

# A LARGE SUBPOPULATION OF AVIAN T CELLS EXPRESS A HOMOLOGUE OF THE MAMMALIAN T $\gamma$ / $\delta$ RECEPTOR

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Thymus-derived cells were first shown in birds to be a separate lineage of antigen-specific lymphocytes that do not produce immunoglobulins (1). The nature of the TCR has since been defined in mammals. The TCR expressed by most T cells in mice and humans is a heterodimer of  $\alpha$  and  $\beta$  glycoprotein chains (2-4). A second TCR, composed of  $\gamma$  and  $\delta$  chains, has recently been identified on a low percentage of thymocytes and peripheral T cells in mice and humans (5-11). The  $\gamma$  chain genes are rearranged and expressed before the  $\alpha$  and  $\beta$  chain genes during murine thymic ontogeny (9, 10, 12-14), suggesting a role for the  $\gamma$ / $\delta$  receptor in T cell development.

Both the TCR- $\alpha$ / $\beta$  and - $\gamma$ / $\delta$  are physically associated with three or more cell surface proteins to form a TCR/T3 complex in mammals (5, 8, 15, 16). mAbs to the mammalian T3 molecules, called cluster-determinant 3 (CD3), can thus be used to immunoprecipitate the entire TCR/T3 complex when the cell surface proteins are solubilized with a relatively mild detergent such as digitonin (16). In an earlier study, we described a mAb (CT3) to chicken T3 molecules and used it to identify an associated TCR heterodimer (17). We have since produced mAbs to chicken CD4 and CD8 homologues (CT4 and CT8; reference 18). In this report we describe an antibody named TCR1 that identifies a T3-associated heterodimer that resembles the mammalian TCR- $\gamma$ / $\delta$ .

## Materials and Methods

*Chickens.* Outbred white Leghorn chickens and fertile eggs were obtained from local commercial hatcheries.

*Lymphocyte Isolation and Analysis.* Mononuclear white blood cells were isolated by low speed centrifugation of heparinized blood samples. Thymus, spleen, and bursa were physically disrupted using a loose-fitting glass homogenizer and the cells were suspended in HBSS supplemented with 5% FCS and 0.1% NaN<sub>3</sub>. For indirect immunofluorescence staining, 1-2  $\times$  10<sup>6</sup> viable cells were incubated with the TCR1 hybridoma supernatant or a control mouse antibody of irrelevant specificity, washed, then incubated with FITC-

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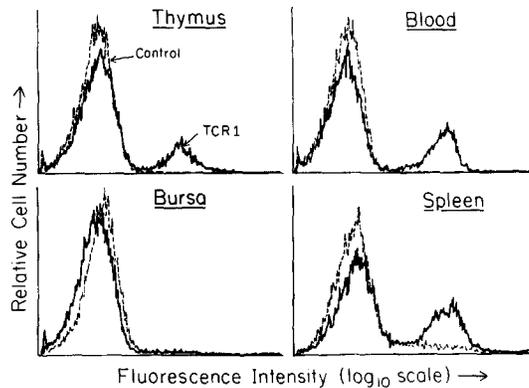


FIGURE 1. Tissue distribution of TCR1-reactive cells. Indirect immunofluorescence of viable cells with the TCR1 antibody (*solid lines*) and a control antibody of the same isotype (*dashed lines*).

goat anti-mouse Ig antibody (Southern Biotechnology Associates, Inc., Birmingham, AL). Antibodies used in fluorochrome conjugations were purified by chromatography on protein A-agarose columns (Genzyme, Boston, MA; reference 19). Conjugation of phycoerythrin (PE)<sup>1</sup> to the TCR1 and CT3 mAbs was performed by Southern Biotechnology Associates, Inc., and the conjugation of FITC to TCR1 was as described (20). Relative fluorescence intensities were measured by automated flow cytometry using a FACS IV instrument (Becton-Dickinson Immunocytometry Systems, Mountain View, CA) equipped with a 2-W argon laser. Lymphocyte incorporation of [<sup>3</sup>H]thymidine was evaluated as described (17).

**Biochemical Analysis of Cell Surface Antigens.** Viable lymphocytes were surface labeled with Na <sup>125</sup>I using the lactoperoxidase method, and cell surface proteins solubilized in either 0.5% NP-40 lysing buffer (21) or 1% digitonin lysing buffer (16) were examined by the solid-phase immunoprecipitation technique (21). Immunoabsorbed molecules were eluted with 2% SDS with or without 2-ME and separated on 10% SDS gels. Digestion with *N*-glycosidase F (Genzyme) was done according to the method of Tarentino et al. (22).

