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Chicken T-cell receptor β-chain diversity: An evolutionarily conserved D_β-encoded glycine turn within the hypervariable CDR3 domain

(*N* region/junctional diversity/immunologic repertoire/diversity gene segment/complementarity-determining region)

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ABSTRACT **Unlike mammals, chickens generate an immunoglobulin (Ig) repertoire by a developmentally regulated process of intrachromosomal gene conversion, which results in nucleotide substitutions throughout the variable regions of the Ig heavy- and light-chain genes. In contrast to chicken Ig genes, we show in this report that diversity of the rearranged chicken T-cell receptor (TCR) β-chain gene is generated by junctional heterogeneity, as observed in rearranged mammalian TCR genes. This junctional diversity increases during chicken development as a result of an increasing base-pair addition at the V_β–D_β and D_β–J_β joints (where *V*, *D*, and *J* are the variable, diversity, and joining gene segments). Despite the junctional hypervariability, however, almost all functional V_β–D_β–J_β junctions appear to encode a glycine-containing β-turn. Such a turn may serve to position the amino acid side chains of a hypervariable TCR β-chain loop with respect to the antigen-binding groove of the major histocompatibility complex molecule. Consistent with this hypothesis, the germ-line D_β nucleotide sequences of chickens, mice, rabbits, and humans have been highly conserved and encode a glycine in all three reading frames.**

Immunoglobulins (Ig) and T-cell receptors (TCR) are the antigen-recognition molecules on the surfaces of B and T cells, respectively (1–3). Whereas B cells recognize soluble antigens, T cells recognize antigenic peptides associated with cell surface molecules encoded by the major histocompatibility complex (MHC) (4, 5). Genetic analyses of the mammalian Ig and TCR gene families have shown that somatic diversity is generated by junctional and combinatorial diversity during the assembly of functional Ig and TCR genes from variable (*V*), diversity (*D*, for Ig heavy, TCRβ, and TCRδ chains), and joining (*J*) gene segments (1–3). In contrast, birds use distinct molecular mechanisms to generate somatic diversity in their Ig loci. Instead of using junctional and combinatorial variation to create somatic Ig gene diversity, chickens rearrange only single *V* and *J* segments for both the heavy (H)- and the light (L)-chain Ig loci (6, 7). After rearrangement, the V_H and V_L segments undergo sequence diversification by intrachromosomal gene conversion using families of *V* pseudogene segments as sequence donors (6–10). Given these differences between mammalian and avian Ig gene diversification, it was of interest to determine the molecular mechanisms for the diversification of chicken TCRβ genes.

The recent description of chicken TCRβ cDNA and genomic sequences revealed evolutionarily conserved structural features of the TCRβ chains in avian and mammalian species (11). The germ-line repertoire of chicken V_β genes consists of only two V_β families, $V_{\beta1}$ and $V_{\beta2}$ (12, 13), which bear structural similarities to the mammalian $V_{\beta I}$ and $V_{\beta II}$ subgroups (14). The chicken $V_{\beta1}$ family consists of six nearly identical V_β genes (average amino acid identity, 97%), and the $V_{\beta2}$ family consists of three to five genes (average amino acid identity, 95%), depending on the chicken strain examined. Unlike chicken Ig *V* segments, all chicken V_β segments that have been sequenced contain heptamer and nonamer elements. Multiple distinct rearrangements of the TCRβ locus are detected when Southern blots of chicken thymic and T-cell-line DNA are probed with $V_{\beta1}$ and $V_{\beta2}$ probes (11–13), suggesting that most or all chicken V_β genes are functional. Comparison of cDNA sequences revealed three unique J_β sequences (11), and genomic screening with an oligonucleotide probe identified a fourth J_β gene segment (13). Sequence alignments of TCRβ cDNA and genomic clones revealed 12–25 nucleotides at the V_β–J_β junction that were not encoded by the germ-line V_β and J_β segments, and suggested the presence of D_β segments (11).

