Chicken CD4, CD8αβ, and CD8αα T Cell Co-Receptor Molecules

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ABSTRACT New knowledge has recently been obtained about the evolutionary conservation of CD4, CD8αα, and CD8αβ T cell receptor (TCR) co-receptor molecules between chicken and mammals. This conservation extends from biochemical structure and tissue distribution to function. Panels of monoclonal antibodies and polyclonal antisera against different epitopes of chicken CD8 and CD4 molecules have proven their value in several recent studies. Chicken CD8 allotypes and homozygous strains carrying these allotypes have been established and these strains provide excellent models for further studies. The extensive polymorphism of CD8α in chickens has not been observed in any other species, suggesting that CD8α and CD8β have evolved under different selective pressure in the chicken. A large peripheral blood CD4+CD8+ T cell population in chicken resembles that observed in some human individuals but the inheritance of peripheral blood CD4CD8αα T cells in the chicken is a unique observation, which suggests the presence of a single gene responsible for CD8α, but not CD8β, specific expression. Despite these unique findings in chicken, the data on CD4, CD8αα, and CD8αβ molecules show that they have evolved before the divergence of mammalian and avian branches from their reptilian ancestors.

(Key words: CD4, CD8, T cell, evolution, chicken)

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INTRODUCTION

CD4 and CD8 are nonpolymorphic molecules that act as co-receptors for the polymorphic T cell receptor (TCR) in recognition of antigens (Littman, 1987; Roitt, 1993). T cells that express CD4 recognize antigens in context with MHC class II, whereas T cells that express CD8 recognize antigens in the context with MHC class I. During antigen recognition, CD4 binds to the extracellular domains of MHC class II (König et al., 1992, 1995) and CD8 binds to the extracellular domains of MHC class I (Norment et al., 1988; Salter et al., 1990). Both CD4 and CD8 co-receptors bind intracellularly to p56ck tyrosine kinase, which is essential for T cell activation through the TCR/CD3 complex (Veillette et al., 1992). In addition, both CD4 and CD8 increase the affinity of MHC/TCR binding (Janeway, 1992). CD4 is a monomer but CD8 is expressed either as an αα-homodimer or αβ-heterodimer (DiSanto et al., 1988; Norment and Littman, 1988; Farnes, 1989). CD8αβ is expressed on normal cytotoxic T cells, whereas selective CD8αα expression has been described in some Natural Killer (NK) (DeToto et al., 1992), γδT (Poussier and Julius, 1994) and CD4+ T cell subpopulations (Morrisey et al., 1995; Reinmann and Rudophi, 1995). The chicken, Gallus gallus domesticus, is a representative of the evolutionary most distant species from man and other mammals in which both CD4 and CD8 homologs have been described (Chan et al., 1988). However there are several recent discoveries that show unique features differing from those of mammals.

This review will explore the new data about CD4, CD8αα, and CD8αβ T cell co-receptor expression, structure, and function in the chicken. The aim is to provide an insight into the evolutionary processes that have preserved the immune system between aves and mammals as well as elucidate the unique features of chicken CD4 and CD8. Better knowledge of CD4 and CD8 will undoubtedly facilitate our understanding of the role of these co-receptors in the poultry immune defense.

AVIAN T CELLS

In birds, both T cell and B cell precursors originate in the bone marrow; T cells develop in the thymus and B cells develop in the bursa of Fabricius (Glick et al., 1956; Cooper et al., 1965; Toivanen et al., 1972a,b). The thymus...
originate as an epithelial outgrowth of the third and fourth pharyngeal pouches in 5-d-old embryos. The mature thymus develops as distinct lobes lining the jugular veins (Le Douarin, 1984). Multipotent stem cells from the mesenchyme will enter the thymus in separate waves to produce the immature thymocytes (Le Douarin and Jotereau, 1975; Lassila et al., 1978; Houssaint et al., 1991). In early embryos, some yolk sac cells can also maintain hematopoiesis but all lymphoid precursors seem to be of intra-embryonic origin (Lassila et al., 1978; Lampisuo and Lassila, 1994; Lampisuo et al., 1995). It seems that chicken β2-microglobulin can direct the stem cells to embryonic thymus (Dunon et al., 1990). Those features that mark the intrathymic development of T cells are generally conserved between chicken and mammals (Cooper et al., 1991; Davidson and Boyd, 1992). From the 14th embryonic d on, the lymphoid cells will migrate to the periphery (Cooper et al., 1991). As peripheral lymphoid organs the peripheral blood, spleen, and intestine resemble those of mammals.

Mature T cells in the chicken express either the TCRβ or TCRαβ (Chen et al., 1988; Sowder et al., 1988; Char et al., 1990; Vainio et al., 1990). The function of αβ T cells in chicken is very similar to that of mammals (Cooper et al., 1991). However, the variable TCRβ 1 (Vβ1), but not Vβ2, expressing T cells seem to preferentially migrate to chicken intestine, where they provide help to mucosal B cells for IgA production (Char et al., 1990; Cihak et al., 1991). The genes encoding TCR Vβ1 and Vβ2 have been cloned (Tjoelker et al., 1990; Lahti et al., 1991) as well as the genes encoding TCRα (Göbel et al., 1994a). It seems that the chicken TCRα and TCRβ loci are relatively simpler than in mammals, as they contain only two V subfamilies. In mouse and in human, γδ T cells are a minor subset, whereas in chicken they can consist of up to 50% of peripheral T cells (Sowder et al., 1988). The chicken has therefore been a valuable model for studying γδ T cells and their function (Arstila and Lassila, 1993; Arstila et al., 1993). Recently, TCRγ genes were cloned in the chicken (Six et al., 1996). It seems that the high frequency of γδ T cells in the chicken is associated with more complex TCRγ and TCRδ loci when compared to mice or humans. Together, these results show the emergence of αβ and γδ T cell sublineages before the divergence of avian and mammalian species in evolution. Also, genes encoding chicken CD3 have been cloned (Göbel et al., 1994b; Göbel and Fluri, 1997). The MHC class I and class II have also been described in detail in the chicken (Simonsen et al., 1982; Simonsen, 1985; Kaufman et al., 1994) as well as MHC class II restriction of APC and T cells (Vainio et al., 1988). The chicken is a valuable animal model for the study of the evolution of mHC and TCR molecules and the antigen recognition process.

