

Thymic Origin of Embryonic Intestinal γ/δ T Cells

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Summary

Current evidence suggests both thymic and extrathymic origins for T cells. Studies in mice favor an in situ origin for a prominent population of intestinal intraepithelial lymphocytes that express γ/δ T cell receptor (TCR). This developmental issue is explored in an avian model in which the γ/δ lymphocytes constitute a major T cell subpopulation that is accessible for study during the earliest stages of lymphocyte development. In the chick embryo, cells bearing the γ/δ TCR appear first in the thymus where they reach peak levels on days 14–15 of embryogenesis, just 2 d before γ/δ T cells appear in the intestine. Using two congenic chick strains, one of which expresses the ov antigen, we studied the origin and kinetics of intestinal colonization by γ/δ T cells. The embryonic γ/δ^+ thymocytes homed to the intestine where they survived for months, whereas an embryonic γ/δ^- thymocyte population enriched in thymocyte precursors failed to give rise to intestinal γ/δ^+ T cells. Embryonic hemopoietic tissues, bone marrow, and spleen, were also ineffective sources for intestinal γ/δ^+ T cells. Intestinal colonization by γ/δ^+ thymocytes occurred in two discrete waves in embryos and newly hatched birds. The data indicate that intestinal γ/δ T cells in the chicken are primarily thymic migrants that are relatively long-lived.

Lymphocytes bearing γ/δ TCR are preferentially localized in the intestinal epithelium in both birds and mammals (for review see references 1 and 2). Although intraepithelial lymphocytes (IEL)¹ of the small intestine are anatomically positioned to be the first line of cellular defense against enteric pathogens, the true function of γ/δ IEL remains unclear. Cytolytic capacity can be demonstrated, but specificity and MHC restriction patterns have not been defined for γ/δ IEL (3, 4). Their localization in the intestinal epithelium is independent of normal microbial colonization (5). It is interesting that γ/δ IEL have the capacity to reverse oral tolerance when adoptively transferred, although direct antigen reactivity of these γ/δ IEL has not been demonstrated (6, 7).

The elucidation of γ/δ T cell origin and migration characteristics should facilitate understanding of the generation of distinct TCR repertoires observed in different anatomical sites (8). Although the thymus is clearly an important source of γ/δ T cells (2), experiments conducted in immunocompromised mice have suggested that a significant proportion of the γ/δ IEL may be generated extrathymically. Cells from bone marrow or day 15 fetal liver infused into irradiated, thymectomized mice gave rise to γ/δ IEL, and the IEL in nude athymic mice are predominantly γ/δ TCR⁺ (9–11). On analyzing the generation of CD8⁺ γ/δ^+ IEL in thymectomized mice depleted of CD8⁺ cells and showing that IEL contained mRNA for the recombinase activating gene (RAG-

1) protein required for TCR rearrangement, Guy-Grand et al. (12, 13) proposed that most γ/δ^+ IEL are derived in situ from precursors of extrathymic origin.

The developmental origin of γ/δ IEL is an important issue since these cells are the first lymphoid cells to appear in the intestine and may play important immunological roles such as control of oral tolerance, control of bacterial colonization, and elimination of damaged epithelial cells. The data on γ/δ intestinal T cell origin have been obtained in young or adult mice, but there is no information concerning the origin of γ/δ IEL during embryogenesis and the first weeks of life. The size of the mouse embryos practically precludes such study. The avian model system, by contrast, offers significant experimental advantages for exploration of this issue. Cells bearing the γ/δ TCR appear first in the chick embryo thymus where they reach a peak on days 14–15 of incubation, just 2 d before γ/δ T cells appear in the intestine (14–16), and studies of chick-quail chimeras suggest that embryonic γ/δ and α/β T cells are generated exclusively in the thymus (17). In the present studies, we have used two congenic chicken strains to analyze the intestinal colonization by γ/δ T cells before and after hatching. The data show that avian γ/δ IEL are primarily of thymic origin and indicate that this colonization occurs by waves of thymic γ/δ migrants having relatively long life spans.

Materials and Methods

Animals. Embryos of White Leghorn chicken strain H.B19 were derived from animals kept at our institute's farm in Gipf-Oberfrick,

¹ Abbreviations used in this paper: IEL, intraepithelial lymphocyte; Tx, thymectomized recipient.