We have examined the molecular mechanisms for the generation of diversity in the chicken TCRβ locus by identifying the germ-line chicken D_β gene segment and by analyzing rearranged chicken TCRβ genes isolated from the developing thymus and mature spleen.† We observe that chicken TCRβ diversity is generated by junctional variability, rather than by gene conversion, as in chicken Ig genes. Further, the junctional diversity increases during development due to increased random base-pair (*N*-nucleotide) addition. Within the junctional variability, however, most rearranged chicken TCRβ genes have at least one glycine residue, which is encoded in the germ-line by an evolutionarily conserved D_β segment. The implications of a conserved glycine for TCR structure and antigen recognition are discussed.

MATERIALS AND METHODS

Isolation of Rearranged Chicken TCRβ Genes. Polymerase chain reaction (PCR; ref. 15) amplification, cloning, and sequencing of rearranged $V_{\beta1.1}$–D_β–$J_{\beta3}$ genes from 4-week thymus DNA were performed as described (16). Thirty cycles of amplification (1.5 min at 94°C, 3 min at 72°C) were performed in a Coy thermal cycler. PCR primers included a

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Abbreviations: CDR, complementarity-determining region; MHC, major histocompatibility complex; TCR, T-cell antigen receptor; TdT, terminal deoxynucleotidyltransferase.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M73064).

sense primer at the 5′ end of $V_{\beta 1.1}$ (5′-CCCCGTCGACGTTCAGACAAAGAGAGTGTAATCCA-3′) and an antisense primer in the 3′ flanking region of $J_{\beta 3}$ (5′-GGAAGCGGCCGCAGGTGAGGGAGATGACAAGCACAGAGCAGG-3′). *Sal* I and *Not* I restriction sites (underlined) at the 5′ ends of the primers facilitated cloning into the pBluescript SK(−) plasmid vector (Stratagene).

Identification of the Chicken Germ-Line D_β Gene Segment. An oligonucleotide probe (5′-GGGACAGGGGGATC-3′) spanning the putative chicken D_β gene segment (11) was end-labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase (17). Southern blots containing restriction digests of cloned genomic fragments of the germ-line chicken TCRβ locus (11) were prehybridized in 10× Denhardt's solution/0.9 M NaCl/90 mM Tris·HCl, pH 8/0.6 mM EDTA/0.1% SDS at 42°C for several hours. Blots were hybridized at 42°C overnight in the same solution after addition of labeled probe (10^6 dpm/ml). Blots were washed twice at room temperature and twice at 42°C in 0.9 M NaCl/0.09 M trisodium citrate, pH 7/0.1% SDS. A 1.8-kilobase (kb) *Hin*dIII genomic fragment positive for hybridization with the D_β oligonucleotide probe was subcloned into pGEM-7Zf(+) (Promega) and sequenced using primers specific for the SP6 and T7 sites of the vector and internal sequences.

RESULTS

Chicken TCRβ Diversity Is Focused at the V_β–J_β Junction. To assess the diversity of the chicken TCRβ repertoire, gene rearrangements involving the $V_{\beta 1.1}$ and $J_{\beta 3}$ genes were cloned from the thymus of a 4-week-old chicken after PCR amplification (15, 16). Comparison of these clones to the germ-line V_β and J_β sequences reveals no sequence variation within the V_β or J_β regions, but extensive variation is observed at the V_β–J_β junction (Fig. 1*A*), which encodes the third complementarity-determining region (CDR3) of the TCRβ chain. In contrast, rearranged chicken Ig genes isolated from the bursa of Fabricius at this age display multiple blocks of nucleotide substitutions throughout the *V* regions as a result of intrachromosomal gene conversion (6–10). Sequence variation of V_β gene segments as a result of gene conversion or somatic mutation does not appear to contribute to the TCRβ repertoire.

