DEVELOPMENTALLY REGULATED EXPRESSION OF T CELL RECEPTOR β CHAIN VARIABLE DOMAINS IN IMMATURE THYMOCYTES

By RALPH C. BUDD, GUIDO C. MIESCHER,* RAWLEIGH C. HOWE, ROSEMARY K. LEES, CLAUDE BRON,‡ AND H. ROBSON MacDONALD

*From the Ludwig Institute for Cancer Research, Lausanne Branch; the *Department of Molecular Biology, Swiss Institute for Experimental Cancer Research; and the ‡Institute of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland*

The process by which developing T lymphocytes are selected within the thymus is basic to the acquisition of T cell tolerance and MHC restriction of antigen recognition. As repertoire determination likely occurs based upon the specificity of TCR molecules (1), identification of the stage at which surface TCR first appears on immature thymocytes is fundamental to studies of the selection mechanism.

The genes encoding the disulfide-linked α- and β-chain subunits of the TCR resemble Ig genes in their structure, requirement for DNA rearrangement before expression, and developmental regulation (2). Thus, immature thymocytes, lacking expression of the differentiation antigens CD4 (L3T4 in mouse) and CD8 (Lyt-2), express high levels of β-chain transcripts but very low levels of α-chain transcripts, and no surface TCR protein has been observed on these cells (3–6). However, $CD4^-,8^-$ thymocytes contain cells at various stages of differentiation (7–9). We show here that a minor (10%) subset of $CD4^-,8^-$ thymocytes transcribes abundant amounts of α-chain and β-chain mRNA and expresses low levels of surface TCR protein. Interestingly, these early T cells show a preferential use of a particular β-chain variable segment gene family ($V_{β8}$) at both the RNA and protein levels.

Materials and Methods

Anti-TCR mAbs. mAbs F23.1 (10) and KJ16-133 (KJ16) (11) have been described. F23.1 recognizes the protein products of all three members of the $V_{β8}$ gene family, whereas KJ16 recognizes only $V_{β8.1}$ and $V_{β8.2}$ (12).

Fresh and Cultured Cell Populations. Thymocytes (50×10^6/ml) from groups of 15–30 C57BL/6, DBA/2, CBA, NIH III, and SJL mice were twice treated by incubation at 37°C for 15 min with a mixture of rat IgM mAbs directed against Lyt-2 (3.168.8.1) and L3T4 (LICR.LAU.RL172.4) as described (13), with or without rat IgM mAb B2A2 (7), all at a 1:10 final dilution of hybridoma culture supernatant. Rabbit complement was then added to a 1:20 final dilution for an additional 60 min. Treated cells were passed over Ficoll-Hypaque and washed twice. Viability was >95% and cell recovery was $0.8 \pm 0.3\%$ (mean ± SD of six experiments) for $CD4^-,8^-$ thymocytes and $0.1 \pm 0.02\%$ for $B2A2^-$

$CD4^-,8^-$ thymocytes. Cultured cells (10^6/ml) were stimulated with PMA (10 ng/ml) and ionomycin (250 ng/ml) and expanded in IL-2-supplemented medium for 7 d.

Flow Microfluorometry (FMF).   For FMF analysis, cells were stained with F23.1 or KJ16 mAbs followed, respectively, by FITC-conjugated goat anti–mouse Ig (GAM-FITC) or goat anti–rat Ig (GAR-FITC). Samples were passed on a flow cytometer (FACS II; Becton Dickinson & Co., Mountain View, CA) gated to exclude nonviable cells. At least 10^4 cells were accumulated using logarithmic (fresh cells) or linear (cultured cells) amplification of fluorescence intensity. Control fluorescence was determined by passing a sample of cells stained with either GAM-FITC or GAR-FITC alone.

Immunoprecipitation and Gel Electrophoresis.   Cells were surface iodinated by the lacto-peroxidase/glucose oxidase method, lysed in 0.5% NP-40, 0.5% deoxycholate, and immunoprecipitated with F23.1 mAb conjugated to Sepharose 4B (3 mg IgG/ml beads) as described in detail elsewhere (14). Immunoprecipitates were run on 10% (nonreduced) or 12.5% (reduced) SDS-PAGE and autoradiographed.

