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# ABBREVIATIONS

The following abbreviations may be used without definition in *Poultry Science*. Plural abbreviations do not require "s". Chemical symbols and three-letter abbreviations for amino acids do not need definition. Units of measure, except those shown below, should be abbreviated as listed in the *CRC Handbook for Chemistry and Physics* (CRC Press, 2000 Corporate Blvd., Boca Raton, FL 33431) and do not need to be defined.

| | |
|---|---|
| A | adenine |
| AME | apparent metabolizable energy |
| $AME_n$ | nitrogen-corrected apparent metabolizable energy |
| ANOVA | analysis of variance |
| B cell | bursal-derived, bursal-equivalent derived cell |
| bp | base pairs |
| BSA | bovine serum albumin |
| BW | body weight |
| C | cytosine |
| cDNA | complementary DNA |
| cfu | colony-forming units |
| CP | crude protein |
| cpm | counts per minute |
| CV | coefficient of variation |
| d | day |
| df | degrees of freedom |
| DM | dry matter |
| DNA | deoxyribonucleic acid |
| EDTA | ethylenediaminetetraacetate |
| ELISA | enzyme-linked immunosorbent antibody assay |
| g | gram |
| *g* | gravity |
| G | guanine |
| GAT | glutamic acid-alanine-tyrosine |
| h | hour |
| HPLC | high-performance (high-pressure) liquid chromatography |
| ICU | international chick units |
| Ig | immunoglobulin |
| i.m. | intramuscular |
| i.p. | intraperitoneal |
| IU | international units |
| i.v. | intravenous |
| kb | kilobase pairs |
| kDa | kilodalton |
| L | liter* |
| m | meter |
| μ | micro |
| *M* | molar |
| ME | metabolizable energy |
| $ME_n$ | nitrogen-corrected metabolizable energy |
| MHC | major histocompatability complex |
| mRNA | messenger ribonucleic acid |
| min | minute |
| mo | month |
| MS | mean square |
| *N* | normal |
| n | number of observations |
| NRC | National Research Council |
| NS | not significant |
| PAGE | polyacrylamide gel electrophoresis |
| PBS | phosphate-buffered saline |
| ppm | parts per million |
| pfu | plaque-forming units |
| r | correlation coefficient |
| $r^2$ | coefficient of determination, simple |
| $R^2$ | coefficient of determination, multiple |
| RIA | radioimmunoassay |
| RNA | ribonucleic acid |
| rpm | revolutions per minute |
| s | second |
| s.c. | subcutaneous |
| SD | standard deviation |
| SDS | sodium dodecyl sulfate |
| SE | standard error |
| SEM | standard error of the mean |
| SRBC | sheep red blood cells |
| T | thymine |
| TBA | thiobarbituric acid |
| T cell | thymic-derived cell |
| TME | true metabolizable energy |
| $TME_n$ | nitrogen-corrected true metabolizable energy |
| Tris | tris(hydroxymethyl)aminomethane |
| TSAA | total sulfur amino acids |
| U | uridine |
| USDA | United States Department of Agriculture |
| vol/vol | volume to volume |
| vs | versus |
| wt/vol | weight to volume |
| wt/wt | weight to weight |
| wk | week |
| $\bar{x}$ | mean |
| yr | year |

*Also capitalized with any combination, e.g., mL.



# POULTRY SCIENCE



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# T Cell Development in the Chicken[1]

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**ABSTRACT** This review summarizes our current view of γδ and αβ T cell development in the chicken. In it we emphasize the functional interplay between the γδ and αβ T cell subpopulations.
(*Key words*: T cell receptors γδ and αβ, Vβ usage, T cell subpopulations, accessory molecules, monoclonal antibodies)

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## INTRODUCTION

Many features of T cell development in birds and mammals are very similar. The T cell receptors (TCR) and accessory molecules defined for mammalian T cells are well conserved in birds. The analysis of avian T cell development using monoclonal antibodies against these cell surface molecules also reveals that the central features of T cell development in mammals are also conserved in the chicken. On the other hand, the avian T cell repertoire is much less complex, and the avian embryo more assessible for experimental manipulation. These and other unique features make the avian model system an informative one for study of T cell development and function.