Identification of the Germ-Line Chicken D_β Gene Segment. The V_β–J_β junctions isolated from thymus DNA of a 4-week-old chicken (Fig. 1*A*) revealed a 12-bp core sequence that could represent a germ-line D_β segment. An oligonucleotide probe spanning this core sequence hybridized to a 1.8-kb *Hin*dIII fragment isolated from a genomic clone encompassing the 20-kb region 5′ of the J_β cluster. Nucleotide sequencing of this genomic fragment revealed a single germ-line D_β segment (Fig. 1*B*). Rearrangement of only one D_β segment with the four J_β segments was detected when Southern blots of thymic DNA were hybridized with probes located 5′ of D_β (data not shown). Comparison of the chicken, mouse (21, 22), rabbit (20), and human (18, 19) germ-line D_β segments revealed 100% nucleotide sequence identity for the first 11 bp of chicken D_β and the human and mouse $D_{\beta 1.1}$ gene segments, one nucleotide substitution in mouse $D_{\beta 2.1}$ and rabbit $D_{\beta 2}$, and a 3-bp insertion in human $D_{\beta 2.1}$ (Fig. 1*B*). There is some variation in the sequence and length at the 3′ ends of the germ-line D_β segments. Except for the conserved heptamer and nonamer elements of the recombination signal sequence, there is no significant sequence homology in the 5′ or 3′ flanking regions of the genomic D_β gene of chicken compared with mouse, rabbit, and human (Fig. 1*B*).

Non-Germ-Line-Encoded Nucleotide Additions to the V_β–D_β and D_β–J_β Junctions. Alignment of the germ-line D_β segment with our initial rearranged TCRβ clones (Fig. 1*A*) revealed a number of non-germ-line base pairs at the V_β–D_β and D_β–J_β junctions, suggesting that *N*-nucleotide addition may account for a large part of the TCRβ junctional diversity. *N* nucleotides are template-independent G+C-rich polynucleotide additions to *V(D)J* joints of mammalian Ig and TCR genes, believed to be mediated by terminal deoxynucleotidyltransferase (TdT) (23–25). This is in contrast to rearranged chicken Ig genes, which lack *N* nucleotides at *V(D)J* joints (6–10). To assess the contribution of *N* nucleotides to TCRβ diversity, additional rearranged $V_{\beta 1.1}$–D_β–$J_{\beta 3}$ clones were isolated from thymocyte DNA prepared from 18-day embryos (early) and from a chicken 4 weeks after hatching (late). Considerable junctional diversity is seen at the 3′ end of $V_{\beta 1.1}$, both ends of D_β, and the 5′ end of $J_{\beta 3}$ at each developmental stage (Fig. 2*A*). The amino acid sequence encoded by the rearranged $V_{\beta 1.1}$–D_β–$J_{\beta 3}$ genes (Fig. 2*B*) shows that the junctional variation at the 3′ end of V_β and at the 5′ end of J_β does not extend more than three amino acids into $V_{\beta 1.1}$ or $J_{\beta 3}$.

The average number of *N* nucleotides observed at chicken V_β–D_β and D_β–J_β junctions in 18-day clones is 1.1 ± 0.27 (mean ± SEM). Rearranged TCRβ genes cloned from the thymus at 4 weeks of age have significantly longer *N* sequence insertions, with an average of 5.1 ± 0.41 (mean ± SEM, $P < 0.001$) nucleotides per joint (Fig. 2*A*). Specific mononucleotide and possibly dinucleotide additions are ev-

A

	$V_{\beta 1.1}$	N-D_β?-N	$J_{\beta 3}$
germline	GTCCTACTCAATGACTCAGGCACTTATTTCTGTGCTAAGCAAGATA		CAAACACACCACTGAACTTTGGACAGGGCACTCGTCTGACAGTGCTTG
4wk #41	----------	CGAGACAGGGAGGC	----------
4wk #42	----------	GGAGGGACAGGGGATCGTTCT	----------
4wk #43	----------	CAGGGGGATCGGGTCCCG	----------
4wk #44	----------	CAGGGGGATCCGGGTC	----------