Northern Blot Analysis.   The procedures have been described in detail (15). Briefly, RNA probes complementary to mRNA were labeled with [^{32}P]UTP (410 Ci/mmol) by in vitro transcription from linearized SP6 plasmids containing TCR constant (C) domain fragments $C\alpha$ and $C\beta$ and the variable (V) domain fragments $V_{\beta8.1}$ and $V_{\beta14}$. For Northern blot analysis, 10 μg RNA were glyoxylated, fractionated on 1.5% agarose gels, and transferred to Hybond nylon filters before hybridization with labeled probes. Quantitation of hybridizing bands was done by densitometric scanning of autoradiograms.

Results and Discussion

Adult murine $CD4^-,8^-$ thymocytes represent a heterogeneous population as defined by the expression of several surface antigens including B2A2 (90%), Ly-1 (20% bright), Pgp-1 (25%), and IL-2R (50%) (7–9, 13). Preliminary experiments using two-color FMF indicated that the B2A2$^-$ subset of $CD4^-,8^-$ thymocytes was enriched for cells expressing a low surface density of the TCR as defined by mAbs F23.1 and KJ16. After purification, B2A2$^-$ thymocytes uniformly expressed high surface levels of Pgp-1 and Ly-1, in agreement with others (7), but lacked IL-2R. Furthermore, the purified B2A2$^-$ subset expressed the F23.1 and KJ16 determinants on 48 and 38% of cells, respectively, as compared with 26% F23.1$^+$ and 18% KJ16$^+$ on mature lymph node (LN) T cells (Fig. 1). B2A2$^-$ thymocytes and LN cells from SJL mice did not express F23.1 or KJ16, consistent with the deletion of the TCR $V_{\beta8}$ gene family in this strain (16).

To perform further biochemical and molecular studies, B2A2$^-$ thymocytes were expanded in culture using a combination of PMA, ionomycin, and IL-2. Under these conditions, there was no change in the proportions of F23.1$^+$ and KJ16$^+$ cells, although the surface density of these epitopes increased somewhat (Fig. 1). The vast majority of proliferating cells retained their $CD4^-,8^-$ phenotype with <5% $CD4^+$ or $CD8^+$ cells observed. Immunoprecipitation of ^{125}I–surface-labeled cells using F23.1 mAb revealed a protein of M_r 85,000 (nonreduced; M_r 38,000, reduced) on cultured LN cells and B2A2$^-$ thymocytes, which was only weakly present on unselected $CD4^-,8^-$ thymocytes and undetectable on SJL LN cells (Fig. 2).