## Results

**Tissue Distribution of TCR1<sup>+</sup> Cells.** The TCR1 hybridoma was made by fusing a plasmacytoma variant (23) with lymph node cells from a BALB/c mouse immunized with thymocytes and Ig-negative blood lymphocytes from outbred chickens. The antibody reacted by indirect immunofluorescence with a subpopulation of Ig-negative lymphocytes. In tissues from adult chickens, TCR1 reacted with ~10% of thymocytes, 15% of circulating lymphocytes, 25% of spleen cells, and <1% of bursal and bone marrow cells (Fig. 1). TCR1 was unreactive with granulocytes, erythrocytes, platelets, macrophages, B cells, and 11 T cell lines derived from birds with Marek's disease.

**Biochemical Characteristics of the TCR1 Antigen.** The TCR1 molecule was analyzed by immunoprecipitation of surface <sup>125</sup>I-labeled blood mononuclear cells and electrophoretic migration in SDS polyacrylamide gels. From nonreduced samples of NP-40-solubilized cells, the TCR1 antibody precipitated a single broad band with an *M<sub>r</sub>* of 90,000 (Fig. 2, lane *e*). After reduction, two bands of 50,000 and 40,000 *M<sub>r</sub>* were seen (lane *h*). This pattern was similar to that for TCR molecules coprecipitated with the CT3 antibody from digitonin-solubilized cells (lanes *c* and *g*). The T3 complex, precipitated alone from NP-40 lysates, is

<sup>1</sup> Abbreviation used in this paper: PE, phycoerythrin.

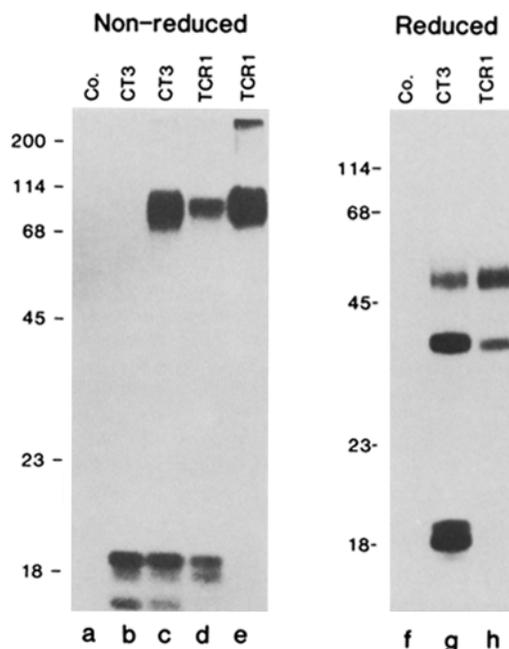


FIGURE 2. Biochemical analysis of TCR1- and CT3-reactive molecules. Mononuclear white blood cells were surface labeled with Na  $^{125}$ I and then solubilized in either 0.5% NP-40 lysing buffer (lanes *b*, *e*, *f*, and *h*) or 1% digitonin lysing buffer (lanes *a*, *c*, *d*, and *g*). Molecules isolated by immunoadsorption were separated on 10% SDS gels. Relative molecular masses of standards ( $\times 10^{-3}$ ) are indicated on the left of each gel pattern.

shown in lane *b*. When TCR1 was used in immunoprecipitation of digitonin-solubilized cells, the T3 complex coprecipitated with the TCR1 reactive molecules (lane *d*) suggesting a physical association of T3 and TCR1. To test this possibility, sequential immunoprecipitations of digitonin-solubilized proteins were performed. The TCR1 antibody did not precipitate any material from cell lysates cleared previously with the CT3 antibody, while lysates cleared first of TCR1 reactive molecules retained material with an  $M_r$  of 90,000 that could be precipitated with the CT3 antibody, representing other TCR molecules (data not shown). These results, and identical results obtained with thymocytes, suggested that the TCR1 molecules are T3-associated heterodimers which are expressed on a subpopulation of CT3<sup>+</sup> lymphocytes.

To determine if the protein backbones of the molecules reactive with TCR1 are the same as those of most CT3-associated receptors, we performed *N*-glycosidase F digestions of TCR1 and CT3 immunoprecipitates. After deglycosylation, two polypeptides of 35,000 and 32,000  $M_r$  were resolved from TCR1 immunoprecipitates (Fig. 3, lane *b*), whereas CT3 immunoprecipitates yielded major deglycosylated proteins of 34,000 and 29,000  $M_r$  (Fig. 3, lane *f*) with deglycosylated proteins of  $M_r$  35,000 and 32,000 being minority elements visible on original autoradiographs. The results indicated that the TCR1 antibody recognizes a different heterodimer than the TCR heterodimer present on the majority of chicken T cells.