B

	nonamer	heptamer	D_β	heptamer	nonamer
Ch Dβ	ACACAGGTGGAATTGTTTTTGTACGAGACTGTACCGTTGTG	GGGACA	GGGGGATC	CACAATGATTCA//TTTCCCAGGAGGTCTTTACAAAAACCTGTAT/TTGCATAAGCCA	
Hu Dβ1.1	C--TG--A--GGC----------A-AG-----A-A-----	------	-----C//	----------AC-C-ACGG-A-/AC----------C-C-GGC-GTCCCAA-T	
Mu Dβ1.1	CT-TG--A--GTCCT--------TA-AG-----A-A-----	------	-----C//	---GG-------AT-C-ATGG-A-/-C----------AT-C-G-CTGTCCCAAGG	
Mu Dβ2.1	TGTGG--AA---ACT--------TC-CGA----A-A-----	-----T	-----GG-	----------AC-GGAAG---T/-CT----------G--C--CCCAAA-A--CA-C	
			AGC		
Hu Dβ2.1	-GCAG--A----ACA--------TC-TGG----A-A-----	-----T^	-----GG/	---G--------GG-AGAGG---T/-CT----------A-CC-GATGCAGTA-GCATC	

FIG. 1. (*A*) Nucleotide sequence alignment of rearranged chicken TCRβ genes with germ-line $V_{\beta 1.1}$ and $J_{\beta 3}$ segments (11). Dashes indicate identity with the germ-line sequence. A 12-base-pair (bp) core sequence for the putative D_β segment is underlined. (*B*) Alignment of nucleotide sequences of the chicken (Ch), human (Hu) (18, 19), rabbit (R) (20), and murine (Mu) (21, 22) genomic D_β segments. Dashes indicate identity with the top line of sequence, and slashes denote gaps introduced for alignment. The D_β region is boxed, and conserved heptamer and nonamer elements of the recombination signal sequence are underlined.