Northern blot analysis of RNA from cultured B2A2$^-$ thymocytes demonstrated the presence of full-length TCR α and β transcripts (Fig. 3). Furthermore, using a $V_{\beta8.1}$ probe that hybridizes to all three members of the $V_{\beta8}$ family (17), we confirmed the overexpression of this gene family by B2A2$^-$ thymocytes (Fig. 3). When normalized to the levels of full-length C_β message, the relative proportion
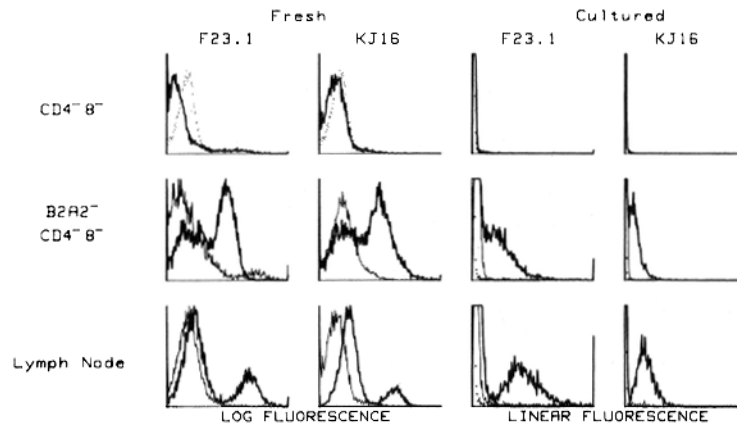
FIGURE 1.   FMF analysis of TCR determinants expressed by immature thymocytes and mature T cells. Fresh or PMA/ionomycin-cultured CD4$^-$,8$^-$ thymocytes, B2A2$^-$ CD4$^-$,8$^-$ thymocytes, or LN cells from C57BL/6 (*thick lines*) or SJL (*thin lines*) mice were stained with mAbs F23.1 or KJ16 followed by appropriate fluoresceinated anti-Ig reagents. Dotted lines represent staining with the fluorescent conjugates alone. Note that histograms of fresh and cultured cells are displayed with logarithmic and linear fluorescence amplification, respectively.
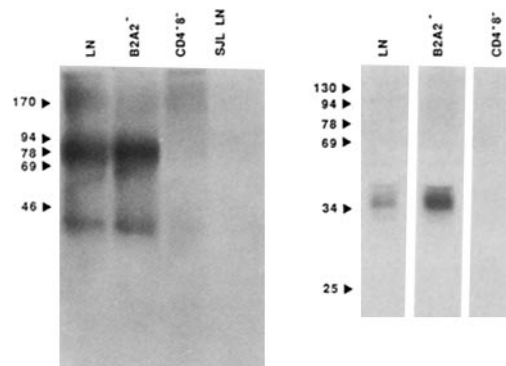

FIGURE 2.   Immunoprecipitation of TCR molecules from cultured C57BL/6 LN, B2A2$^-$ CD4$^-$,8$^-$ thymocytes (B2A2$^-$), unselected CD4$^-$,8$^-$ thymocytes (CD4$^-$,8$^-$), or SJL LN. Immunoprecipitates were analyzed by SDS-PAGE under nonreducing (*left panels*) or reducing (*right panels*) conditions.
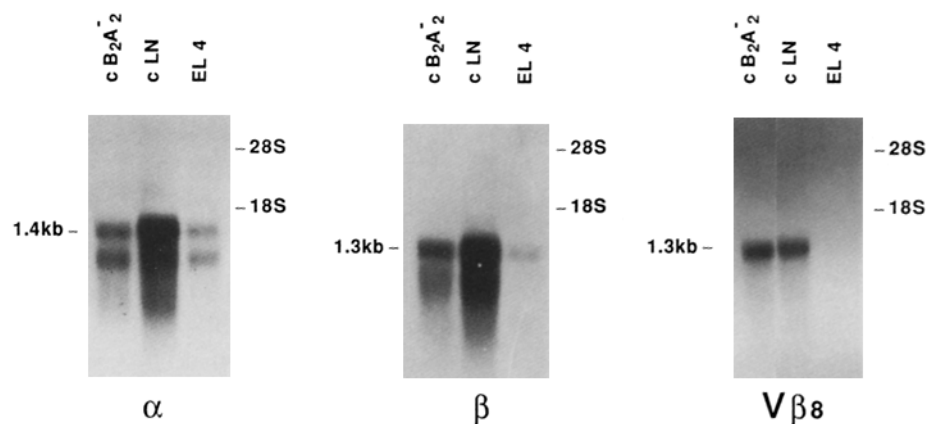

FIGURE 3.   Analysis of C$_\alpha$, C$_\beta$, and V$_{\beta 8}$ mRNA levels in cultured (*c*) B6 LN cells, B2A2$^-$ CD4$^-$,8$^-$ thymocytes (B2A2$^-$), or EL4 thymoma cells.