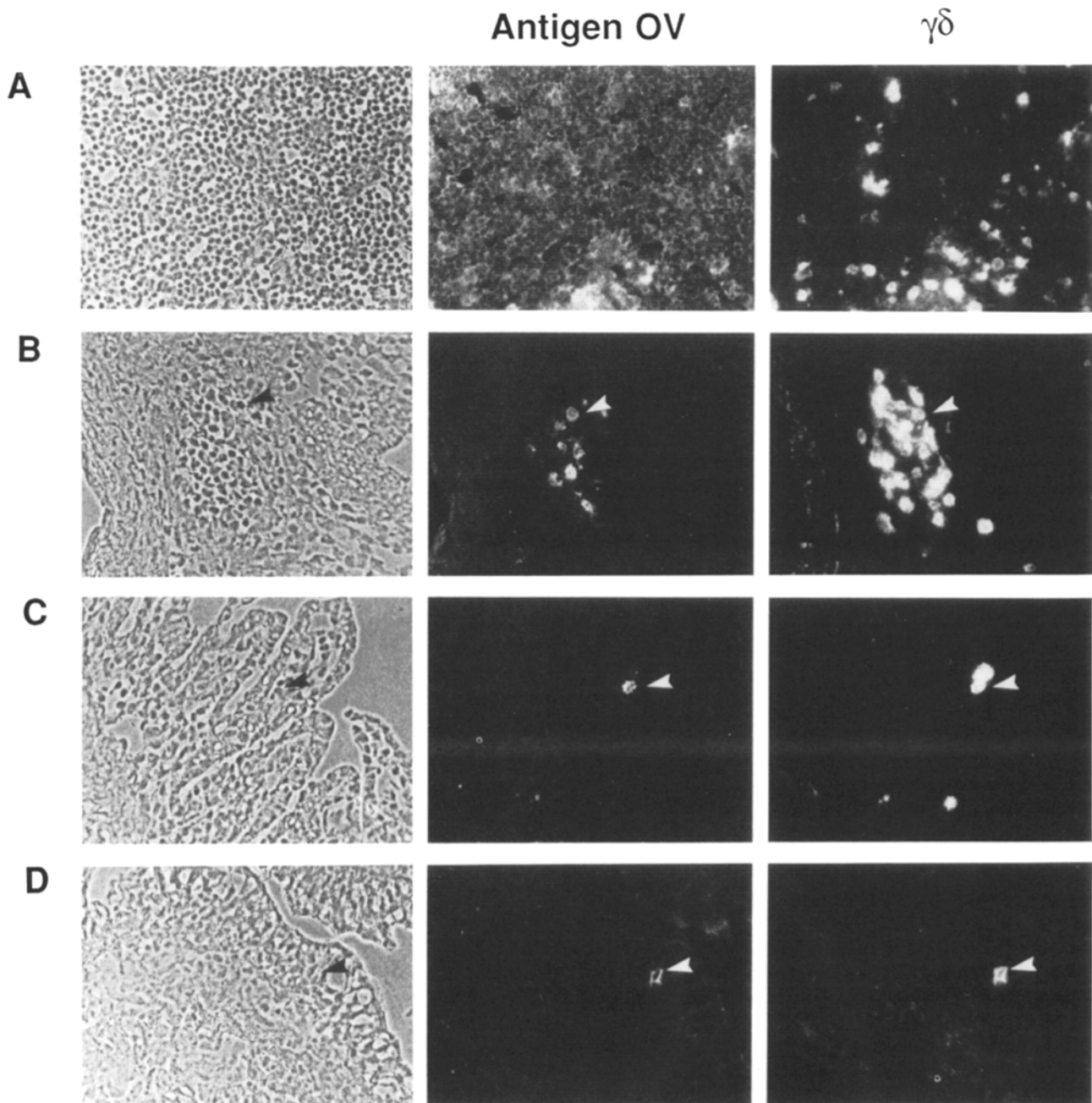


Figure 1. Embryonic γ/δ thymocytes home to the intestine. Double immunofluorescence staining of frozen tissue section. (A) Thymus from day 16 H.B19ov⁺ embryos. (B and C) Small intestine from day 18 H.B19ov⁻ embryos injected at day 16 with day 14 ov⁺ thymocytes (B) illustrating submucosal lymphoid aggregates (chickens do not have true Peyer's patches, [8]), (C) correspond to intraepithelial lymphocytes, and (D) small intestine of a 1-mo-old chicken that was thymectomized 2 d after hatching and immediately injected with 14-d-old ov⁺ splenocytes. (Arrowheads) Donor IEL (ov⁺ γ/δ ⁺). $\times 270$.

Switzerland. Fertilized eggs were incubated at 38°C and 80% humidity in a ventilated incubator. The H.B19 strain was subdivided into congenic lines (H.B19ov⁺ and H.B19ov⁻). They can be distinguished by the ov antigen which is present on thymocytes and T cells only in H.B19ov⁺ animals, and which is recognized by the mAb 11-A-9 (18–20). The experimental animals were treated according to Swiss government veterinary guidelines.