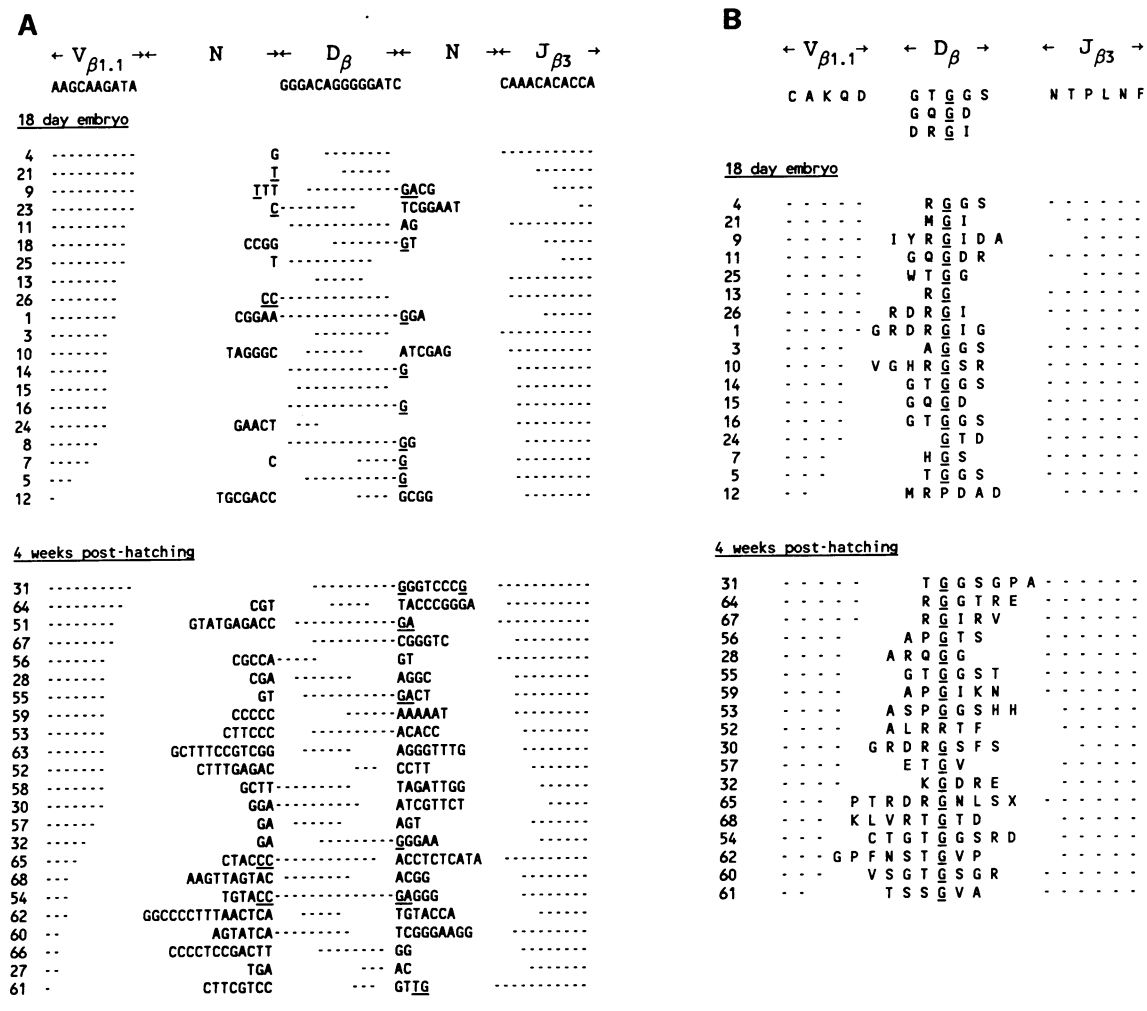


FIG. 2. (*A*) Nucleotide sequences of 43 rearranged $V_{\beta 1.1}$–D_β–$J_{\beta 3}$ junctions, isolated from 18-day embryonic and 4-week post-hatching thymus DNA. Clones were picked and numbered at random and are listed in order of decreasing length of germ-line V_β and J_β sequence. Sequences are aligned with the germ-line $V_{\beta 1.1}$, D_β, and $J_{\beta 3}$ sequences (top line). *N* nucleotides (23–25) are indicated, and possible *P* regions (16, 26) are underlined. Dashes indicate identity with the top line of sequence. Similar results were observed in 20 rearranged $V_{\beta 1.1}$–D_β–$J_{\beta 4}$ genes (16 in-frame, 4 out-of-frame). (*B*) Amino acid sequences of rearranged in-frame chicken $V_{\beta 1.1}$–D_β–$J_{\beta 3}$ junctions isolated from 18-day embryonic and 4-week post-hatching thymus DNA. Dashes indicate identity with the top line of sequence. The germ-line D_β-encoded glycine residues are underlined.

ident at the ends of some full-length V_β, D_β, and J_β segments, which appear to be palindromic to the coding-region nucleotides adjacent to the signal-sequence heptamer element. Originally reported for rearranged chicken Ig light-chain genes (16), the generality of specific palindromic nucleotide additions (*P* regions; ref. 26) has been extended to mammalian TCR (26) and Ig heavy-chain (27–29) genes and to chicken TCRβ genes (this report).

Out-of-frame and in-frame junctions are observed at both developmental time points with the same average number of *N* nucleotides (Fig. 2*A*). At embryonic day 18, there are 1.1 *N* nucleotides per in-frame joint (n = 48) and 1.3 per out-of-frame joint (n = 10); at 4 weeks post-hatching, there are 4.9 *N* nucleotides per in-frame joint (n = 54) and 5.9 per out-of-frame joint (n = 14).

