contrast, α -chain expression appears to occur later in thymocyte ontogeny and, therefore, could control surface expression of the antigen receptor and T3.

If this conclusion is correct, then α -chain expression would be expected to be low or undetectable in the immature thymocyte population that lacks T3. In order to assess this, total RNA was prepared from a purified T3-negative thymocyte population predominantly composed of immature thymocytes (6, 7). Sequences corresponding to the β -chain gene were abundant in T3-negative thymocytes (Fig. 3, lane F); α -chain transcripts were, however, barely detectable (Fig. 3, lane E). Both α - and β -chain sequences were easily visible in RNA isolated from total thymocytes (Fig. 3, lanes C and D).

When corrections were made for the different sizes and specific activities of the α - and β -chain probes, densitometric scans of several exposures of the blot hybridization shown in Fig. 2 revealed that the β -chain 1.3-kb transcript was expressed in Jurkat at about twice the level of the 1.4-kb α -chain transcript and that β -chain transcripts were also more abundant than α -chain transcripts in total thymocytes (Fig. 3), whereas in peripheral blood T cells and the helper T-cell clone, the α -chain transcript was about 2-fold higher than the β -chain transcript. The level of expression of α - and β -chain transcripts in peripheral blood T cells was about 15–30% of their expression in Jurkat. The high level of thymic versus peripheral T-cell expression of β -chain transcripts has been described (9).

DISCUSSION

T-lineage tumors have been placed on a scheme of thymic ontogeny as defined on the basis of reactivity with monoclo-

FIG. 2. Expression of α -chain and β -chain transcripts. Labeled insert (6×10^8 cpm/ μ g) from the α -chain plasmid pJ6a2 (Upper) was hybridized to a blot containing 5 μ g of poly(A)⁺ RNAs from: peripheral blood T cells, obtained by culturing phytohemagglutinin-stimulated human peripheral blood lymphocytes (HPBL lane) for 10 days in the presence of interleukin 2 (80% T3-positive, 70% T8-positive); HUT 78; MOLT 4; HSB2; Jurkat (mRNA preparations from three independent cultures); BR18 (lymphoblastoid B-cell line); and Maja (lymphoblastoid B-cell line) as described. The blot was then stripped, and rehybridized with labeled insert (3×10^8 cpm/ μ g) from a β -chain plasmid pB400 composed of the majority of the $C_{\beta 1}$ constant region coding sequence (13) (Lower). Total RNA from RPMI 8402 (20 μ g) was electrophoresed and hybridized to α - and β -chain probes in a separate experiment. The α -chain exposure (Upper) is considerably longer than that for the β chain (Lower) to emphasize the lack of α -chain transcripts in some cell lines. When densitometric scans of several exposures, within the linear response range, of both experiments were performed and corrections made for the different sizes and specific activities of these two probes, we found that the α -chain mRNA level in HPBL cells was about twice that of the β chain.

nal antibodies directed at specific T-cell glycoproteins (6, 7). The surface phenotypes of the tumor lines used in the present study are shown in Table 1. The most phenotypically immature T-lineage tumor lines (RPMI 8402, HSB2, and MOLT 4),

FIG. 3. Expression of an α -chain transcript in a human helper T-cell clone and T3-negative thymocytes. The conditions for blot-hybridization analysis were as described; 10 μ g of total RNA per lane was used. Lanes: A, C, and E, hybridization with the pJ6a2 insert (2×10^8 cpm/ μ g); B, D, and F, hybridization with the pB400 insert (2×10^8 cpm/ μ g). The RNA was isolated from: the helper clone HA1.7 (lanes A and B), total human thymocytes (lanes C and D), and T3-negative human thymocytes prepared by complement-dependent cytotoxicity with a monoclonal anti-T3 antibody (UCHT1; a kind gift from P. Beverley) (lanes E and F). The starting thymocyte population was assessed as 36% T3-positive. No brightly staining cells were observed after complement-mediated lysis.

which possess high levels of β -chain transcripts but do not express α -chain mRNA sequences (or barely detectable in the case of MOLT 4), fail to express detectable levels of T3 glycoprotein or the Ti heterodimer. In contrast, Jurkat, which exhibits the surface phenotype of a mature thymocyte, expresses surface T3 and Ti and easily detectable α -chain mRNA. HPBALL, which possesses a similar surface phenotype to Jurkat, also expressed equivalent levels of α - and β -chain transcripts (data not shown). We conclude that α -chain expression occurs late during thymocyte differenti-

Table 1. Surface phenotypes of T-lineage tumors

Lines	OKT series of antibodies							Other markers			Stage of ontogeny	
	1	3	4	6	8	9	10	11	Ia	TdT		Ti
HSB2	-	-	-	-	-	+	+	-	-	-	-	Stage I
RPMI 8402	+	-	-	-	-	+	+	-	-	+	ND	Stage I
MOLT 4	+	-	±	+	±	+	+	+	-	-	-	Stage I-stage II
Jurkat	+	+	+	-	+	±	+	+	-	±	+	Stage II-stage III
HUT 78	+	+	+	-	-	ND	ND	-	+	±	+	Peripheral T cell

The phenotypes were taken from Greaves *et al.* (6), except for HUT 78, whose phenotype was determined by M. F. Greaves (personal communication). For each line T3 was assessed by using the fluorescence-activated cell sorter on the same cell batch used for mRNA isolation. Surface Ti was assessed by diagonal NaDod-SO₄/polyacrylamide gel electrophoresis of surface-labeled cells. The symbols used are: +, strong staining; ±, weak staining or staining on <50% of cells; -, <5% of cells staining; ND, not determined. The stage of ontogeny was determined from Greaves *et al.* (6) and Reinherz *et al.* (7).

ation and may control the expression of Ti (and T3) at the thymocyte cell surface. However, one obvious caveat is that the stage at which mRNA for the three T3 polypeptides is first expressed in the cytoplasm is unknown. In contrast, β -chain expression most probably occurs at an early stage of differentiation, since β -chain transcripts were detected in every T-lineage tumor examined.