**Immunophenotype of TCR1<sup>+</sup> Cells.** T $\gamma/\delta$  cells in humans and mice characteristically lack the CD4 and CD8 surface markers (5, 8, 11, 24). TCR1<sup>+</sup> cells were therefore examined by two-color immunofluorescence with the mAbs TCR1, CT3, CT4, and CT8. As expected from the preceding data, the TCR1<sup>+</sup> cells expressed the CT3 antigen (Fig. 4A). While all of the CT4<sup>+</sup> and CT8<sup>+</sup> cells also

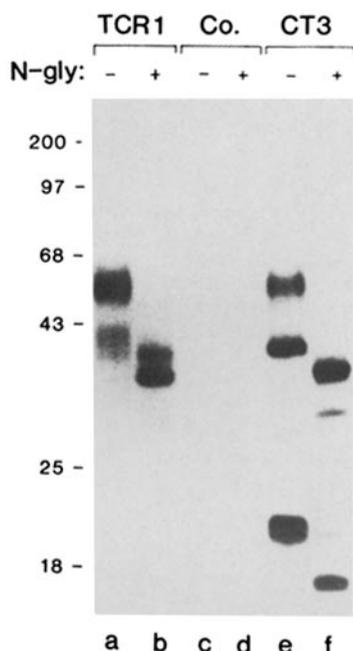


FIGURE 3. Deglycosylation of CT3 and TCR1 immunoprecipitates. CT3 immunoprecipitates from surface  $^{125}\text{I}$ -labeled and digitonin solubilized cells (lanes *e* and *f*) and TCR1 immunoprecipitates from NP-40 solubilized cells (lanes *a* and *b*) or the control samples (lanes *c* and *d*) were treated with *N*-glycosidase F (lanes *b*, *d*, and *f*), then separated by SDS-PAGE under reducing conditions. The undigested samples were run in parallel (lanes *a*, *c*, and *e*).

expressed CT3, a subpopulation of CT3<sup>+</sup> cells were CT4<sup>-</sup>,CT8<sup>-</sup> (Fig. 4B), and these cells were TCR1<sup>+</sup> (Fig. 4C). The frequency of CT3<sup>+</sup> cells ( $85 \pm 5\%$ ; mean  $\pm 1$  SD) among blood lymphoid cells was equal to the sum of the frequencies of CT4<sup>+</sup> ( $49 \pm 12\%$ ), CT8<sup>+</sup> ( $19 \pm 5\%$ ), and TCR1<sup>+</sup> ( $16 \pm 6\%$ ) cells, a further indication that the CT4, CT8, and TCR1 molecules mark discrete T cell subpopulations.

**Ontogeny of TCR1<sup>+</sup> Cells.** Ontogenetic studies revealed expression of TCR1 on a small percentage of thymocytes by embryonic day 11 (Fig. 5A). The TCR1<sup>+</sup> thymocytes increased to  $\sim 30\%$  by embryonic day 15, then declined to  $\sim 5\%$  by hatching. The TCR1<sup>+</sup>,CT3<sup>+</sup> thymocytes, which were CT4<sup>-</sup>,CT8<sup>-</sup>, gradually increased in frequency after hatching to reach an adult level of  $\sim 10\%$ . TCR1<sup>-</sup>,CT3<sup>+</sup> thymocytes appeared after embryonic day 15 then quickly increased to greatly exceed the level of TCR1<sup>+</sup>,CT3<sup>+</sup> thymocytes (Fig. 5, A and B). TCR1<sup>+</sup> and CT3<sup>+</sup> lymphocytes were infrequent in the spleens of embryos (Fig. 5A). Splenic population with CT3<sup>+</sup> cells was initiated around the time of hatching, and adult levels were acquired over the first weeks of life.

Immunofluorescence analysis indicated that the first embryonic wave of intrathymic T cells express TCR1 and CT3 at cell surface levels comparable to those characteristic of mature T cells (compare Figs. 1 and 5B). By contrast, the CT3 levels on the TCR1<sup>-</sup> population of thymocytes were relatively low, a pattern of expression noted in the development of discrete subsets of TCR<sup>+</sup>- $\alpha/\beta$  cells in mice (24–26).