A Conserved Glycine Residue Occurs Within the Hypervariable CDR3 Domain. Early and late chicken TCRβ gene rearrangements display extreme junctional diversity at the V_β–D_β and D_β–J_β junctions (Fig. 2*A*). However, one striking feature of CDR3 is the presence of at least one D_β-encoded glycine (Fig. 2*B*). The amino acid sequences of all germ-line chicken and mammalian D_β segments include at least one glycine in all three reading frames (Fig. 1*B*), and all three D_β reading frames are used in rearranged chicken (Fig. 2*B*) and mammalian (30–32) TCRβ genes. Thirty-three of 35 in-frame junctions (94%) contain at least one D_β-encoded glycine (Fig. 2*B*).

In contrast to the conserved glycine in most of the V_β–D_β–J_β junctions examined, the amino acid sequences of CDR3 on each side of the central D_β-encoded glycine residue are hypervariable. At position −1 from the central glycine, only three different amino acid residues are encoded by the germ-line D_β, yet 10 of the 20 possible amino acids are found in the 51 in-frame TCRβ rearrangements we examined (Fig. 2*B*). At position −2, where two different amino acids are encoded by D_β, 14 different amino acids are observed. Similarly, at positions +1 and +2, rearranged genes encode 12 and 11 different amino acid residues, respectively.

Chicken TCRβ Diversity in the Mature Spleen. The contribution of early and late TCRβ gene rearrangements to the TCR repertoire in the periphery was determined by isolating rearranged TCRβ genes from spleen DNA of a 6-week-old chicken. The nucleotide and amino acid sequences of rearranged splenic $V_{\beta 1.1}$–D_β–$J_{\beta 3}$ genes (data not shown) reveal that most in-frame junctions (12 of 14) include a D_β-encoded glycine residue. Both early and late TCRβ clones, characterized by little or no *N*-nucleotide addition and extensive *N*-nucleotide addition, respectively, appear to contribute to the adult splenic T-cell repertoire.

DISCUSSION

In this report we demonstrate that in contrast to the chicken Ig loci, in which somatic diversity is generated by gene conversion, somatic diversity is generated in the chicken TCRβ locus by junctional variation during *V(D)J* joining. Much of the TCRβ diversity is also generated by the addition of nontemplated *N* nucleotides, and the length of *N*-sequence addition at V_β–D_β and D_β–J_β junctions increases during development. It appears unlikely that selection at the protein level is responsible for the appearance of longer *N* sequences, because out-of-frame junctions are observed at both developmental time points with the same average number of *N* nucleotides as in-frame joints (Fig. 2*A*). These results suggest that the neonatal TCRβ repertoire in the thymus is different from that generated later in life and is encoded primarily by the germ-line TCRβ gene segments.

The observation that *N*-segment length increases during development suggests that the expression of TdT, the enzyme believed to mediate *N*-nucleotide addition to Ig and TCR genes, may be developmentally regulated in the chicken thymus. TdT levels increase gradually in the developing thymus of mice and rats (33, 34), and the length of *N*-nucleotide addition increases during development in rearranged mammalian Ig heavy-chain (27–29) and TCR (26, 35) genes. Developmentally regulated *N*-sequence addition therefore appears to be an evolutionarily conserved mechanism for generating diversity in vertebrate Ig and TCR genes. The lack of *N* segments in rearranged chicken Ig genes (6–10) is probably due to developmentally programmed Ig gene rearrangement (36) prior to expression of TdT in chicken lymphoid stem cells.