DISCOVERY OF CHICKEN CD4 AND CD8

CT4 and CT8 monoclonal antibodies (mAb) reactive with chicken CD4 and CD8 homologs have been described (Chan et al., 1988). CT4+ T cells produced soluble T cell growth factors, whereas CT8+ T cells are capable of lysing certain hybridoma cell line. The majority of thymocytes expressed both CD4 and CD8 homologs, whereas spleen and peripheral blood T cells expressed either but not both. However, the molecular weight (Mr) of the putative CT4 antigen (64 kDa) was slightly higher than that of human (50 kDa) or mouse (60 kDa) (Terhorst et al., 1980; Dialynas et al., 1983). CT8 precipitated a dimer with a Mr of 34 kDa but it was not known whether it was a homodimer or a heterodimer as in mice (34 and 38 kDa) (Ledbetter et al., 1981; Johnson et al., 1985). In addition, no variant (CD8α') precipitate was observed in the thymus, as has been demonstrated in the mammals (Snow and Terhorst, 1983). In humans and rats, the monocytes express CD4 (Stewart et al., 1986), but the CD4 homolog was not observed in chicken monocytes. In conclusion, the characterization of the two mAb CT4 and CT8 suggested a similar tissue distribution and function of CD4 and CD8 homologs in the chicken as in mammals; however, several differences were also observed. The chicken is evolutionarily the most distant species in which CD4 and CD8 homologs have been described and is an ideal model for the study of the evolutionary conservation of CD4 and CD8.

CHICKEN CD4

Structure, Function, and Tissue Distribution of Chicken CD4

A panel of mouse mAb and polyclonal rabbit antisera detecting different cell surface epitopes of chicken CD4 are currently available (Table 1). The CD4 specificity of the mAb and rabbit sera has been proven by tissue distribution, immunoprecipitation, and inhibition by other CD4 mAb binding. Two-color immunofluorescence analysis has demonstrated that chicken peripheral αβ T cells are either CD4 or CD8 single positive but they are double positive in the thymus (Chan et al., 1988). In contrast, some γδ T cells are CD8+ but all are CD4- in the thymus and peripheral tissues. Unlabeled antibody competition analysis by flow cytometry demonstrated that several different epitopes on chicken CD4 are recognized by several CD4 antibodies (Luhtala et al., 1993). All antibodies and rabbit sera precipitate a monomeric protein from surface-labeled chicken thymocytes and T cells with a Mr of 64 kDa as analyzed by SDS-PAGE. Under reducing conditions, a slight increase in the electrophoretic mobility of chicken CD4 has been observed, demonstrating the presence of intramolecular disulfide bridges (Luhtala et al., 1993, Vainio et al., 1995). Removal of N-linked carbohydrates by endoglycosidase F treatment increases the electrophoretic mobility and demonstrates that the core protein has a Mr of 45 kDa. The molecular structure of chicken CD4 is summarized in Figure 1 (CD4 is in the middle). Functional
Table 1. Characteristics of anti-CD4 monoclonal antibodies (mAb) and rabbit sera.

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1+ = result confirming CD4 specificity, = no inhibition, +/- = partial binding inhibition.
2IF = typical immunofluorescence staining pattern with anti-CD8 mAb: double-positive in thymus and CD4 or CD8 single-positive in peripheral blood and spleen.
3ippt = 64 kD immunoprecipitate under reduced conditions from thymocyte lysates.
4COS = Recognizes COS-7 cells transfected with chicken CD4 cDNA (R. Koskinen and O. Vainio, manuscript in preparation).
52482 and 2483 are polyclonal rabbit sera raised against the chicken CD4 molecule. They detect chicken CD4 cDNA transfected COS-7 cells as well as immunoprecipitate CD4. Rabbit antiserum was used to inhibit the binding of anti-CD4 mAb, whereas anti-CD4 mAb were used to inhibit binding of biotinylated mAb 2±6. 2439 rabbit serum is a negative control. In both assays, bound mAb was detected by immunofluorescence.
6Luhtala et al., 1993.
7Luhtala et al., 1997.
8Chan et al., 1988.

Studied show that chicken CD4 gene expression is down-modulated when thymocytes are stimulated in vitro with phorbol esters. The anti-CD4 antibodies inhibit antigen-induced cellular proliferation of a keyhole limpet hemocyanin (KLH)-specific T cell line and synergized the blocking of T cell proliferation with anti-class II MHC-specific antibodies. In addition, the in vivo graft-vs-host reaction in chicken is dependent solely on donor CD4+ T cells (Tsuji et al., 1996; Luhtala et al., 1997a). These results show that the molecular structure and tissue distribution of CD4 as well as the functional capacity of CD4+ T cells are similar in chicken and mammals.

Evolutionary Conservation of Chicken CD4

The present results (Chan et al., 1988; Luhtala et al., 1993; Luhtala, 1997), indicate that the tissue distribution of chicken CD4 expression is very similar to that of mammals. In addition, biochemical and functional similarities between mammalian and chicken CD4 molecules are remarkable. Two N-linked oligosaccharide chains in human CD4 are added after translation and this is essential for the transport of CD4 to the cell membrane (König et al., 1988). Earlier immunoprecipitation experiments have demonstrated that chicken CD4 is significantly larger than human CD4. However, after removal of N-linked carbohydrates, the core protein of chicken CD4 was about Mr of 45 kDa, which is similar to that of human (Luhtala et al., 1993). This suggests that the more pronounced glycosylation of chicken CD4 results from additional glycosylation sites (Figure 1), as is the case when comparing the chimpanzee and human CD4 molecules (Camerini and Seed, 1990). The presence of seven potential N-glycosylation sites have recently been confirmed by cloning and sequencing the chicken CD4 cDNA (Koskinen et al., 1997).