## T CELL RECEPTORS AND ACCESSORY MOLECULES

Monoclonal antibodies (mAb) have been produced against a variety of functionally important molecules expressed on the surface of chicken T cells (Chen *et al.*, 1991), and most of these have well-defined mammalian counterparts. The chicken T cell receptors can be divided into three subgroups, each of which can be identified by a specific mAb. All of the γδ T cells are recognized by the TCR1 mAb (Sowder *et al.*, 1988), whereas two discrete subsets of αβ T cells can be identified by the TCR2 and TCR3 mAb (Chen *et al.*, 1988; Cihak *et al.*, 1988; Char *et al.*, 1990). All three receptor molecules are disulfide-linked heterodimers that are noncovalently associated with a CD3 protein complex to form a signal transduction unit. The avian CD3 complex contains chains similar to the mammalian CD3 γ, δ, ε, and ζ chains (Chen *et al.*, 1986; Göbel *et al.*, unpublished data), but only the CD3 gene that encodes a 19-kDa chain has been cloned so far (Bernot and Auffray, 1991; Lahti *et al.*, St. Jude Children's Research Hospital, Memphis, TN 38101-0318, personal communication). The sequence of this chicken CD3 protein has homology with both the mammalian CD3 γ and δ chains.

The CD4 and CD8 coreceptors have also been identified in the chicken (Chan *et al.*, 1988). The CD4 molecule is a single peptide and CD8 is a disulfide-linked dimer. Each molecule is associated with a cellular tyrosine protein kinase that is homologous to the mammalian $p56^{lck}$ (Veillette and Ratcliffe, 1991). As in mammals, both CD8α and β chains are expressed in the chicken to form CD8αα homodimers and CD8αβ heterodimers

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(Kong *et al.*, 1994; Young *et al.*, Institute for Animal Health, Newsbury, Berkshire RG16 ONM, U.K., personal communication). The molecular weights, tissue distribution, and function of the TCR/CD3, CD4, and CD8 molecules are all very similar to their mammalian counterparts (Chen *et al.*, 1990; Cooper *et al.*, 1991).

## THREE SUBPOPULATIONS OF T CELLS

The avian thymus is colonized with thymocyte precursors in waves during embryogenesis, and the thymocyte progeny of each sequential wave overlap each other (Coltey *et al.*, 1987). During T cell ontogeny, the three subpopulations of T cells appear in the order TCR1, TCR2, and TCR3 (Char *et al.*, 1990). Analyses of chicken thymocyte development in chick-quail chimeras reveal that all three subsets of T cells are derived from each wave of thymocyte precursors (Coltey *et al.*, 1989). Migration of the three T cell subsets to the periphery also follows the same TCR1, TCR2, an TCR3 order (Char *et al.*, 1990), but the TCR2 cells become the predominant population in mature chickens.

## UNIQUE FEATURES OF TCR1 ($\gamma\delta$) CELLS

Unlike human and mouse, in which $\gamma\delta$ cells comprise a minor subset of T lymphocytes in the circulation (Haas *et al.*, 1993), the chicken has a relatively large subset of $\gamma\delta$ T cells (Sowder *et al.*, 1988). The frequency of TCR1 cells is usually 20 to 25% of the total blood T cells, but can reach approximately 50% in chickens of 6 mo of age (Cihak *et al.*, 1993). The high frequency of avian $\gamma\delta$ T cells and the availability of the anti-$\gamma\delta$ mAb has allowed extensive characterization of the properties of $\gamma\delta$ T cells in the chicken (Table 1).