These conclusions depend upon the assumption that the surface phenotype of a T-lineage tumor allows an accurate identification of the maturation stage from which that tumor was derived. The exact lineage interrelations of the various surface phenotypes are unclear; however, several lines of evidence indicate that at least early lineage (stage I) thymic tumors are accurate models for the immature thymocyte precursors of peripheral T cells (6, 7). Moreover, blot-hybridization analysis of mRNA from T3-negative human thymocytes detected high levels of β -chain transcripts but virtually no α -chain transcripts, a result that is consistent with the studies using T-lineage tumors and that strongly supports the idea of α -chain control of T3 expression.

The level of expression of α -chain mRNA sequences in the Sezary cell leukemia HUT 78 was less than 1% that of β -chain transcripts. HUT 78 cells express T3 antigen and, consequently, would be expected to express Ti. In this context, the thymic tumor line Jurkat, total human thymocytes, peripheral blood T cells, and a human functional helper cell clone, which were positive for T3, expressed easily detectable amounts of α -chain transcripts. Two dimensional NaDodSO₄ gel electrophoresis of surface-labeled HUT 78 cells has failed to detect a heterodimer-like Ti protein (19). From the evidence presented here it is possible that the α chain does not constitute part of any Ti structure on HUT 78 cells. Therefore, T3 may be expressed on the surface of these cells in the absence of the $\alpha\beta$ heterodimer but may be associated with an additional Ti-like structure in which the β chain exists as a dimer or complexed with a third α -chain-like polypeptide. The unusual nature of the putative Ti structure on this tumor line may not be of physiological significance; alternatively, it may represent a normal stage in ontogeny.

Studies in the mouse and human systems have revealed that the functional variable-diversity-joining-constant region (V-D-J-C) β -chain transcripts are 1.3 kb in length, whereas the 1.0-kb mRNA corresponds to transcripts from incompletely rearranged loci lacking variable regions (13, 20, 21). Similar aberrant rearrangements may also be a characteristic of α -chain genes, since a lower transcript has been detected in mouse T cells (10, 11). It is clear, however, from the blot-hybridization analysis presented here that the level of expression of aberrant transcripts is considerably lower for α chains than for β chains. Thus, the only α -chain transcript detected in this study was the 1.4-kb productive transcript described previously by Sim *et al.* (12). Southern hybridization analysis with a variety of restriction enzymes has failed to provide evidence for more than one human α -chain constant region (unpublished observation). The high level of aberrant β -chain, as opposed to α -chain, transcripts might reflect, therefore, the presence of two β -chain constant region genes per haploid genome or be a consequence of an intrinsically greater accuracy of the α -chain rearrangement machinery. However, as α -chain gene rearrangement probably follows that of β chain, it seems that the shut down of gene rearrangement machinery following productive α -chain mRNA appearance is likely to account for this difference.

Incomplete T-cell receptor gene rearrangements may occur in B cells because B-cell sequences that cross-hybridized with the α -chain probe were consistently observed in our blot-hybridization analyses (Fig. 2). They also were detected at longer exposures using the β -chain probe (data not shown). These transcripts were about 200–300 base pairs smaller than the productive α - and β -chain transcripts expressed in T cells

and appeared to be specific, since they were not observed either in RNA from other tissues (erythroid, macrophage, or epithelial) or (in the case of the α chain) in T-cell lines not expressing 1.4-kb transcripts but were found in six lymphoblastoid B-cell lines tested. The levels of these B-cell transcripts varied considerably in different lymphoblastoid B-cell lines (see for example Fig. 2 *Upper*, BR18 and Maja lanes). Their origin is unclear; however, they may be a consequence of the similar mechanisms that are thought to mediate rearrangement of T-cell receptor and B-cell immunoglobulin genes, as evidenced by the similar recognition sequences associated with the corresponding gene segments (22, 23).

In conclusion, we have shown that mRNA encoding the α chain appears later in T-cell ontogeny than mRNA encoding the β chain. This result is in agreement with studies using fractionated mouse fetal and adult thymocyte populations and peripheral T cells (24). The human T-cell surface antigen T3 is closely associated with the antigen receptor on the cell surface (4, 5). We find that α -chain mRNA expression in thymus leukemia cell lines and in fractionated human thymocytes is correlated with the presence of T3. Therefore, α -chain expression may control the expression of the T3–Ti complex at the thymocyte cell surface. This hypothesis can be tested directly by transfection of a rearranged α -chain gene into an immature (stage I) thymic tumor line in order to induce the surface appearance of the T3–Ti structure.

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