Cutting Edge: The Chicken TCR ζ-Chain Restores the Function of a Mouse T Cell Hybridoma

Thomas W. F. Göbel and Luca Bolliger

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The TCR/CD3 complex has been intensively studied in mammals, but it has been difficult to isolate homologues in other vertebrates. Here, we characterize the chicken ζ-chain, the first nonmammalian homologue identified. The comparison of mammalian and chicken ζ-proteins revealed high identity of the transmembrane and the C-terminal cytoplasmic domains. Transfection of a mouse ζ-deficient cell line, with the chicken ζ gene, restored surface expression of the murine TCR/CD3 complex. The chicken ζ-chain was stably associated with the mouse TCR/CD3 components and fully restored its signaling capacity upon stimulation with Ab, superantigen, and peptide Ag. This is the first report of a nonmammalian TCR component that is capable of fully restoring a mammalian TCR in every aspect analyzed, thus demonstrating the enormous selective pressure to maintain the ζ-chain as a structural and signaling component over a period of 300 million years. The Journal of Immunology, 1998, 160: 1552–1554.

Materials and Methods

PCR amplification and DNA sequence analysis

The Marathon cDNA amplification kit (Clontech, Palo Alto, CA) was employed for cDNA synthesis and adaptor ligation from chicken T cell-devoid mRNA. The adaptor-ligated cDNA was amplified using an adapter-specific primer and a degenerate primer encoding the amino acid sequence YDAHMQ (5′-TGCTATA/GTGIAA/GIGCA/GTCA/GTAA-3′), with the cycling conditions: 95°C/5 s; 50°C/30 s; 72°C/2 min for 35 cycles followed by a 72°C extension for 10 min. 3′ rapid amplification of cDNA ends was employed to amplify a full length cDNA. The PCR products were subcloned and sequenced with the ABI PRISM dye terminator cycle sequencing ready reaction kit (Perkin-Elmer, Foster City, CA) on an ABI 373A STRETCH sequencer.

DNA transfections and analysis of transfectants

The chicken ζ cDNA was amplified with specific oligonucleotides and ligated into EcoRI cut LSXP vector. An amount equal to 10 μg of purified LSXP DNA was used to transiently transfec 2.5 × 10^6 Bosc23 cells by a standard calcium-phosphate procedure. Following an overnight transfection, cells were provided with 6 ml of fresh 10% FCS Iscove’s modified Dulbecco’s medium and 1 ml of the supernatant harvested 24 h later was subsequently used to infect 10^7 MA5.8 cells (14) in the presence of 40 μg/ml DEAE-dextran. After 12 h incubation, 3 mg/ml puromycin was added to select for infected cells. Quantification of the TCR surface expression was conducted by flow cytometry using mAbs H57-597 (anti-mouse TCRβ), 2C11 (anti-mouse CD3ε), and KJ25 (anti-mouse TCRγ/δ; all PharMingen, San Diego, CA). Surface biotinylation and immunoprecipitations were performed according to standard procedures.

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Evolutionary comparison of the TCR/CD3 proteins mainly focused on the αβ and γδ TCR chains. In fact, nonmammalian TCR genes have first been isolated in the chicken (8, 9) and recently in other species, including cartilaginous fish (10). In contrast, the information about the signal transduction units of the TCR/CD3 complex of nonmammalian species is limited to a chicken and Xenopus laevis CD3 protein with equal homology to mammalian CD3γ and CD3ε (11, 12) and the chicken CD3ε chain (13).

To draw analogies, in particular for the signaling pathways, to the mammalian TCR/CD3 complex, the gene encoding the chicken ζ-chain was isolated and characterized in mouse ζ-chain-deficient cells. Unexpectedly, the chicken ζ-chain was able to rescue the surface expression and function of the mouse TCR. These experiments demonstrate that the components involved in the signal transduction of the TCR/CD3 complex are extremely well conserved and that nonmammalian species use similar modular signaling units.

Cutting Edge

Thomas W. F. Göbel2 and Luca Bolliger

Basel Institute for Immunology, Basel, Switzerland

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1 The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche & Co. Ltd., Basel, Switzerland

2 Address correspondence and reprint requests to Dr. Thomas Göbel, Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005 Basel, Switzerland. E-mail address: goebel@bii.ch

3 Abbreviations used in this paper: ITAM, immunoreceptor tyrosine-based activation motif; CY, cytoplasmic; EC, extracellular; TM transmembrane; NR, nonreducing; R, reducing.

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incubated with the indicated amounts of Staphylococcus aureus A toxin (Sigma, Brunschwig, Switzerland) at 5 × 10^5 cells/ml. An amount equal to 50 μl DAP3 cells was incubated with 5 × 10^5 transfected cells for 20 h. The Ag stimulation was performed similarly with cytochrome c peptide-pulsed LK35.2 cells. The culture supernatants were assayed in triplicates for IL-2 production using the standard HT-2 bioassay (16).

**Results and Discussion**

Commonly evolved features of ζ-chains

The gene encoding the chicken ζ-chain was isolated with a degenerate PCR-RACE approach utilizing a primer specific for the highly conserved YAQLHMQ motif. The 1598-bp-long chicken CY domain cDNA clone is 50% identical to the human homologue and contains a 42-bp 5′ untranslated region, a 501-bp open reading frame, and a 1055-bp 3′ untranslated region 4. The 166-amino acid-long protein has a M_r of 16,235, an isoelectric point (pI) of 9.4, and lacks N-glycosylation sites. The comparison to the mammalian CY domain sequences allows the assignment of a 22-amino acid signal peptide sequences. Predicted peptide sequences inferred from respective cDNA sequences were aligned using the CLUSTAL program (DNASTAR Inc., Madison, WI). The signal peptide is not shown. The location of the TM domain is indicated with a bar above the sequence.