**Signal Transduction by the TCR1/T3 Complex.** The capacity of TCR1<sup>+</sup> cells to proliferate in response to cross-linkage of their receptors was examined using CT3 and TCR1 antibodies conjugated to Sepharose 4B beads (17). Each immobilized antibody was capable of stimulating DNA synthesis in a TCR1<sup>+</sup> population

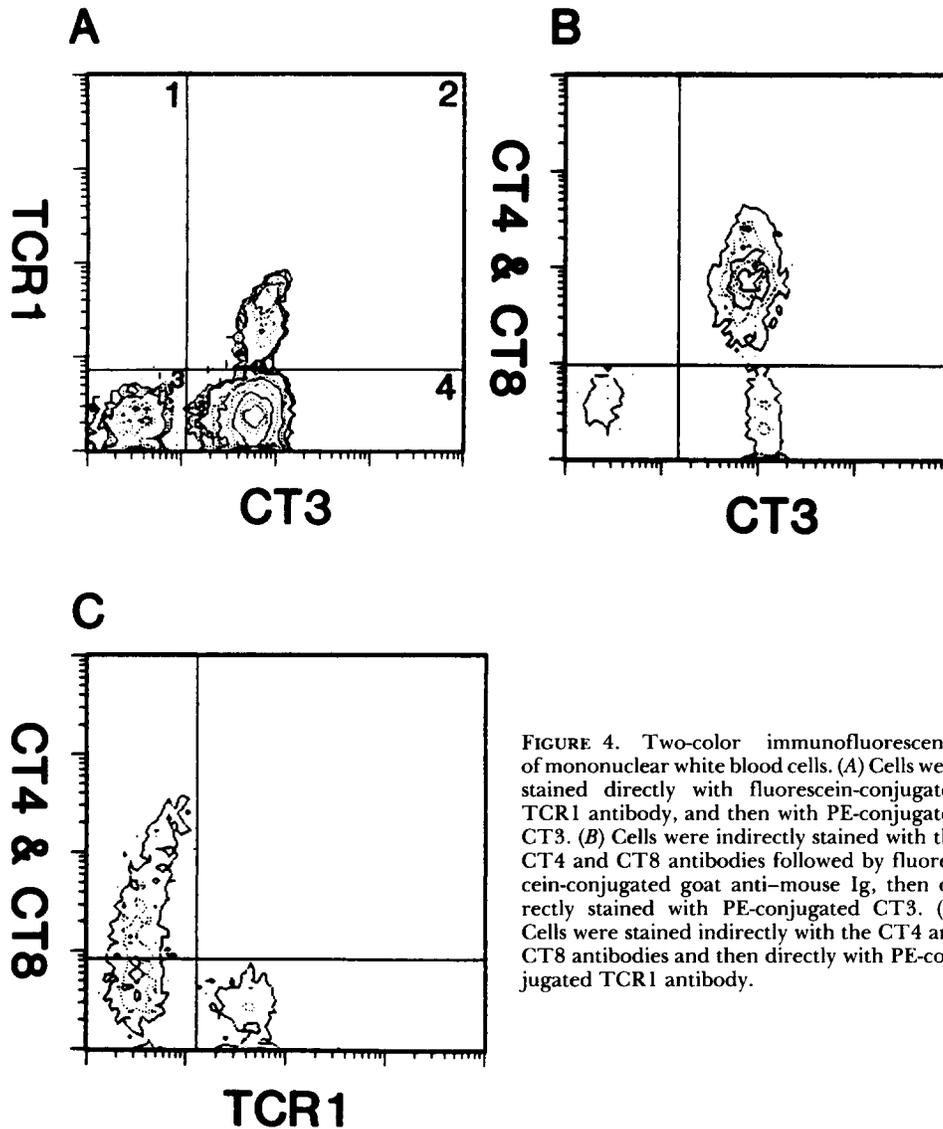
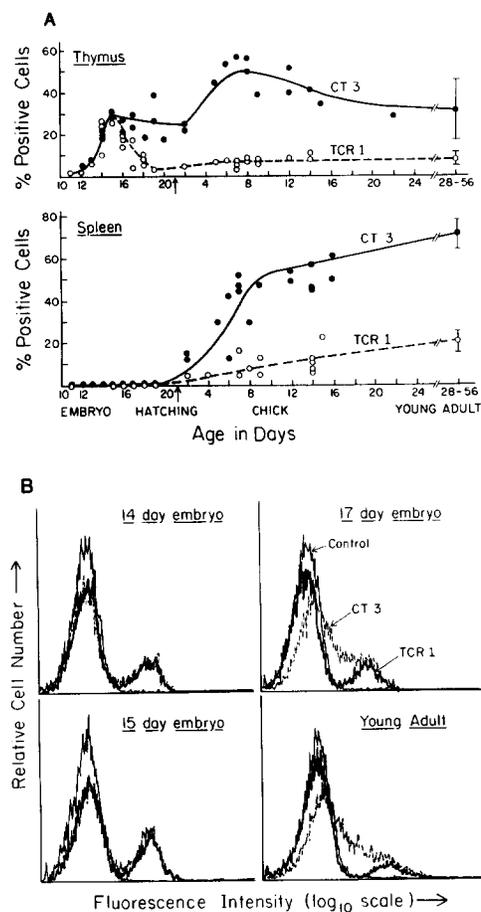