It has been shown that chicken T cells stimulate B cells as in mammals (Vainio et al., 1984; Lassila et al., 1988). In addition, the antigen-driven proliferation of a chicken CD4+ T cell line is blocked in vitro with anti-MHC class II and anti-CD4 antibodies (Luhtala et al., 1993). This antibody blockage indicates that chicken CD4 is also involved in the antigen recognition process of CD4+ T cells. Chicken CD4+ T cells can stimulate γδT cells (Arstila et al., 1993, 1994) and they are responsible for pokeweed mitogen (PWM)-induced lymphokine production by splenic T cells (Chan et al., 1988). Chicken CD4 is physically associated with the p56ck homolog and also the cytoplasmic interaction sites have been well conserved (Veillette and Ratcliffe, 1991; Chow, 1992; Koskinen et al.,...
CHICKEN CD4, CD8αβ, AND CD8αα

1997). These findings suggest that the signalling components of the CD4 co-receptor are conserved between avian and mammalian species. This idea is supported by finding that the PMA-induced activation of thymocytes leads to the down modulation of chicken CD4 (Luhtala et al., 1993). The graft-vs-host capacity of chicken lymphocytes is dependent on donor CD4+ T cells (Tsui et al., 1996; Luhtala et al., 1997a), indicating that CD4+ T cell helper function is essential for vertebrate immune responses and has been conserved in the chicken.

CHICKEN CD8

Structure, Function, and Tissue Distribution of Chicken CD8

To study the different epitopes of chicken CD8, a panel of mAb and polyclonal rabbit antisera has been created (Table 2). The antibodies and rabbit sera precipitate a Mr of 64 kDa protein under nonreducing conditions and a Mr of 34 kDa dimeric protein under reducing conditions (Chan et al., 1988). Removal of N-linked carbohydrates suggested that one CD8 chain utilized several N-glycosylation sites, whereas no N-glycosylation was detected in the other CD8 subunit (Luhtala et al., 1995; Vainio et al., 1995). No variations in the Mr have been observed between different organs (spleen, thymus, or spleen). As in mammals, chicken CD8β requires CD8α for cell surface expression (DiSanto et al., 1988; Tregaskes et al., 1995). The molecular structure of chicken CD8 is summarized in Figure 1 (CD8αα is on the left and CD8αβ is on the right). The CD8 specificity of mAb and rabbit sera has been conclusively proven by staining COS-cells transfected with plasmids containing CD8αα cDNA (Tregaskes et al., 1995; Luhtala et al., 1995, 1997a, b). Antibody and antisera competition analysis by flow cytometry demonstrated that several different epitopes of chicken CD8 are recognized by these antibodies (Luhtala et al., 1995). Two-color immunofluorescence analysis shows that normal chicken peripheral αβ T cells are either CD4 or CD8 single positive, whereas thymocytes are double positive (Chan et al., 1988). A subpopulation of γδ T cells are CD8 positive in spleen and in intestinal intraepithelial lymphocytes (iIEL). CD8 antibodies inhibit in vitro both TCR Vβ1 specific mAb induced proliferation and the cytotoxic responses of virus infected chicken peripheral blood T cells (Luhtala, 1997), whereas sorted CD8+ T cells can not induce an in vivo graft-vs-host reaction in chicken (Luhtala et al., 1997a).

FIGURE 1. Schematic structure of chicken CD4, CD8αα, and CD8αβ T cell co-receptors. Molecular structure of CD4 is shown in the middle, whereas CD8αα homodimer is on the left and CD8αβ heterodimer is on the right. In the extracellular part of CD8 molecule, disulfide bridges (SS) are shown between CD8 chains as well as the intramolecular disulfide bridges in CD4 and in both CD8 chains. Putative N-glycosylation sites in CD4 and CD8αβ are indicated with symbols (○). The hydrophobic transmembrane parts of CD4, CD8αα, and CD8αβ go through the cell membrane (○○○) that separates the extracellular and intracellular domains of the molecules. Intracellular binding of p56lck to CD4 and to CD8αα.
TABLE 2. Characteristics of anti-CD8 monoclonal antibodies and rabbit sera

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<sup>1</sup>+ = result confirming CD8 specificity, - = no inhibition, +/- = partial binding inhibition, nd = not defined.

<sup>2</sup>IF = typical immunofluorescence staining pattern with anti-CD4 mAb: double-positive in thymus and CD4 or CD8 single-positive in peripheral blood and spleen.

<sup>3</sup>ippt = 32 to 34 kDa immunoprecipitate under reduced conditions from thymocyte lysates.

<sup>4</sup>COS = recognizes COS-7 cells transfected with chicken CD8 cDNA.

<sup>5</sup>2442 and 2448 are polyclonal rabbit sera raised against the chicken CD8 molecule. They detect chicken CD8<sub>a</sub> cDNA transfected COS-7 cells as well as immunoprecipitate CD8<sub>a</sub><sub>b</sub> heterodimer. Rabbit antiserum was used to inhibit the binding of anti-CD8 mAb and an 0-bleed from the immunized rabbits served as a negative control. Anti-CD8 mAb were used to inhibit binding of biotinylated mAb 11±38 and 11±39. In both assays, bound mAb was detected by immunofluorescence.