Injection of Lymphoid Cells into Congenic Chickens. Embryonic day 13 (E13) bone marrow cells, E13 splenocytes, and E14 thymo-

cytes (25×10^6) were injected. Bone marrow and spleen from day 13 embryos do not contain detectable γ/δ ⁺ cells. Injections of sorted populations of E14 thymocytes were also performed. In this case, thymocytes from 14-d-old H.B19ov⁺ embryos were suspended in PBS containing 10% FCS, filtered through a nylon sieve (mesh width of 25 μm ; Nytal P-25 my, SST, Thal, Switzerland) and centrifuged at 255 g for 7 min. Immunofluorescence staining of these relatively fragile cells was performed in 96-well plates, to avoid repeated centrifugation, using the anti- γ/δ antibody TCR1,

Table 1. T Cell Marker Expression by ov^+ Cells Derived from E14 Thymocyte Transplants in ov^- Recipients

Days of development	Percent intestinal cells bearing the donor ov antigen				
	γ/δ^+	α/β^+	CD3 ⁺	CD8 ⁺	CD4 ⁺
18	82 ± 7	0	100 ± 2	37 ± 4	0
19	75 ± 3	0	95 ± 4	41 ± 4	0
22	74 ± 3	0	100 ± 3	40 ± 4	0.5 ± 0.5
28	75 ± 3	0.5 ± 0.5	100 ± 3	42 ± 4	0.5 ± 0.5

Expression of T cell markers was determined by double immunofluorescence staining of gut frozen sections. Each slide contained a section of the duodenum, a section of the ileum, and a section located around the coecal tonsils. Counting was performed on two slides for each animal. 200 ov^+ intestinal cells were counted per slide. Data are expressed in percent ov^+ intestinal cells and correspond to the mean of three animals. Variability corresponds to SE. 0 is defined as <0.1%, i.e., no ov^+ γ/δ^+ T cells were detected in analyzed slides.

and then fluorescein-coupled anti-mouse Ig antibody (Silenus, Hawthorn, Australia). Stained and unstained thymocytes were resuspended in 10% FCS/PBS and sorted using a cell sorter (FACStar plus[®]; Becton Dickinson & Co., Mountain View, CA).

These hemopoietic cells were injected into a large vein at the end of the airsac of 16-d-old H.B19 ov^- embryos. Alternatively, cells (20×10^6) were injected into the jugular vein of H.B19 ov^- chicks just after thymectomy on the second day after hatching. After killing, the neck region of thymectomized chickens was dissected and examined for remnants that would indicate incomplete thymectomy. Those cases in which thymic remnants were detected were excluded from this analysis. Control animals were H.B19 ov^- recipients that were not thymectomized. The different recipients received no irradiation or drug treatment.

Analysis of donor cells in the intestine was performed by FACS[®] analysis and by two-color immunofluorescence staining of frozen tissue sections. For FACScan[®] analysis of intestinal lymphocytes, the small intestine was extensively washed with PBS containing 4 g/liter glucose, minced into small pieces at 4°C, washed 10 min at room temperature with 2 mM DTT/4 g/liter glucose plus PBS, and lymphocytes were detached in 3 mM EDTA/2 mM DTT/4 g/liter glucose plus PBS at 37°C for 15 min (21). Cells were then pelleted by centrifugation and stained.

Immunolabeling. ov , γ/δ , α/β , CD3, CD8, and CD4 antigens were detected using 11-A-9, TCR1, TCR2, CT3, CT8, and CT4 mAbs, respectively (14, 18, 22–25). Except for 11-A-9, which was a mouse IgM (18, 19), all mAbs were mouse IgG1. Second antibodies were fluorescein-labeled sheep anti-mouse IgM and PE or Texas red-coupled anti-mouse IgG1 antibody (Southern Biotechnology Associates, Birmingham, AL). Controls were performed using second antibodies alone and also with tissues of noninjected individuals from the H.B19 ov^- strain.

Frozen sections of embryonic organs were cut to a thickness of 5 μ m on a cryostat (E. Leitz, Wetzlar, Germany), fixed with acetone, rehydrated in PBS containing 1% BSA.

Results

H.B19 ov^+ chickens express ov antigen on the surface of most hemopoietic precursors during embryonic life, but only on T lineage cells and their precursors postnatally (18). Chickens of the congenic strain H.B19 ov^- , which do not express the ov antigen (18–20), were employed as recipients in these experiments. On day 14 of embryogenesis, ~25%

of all thymocytes are γ/δ^+ and <3% are α/β^+ , all of these being ov^+ in the H.B19 ov^+ strain (23 and data not shown). Injection of day 14 H.B19 ov^+ thymocytes into 15- or 16-d-old H.B19 ov^- embryos led to the appearance of γ/δ^+ T cells of the donor ov^+ type in the small intestine within 2 d after injection (Fig. 1). The injected thymocytes homed to the intestine and the spleen, but neither thymus nor bursa were colonized at significant levels (Figs. 1 and 2 and data not shown). The percentage of donor γ/δ^+ T cells in the intestine remained relatively constant until the end of embryogenesis (days 18, 19, and 20).

Donor ov^+ E14 thymocytes homing to the gut expressed CD3 and >75% of these were identifiable as γ/δ^+ (Table

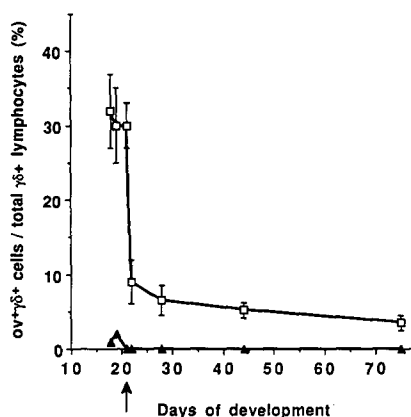


Figure 2. Survival pattern of donor γ/δ^+ cells in intestine. Day 16 H.B19 ov^- embryos were injected with 25×10^6 day 14 H.B19 ov^+ thymocytes and killed at various times after injection. (Arrow) Hatching at day 21. Data are expressed as percent γ/δ^+ IEL that express the donor ov antigen marker and correspond to the mean of two to four animals derived from three independent experiments. Analysis was performed in intestine (\square) and thymus (\blacktriangle). These results were obtained from two-color immunofluorescence analysis of tissue sections throughout development. Each slide contained a section of the duodenum, a section of the ileum, and a section located around the coecal tonsils. Counting was performed on two slides for each animal. 200 and 500 γ/δ IEL were counted per slide of embryonic intestine and newborn chick, respectively. By day 22 (after hatching) these results were confirmed by FACScan[®] analysis. Error bars correspond to SE.

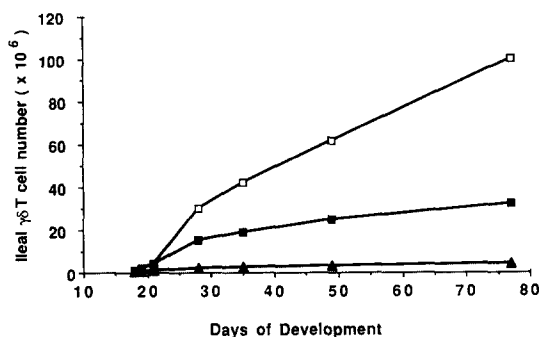


Figure 3. Quantitation of ileal γ/δ^+ T cells during development. Ileal γ/δ^+ T cells were counted on frozen gut sections stained by immunofluorescence. Taking into account the length of the ileum, the area of the ileum sections, and the thickness of these sections, the total number of ileal γ/δ^+ lymphocytes was calculated for the different stages of development. (□) Control animals; (■) chickens thymectomized on the second day after hatching; and (▲) ileal ov^+ γ/δ^+ T cells of H.B19 ov^- animals injected with 25×10^6 day 14 H.B19 ov^+ thymocytes (see Fig. 2). Day 21 corresponds to hatching.

1). Approximately 40% of the ov^+ γ/δ^+ T cells in the intestine were $CD8^+$ and the remainder were $CD4^-CD8^-$, a phenotypic distribution similar to that of γ/δ T cells in the adult intestinal mucosa (15) which has also been observed in human intraepithelial mucosa (26–27). As expected at this early embryonic stage, significant homing of ov^+ α/β^+ E14 thymocytes to the gut was not observed.

The donor γ/δ T cells persisted in the recipient intestine beyond 75 d, although a dramatic decrease in the percentage of donor intestinal γ/δ^+ T cells occurred around hatching (Fig. 2). This decrease might result from dilution by the intestinal arrival of host γ/δ^+ T cells. The quantitative analysis of ileal γ/δ^+ T cells during their development, which revealed a significant increase in the total number of cells occurring in the first days after hatching (Fig. 3), favored this hypothesis. Alternatively, a loss of γ/δ^+ IEL due to the

onset of digestive function could theoretically account for the observed decrease.

To analyze the origin of the posthatch emigrants to the intestine, we injected lymphoid cells into normal and thymectomized chickens after hatching (Table 2). Injection of 14-d-old H.B19 ov^+ embryonic thymocytes into 2-d-old H.B19 ov^- thymectomized chickens led to the development of >20% ov^+ cells among the γ/δ^+ T cell population in the intestine during the first month after the injection. Injection of the same number of ov^+ thymocytes into 2-d-old, non-thymectomized chickens led to only 3–9% of intestinal γ/δ^+ T cells being of the donor type. These results suggest that cells derived from the recipient's thymus compete with the homing of the donor thymocytes. Moreover, γ/δ thymocytes from either 14-d-old H.B19 ov^+ embryos or 2-d-old H.B19 ov^+ chicks colonized the intestine of 2-d-old H.B19 ov^- chicks with the same efficiency (data not shown). The progeny of stem cells of the second wave of thymus colonization (days 12–13 of embryogenesis) mature around the time of hatching (7–8 d later; [17, 28]). Thus, this second wave of γ/δ^+ T cells homing to the intestine after hatching also appears to be thymus derived. In further support of this interpretation, injection of 35×10^6 14-d-old H.B19 ov^+ thymocytes into H.B19 ov^- chickens at 1 wk of age, contributed a very small proportion of intestinal γ/δ^+ T cells ($0.5 \pm 0.5\%$).

Injections of day 13 embryonic splenocytes or bone marrow cells into 16-d-old embryos did not contribute embryonic intestinal γ/δ^+ T cells of donor origin. However, when embryonic day 13 splenocytes and bone marrow cells were injected into newly hatched thymectomized chickens, occasional donor γ/δ^+ T cells could be found in the intestine of 6 of 15 recipients (Table 2 and Fig. 1). These intestinal γ/δ^+ T cells of possible extrathymic origin were detected only 3–4 weeks after injection in thymectomized recipients and never in normal recipients.

To examine further the possibility that thymocyte precursors

Table 2. Intestinal Homing of ov^+ γ/δ^+ T Cells into Thymectomized (Tx) and Normal ov^- Newly Hatched Chickens

Recipient age	Donor-derived γ/δ^+ cells after injection of:					
	E14 thymocytes		E13 bone marrow cells		E13 splenocytes	
	Tx	Control	Tx	Control	Tx	Control
<i>wk</i>						
1	23 ± 6	9 ± 4	ND	0	ND	0
2	30 ± 8	3 ± 2	0	0	0	0
4	20 ± 6	3 ± 2	0.5 ± 0.5	0	0.5 ± 0.5	0
8	8 ± 5	ND	0.5 ± 0.5	ND	0.5 ± 0.5	0

Data correspond to the percent γ/δ IEL that express the donor ov alloantigen marker. Each point corresponds to the mean of two to four animals. E13 and E14 correspond to 13- and 14-d-old embryos. γ/δ donor cells were analyzed by two-color immunofluorescence staining of intestine sections. Each slide contained a section of the duodenum, a section of the ileum, and a section located around the coecal tonsils. Counting was performed on two slides for each animal. 200 γ/δ IEL were counted per slide. Variability corresponds to SE. 0 is defined as <0.1%; no ov^+ γ/δ^+ T cells were detected in analyzed slides. These results were confirmed by FACScan® analysis.

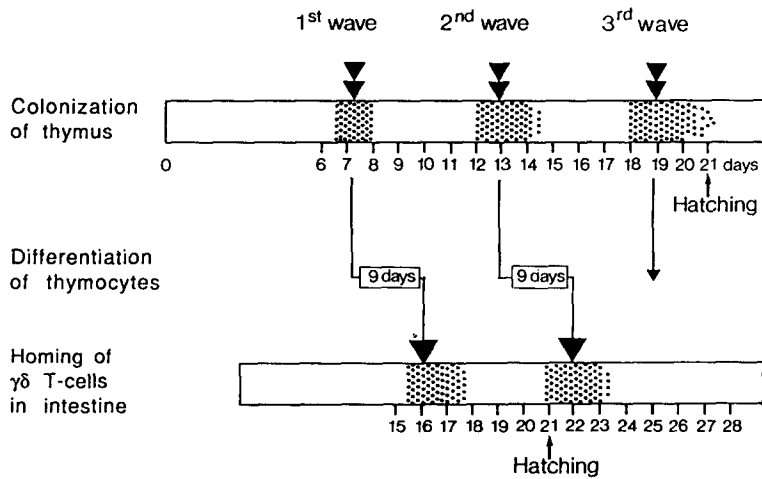


Figure 4. A model of colonization of the chicken intestine by γ/δ T cells. Colonization of the embryonic thymus occurs in three waves and these T cell precursors mature in situ around 9 d later (12). Day 14–16 embryonic γ/δ thymocytes derived from the first wave of thymus colonization seed the intestine at days 16–18 of embryogenesis. Progeny of the second wave of stem cells colonizing the thymus should lead to the second wave of intestine colonization by γ/δ T cells, although we cannot exclude the possibility that some γ/δ thymocytes derived from the first wave of thymus colonization may contribute to the second wave of intestine colonization.

might differentiate extrathymically into γ/δ T cells, donor thymocytes from 14-d-old embryos were separated into γ/δ^- and γ/δ^+ populations. To yield an enrichment of thymocyte precursors, embryos at this development stage were chosen as donors since the second wave of precursor cells enters the thymus between embryonic days 12–14 (see Fig. 4). When sorted γ/δ^- or γ/δ^+ thymocytes were injected into 16-d-old embryos, the γ/δ^+ thymocytes gave rise to γ/δ^+ intestinal lymphocytes of the donor type while the γ/δ^- thymocyte population did not (Table 3). Indeed, when H.B19ov⁻ chickens were injected with ov⁺ γ/δ^- thymocytes, donor intestinal γ/δ^+ T cells were still undetectable 2 mo after hatching.

Discussion

These results provide direct evidence for population of intestinal epithelium by emigrating γ/δ thymocytes. Our data indicate that the homing of γ/δ T cells from the thymus

Table 3. Intestinal Homing of Sorted Congenic γ/δ^+ or γ/δ^- Thymocytes Injected Intravenously

	ov ⁺ γ/δ^+ thymocytes		ov ⁺ γ/δ^- thymocytes
No. of cells injected	1.5×10^6	3×10^6	3×10^6
Intestinal colonization by γ/δ T cells/no. of recipients	2/2	2/2	0/3

Sorted γ/δ^+ and γ/δ^- thymocytes from 14-d-old H.B19ov⁺ embryos were injected in 16-d-old H.B19ov⁻ embryos. Embryos were killed at day 18 and γ/δ donor cells were analyzed by two-color immunofluorescence staining of intestine sections. Each slide contained a section of the duodenum, a section of the ileum, and a section located around the coecal tonsils. Counting was performed on three slides for each animal. 500 γ/δ IEL were counted per slide. By day 22 (after hatching) these results were confirmed by FACScan[®] analysis. Embryos injected with 1.5×10^6 and 3×10^6 ov⁺ γ/δ^+ thymocytes presented 13 and 5% of γ/δ IEL of the donor type. No γ/δ^+ donor cells were detected in embryos injected with ov⁺ γ/δ^- thymocytes.

to the intestine occurs in a developmental pattern that follows the scheme of thymus colonization by waves of precursor stem cells. In chick-quail chimeras, three waves of precursor cells have been shown to enter the thymus during embryonic life as outlined in Fig. 4 (28). Within the thymus, each in turn gives rise to a wave of progeny T cells that includes both γ/δ and α/β subpopulations (17). Using congenic strains of chickens, one of which expresses the marker ov antigen, we found that γ/δ T cells in the first two developmental waves of embryonic thymocytes migrate to the intestine where they populate the epithelial lining. These thymus-derived γ/δ^+ IEL apparently can survive for long periods of time in this environment. In our cell transfer experiments, embryonic ov⁺ γ/δ^+ thymocytes rapidly populated the intestines of ov⁻ embryonic recipients, where they were found to persist into young adulthood. While the present experiments did not explore the fate of γ/δ^+ thymocytes in subsequent waves or the survival of the initial migrants beyond 75 d of age, the data suggest that, once established, this long-lived population γ/δ^+ IELs may require minimal replenishment. Since α/β^+ cells do not appear before E19–20 in intestine and represent <3% of E14 thymocytes (16, 23), the experiments performed here, involving injection of E14 thymocytes, do not allow analysis of the origin of α/β^+ intestinal cells.

One of the major issues addressed in these experiments in the chicken concerns the possible extrathymic origin for γ/δ^+ IEL suggested by studies in mice (9–12). Our results indicate that the avian γ/δ^+ IEL population includes few, if any, γ/δ T cells that are derived from extrathymic sources. In all of the normal recipients and one half of the thymectomized recipients of embryonic (E13) bone marrow or spleen cells, we could not identify γ/δ^+ IEL of donor origin. In some of the thymectomized recipients, however, we found γ/δ^+ IELs of donor origin in very low frequencies at 1–2 mo of age. These γ/δ^+ IELs could be of thymic origin since the donor bone marrow and splenic populations were obtained 1 or 2 d after the appearance of γ/δ^+ thymocytes. Indeed, when the γ/δ^- fraction of 14-d embryonic thymocytes was infused as an enriched source of thymocyte precursors

(Table 3), no γ/δ^+ T cell progeny were found subsequently, whereas the γ/δ^+ thymocytes efficiently populated the intestinal epithelium. We conclude from these observations that the thymus is the prime source of γ/δ^+ IELs in the chicken, at least early in development when the question of cell origin can be experimentally addressed most incisively.

It is interesting that ~25% of the IELs of donor thymocyte origin were noted to be CD3⁺ in tissue sections, but negative for both γ/δ and α/β TCR (Table 1). In these experiments, the entire thymocyte population of 14-d-old embryos was infused in the donor inoculum. The cytoplasmic CD3⁺/TCR⁻ lymphocytes in the chicken have been characterized in other studies as NK cells (29). Thus, the avian thymus may contain precursors of both T and NK cells, as has been proposed for mice and humans (30). Although the rearrangement of TCR genes to become a T cell would appear to require microenvironmental cues provided in the thymus, without the need for TCR gene rearrangement, NK cell progeny can be generated in extrathymic tissues (30, 31).

The selective homing of γ/δ T cells to the intestinal epithelium appears to be a highly conserved property of these cells since this pattern of localization is seen in birds, mammals, and amphibians (2, 32), suggesting an important role in defense of this epithelial barrier. Whereas our experimental results indicate that the thymus is the major source of these cells in the chicken, studies in mice suggest that many γ/δ^+ IEL are extrathymic in origin. In irradiated thymectomized recipients, the γ/δ IEL population can be restored by infusion of bone marrow or fetal (E15) liver cells (9–12), and γ/δ T cells are also acquired in the intestine and skin of nude

mice with severe thymic hypoplasia (33–35). However, it is difficult to be certain of an extrathymic origin for the γ/δ IEL in these experimental models, because the thymic generation of γ/δ T cells begins on day 12 of embryonic life, and the nude mouse has a small vestigial thymus with apparent lymphopoietic function (36). Rearrangements of the V γ 5 gene has been identified by PCR technique in the liver and intestine as early as embryonic day 11, but it is not known that these rearrangements are productive (37). The similarity of the V γ 5/V δ 1 repertoire in the gut and thymus of mouse embryos may imply that most intestinal γ/δ T cells are also thymus derived in the embryonic mouse (38).

In sheep, as in the chicken, the thymus also appears to be the primary source of γ/δ IEL. Thymectomy performed in utero, just a few days after departure of the first thymic emigrants, results in severe and persistent depletion of the γ/δ T cells in lambs (39). Intestinal γ/δ T cells were virtually absent in the thymectomized lambs from birth through the first year of life, in contrast to the abundant population of intestinal γ/δ T cells in normal animals. It is interesting to note that γ/δ^+ cells are predominant IEL in both chicken and sheep, whereas they represent 50% of IEL in mice and no more than 10–15% of IEL in human (2). One possibility is that these numerical differences in γ/δ IEL abundance may reflect differences in the proportion of extrathymic versus thymic-derived γ/δ IEL in these species. Alternatively, the complementary nature of the results in the sheep model and our data in the chicken may suggest the need for reappraisal of the hypothesis of an extrathymic origin for γ/δ T cells during mouse and human development.

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References

1. Viney, J., T.T. MacDonald, and J. Spencer. 1990. Gamma/delta T cells in the gut epithelium. *Gut*. 31:841.
2. Allison, J.P. 1991. Gamma delta T cells. Seminars in Immunology. Vol. 3. W.B. Saunders Company. 129 pp.
3. Lefrançois, L., and T. Goodman. 1989. In vivo modulation of cytolytic activity and thy-1 expression in TCR γ/δ^+ intraepithelial lymphocytes. *Science (Wash. DC)*. 243:1716.
4. Viney, J., P.J. Kilshaw, and T.T. MacDonald. 1990. Cytotoxic α/β^+ and γ/δ^+ T cells in murine intestinal epithelium. *Eur. J. Immunol.* 20:1623.
5. Bandeira, A., T. Motas-Santos, S. Itoharu, S. Degermann, C. Heusser, S. Tonegawa, and A. Coutinho. 1990. Localization of γ/δ T cells to the intestinal epithelium is independent of normal microbial colonization. *J. Exp. Med.* 172:239.
6. Fujihashi, K., T. Taguchi, J.R. McGhee, J.H. Eldridge, M.G. Bruce, D.R. Green, B. Singh, and H. Kiyono. 1990. Regulatory function for murine intraepithelial lymphocytes. *J. Immunol.* 145:2010.

7. Fujihashi, K., T. Taguchi, W.K. Aicher, J.R. McGhee, J.A. Bluestone, J.H. Eldridge, and H. Kiyono. 1992. Immunoregulatory functions for murine intraepithelial lymphocytes: γ/δ T cell receptor-positive (TCR⁺) T cells abrogate oral tolerance, while α/β TCR⁺ T cells provide B cell help. *J. Exp. Med.* 175:695.
8. Lefrançois, L. 1991. Extrathymic differentiation of intraepithelial lymphocytes: generation of a separate and unequal T-cell repertoire. *Immunol. Today.* 12:436.
9. Mosley, R.L., D. Styre, and J.R. Klein. 1990. Differentiation and functional maturation of bone marrow-derived intestinal epithelial T cells expressing membrane T cell receptor in athymic radiation chimeras. *J. Immunol.* 145:1369.
10. Lefrançois, L., R. LeCorre, J. Mayo, J.A. Bluestone, and T. Goodman. 1990. Extrathymic selection of TCR γ/δ ⁺ T cells by class II major histocompatibility complex molecules. *Cell.* 63:333.
11. Bandeira, A., S. Itoharu, M. Bonneville, O. Burlen-Defranoux, T. Mota-Santos, A. Coutinho, and S. Tonegawa. 1991. Extrathymic origin of intestinal intraepithelial lymphocytes bearing T-cell antigen receptor γ/δ . *Proc. Natl. Acad. Sci. USA.* 88:43.
12. Guy-Grand, D., N. Cerf-Bensussan, B. Malissen, M. Malassis-Seris, C. Briottet, and P. Vassali. 1991. Two gut intraepithelial CD8⁺ lymphocyte populations with different T cell receptors: a role for the gut epithelium in T cell differentiation. *J. Exp. Med.* 173:471.
13. Guy-Grand, D., C. Van den Broecke, C. Briottet, M. Malassis-Seris, F. Selz, and P. Vassali. 1992. Different expression of the recombination activity gene RAG-1 in various population of thymocytes, peripheral T cells, and gut thymus-independent intraepithelial lymphocytes suggests two pathways of T cell receptor rearrangement. *Eur. J. Immunol.* 22:505.
14. Sowder, J.T., C.H. Chen, L.L. Ager, M.M. Chan, and M.D. Cooper. 1988. A large subpopulation of avian T cells express a homologue of the mammalian T γ/δ receptor. *J. Exp. Med.* 167:315.
15. Bucy, R.P., C.H. Chen, J. Cihak, U. Lösch, and M.D. Cooper. 1988. Avian T cells expressing γ/δ receptors localize in the splenic sinusoids and the intestinal epithelium. *J. Immunol.* 141:2200.
16. Bucy, R.P., C.L. Chen, and M.D. Cooper. 1990. Ontogeny of T cell receptors in the chicken thymus. *J. Immunol.* 144:1161.
17. Coltey, M., R.P. Bucy, C.H. Chen, J. Cihak, U. Lösch, D. Char, N.M. Le Douarin, and M.D. Cooper. 1989. Analysis of the first two waves of thymus homing stem cells and their T cell progeny in chick-quail chimeras. *J. Exp. Med.* 170:543.
18. Vainio, O., T.V. Veromaa, E. Eerola, and P. Toivanen. 1987. Characterization of two monoclonal antibodies against chicken T lymphocytes surface antigen. In *Avian Immunology*. W.T. Weber and D.L. Ewert, editors. Alan R. Liss Inc., New York. pg. 99.
19. Dunon, D., J. Kaufman, J. Salomonsen, K. Skjoedt, J.P. Thiery, and B.A. Imhof. 1990. T cell precursor migration towards β 2-microglobulin is involved in thymus colonization of chicken embryos. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3315.
20. Houssaint, E., A. Mansikka, and O. Vainio. 1991. Early separation of B and T lymphocyte precursors in chick embryo. *J. Exp. Med.* 174:397.
21. Chai, J.-Y., and H.S. Lillehoj. 1988. Isolation and functional characterization of chicken intestinal intraepithelial lymphocytes showing natural killer cell activity against tumor target cells. *Immunology.* 63:111.
22. Cihak, J., H.W.S. Ziegler-Heitbrock, H. Trainer, I. Schraner, M. Merckenschlager, and U. Lösch. 1988. Characterization and functional properties of a novel monoclonal antibody which identifies a T cell receptor in chickens. *Eur. J. Immunol.* 18:533.
23. Chen, C.H., J. Cihak, U. Lösch, and M.D. Cooper. 1988. Differential expression of two T cell receptors, TcR1 and TcR2, on chicken lymphocytes. *Eur. J. Immunol.* 18:539.
24. Chen, C.H., L.L. Ager, G.L. Gartland, and M.D. Cooper. 1986. Identification of a T3/T cell receptor complex in chickens. *J. Exp. Med.* 164:375.
25. Chan, M.M., C.H. Chen, L.L. Ager, and M.D. Cooper. 1988. Identification of the avian homologues of mammalian CD4 and CD8 antigens. *J. Immunol.* 140:2133.
26. Bucy, R.P., C.L. Chen, and M.D. