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Short Communication

Developmental Expression of IL-2-Receptor Light Chain (CD25) in the Chicken Embryo

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Thymocyte differentiation obeys the same fundamental principles in mammals as in avian species. This parallelism does not only affect the developmentally controlled acquisition of CD3, 4, 8, and TcR isotype expression, but also concerns CD25, the light chain of the interleukin-2 receptor (IL-2R). On chicken thymocytes, surface CD25, which is recognized by the monoclonal antibody INN Ch16, is first observed during day 11 of embryonic life, and peaks at day 14, when it is expressed by about one-third of all lymphoid cells. CD25 is found on subsets of all thymocyte populations as defined by TcR$\alpha\beta$, TcR$\gamma\delta$, 2, CD4, and CD8 expression, cortical or medullary localization, and is also present on a subset of intrathymic nurse-cell lymphocytes. These findings suggest phylogenetic conservation of the IL-2/IL-2R-triggered differentiation pathway previously described for mammalian species, thus underlining its probable functional importance.

KEYWORDS: CD25, IL-2 receptor, thymus, T-cell ontogeny

INTRODUCTION

During the past few years, intensive research activity has allowed to delineate the principles of thymic ontogeny as well as the successive differentiation states of thymus-dependent lymphocytes. According to current understanding, mesenchymal and epithelial elements interact with lymphocyte precursors either via cell contact or soluble mediators, thus propagating or arresting their proliferation and differentiation in terms of positive or negative selection processes (reviewed by Strominger, 1989; Blackman et al., 1990; von Boehmer and Kieselow, 1990). Although immunology owes the discovery of the fundamental dichotomy of the B- and T-cell systems to the chicken (*Gallus domesticus*) (Cooper et al., 1966), until recently the elucidation of avian T-cell differentiation was handicapped due to the paucity of suitable immunological reagents. The availability of monoclonal antibodies (mAbs) specific for the chicken equivalents of CD3 (Chen et al., 1986), CD4, and CD8 (Chan et al., 1988), as well as the T-cell receptor (TcR) $\alpha\beta$ and $\gamma\delta$ isotypes (Chen et al., 1988; Sowder et al., 1988), have recently allowed to characterize the phenotype of thymocytes in the course of embryogenesis. Thus, the first wave of T-cell development, from day 11 to day 14 of incubation (E11 to E14), is marked by an increasing population of predominantly CD3$^+$TcR$\gamma\delta^+$CD4$^-$-CD8$^-$ cells. In a second phase, starting from E14, CD3$^+$TcR$\alpha\beta^+$ cells emerge, the majority of which acquire both CD4 and CD8 surface expression (Chan

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et al., 1988). TcR$\alpha\beta$ and TcR$\gamma\delta$ are expressed on a mutually exclusive basis (Chen et al., 1988). CD3$^+$TcR$\alpha\beta^+$ single positive (CD4$^+$ CD8$^-$ or CD4$^-$CD8$^+$) splenic or peripheral blood lymphocytes (PBL), CD3$^+$TcR$\gamma\delta^+$ double-negative (CD4$^-$CD8$^-$) PBL, and CD3$^+$TcR$\gamma\delta^+$CD4$^-$CD8$^+$ splenocytes become detectable after hatching (Chan et al., 1988; Chen et al., 1986).

Parallel to the characterization of the previously mentioned differentiation antigens, the description of lymphokines also has advanced in the chicken system. Thus, the discovery of chicken equivalents of interleukin-1 (IL-1) and interleukin-2 (IL-2) (Schauenstein and Kroemer, 1987), as well as the production of an mAb directed against the inducible light chain (CD25) of the heterodimeric high-affinity IL-2 receptor (Hála et al., 1986; Schauenstein et al., 1988) have demonstrated that peripheral T-cell activation obeys the same rules in the avian as in the mammalian immune system. In the present report, we addressed the question as to whether the involvement of IL-2 during thymocyte maturation, which is established for the human and the mouse system (Jenkinson et al., 1987; Shimonkevitz et al., 1987; Tentori et al., 1988; Pearse et al., 1989; Toribio et al., 1989; Zuñiga-Pflücker et al., 1990), is phylogenetically conserved. From the data presented herein, it will emerge that the expression of CD25 precedes the acquisition of "classical" T-cell markers in the chicken thymus and that CD25 is present on a quantitatively important minority of all T-cell subpopulations throughout ontogeny. These findings add to the conservation of principle cell-differentiation programs over large evolutionary intervals.