<sup>6</sup>Luhtala et al., 1995.
<sup>7</sup>Luhtala, 1997.
<sup>8</sup>Luhtala et al., 1997a.
<sup>9</sup>Chan et al., 1988.
<sup>10</sup>Paramithiotis et al., 1991.
<sup>11</sup>Tregaskes et al., 1995.
<sup>12</sup>LillehoÈj et al., 1988.
<sup>13</sup>EP42 is specific for chicken CD8<sub>b</sub>, whereas all other mAb are specific for chicken CD8<sub>a</sub>.
<sup>14</sup>M. Luhtala and O. Vainio, unpublished observations.

Evolutionary Conservation of Chicken CD8

The tissue distribution of the avian CD8 molecule is very similar to the mammalian CD8 (Norment and Littman, 1988; Parnes, 1989; Cooper et al., 1991). In addition, the CD8 dimer is a heterodimer that is composed of differentially N-glycosylated chains (Luhtala et al., 1995). The presence of N-glycosylation sites only in chicken CD8<sub>b</sub>, but not in CD8<sub>a</sub>, have been confirmed from the cDNA deduced protein sequences (Tegaskes et al., 1995). In addition, the cytoplasmic binding site for p56<sup>lck</sup> is well conserved in chicken CD8<sub>a</sub> (Figure 1). On the basis of differential CD8 mAb staining, it has been suggested that chicken CD8<sub>a</sub>-homodimer is expressed on sub-populations of peripheral blood NK cells and on intestinal and embryonic spleen γδ T cells (Paramithiotis et al., 1991; Göbel et al., 1994b; Tegaskes et al., 1995) as well as on some chicken Cu T cell lines (C. H. Chen and M. D. Cooper, personal communication: Howard Hughes Medical Institute, Birmingham, AL 35294). Recent findings also demonstrate that in some H.B15 chickens, a large subpopulation of peripheral blood and spleen CD4<sup>+</sup> T cells express only CD8<sub>a</sub> mRNA but not CD8<sub>b</sub> (Luhtala et al., 1997a). It was shown earlier that chicken CD8<sup>+</sup> T cells are responsible for CTL (Chan et al., 1988) responses against an allogenic tumor line and that their presence suppresses rather than helps PWM-induced lymphokine production (Chan et al., 1988). CD8 mAb were also shown to inhibit the ConA- and PHA-induced proliferation of spleen cells. These findings have recently been extended by showing that anti-CD8 mAb can inhibit the CTL response to viral antigens and to TCR<sub>a</sub><sub>b</sub> mAb induced proliferation (Luhtala, 1997). CD8<sub>a</sub><sub>b</sub> T cells do not induce an in vivo graft-vs-host reaction but CD8<sub>a</sub><sub>a</sub> T cells (co-expressing CD4) induce normal alloreaction to the same extent as normal CD4<sup>+</sup> T cells (Luhtala et al., 1997a). Consequently, the CD8<sub>a</sub><sub>a</sub> homodimer can be expressed on normal peripheral CD4<sup>+</sup> T cells, whereas the CD8<sub>a</sub><sub>b</sub> heterodimer expression is restricted to CD8 lineage cytotoxic T cells. These data indicate that the molecular structure and tissue distribution of CD8<sub>a</sub><sub>a</sub> and CD8<sub>a</sub><sub>b</sub> as well as the function of CD8<sub>a</sub><sub>b</sub> heterodimer carrying T cells...
CHICKEN CD8 POLYMORPHISM

Chicken CD8 Allotypes

Polymorphism of chicken CD8 has been suggested to explain why some anti-CD8 mAb do not stain COS cells that were transfected with chicken CD8α or CD8β cDNA carrying pCDM8 plasmids (RPL line 7 or 0; Luhtala et al., 1995). More detailed studies revealed that some anti-CD8 mAb did not stain PBL from certain individuals in the H.B15 strain (mAb 9-8, 11-9, 11-13, 11-30, and 11-38) (Luhtala et al., 1997b), whereas all other anti-CD8 mAb were constantly positive with all these individuals. Three distinctive patterns of mAb staining have been established (Luhtala, 1997, and unpublished data): 1) the

FIGURE 2. Schematic pedigree of different CD8 allotypic chicken strains. Males chickens are indicated as squares and females as circles. Filled symbols indicate the CD8 phenotype, which is positive with monoclonal antibody (mAb) recognizing polymorphism. The number of tested individuals in each generation of the relevant substrain is shown in parentheses. After testing, each generation was bred together to produce the next generation. H.B15.H strain (A) is negative with mAb 11-38, 11-13, 11-30, 11-9, and 9-8; H.B15.HY strain (B) is negative with mAb 11-13, 11-30, 11-9, and 9-8, whereas H.B15.H12 strain (C) is positive with all these mAb. H12 to H7 crossbreedings (D) were performed to identify and select the homozygous founder animals for the H.B15.H12 strain. Data are taken from Luhtala (1997; and unpublished data).
Polymorphism of CD8α and CD8β Genes

The genes encoding chicken CD8α and CD8β have been cloned (Tregakes et al., 1995) and thus it was recently possible to define the structural basis of CD8 polymorphism (Luhtala, 1997; Luhtala et al., 1997b). The three chicken strains studied (RPRL 7, H.B15.H7, and H.B15.H12) have 14 nucleotide differences in the CD8α cDNA sequence, causing eight amino acid replacements in the extracellular part of the molecule (Figure 3). Only two amino acid replacements and four silent mutations were observed in the CD8β cDNA sequence in one (H7) of the chicken strains (Figure 4). No mutations were observed in the partially sequenced signal peptide and transmembrane parts of the CD8α and CD8β genes from the same individuals. The majority of the amino acid differences between L7, H12, and H7 are located in the Ig V-like domain and three of the mutations (aa 50, 54, and 78) are situated in the putative MHC class I binding complementary determining region (CDR) 1 and CDR2 regions of the CD8α. The replacement:substitution (R:S) ratio was 0.58 (7/12) for H7 CD8α, 0.78 (7/9) for H12 CD8α and 0.33 (2/6) for H7 CD8β. The R:S ratio was especially high 1.0 (3/3) in the CDR1 region of CD8α and the same tendency was observed for the CDR2 and CDR3 regions. These results show extensive polymorphism of chicken CD8α but not CD8β.