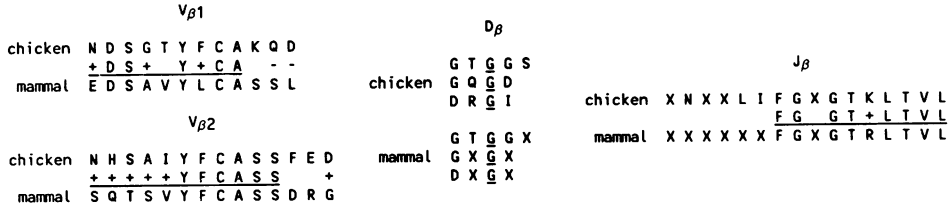
If junctional variability and increasing *N*-sequence addition are the mechanisms used to create CDR3 diversity, and TCRs undergo both positive and negative selection in the thymus, why is a D_β element required, and why is it so highly conserved? The requirement for two joining events involving D_β increases the junctional variability of CDR3, which is encoded at the V_β–D_β–J_β junction. One striking feature of CDR3, however, is the presence of at least one D_β-encoded glycine residue (Fig. 2*B*) within the otherwise hypervariable CDR3 domain. In addition to conservation of the D_β element, comparison of the consensus amino acid sequences for chicken and mammalian $V_{\beta1}$, $V_{\beta2}$, and J_β shows that CDR3 is also flanked by regions encoded by the V_β and J_β segments that have been evolutionarily conserved (Fig. 3*A*). Modeling studies based on high-resolution x-ray structures of the Ig Fv region (37) suggest that these conserved framework regions may be folded into a β-sheet structure.

In the case of the TCR molecule, which appears to have a three-dimensional structure similar to Ig, the conserved regions of V_β and J_β could serve to position the CDR3 domain of the TCRβ chain with respect to the antigen-binding groove of the MHC molecule (2, 38). The CDR3 domains of the TCRα and -β chains may contact antigen/MHC along the antigen-binding groove of the MHC molecule, whereas the CDR1 and CDR2 domains may interact predominately with the α-helices of the MHC molecule (Fig. 3*B*). If the TCRβ CDR3 encodes a loop that is positioned on the MHC molecule in such a way as to interact with peptide antigen, the α-carbon backbone of the TCRβ chain may be required to extend into, along, or across the groove and then turn back. We favor a model in which the loop extends along either side of the antigen-binding groove, because this position can accommodate the variable lengths of CDR3 observed in early and late TCRβ clones. The presence of one or more glycine residues may provide enough flexibility to allow the loop to cross over antigen positioned within the groove between the two MHC α-helices. Such a loop may be formed by a type II or glycine turn, in which all of the α-carbons of adjacent amino acids in the turn are oriented in one plane (39, 40). Glycine residues are also preferentially located in 2-, 4-, and 5-residue loops associated with β-hairpins in globular proteins (41) and in the longer ω loop structure, which is 6–16 residues long (42). That most chicken TCRβ rearrangements include at least one D_β-encoded glycine residue supports the hypothesis that a glycine-containing turn or loop may be important for the structure of the TCRβ CDR3 domain.

Interestingly, most rearranged human and murine TCRδ genes have a D_δ-encoded glycine (35, 43–45). In addition, the preferred reading frame of the murine Ig heavy-chain D_{SP2} and D_{FL16} gene segments includes a glycine codon (27–29, 46, 47). These observations suggest that a *D*-encoded glycine-containing turn may be important for the structure of the antigen-binding CDR3 loop in TCRβ, TCRδ, and Ig heavy chains.

Of the two clones we observed in the thymus that lacked a glycine in CDR3 (Fig. 2*B*), one (clone 12) encodes a proline residue, which would provide a kink in the α-carbon backbone to allow formation of a CDR3 loop. The observed bias for the addition of G nucleotides by TdT during TCR and Ig gene rearrangement (23–25) may provide an additional mechanism