FIGURE 4. Two-color immunofluorescence of mononuclear white blood cells. (A) Cells were stained directly with fluorescein-conjugated TCR1 antibody, and then with PE-conjugated CT3. (B) Cells were indirectly stained with the CT4 and CT8 antibodies followed by fluorescein-conjugated goat anti-mouse Ig, then directly stained with PE-conjugated CT3. (C) Cells were stained indirectly with the CT4 and CT8 antibodies and then directly with PE-conjugated TCR1 antibody.

of blood lymphocytes depleted of IgM<sup>+</sup>, CT4<sup>+</sup>, and CT8<sup>+</sup> cells by a FACS. TCR1 induced a 15-fold increase in [<sup>3</sup>H]thymidine uptake (5,212 cpm, mean value) on the third day of culture, while CT3 induced a 25-fold increase (8,929 cpm) over control values (356 cpm).

#### Discussion

The data indicate that the TCR1 antibody identifies a cell surface heterodimer associated with CT3 molecules that together can serve as a signal-transducing receptor complex. This T cell heterodimer appears to be the avian homologue of the mammalian TCR- $\gamma/\delta$  since (a) its polypeptide chain cores differ in  $M_r$  from those of CT3-associated heterodimers on most chicken T cells, and resemble



**FIGURE 5.** (A) Ontogeny of TCR1<sup>+</sup> (○) and CT3<sup>+</sup> (●) thymocytes and splenic cells analyzed by cell surface immunofluorescence. (B) Comparison of FACS profiles of thymocytes stained with TCR1 (—), CT3 (---), or a control antibody (—) at different times during development. Cell pools derived from at least three embryos were used for each time point during the embryonic period, and cells from one chicken for each point after hatching.

murine TCR- $\gamma/\delta$  polypeptides (8–10); (b) TCR1 is the receptor used by the first embryonic wave of T cells; and (c) TCR1 is expressed by a discrete subpopulation of circulating T cells lacking both CT4 and CT8 molecules. While this conclusion will require confirmation at primary protein structural and gene levels, the information revealed by this unique panel of mAbs suggests remarkable conservation of the developmental pattern of T cell subpopulations and their receptors in birds and mammals.

The full flowering of the TCR1/CT3 complex on the cell surface of the very earliest T cells identifiable in the embryonic thymus is an unexpected observation with possible functional implications. Because TCR1<sup>+</sup> cells could not be found elsewhere in chick embryos of this age, we assume that they are generated in the thymic microenvironment from lymphoid progenitors that migrated into the epithelial anlagen several days earlier (27).

The size of the TCR1<sup>+</sup>,CT3<sup>+</sup> subpopulation is surprisingly large. ~20% of circulating T cells and 30% of splenic T cells exhibit this phenotype in the adult chicken suggesting that the  $\gamma/\delta$  T cell subpopulation may play a very important role in the development and function of the immune system in birds.

Unresolved questions about the functions and lineal relationships of the  $\alpha/\beta$  and  $\gamma/\delta$  subpopulations of T cells should be amenable to analysis with the TCR1 antibody.

### Summary

This report describes an avian TCR molecule, TCR1, whose molecular characteristics, signal-transducing property, and tissue distribution suggest that it is a homologue of the mammalian TCR- $\gamma/\delta$ . TCR1<sup>+</sup> cells are the first to be generated in the thymus during ontogeny, preceding other T3<sup>+</sup> cells by ~3 d. Unlike their mammalian counterpart, TCR1<sup>+</sup> cells constitute a relatively large subpopulation of peripheral T cells in mature chickens. These results suggest a phylogenetically important role for this receptor in T cell development and function.

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