**TABLE 1. Special features of avian T cell receptor 1 (TCR 1) cells**

| | |
|---|---|
| 1. | Large subpopulation of T cells |
| 2. | Characteristic *intrathymic* developmental pattern<br>Short cortical transit time<br>High level of TCR1/CD3 expression<br>Not easily aborted via TCR1-mediated signals |
| 3. | Preferential homing to intestinal epithelium and splenic red pulp |
| 4. | Acquire CD8 in the periphery |
| 5. | Cytotoxic capability, but lack graft-vs-host potential |
| 6. | Require exogenous growth factors |

The $\gamma\delta$ thymocytes are unlike the $\alpha\beta$ T cells in that they express high levels of their TCR complex from the earliest time in appearance in the thymic cortex, and these $\gamma\delta$ receptors are relatively difficult to modulate by receptor cross-linkage (George and Cooper, 1990). Whereas $\alpha\beta$ thymocytes take several days to migrate from cortex to medulla, during which they undergo extensive proliferation and selection, $\gamma\delta$ cells rapidly traverse this compartment and soon exit from the thymus (Bucy *et al.*, 1990). These results suggest that $\gamma\delta$ T cells may not undergo the same selection pressures as $\alpha\beta$ thymocytes.

The distribution patterns differ for $\alpha\beta$ and $\gamma\delta$ cells in peripheral lymphoid tissues (Bucy *et al.*, 1988). In the spleen, the $\gamma\delta$ cells are located predominantly in the sinusoidal areas. In the intestine, they are preferentially localized in the epithelium. In contrast, both TCR2 and TCR3 cells home to the periarteriolar lymphatic sheaths in spleen, and TCR2 cells are located mainly in the lamina propria of the intestine. Interestingly, TCR3 cells are rarely found in the intestine.

The majority of the $\gamma\delta$ cells in thymus and blood are CD4-CD8- (Sowder *et al.*, 1988), although a small subset of them may express CD8 or CD4 coreceptors (unpublished data; Davidson *et al.*, 1992). However, when the $\gamma\delta$ cells migrate into the spleen and intestine, most of them begin to express CD8.

The biological function of $\gamma\delta$ T cells is still unclear, but they are clearly capable of cytotoxic activity *in vitro*. Using a redirected cytotoxicity assay, $\gamma\delta$ T cells were shown to specifically lyse anti-CD3 hybridoma cells (Chan *et al.*, Rutgers University, Piscataway, NJ 08855-6268, personal communication). The CD8+ $\gamma\delta$ T cells may also be involved in down-regulation of immune responses (Quere *et al.*, 1990). However, they are incapable of inducing graft-vs-host (GVH) reactions,

whereas both the TCR2 and TCR3 subpopulations of CD4+ αβ T cells are capable of GVH activity (Char *et al.*, Baylor College of Medicine, Division of Neurosciences, Houston, TX 77030-3498, personal communication).

## DEPENDENCE OF γδ T CELL GROWTH ON αβ T CELLS

During studies on the developmental origin of γδ T cells, we examined the long-term effects of thymectomy on the development of T cells. Neonatal thymectomy resulted in a dramatic and persistent decrease of TCR1 cells to a frequency of 5% or less of blood T cells, whereas the frequencies of TCR2 and TCR3 cells were not altered significantly (Chen *et al.*, 1989; Cihak *et al.*, 1993). This observation suggests that expansion of the γδ population in the periphery requires continual seeding of thymic γδ T cells. Moreover, unlike the αβ cells that exhibit follicular growth, γδ cells do not. Instead they are randomly distributed in the peripheral tissues predominantly as single cells (Bucy *et al.*, 1990). These results imply that the γδ T cells differ strikingly from αβ T cells in their proliferative characteristics.