A



B

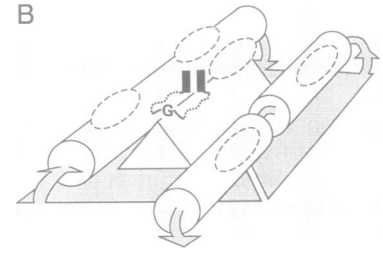


FIG. 3. (*A*) Comparison of consensus amino acid sequences for chicken and mammalian $V_{\beta1}$, $V_{\beta2}$, D_β, and J_β segments (11, 37). X indicates a position at which no consensus residue has been identified. Alignments were made based on the PAM250 amino acid similarity matrix using the AALIGN program (DNASTAR). A positive relationship (+), a 0-value relationship (blank space), a negative relationship (−), and identity between residues are indicated in the line between the chicken and mammalian consensus sequences. Conserved V_β and J_β regions and the central glycine of D_β are underlined. (*B*) Schematic model of TCR/antigen/MHC interaction (2, 38). The MHC molecule is depicted as two α-helical regions atop a β-pleated sheet, and the antigen is denoted by the triangular prism. Potential sites of TCRα- and -β-chain CDR1 and CDR2 interaction with the MHC molecule, and of the TCRα-chain CDR3 interaction with antigen/MHC, are depicted as dashed ovals. The ends of the conserved V_β and J_β regions (see *A*), symbolized by the filled rectangles, may provide anchor points by which the hypervariable loop of the TCRβ chain (dotted line) is fixed in position. To account for the variable length of the CDR3 loop (Fig. 2), the loop is depicted as extending away from the center of the MHC antigen-binding groove. However, the loop may extend into, along, or across the antigen-binding groove and may interact with antigen and/or both α-helices of the MHC molecule. Within the hypervariable TCRβ loop, the G symbolizes the conserved glycine residue encoded by the D_β segment.

to ensure that CDR3 encodes a glycine or proline turn, because addition of G nucleotides to the sense strand of V_β or D_β, or to the antisense strand of D_β or J_β, results in the creation of glycine codons (GGN) or proline codons (CCN), respectively.

Clone 52 in Fig. 2*B* lacks both glycine and proline in CDR3. However, because TCR gene rearrangement is ongoing in the thymus (2), one might speculate that this clone represents a T cell that has not undergone positive selection for antigen/MHC binding. One prediction of this model would be that most rearranged TCRβ genes isolated from peripheral T cells (e.g., spleen) might have a glycine turn in CDR3, having undergone selection for interaction with antigen/MHC via the CDR3 loop. Most in-frame rearranged TCRβ genes isolated from the splenic DNA from a 6-week-old chicken do include a D_β-encoded glycine (data not shown). Interestingly, each of the two splenic TCRβ clones lacking a glycine turn has a very short CDR3 (3 or 4 amino acids encoded by N–D_β–N), suggesting that in contrast to clones that encode larger CDR3 domains, the CDR3 domain of these TCRβ chains may not extend into or along the antigen-binding groove. Inspection of human TCRβ sequences reveals that those sequences with short CDR3 domains may also lack a glycine turn; however, nearly all human TCRβ sequences with longer CDR3 domains encode a glycine or proline in CDR3 (37).

In summary, although there appears to be limited diversity in the germ-line elements that encode the chicken TCRβ chain, extensive junctional diversity is created during TCRβ gene rearrangement in the chicken thymus. Junctional diversity increases during development as a result of increasing lengths of N-nucleotide addition at both the V_β–D_β and D_β–J_β junctions. Increased N-sequence addition during development expands the TCRβ chain repertoire by the addition of amino acids along each side of the hypervariable CDR3 loop. The conservation of the amino acid sequence at the 3′ end of V_β, at the 5′ end of J_β, and in the D_β segment suggests that the hypervariable portion of CDR3 may be positioned with respect to the antigen-binding groove of the MHC molecule by the conserved structural features of the V_β and J_β sequences and a glycine-containing turn encoded by D_β. This glycine turn is conserved in the nucleotide sequence of the germ-line D_β segment of chickens, mice, rabbits, and humans.

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