Due to the high identity of mouse and chicken ζ-chains, the ζ-deficient and TCR surface-negative MA5.8 cell line (14) was transfected with either the chicken ζ gene (MA5.8-ζc) or the wild-type mouse ζ gene (MA5.8-mζ). Both the mouse and the chicken ζ-chain similarly restored TCR expression (Fig. 2). The variation in TCR/CD3 levels in both MA5.8-mζ and MA5.8-ζc cells was due to the bulk infection procedure and was not observed in stable clones (data not shown). Potential epitope alterations as tested with different TCR/CD3-specific mAb (anti-CD3ε, anti-ζ, anti-Vβ3) could not be detected (Fig. 2, and data not shown). Following surface biotinylation of MA5.8-ζc cells, the chicken ζ-chain homodimer (34- and 36-kDa NR; 17-kDa R) was communoprecipitated along with the mouse TCR-αβ (90-kDa NR; 40- and 50-kDa R), CD3ε (23-kDa NR; 25-kDa R), CD3δ (27-kDa NR; 28-kDa R) and CD3γ (23-kDa NR; 21-kDa R; Fig. 3). Thus the chicken ζ-chain is able to restore mouse TCR surface expression by stably assembling with all mouse TCR/CD3 components.

**Chicken ζ restores signal transduction of a mouse TCR**

The functional capabilities of this “xenogenic” TCR complex were tested with mAb, superantigen, or Ag stimulation. Both the amino acid identity of the human and chicken CY domains, whereas a comparison of the three ITAMs yields 67%, 81%, and 87% identity, respectively. Interestingly, the 8-amino acid spacing of the second ζ-chain ITAM has also been conserved. The chicken ζ-chain also harbors a putative GTP/GDP binding site located between the second and third ITAM (17). In summary, areas of high amino acid identity in the chicken ζ-chain support the functional evolution of commonly used motifs.

**The chicken ζ-chain associates with the mouse TCR/CD3 complex**

The ζ-chain of mouse TCR/CD3 complex

The functional capabilities of this “xenogenic” TCR complex were tested with mAb, superantigen, or Ag stimulation. Both the...
FIGURE 4. The chicken ζ-chain restores the function of the mouse TCR. IL-2 secretion of untransfected MA5.8 cells, MA5.8-μm cells, and MA5.8-μc cells upon triggering with increasing concentrations of a plate-bound mouse TCRβ-specific mAb (A), the superantigen staphylococcal enterotoxin A (SEA) (B), apocynochrome c peptide Ag (C), and soluble Thy-1-specific mAb supernatant (D). One representative experiment of four is shown using three independent bulk infections.

MA5.8-μm cells and MA5.8-μc cells responded equally well to cross-linking of their TCR/CD3 complex with plate-bound mAbs, whereas the nontransfected MA5.8 cells showed only residual stimulation with high amounts of mAb (Fig. 4A). Using the superantigen staphylococcal enterotoxin A (SEA), the MA5.8-μm cells were more efficiently stimulated than the MA5.8-μc cells, whereas the MA5.8 control cells were not stimulated (Fig. 4B). Most importantly, when stimulated with the nominal peptide Ag and APCs, the MA5.8-μm cells and the MA5.8-μc cells responded equivalently across the entire dose-response curve (Fig. 4C). Previous experiments have suggested a ζ-chain-independent T cell activation pathway mediated through the CD3ζ-ε ITAMs (18). To prove the true signaling capacity of the chicken ζ-chain in the context of the murine TCR, anti-Thy-1 stimulation as a strictly ζ-chain ITAM-dependent T cell activation (18) was compared between MA5.8-μm cells and MA5.8-μc cells. Both cell lines responded equally well to increasing concentrations of anti-Thy-1 mAb, thus clearly demonstrating that the chicken ζ-chain ITAMs are important to functionally restore the mouse TCR (Fig. 4D). Moreover, the early intracellular signaling events as detected by increased TCR associated tyrosine phosphorylation appeared to be ITAMs important to functionally restore the mouse TCR (Fig. 4E). To prove the true signaling capacity of the chicken ζ-chain ITAMs is composed of at least two independent signaling modules, the CD3ζ-ε ITAM complex is composed of at least two independent signaling modules.

This is the first report where a nonmammalian TCR component fully restores the assembly and function of a mouse TCR. The extraordinarily high conservation of the ζ-chain over a period of 250 to 300 million years, when the avian and mammalian lineages diverged (19), illustrates the importance of the ζζ homodimer for the assembly, transport, and function of the TCR/CD3 complex. A gradient of conservation with low EC but relatively high CY identity is evident when the chicken and mammalian ζ, TCR and CD3 proteins are compared. The low EC conservation of the TCR and the CD3 proteins throughout evolution most likely reflects the adaptation to different selective pressures in various organisms determined by the structure of self MHC molecules and the antigenic environment. In contrast, due to the lack of intrinsic enzymatic activity, the CY regions have been highly conserved to bind downstream signaling modules, like SH2 and SH3 domains. In chickens and Xenopus laevis, there is good biochemical and genetic evidence that only two CD3 genes, CD3δ and CD3ε, exist (11, 12, 20). The nonmammalian TCR/CD3 complexes contain only the CD3ζε heterodimer and ζζ homodimer as signaling modules, indicating that these two independent dimers are sufficient for TCR function. Despite these differences, our results demonstrate that the chicken ζζ homodimer is not altering the composition of the mouse TCR/CD3 complex. In conclusion, the chicken is the only nonmammalian species where all components of the TCR/CD3 complex have now been characterized, thus providing an excellent model for functional and structural phylogenetic analyses.

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