Because of their high frequency in the chicken, it is relatively easy to analyze the growth requirements of normal γδ T cells. When TCR1, TCR2, and TCR3 cells are purified by negative selection and their proliferative responses compared, the TCR1 cells cannot respond well to mitogens or TCR ligation, except in the presence of αβ T cells. In contrast, the αβ T cells can grow very well alone (Kasahara *et al.*, 1993). The TCR1 cells fail to produce adequate amount of interleukin (IL)-2 and they proliferate in response to receptor ligation only in the presence of exogenous cytokines, including IL-2. Furthermore, only the CD8+ subpopulation of γδ T cells responds to the dual stimulation of receptor ligation and exogenous growth factors. The CD8+γδ T cells are relatively large and express MHC Class II on their surface, indicating a state of activation. Because activated T cells can process and present antigen (Wyss-Coray *et al.*, 1993), we suggest that a two-way interaction between γδ and αβ T cells may result in mutual regulatory roles of these two subpopulations in the immune response (Kasahara *et al.*, 1993). Analysis of this interaction may be essential for understanding the biological function of γδ T cells.

## TWO DISTINCT SUBPOPULATIONS OF αβ T CELLS

### *TCR2 and TCR3 Cells Differ in Function*

In addition to their differences in ontogeny and tissue distribution, the two αβ T cell subpopulations that express TCR2 or TCR3 receptors also exhibit functional differences (Table 2). Both TCR2 and TCR3 cells are capable of GVH alloreactivity, but they vary in their GVH potential depending on donor and recipient MHC combinations

**TABLE 2. Comparison of chicken T cell receptor (TCR) TCRα and TCRβ chains**

| Variable | TCRα | TCRβ |
|---|---|---|
| cDNA, kb | 1.7 | 1.3 |
| Amino acids | 257 | 273 |
| Predicted molecular weight, kDa | 28 | 31 |
| Predicted isoelectric point | 5.0 | 8.5 |
| Possible N-glycosylation sites | 1 | 4 |
| Homology to mammals, % | | |
| Constant region | 26 | 35 |
| Joining region | 35 | 47 |
| Diversity region | . . .[1] | + |
| Variable region | 28 | 22 (Vβ1)<br>46 (Vβ2) |

[1]No diversity region in TCRα.

(Char *et al.*, personal communication). This may suggest repertoire differences in the TCR2 and TCR3 populations.

When TCR2 cells are suppressed by embryonic injection of anti-TCR2 monoclonal antibody and subsequent thymectomy, the treated chickens acquire increased levels of TCR3 cells but are deficient in TCR2 cells (Chen *et al.*, 1989; Cihak *et al.*, 1991). These TCR2-depleted birds appear healthy and they can respond normally to many T cell-dependent and T cell-independent antigens (unpublished data). Their serum IgG and IgM concentrations are also normal but their capacity for IgA production is severely compromised (Cihak *et al.*, 1991). Secretory IgA concentrations in bile and lung lavage fluid are reduced 1,000- to 10,000-fold, and secretory IgA antibodies are not produced in response to mucosal immunization. These results indicate the importance of TCR2 cells in IgA production.

### *TCRβ Genes*

Definition of the chicken TCRα and TCRβ genes has provided insight into the molecular basis for the differences in the two αβ T cell subsets. The cloning of the TCRβ chain was achieved by cross-hybridization of a chicken cDNA library with fragments of a mixture of mammalian TCRβ DNA under low stringency conditions (Tjoelker *et al.*, 1990). A chicken TCRβ cDNA encodes a protein of approximately 300 amino acids including leader (L), variable (V), joining (J), diversity (D), and constant (C) regions (Figure 1). Although chicken and mammalian TCRβ chains display only approximately 30% overall amino acid sequence identity, a number of conserved structural features are observed. These include consensus amino acids that are found in the most mammalian TCRβ chains, the cysteine residues that form intra- and interdisulfide bonds, and a positively charged lysine that is thought to form a salt bridge with a negatively charged amino acid of CD3 molecules in the transmembrane domain (Bernot and Auffray, 1991).