RESULTS AND DISCUSSION

CD25 Expression on Avian Thymocytes

INNCh16, which competetively inhibits the IL-2 response and the IL-2 adsorbtive capacity of activated T lymphocytes (Schauenstein et al., 1987), detects a membrane protein of an apparent molecular mass of 48–50 kD (Hála et al., 1986), that is, close to the 55 kD IL-2R light chain (CD25) of rodents or humans (Smith, 1989). As in mammals, CD25 is scarcely detectable on freshly isolated peripheral T cells, but is induced by short-term culture in the presence of T-cell mitogen (Kroemer et al., 1988; and Fig. 1). INNCh16 stains a significant portion of fetal thymocytes, although relatively weakly as compared to peripheral T lymphocytes activated *in vitro* with Concanavalin A (Fig. 1). Among these CD25$^+$ cells, both small and blastlike thymocytes are encountered.

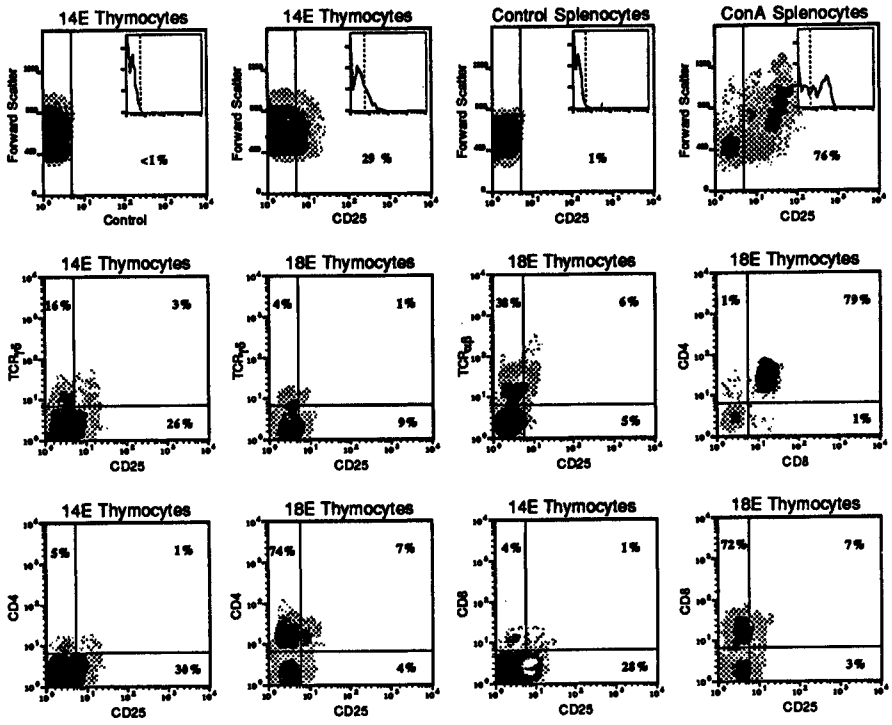

FIGURE 1. Cell-surface expression of CD25 on embryonic thymocytes. In the *upper panel*, FACS scattergrams and frequency distributions (*inserts*) of 14E thymocytes, fresh splenocytes derived from a 4-week-old animal, or splenocytes activated for 18 h with ConA stained with INNCh16 (specific for CD25) plus anti-IgM-FITC are shown. As a control, INNCh16 was omitted from the staining procedure. Note the elevated fluorescence intensity of CD25$^+$ ConA blasts as compared to thymocytes. In the *two lower panels*, FACS scattergrams of 14E or 18E thymocytes stained with INNCh16 plus anti-IgM-FITC in combination with monoclonal antibodies specific for TcR$\gamma\delta$ or TcR$\alpha\beta$ (biotin-labeled TcR1, TcR2; stained by means of PE-streptavidin conjugate), CD4 (CT4, PE-labeled), and CD8 (CT8, PE-labeled) are shown. CD4 (CT4, PE-labeled) and CD8 (CT8, TRITC-labeled) are coexpressed, whereas TcR$\gamma\delta$ and TcR$\alpha\beta$ are found on mutually exclusive subpopulations. Numbers indicate the percentage of cells found in the corresponding sector.