In order to define the molecular basis of CD8 polymorphism and to confirm the specificity of the mAb, a novel method has been developed for expressing a cDNA encoding the extracellular parts of chicken CD8α and CD8β in COS cells (Luhtala et al., 1997b). The cDNA encoding the extracellular part of CD8α or CD8β cDNA

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<th>CD8α H7</th>
<th>CD8α H12</th>
<th>CD8α L7</th>
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<tr>
<td>SSNNINILYC</td>
<td>EIFTMAP</td>
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<td>171</td>
<td>181</td>
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was amplified from several individuals of the H7 and H12 substrains and ligated to the pCDM8 expression vector pEP42 by using different restriction sites present in the cDNA sequences. These novel plasmids were used to transfect COS cells and the cells were stained with different CD8-specific mAb. The results show that the amino acid changes responsible for the allelic variation, detected by mAb 9-8, 11-9, 11-13, and 11-30, are all situated on CD8α. Other CD8α- and one CD8β-specific mAb stained the cDNA transfectants from all allotypic chicken strains (Luhtala, 1997). The transfection results confirm that there are three different allotypes of chicken CD8α. Four mutated amino acids in the chicken CD8α protein that are responsible for distinct patterns of staining with CD8 mAb were defined. Either proline-44, alanine-82, or both were responsible for mAb 11-13 and 11-30 binding to RPRL Line 7 and H.B15.H12 allotypes. Whereas phenylalanine-78, valine-126, or both were responsible for mAb 11-9 and 9-8 binding to the H.B15.H12 allotype.

**Unique Polymorphism of Chicken CD8α**

The three chicken strains so far studied (RPRL 7, H.B15.H7, and H.B15.H12) have 14 nucleotide differences in the CD8α cDNA sequence causing 8 amino acid replacements in the extracellular part of the molecule (Luhtala et al., 1997b). Several of these substitutions occur in the putative MHC class I binding sites corresponding to mammalian CDR1 (residues 50 and 54) and CDR2 (78) domains (Tregaskes et al., 1995; Luhtala, 1997). These replacements change the biochemical properties of these residues and it is thus possible that they affect the MHC class I binding as shown in mammals (Sanders et al., 1991; Giblin et al., 1994). Avian CD8α has more negatively charged amino acids in the putative MHC class I binding sites than human CD8α. However, the corresponding CD8α interaction site in chicken MHC class I has more positively charged amino acids than human MHC class I (Tregaskes et al., 1995; Luhtala et al., 1997b). This difference has led to speculations that compensatory changes in the avian CD8α and MHC class I binding regions, when compared with mammalian CD8α and MHC class I binding regions, might be a result of a ligand-receptor pair mutating under co-evolutionary pressure (Kaufman et al., 1992; Luhtala, 1997). It will be interesting to see whether the CD8/MHC class I interaction is affected by the CD8α polymorphism we observed in the chicken. Extensive polymorphism of CD8α has not been found in any other species and it suggests that CD8α and CD8β have evolved under different selective pressures in the chicken.

It seems that CD8α polymorphism is much higher in chickens than in other species, whereas CD8β is more conserved (Youn 1988a,b; Luhtala et al., 1997b). This hypothesis is further supported by the fact that polymorphism of CD8α has recently been observed in additional

![FIGURE 4](image-url)  
**FIGURE 4.** CD8β amino acid sequence alignments of different chicken CD8 allotypes. CD8β sequences from both H.B15.H12 (H12) and H.B15.H7 (H7) substrains are compared to RPL line 0 (0) sequences and are based on data from Luhtala et al. (1997b; EMBL Y 11474 and Y 11475). Amino acids situated in the signal peptide and intracellular parts of the molecule are underlined. Identical residues are indicated with dashes and the altered amino acids are shown in boldface type. Numbering of the amino acids is according to RPL line 0 sequence (Tregaskes et al., 1995).
chicken strains (M. Luhtala and O. Vainio, unpublished observations), which suggests that there has been a selection for CD8α polymorphism in the chicken. This hypothesis is supported by a higher R:S ratio in CD8α (0.57) than in CD8β (0.33). The accumulation of mutations to CDR regions of chicken CD8α also suggests a selective pressure. It is tempting to speculate that this selection may be driven by adaptation to pathogens, perhaps retroviruses (Murphy, 1993, 1994a,b), that infect T cells and could use CD8α as a receptor. This adaptation would be comparable to some human MHC class I alleles that have selective advantage as Epstein-Barr virus escape mutants (DeCampos-Lima et al., 1993). Alternatively, the differences in the rates of variation between CD8α and CD8β may reflect different levels of constraint for conservation of function in thymic T cell selection. Fitting to this hypothesis, CD8β knock-out mice have a failure in positive selection of CD8+ T cells (Fung-Leung et al., 1991). Interestingly, β-2 microglobulin diversity has also been described in individual rainbow trout (Shum et al., 1996). It could be that some species might acquire additional TCR/MHC I antigen recognition capacity by diversity in β2-microglobulin or CD8. A similar polymorphism to that described in chicken CD8 has been shown in the CD4 of miniature swine (Sundt et al., 1992). Pigs have 11 amino acid replacements between two CD4 allotypic strains and the mutations accumulate in the CDR2 region of the molecule (Gustafsson et al., 1993). The CDR2 region has earlier been implicated as the HIV binding site for human CD4 and therefore CD4 polymorphism in pig might be a selective mechanism to alter a receptor for an unknown virus. Whether CD8α polymorphism in chicken has any role in extending the antigen recognition capacity of TCR or in evading viral infections remains to be seen.

**PERIPHERAL CD4+CD8+ T CELLS**

**Peripheral Blood CD4CD8αα T Cells in the Chicken**

Only a few peripheral blood CD4+CD8+ cells are observed in normal humans as well as in normal chicken (Luhtala, 1997). However, in certain H.B15 individuals, a large proportion of T cells in peripheral blood (20 to 40%), spleen (10 to 20%) and intestinal epithelium (5 to 10%) co-expressed CD4 and CD8α, but not CD8β (Luhtala et al., 1997a). This co-expression has been confirmed with several CD4 and CD8 specific mAb. Because it was not clear whether the absence of CD8β staining in these CD4+CD8+ T cells was due to stearic hindrance, an altered CD8β mAb epitope, or a lack of CD8β expression, a reverse transcriptase polymerase chain reaction (RT-PCR) analysis for chicken CD8α and CD8β was developed. CD4+ T cells were sorted from peripheral blood and RT-PCR experiments demonstrated that only CD8α mRNA was expressed in peripheral CD4+ T cells. The phenotype of these cells was further studied by immunofluorescence and they expressed CD3, CD28, and either TCR Vβ1 or Vβ2 molecules. In addition, chicken CD4CD8αα peripheral T cells expressed normal levels of MHC class I, ChT1, and CD5 antigens but were surface negative for mAb recognizing γδ TCR, Ig, MHC class II, and CD25.

Because PBL CD4CD8αα cells are distinct from conventional αβ T cells, their functional capacity has been tested (Luhtala et al., 1997a). These T cells respond to mitogenic stimuli, to mAb crosslinking of the CD3/TCR complex, and to CD28 co-stimulation. Furthermore, sorted CD4CD8αα cells induced a significant graft-vs-host reaction, similar to that of normal CD4+ T cells when injected into 14-d-old MHC incompatible H.B2 embryos. To analyze whether the phenotype of CD4CD8αα cells was stable Ov-antigen (ChL12; Houssaint, 1991) congeneric donors and recipients were used to follow the ChL12+ donor cells which accumulated in enlarged spleens of ChL12− recipients. Three color fluorescence analysis demonstrated that all donor cells in splenomegalic spleens expressed CD4 and that the CD4CD8αα phenotype was also stable after in vivo graft-vs-host stimulation (Luhtala, 1997). CD4CD8αα T cells seem to be of thymic origin, as they can be detected among FITC-labeled recent thymic emigrants (as described by Katevuo and Vainio, 1996) and among donor thymocytes capable of a graft-vs-host reaction (M. Luhtala and O. Vainio, unpublished observations). Together, these data demonstrate the stable co-expression of CD8α on peripheral blood CD4+ T cells in chicken.

**Inheritance of Peripheral Blood CD4CD8αα T Cells in the Chicken**

To study the inheritance of the CD4CD8αα phenotype in the chicken, a CD4CD8αα male was mated with a female of the same phenotype (HX, Figure 5A). The majority (9/12) of their offspring had a similar CD4CD8αα T cell population in the periphery as the parental animals, whereas the rest were normal and had less than 2% of these cells (Luhtala et al., 1997a). For the next generation, 2 males and 2 females with the CD4CD8αα phenotype were mated individually and 100% (39/39) of their offspring were of the same phenotype. The mean percentage of CD4CD8αα T cells in PBL was 32% in first progeny and 33% in the second progeny. Second progeny animals were further bred together and all of their offspring (41/41) were of the CD4CD8αα phenotype. In addition, two males with CD4CD8αα phenotype were backcrossed to H.B15 animals that had a normal phenotype (H99, Figure 5B). This backcross resulted in 100% (40/40) of the offspring with a CD4CD8αα phenotype in PBL (Luhtala, 1997). When these backcrossed animals were further bred together, the normal CD4 phenotype emerged again in their offspring (8/30). This result shows that both heterozygous and homozygous animals for the trait have the same CD4CD8αα phenotype and that sex has no role in the inheritance. Cross breeding to normal chickens suggested that the CD4CD8αα T cell population in spleen
and peripheral blood is inherited in a dominant Mendelian manner. Interestingly, a minor (2 to 5%) intestinal CD4CD8\(\alpha\alpha\) T cell population was observed independently of the Mendelian inheritance of peripheral blood and spleen CD4CD8\(\alpha\alpha\) T cells (M. Luhtala and O. Vainio, unpublished data). For further breedings, only homozygous parents (Figure 5C), that produced 100% PBL CD4CD8\(\alpha\alpha\) phenotype siblings in crosses with H.B15.H12 animals (9/12), were selected (Luhtala, 1997). Breeding experiments demonstrate the dominant inheritance of CD8\(\alpha\) expression on peripheral blood CD4+ T cells and establish a homozygous HB15.CD8\(\alpha\alpha\) strain (Figure 5D; Luhtala, 1997, and unpublished data).

**Peripheral Blood CD4+CD8+ T Cells in Other Species**

Current dogma states that mature lymphocytes are either CD4+ or CD8+ and that only immature T cells in the thymus can express both CD4 and CD8. Although CD4+CD8+ T cells are usually rare (< 3%) in human peripheral blood (Blue *et al*., 1985; Ortolani *et al*., 1993),
higher proportions (15%) can be observed in certain pathological conditions and in some normal blood donors (Kay et al., 1990; Furukawa et al., 1992; Macchi et al., 1993; Murakami et al., 1993; Sala et al., 1993; Prince et al., 1994). It seems that the minor human CD4+CD8+ T cell population expresses only the CD8α homodimer (Moebius et al., 1991) and that these cells carry a functional TCRαβ/CD3 complex (Prince et al., 1994). In addition, some of CD4+CD8+ T cells (>10%) express HLA-DR, CD25, CD11b, CD38 molecules, whereas the majority of them (>80%) express CD28.

Larger CD4+CD8+ T cell populations have also been observed in the peripheral tissues of adult swine: peripheral blood (10 to 50%), spleen (20 to 30%) and lymph nodes (10 to 13%), (Pescovitz et al., 1985, 1994; Saalmuller et al., 1989; Zuckerman and Gaskins, 1996). In these studies, CD8 expression on CD4+ T cells was a general phenomenon seen in older individuals and in several strains. Interestingly, the number of CD4CD8αα T cells in PBL increased with age (Zuckerman and Husemann, 1996), in contrast to that observed in humans. It has been suggested that porcine CD4+CD8αα are of thymic origin as they do not express thymocyte markers (CD1) but, after thymectomy, the expression of “memory or antigen experienced” phenotype markers will dominate. It is not known whether porcine peripheral CD4+CD8+ T cells express the CD8αα homodimer or CD8αβ heterodimer, CD25, or CD8αβ antigens.

The peripheral blood CD4CD8αα T cell population in the H.B15.CD8αα chicken strain (Luhtala, 1997; Luhtala et al., 1997a) is similar to human CD4+CD8+ T cell population (22%) in several parameters for the following reasons: 1) only a few normal individuals have large CD4+CD8αα αβ T cell population, 2) CD4+CD8+ T cells respond normally to mitogenic or TCR stimuli, 3) CD4+CD8+ T cells lack typical thymocyte surface markers but express CD28, and 4) CD4+CD8+ T cells do not express CD25 or MHC II and show no other indication of previous activation. Results using the chicken further suggest that the large CD4+CD8+ T cell population observed in humans might express only the CD8αα homodimer and that they could be functionally CD4+ T cells. Breeding experiments show the inheritance of PBL CD4CD8αα T cells and provide a novel animal model to study peripheral blood CD4+CD8+ T cells.

Intestinal CD4+CD8+ T Cells

It has been shown in the mouse and in the rat that a subset of iIEL co-express CD4 and CD8 (Mosley et al., 1990; Lefrancois, 1991; Poussier and Julius, 1994; Lefrancois and Puddington, 1995). It seems that in mouse intestinal epithelium some CD4+ T cells co-express CD8 as an αα-homodimer. When peripheral CD4+ T cells are grafted from a normal to a SCID mouse, a subpopulation of the CD4+ T cells homing to the microenvironment of the intestinal epithelium (30 to 40%) start to re-express CD8α in vivo (Morrisey et al., 1995; Reinmann and Rudolph, 1995). A CD4+CD8+ population has also been observed in some mucosal associated lymphoid tissue of pigs (Zuckermann and Gaskins, 1996). A similar iIEL CD4CD8αα T cell population has been observed in H.B15.CD8αα strain chickens as well as in normal individuals (Luhtala, 1997; Luhtala et al., 1997a; M. Luhtala and O. Vainio unpublished results). This result suggests an evolutionary conservation of the intestinal CD4CD8αα T cell population between aves and mammals.

Peripheral blood CD4CD8αα T cells seem to be inherited under the regulation of single dominant gene (Luhtala et al., 1997a). However this gene is not responsible for CD4CD8αα T cells in the intestine, as this population is present in all individuals (Luhtala, 1997 and unpublished data). Several studies demonstrate that an intestinal CD4CD8αα T cell population in pigs, mice, and rats is also present in all individuals (Moseley et al., 1990; Takimoto et al., 1992; Zuckerman and Gaskins, 1996). It has been shown in rats that both age and the presence of gastrointestinal flora affect the appearance of intestinal CD4+CD8+ T cells (Takimoto et al., 1992). Similarly, CD4+CD8+ PBL T cells in pigs also increase with age (Zuckermann and Husmann, 1996). These findings suggest that antigen exposure in the intestine might induce CD8α expression in some CD4+ T cells. Whether age or other factors affect iIEL CD4CD8αα T cells in chicken will be an interesting field to study. Based on these results it has been suggested that peripheral blood and iIEL CD4CD8αα T cell populations are of different origin (Luhtala, 1997). However, similar mechanisms can still result in CD8α expression on CD4+ T cells in both cases. The mechanisms causing CD4 and CD8α co-expression in the intestine remain to be solved.

Function of Peripheral CD4+CD8+ T Cells

Peripheral blood CD4CD8αα T cells in chicken are functionally normal T cells, as they proliferate in response to mitogens and signals delivered via the αβT cell receptor as well as via the CD28 co-receptor (Luhtala et al., 1997a). Most importantly these CD4CD8αα T cells induce an in vivo alloreaction comparable to that of normal CD4+ T cells providing direct evidence for peripheral blood CD4+CD8+ T cell functioning as helper CD4+ T cells. It seems that expression of CD8α does not interfere with the function of peripheral blood CD4+ T cells.

Earlier studies on human T cell clones in vitro have shown that PBL CD4+CD8+ T cells can be either CD8 lineage cells re-expressing CD4 (Richardson et al., 1986; Lusso et al., 1991) or CD4 lineage cells re-expressing CD8 (Blue et al., 1986; Paliard et al., 1988). However, very little is known about CD4+CD8+ T cells in vivo. Several observations suggest that peripheral CD4+CD8+ T cells function as normal T cells. Human peripheral blood CD4+CD8+ T cells have been shown to respond to signals delivered by mitogens or via CD3/TCR complex in vitro (Prince et al., 1994). The specific function of PBL CD4+CD8+ T cells present in normal blood donors remains to be discovered.
However, the presence of CD4+CD8+ T cells in places of inflammation (Burdick et al., 1984; DeMaria et al., 1987) suggest a role in immunoregulation.

Porcine CD4+CD8+ T cells respond to mitogenic stimuli as well as proliferate in response to specific antigens (Zuckerman and Gaskins, 1996). In addition, it has been also shown that both CD4 and CD8 mAb inhibit antigen specific proliferation of porcine peripheral blood CD4+CD8+ T cells, suggesting that they both are involved in this process. The same studies suggest that CD8 expression is an activation/memory T cell marker in porcine CD4+ T cells. Although these observations are elusive, they are in line with recent observation of CD4 lineage function for CD4+CD8+ T cells in the chicken (Luhtala et al., 1997a). All pigs have CD4+CD8+ T cells and these cells differ also in other respects from those seen in the human and in chicken.

It has been shown that CD4 PBL cells can start to express CD8α in vivo for mouse T cells migrating to iIEL (Morrisey et al., 1995; Reinmann and Rudolphi, 1995). Intestinal IEL CD4CD8α T cells seem to be capable of inducing inflammatory bowel disease as well as normal CD4+ T cells (Davidson et al., 1996). However, some studies have also suggested that intestinal CD4+CD8+ T cells might be cytolytic (Gramzinzki et al., 1993). Recently, it was shown that the cytokine expression pattern of mouse intestinal IEL CD4CD8α T cells resembles that of CD4+ helper T cells and that these IEL CD4CD8α T cells are also capable of inducing Th2 type help to B cells in vitro (Fujihashi et al., 1993a,b). However, in mammals the role of CD4+CD8+ T cells in normal immune response is as elusive in iIEL as it is in PBL.

**Origin of Peripheral CD4+CD8+ T Cells**

The genetic background has been shown to play a role in determining the peripheral blood CD4:CD8 ratio in inbred mice or chicken strains as well as in normal blood donors (Kraal et al., 1983; Amadori et al., 1995). These findings suggest that genes regulating CD4 and CD8 expression might be evolutionarily conserved but these genes are poorly known. Some experiments have suggested that DNA methylation or viral infections can induce CD4 expression on CD8+ T cells (Richardson et al., 1986; Lusso et al., 1991). In these cases it might be that a CD4 silencer, situated in the first intron of the gene, was somehow inactivated (Sawada et al., 1994; Donda et al., 1996). However, very little is known about CD8α and CD8β co-receptor regulation in CD4+ T cells. A recent study shows that the number of intestinal CD4+ CD8+ T cells is dramatically reduced in β2 microglobulin-deficient mice but unaffected in TAP 1-deficient mice (Sydora et al., 1996). β2 microglobulin is essential for surface expression of both classical and nonclassical MHC class I molecules but TAP 1 affects mainly antigen presentation by classical MHC class I molecules. Therefore these data suggest that the nonclassical class I molecules expressed in the intestine might play a role in the development of intestinal CD4+CD8+ T cells. Consequently, the expression of nonclassical class I molecules outside the intestine, or seeding of intestinal CD4+CD8+ T cells to peripheral blood, might also result in peripheral blood CD4+CD8+ T cell population in chicken (Luhtala, 1997c; Luhtala et al., 1997a). Two recent transgenic mouse models provide additional putative regulatory elements for CD4 and CD8 co-expression in peripheral T cells. Constitutive expression of c-myc or v-rel on thymocytes leads to an increase in thymocytes with a CD4+CD8low phenotype and in a large splenic T cell population co-expressing CD4 and CD8 (Broussard-Diehl et al., 1996; Carrasco et al., 1996). Whether either of these transgenic models has any relevance to the origin of peripheral blood CD4+ CD8+ T cells in the chicken remains to be seen. However, it is evident that expression of CD8α alone is not sufficient to activate the CD8 silencers in these chickens.

There are at least three possibilities for CD8α expression on peripheral CD4+ T cells in the chicken (Luhtala, 1997); 1) T cells of extra thymic origin may escape normal selection and co-receptor exclusion mechanisms, 2) defective CD8α co-receptor exclusion from CD4 lineage committed T cells in the thymus, and 3) post-thymic re-expression of CD8α on CD4+ T cells. CD8 expression on peripheral CD4+ T cells has been shown in vitro for human (Blue et al., 1986; Paliard et al., 1988) and porcine T cells (Zuckerman and Husmann, 1996), whereas in vivo only for mouse T cells migrating to iIEL (Morrisey et al., 1995; Reinmann and Rudolphi, 1995). The stable phenotype and the spontaneous and the hereditary nature of peripheral CD4CD8α T cell population in chicken do not favor the CD8α re-expression hypothesis. Extra-thymic maturation of T cells has, so far, not been observed in the chicken (Dunon et al., 1993, 1995) and we show that CD4CD8α T cells are among the recent thymic emigrants in H.B15.CD8α chicken (Luhtala, 1997). These results agree with the hypothesis of the thymic origin of peripheral CD4CD8α T cells. Only CD8α is detected on CD4+ T cells in peripheral tissues and this implies that at least CD8β co-receptor exclusion occurs normally. Recent results in chicken would fit to a single gene phenomenon where putative CD8α silencer is inactivated in vivo in a similar way that inactivation of a CD4 silencer has been demonstrated in vitro (Lusso et al., 1991; Sawada et al., 1994). The chicken is, so far, the only animal model where hereditary co-expression of CD4 and CD8α has been demonstrated (Luhtala 1997 and unpublished data; Luhtala et al., 1997a) and the Mendelian dominant inheritance provides a unique opportunity to study the genes regulating CD8α expression in peripheral CD4+ T cells.

**CONCLUDING REMARKS**

Although birds are evolutionarily distant from mammals, it is striking that the antigen specific immune system seems remarkably homologous. All lymphocyte populations (T, B, and NK) have been conserved during evolution. Molecules responsible for antigen presenta-
tional (MHC I and MHC II), antigen recognition (TCRβ, TCRγδ, CD3, and Ig) as well as co-receptor molecules (CD28, B7, CD4, and CD8) have all been cloned in the chicken and have well conserved functional motifs when compared to mammals. This demonstrates the importance of CD4 and CD8 co-receptors as well as these other molecules in normal immune responses. Unique features of chicken CD4, CD8αβ, and CD8αα provide a challenge for scientists working on evolutionary immunobiology as well as on poultry science. Understanding the role of these unique features can help in understanding and solving the problems in avian immunobiology and infectious diseases.

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REFERENCES