TABLE I

Comparison of F23.1 and KJ16 Expression in Different H-2 Haplotypes

Cells	C57BL/6 (H-2^b)		DBA/2 (H-2^d)		CBA (H-2^k)		NIH III (H-2^q)	
	F23.1$^+$	KJ16$^+$	F23.1$^+$	KJ16$^+$	F23.1$^+$	KJ16$^+$	F23.1$^+$	KJ16$^+$
	%		%		%		%	
B2A2$^-$ thymocytes (A)	46.3	35.9	43.7	34.5	59.0	46.0	54.8	37.7
Lymph node (B)	25.7	17.5	35.4	18.8	31.6	21.6	19.7	12.6
Ratio A/B	1.80	2.05	1.23	1.84	1.87	2.13	2.78	2.99

Cultured B2A2$^-$ thymocytes or unseparated LN cells from the indicated mouse strains were stained with F23.1 (revealed by GAM-FITC) or KJ16 (revealed by GAR-FITC). Data are expressed as percent positive cells (after subtraction of control samples stained with the fluorescent conjugate alone).

of $V_{\beta 8}$ message was five- to sixfold higher in B2A2$^-$ thymocytes compared with LN T cells. In contrast, B2A2$^-$ thymocytes expressed threefold less $V_{\beta 14}$ message than LN T cells (data not shown). Thus, not all V_β gene segments are overexpressed in B2A2$^-$ thymocytes.

It was possible that the high proportion of F23.1$^+$/KJ16$^+$,B2A2$^-$ thymocytes in C57BL/6 mice resulted from a self-MHC–related selective process (1). B2A2$^-$ thymocytes from different strains of mice with diverse H-2 haplotypes were consequently examined for expression of F23.1 and KJ16. Table I shows that in the four strains studied, B2A2$^-$ thymocytes contained on average 1.8-fold and 2.2-fold higher proportions, respectively, of F23.1$^+$ and KJ16$^+$ cells than LN T cells of the same mice. Thus, the selective use of $V_{\beta 8}$ genes in B2A2$^-$ thymocytes is not MHC associated and would rather appear to be a developmentally regulated event. An alternative possibility, namely that expression of TCR chains is not allelically excluded in B2A2$^-$ thymocytes, seems unlikely in view of the fact that no such phenomenon has been observed at the protein level in either B or T cells (18).

The preferential usage of a particular TCR V_β gene family by early T cells has parallels with the ontogeny of Ig gene expression. Early B cell lines (18), as well as colonies of normal pre-B cells (19), exhibit selective use of certain families of V_H genes. A model has been proposed for a single recombinase common to Ig and TCR variable region genes, as both have similar recombination recognition sequences (20). Our data lend further support to such a model in that they indicate that TCR V_β genes, like V_H genes, are expressed (and thus presumably rearranged) in a developmentally controlled sequence. Whether such developmental control is related to proximity of certain V_β genes to the DJ cluster, as is the case for V_H expression in early B cell lines (18), cannot be answered until further information on the genomic localization of TCR V_β genes becomes available.

B2A2$^-$ thymocytes are likely to be among the earliest T cells to express surface TCR protein (before CD4 or CD8), and as such, may represent a pivotal stage in T cell differentiation. Preliminary observations indicate that B2A2$^-$ thymocytes can be induced to proliferate in response to stimulation by F23.1 mAbs. The ability of these cells to differentiate further in vivo and in vitro should provide vital clues to the lineages of T cell subsets as well as to the fundamental process of thymic selection.

Summary

A minor subset of immature (CD4$^-$,8$^-$) thymocytes that lack expression of the B2A2 antigen was found to express low levels of surface TCR protein as detected by mAbs F23.1 and KJ16 (reacting with protein products of the V$_{\beta 8}$ gene family). Interestingly, F23.1/KJ16 determinants were expressed on a two- to three-fold higher proportion of B2A2$^-$ thymocytes than mature lymph node T cells in four independent haplotypes. When expanded in short-term culture with PMA and calcium ionophore, B2A2$^-$ thymocytes retained their overexpression of F23.1/KJ16 determinants and showed a fivefold elevated level (relative to lymph node) of V$_{\beta 8}$-specific mRNA. Taken together, these findings suggest that expression of TCR V$_\beta$ genes, like Ig genes, is developmentally regulated.

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