Ontogenic Profile of CD25 Expression

As depicted in Fig. 2, CD25 expression in the thymus is first detectable on day 11 of embryonic life (11E), reaches its maximum at 14E, gradually declines until 20E, and peaks for a second time in the postnatal period. This kinetics contrasts with the monophasic transient IL-2R light-chain expression observed in the mouse fetus (Shimonkevitz et al., 1987) and can be speculatively related to the discontinuous thymic colonialization by lymphoid stem cells (Coltey et al., 1989). Both the kinetics and the high percentage of CD25 expression—up to one-third of all thymocytes—suggest the possibility that the majority of developing T lymphocytes transiently express CD25 during intrathymic maturation. Although at a much lower level, $CD25^+$ expression is also detectable on a minor fraction of the embryonic splenocytes (Fig. 2), among which no surface $CD3^+$ cells are detectable until hatching, but cytoplasmic $CD3^+$ surface $CD3^-TcR^-CD4^-CD8^+$ lymphocytes develop as a thymus-independent lineage (Bucy et al., 1989). These so-called TcR0 cells probably represent avian natural killer-cell equivalents (Bucy et al., 1990) and expand when the chicken embryo undergoes graft-versus-host reactions, where they express CD25 (Fedecka-Bruner et al., in preparation). In the posthatching phase, very few cells in the spleen, and virtually none in peripheral blood, express CD25 (Fig. 1).

$CD25^+$ Thymocyte Populations

$CD25^+$ cells are first found in the thymic cortex (Fig. 3A). Corticomedullary transfer takes place before day 18, when medullary CD25 expression may be discerned (Fig. 3B). Double-staining experiments revealed the existence of $CD25^+$ cells within the double-positive ($CD4^+CD8^+$), double-negative ($CD4^-CD8^-$), $TcR\gamma\delta^+$, $TcR\alpha\beta^+$, and $TcR\alpha\beta^-\gamma\delta^-$ thymocyte subpopulations (Fig. 1, Table 1). $CD25^+$ 14E thymocytes are predominantly negative for T-cell markers, whereas 4 days later (18E), most $CD25^+$ cells belong to the double-positive stage, although the bulk of $CD4^+CD8^+$ thymocytes is $CD25^-$ (Table 1). Thus, CD25 expression in the chicken thymus shows an overall distribution analogous to that described in mammalian species with the only exception that in the chicken CD25 is expressed on a subset of double-positive thymocytes, which is not the case in mammals (Jenkinson et al., 1987; Toribio et al., 1988, 1989; Pearse et al., 1989). $CD25^+$ cells were encountered within a subpopulation ($24 \pm 5\%$) of intrathymic nurse-cell (TNC) lymphocytes (Figs. 3C and 3D). These cells might represent functionally active and relatively mature T lymphocytes, since INNCh16 blocks the capacity of isolated intra-TNC lymphocytes to mediate graft-versus-host reactions in the chorionallantoic membrane assay (Penninger et al., 1989, 1990).

CONCLUDING REMARKS

In mammals, the IL-2/IL-2R system is thought to play a pivotal role in thymocyte proliferation and differentiation. Lymphoid cells committed to either the NK or T-cell lineages acquire the p70/75 heavy

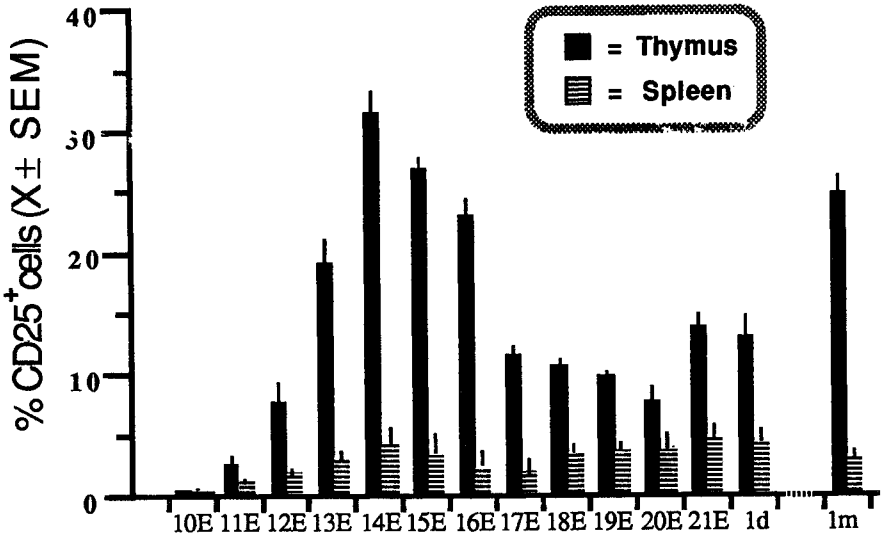

FIGURE 2. Ontogeny of CD25 expression in thymus and spleen. Cytofluorometric analysis was performed on cells stained with INNCh16 plus anti-IgM-FITC, as shown in Fig. 1. Splenocytes (striped columns) or thymocytes (black columns) were derived from 10- to 21-day-old embryos or from hatched animals. For each point, a minimum of five animals was analyzed. Cells were incubated with the vital dye acridin orange and positive cells were excluded from FACS analysis.

TABLE 1
Phenotype of Embryonic CD25$^+$ Thymocytes[a]

Phenotype	% (X ± SEM) positives among			
	14E		18E	
	Total thymocytes	CD25$^+$ cells	Total Thymocytes	CD25$^+$ cells
CD25$^+$	31 ± 3	100	11 ± 2	100
CD4$^+$	5 ± 1	8 ± 2	83 ± 5	63 ± 5
CD8$^+$	5 ± 2	9 ± 3	79 ± 4	64 ± 6
CD4$^+$/CD8$^+$	5 ± 1	8 ± 2	82 ± 6	66 ± 4
TcR$\alpha\beta^+$	< 2	ND	42 ± 3	49 ± 7
TcR$\gamma\delta^+$	18 ± 3	9 ± 3	5 ± 2	15 ± 5
TcR$\alpha\beta^+$/TcR$\gamma\delta^+$	ND	ND	49 ± 5	64 ± 5
CD4$^+$/CD8$^+$CD4$^+$/TcR$\alpha\beta^+$/TcR$\gamma\delta^+$	ND	ND	86 ± 4	79 ± 6

[a]Thymocytes of 14- or 18-day-old embryos were stained with specific mAbs in combination with class-specific anti-IgM antiserum (FITC-labeled; for INNCh16, specific for CD25) or anti-IgG1-texas red conjugate (for all other mAbs) followed by cytofluorometric analysis. Mean values of at least three independent experiments are shown.

chain of the IL-2R (intermediate affinity IL-2R) on a prethymic stage. After migration into the thymus, interaction with stromal cells induces IL-2 and IL-2R light-chain (CD25) expression on pro-T cells (that still lack components of the CD3/TCR complex, CD4, and CD8), thus allowing formation of high-affinity heterodimeric IL-2R and autocrine proliferation via the IL-2/IL-2R pathway (Toribio et al., 1989). The inducibility of both IL-2 and CD25 is developmentally controlled, that is, is restricted to certain thymocyte populations (Boyer et al., 1989; Carding et al., 1989). Under *in vitro* culture conditions, IL-2 is capable of inducing CD3, TcR, CD4, and CD8 surface expression on immature thymocytes (Toribio et al., 1988). At the present stage, it is still a matter of debate whether the IL-2/IL-R-mediated autocrine pathway of T-cell differentiation is an abortive or a productive one, that is, whether such cells ultimately give rise to functional peripheral T cells. Other cytokines, including IL-1, IL-3, IL-4, IL-6, IL-7, TNF-α, and GM-CSF, also have been implicated in thymic lymphopoiesis (reviewed by Martínez-A., 1990). Some findings have shed doubts on the obligatory involvement of the IL-2/IL-2R pathway in thymocyte maturation. In the human system, defects in IL-2 production by peripheral T cells have been observed that are not accompanied by major numeric T-cell deficiencies (Chatila et al., 1989; Disanto et al., 1990) and, in the rat, the expression of CD25 and IL-2 in double-negative cells has been contested (Takács et al., 1988). The fact that CD25 expression follows an analogous kinetics and distribution in the mammalian and the avian embryo suggests conservation of the IL-2-mediated thymocyte-differentiation pathway over about 270 $\times 10^6$ years of independent evolution, thus underlining the probable functional importance of CD25 in thymic ontogeny. In this context, it is tempting to speculate that this early pro-T cell growth-promoting function of IL-2 might be the "original" one

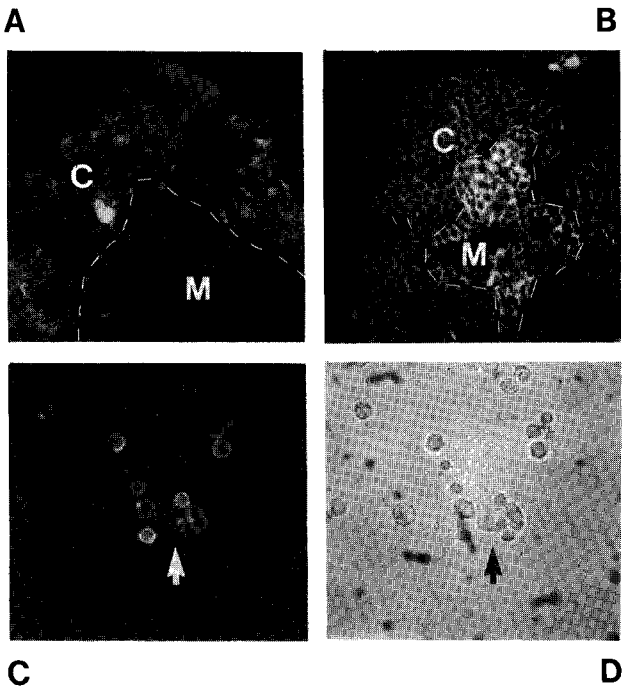

FIGURE 3. Histological analysis of CD25 distribution. Thymic sections of 14 (A, magnification 1×120) or 18-day (B, 1×300) old donors were stained with INNCh16 plus anti-Ig-FITC conjugate. The dashed line marks the boundary between thymic medulla (M) and cortex (C). In the lower part of the figure, the staining pattern of intrathymic nurse cell (TNC) lymphocytes (TNC marked by arrow) with INNCh16 (C) is compared to its transillumination picture (D).

and that the involvement of IL-2 in peripheral T, NK, and B-cell physiology might have been acquired later during phylogeny. Investigations concerning more primitive vertebrate and invertebrate species might shed light on this issue.

MATERIALS AND METHODS

Animals and Cell Preparations

Thymi and spleens from normal White Leghorn chick (*Gallus domesticus*) embryos (eggs incubated at 38°C) or up to 4-week-old individuals were teased through 200-mesh sieves and cells suspended in ice cold PBS (pH=7.2) supplemented with calf serum (10%). Viable cells were isolated by centrifugation of a Ficoll Hypaque^R (Pharmacia, Uppsala, Sweden) gradient. In one experiment, splenocytes from 4-week-old donors (5×10^6/ml) were cultured overnight in RPMI1640 (Seromed, Munich, FRG) supplemented with penicillin (100 U/ml), streptomycin (5 μg/ml), and concanavalin A (ConA, 2.5 μg/ml, Sigma, St. Louis, MO, USA) as described (Kroemer et al., 1988). Thymic nurse cells were isolated by Percoll^R (Pharmacia) density-gradient centrifugation of collagenase type IV-digested thymic tissue and mounted on slides as described (Wick and Oberhuber, 1986).

Immunocytohistochemistry

The mAbs INNCh16 (IgM, specific for CD25) (Hála et al., 1986), CT4 (IgG1, specific for CD4), CT8 (IgG1, specific for CD8) (Sowder et al., 1988), TcR1 (IgG1, specific for the γ/δ TcR) (Chen et al., 1986), and TcR2 (IgG1, specific for the α/β TcR (Chen et al., 1988) were used conjugated to phycoerythrin (PE) or fluorescein isothiocyanate (FITC) for direct fluorescence. For indirect staining, cells or tissue sections were incubated with unconjugated mAbs followed by FITC-conjugated antimouse IgM or texas red-conjugated antimouse IgG1 or with biotinylated mAbs followed by FITC or PE-conjugated streptavidin (Seromed, Blackthorn, UK). Automated flow cytometry was performed using the FACS II or FACS-Scan instruments (Becton Dickinson, Sunnyvale, CA). For monocolor fluorocytometry, cells were incubated with the vital dye acridin orange and stained cells were excluded from analysis. Results were expressed as percentage stained cells (mean values ± standard error of the mean, SEM) and significance was calculated using the Student t test.

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