The TCRβ locus contains mammalian-type, V, D, J, and C segments (Tjoelker *et al.*, 1990; Cooper *et al.*, 1991). The exon structure of the C region is virtually identical with that of mammals. The genomic V, D, and J elements are flanked by classical heptamer-nonamer recombination signal sequences. As in mammals, the TCRβ repertoire in the chicken is created by ordered recombination of V-D-J segments. However, the chicken TCRβ locus is much simpler in that it contains only two Vβ families and does not feature a duplication of the J and C regions (Tjoelker *et al.*, 1990; Lahti *et al.*,

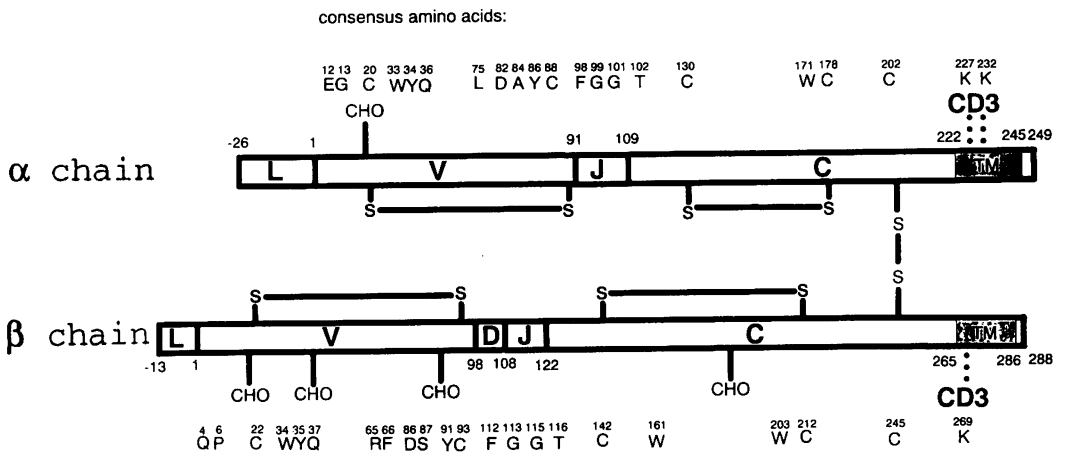


**FIGURE 1.** Sketch depicts overall T cell receptor (TCR) TCRαβ structure and indicates disulfide bonds, possible N-glycosylation sites (CHO), leader (L), variable (V), diversity (D), joining (J), and constant (C) regions. The consensus amino acid residues and their positions are listed by protein structure.

1991). Within each Vβ gene family, most chicken strains contain approximately 6 members of the Vβ1 and 3 to 5 members of the Vβ2 family, although 17 Vβ1 members have been identified in the chicken strain H.B19 (Dunon *et al.*, 1994). Likewise, only four relatively similar J segments are identified. Thus there is a limited capacity for combinatorial diversity in the avian TCRβ repertoire. Instead, the CDR3 sequences formed by VD and DJ recombination with N-sequence additions are unique for each TCRβ (McCormack *et al.*, 1991).

### *TCRα Genes*

The chicken TCRα genes have only recently been identified. The TCRα proteins were isolated by antibody affinity chromatography and peptide sequences were determined. Degenerate oligonucleotide probes were then used to identify TCRα cDNA. A TCRα cDNA clone consists of 1.7 kb containing a 375-bp 5′ untranslated region, a 503-bp 3′ untranslated region, and an open reading frame of 825 bp (Göbel *et al.*, 1993). The predicted 275 amino acid TCRα chain contains V, J, and C regions. Although chicken TCRα shares only 26% overall homology with its mammalian counterpart, most of the consensus amino acids thought to be important for structural integrity of the mammalian TCRα chains are conserved (Figure 1). Genomic analysis reveals multiple J segments and at least one Vα family that contains approximately 25 members. The classical heptamer and nonamer recombination signal sequences and length of the spacer between them are conserved. In contrast to the mammalian and avian TCRβ loci, however, a single exon encodes the avian Lα and Vα. A comparison of TCRα and β chains is shown in Table 3.

***TCR2 and TCR3 Cells Use Distinct Vβ Families.*** The relationship between TCR gene usage and the TCR2 and TCR3 sublineages defined by mAb was analyzed by Northern blotting. Both TCR2 and TCR3 cells contain Cα and Cβ mRNA, confirming that they are subsets of αβ T cells. Although the same Dβ and Jβ are shared by both subsets, the TCR2 cells contain only Vβ1 transcripts and TCR3 cells contain only Vβ2 mRNA (Lahti *et al.*, 1991). Furthermore, TCR2 cells undergo V-D-J joining by deletional rearrangement, whereas TCR3 cells undergo V-D-J joining by inversional rearrangement (Table 2). This might explain why the Vβ1 gene segment rearranges prior to Vβ2 segment during ontogeny. Interestingly, mammalian TCRβ chain sequences can be subdivided into two subgroups, VβI and VβII, based on the structural similarities of the proteins (Schiffer *et al.*, 1992), and these same structural features are conserved in chicken Vβ1 and Vβ2 (Tjoelker *et al.*, 1990).

**TABLE 3. Two distinct αβ T cell subpopulations in the chicken[1]**

| Characteristic | TCR2 | TCR3 |
|---|---|---|
| Appearance during ontogeny | | |
| Thymus | E14[2] | E17 |
| Spleen | E19 | D2 |
| Phenotype | | |
| CD4:CD8 ratio | 2:1 to 3:1 | 4:1 |
| Tissue homing pattern | | |
| Spleen | Periarteriolar sheaths | Periarteriolar sheaths |
| Intestine | Lamina propria | Rarely identified |
| Function | | |
| Helper activity for IgA production | Yes | No |
| Graft vs host | Quantitative differences depending on MHC pairing | Quantitative differences depending on MHC pairing |
| TCRβ[2] usage | Vβ1 (1.1 to 1.17) | Vβ2 (2.1 to 2.5) |
| Mechanism of Vβ rearrangement | Deletion | Inversion |

[1]TCR = T cell receptor.

[2]Embryonic day 14.

The Vα usage by TCR2 and TCR3 cells may also contribute to their differences in function. To examine the utilization of Vα segments by TCR2 and TCR3 cells, a panel of the defined cell lines was examined by Northern blot analysis. Under very stringent conditions 2 of 10 cell lines reacted with the Vα1 probe cloned from TCR2 cell line UG9 (Göbel *et al.*, 1993). Analysis of $V\alpha^-$ cDNA clones has revealed multiple Vα families in the chicken. Homology between the Vα1 and Vα2 members is only around 24%. Interestingly, both TCR2 and TCR3 cells can use Vα2 family members, suggesting that Vβ1 and Vβ2 genes themselves govern functional differences in TCR2 and TCR3 T cells.

## CONCLUDING REMARKS

Comparative studies reveal striking conservation of T cell development in avian and mammalian species. The relatively high frequency of γδ T cells in the chicken and the experimental accessibility of the embryo make birds a valuable model for study of the early divergence in the αβ and γδ T cell lineages as well as the physiological role of γδ T cells. The two subsets of αβ T cells recognized by TCR2 and TCR3 mAb express prototypic VβI and VβII genes, and they differ in their ontogeny, tissue distribution, and function. It is not yet known whether or not the two αβ subpopulations utilize different Vα genes. The relative simplicity of the avian αβ TCR gene loci may thus reveal basic principles in T cell physiology that are difficult to appreciate in more complex mammalian systems. Finally, our studies in the chicken model suggest that γδ and αβ T cells are functionally interdependent.

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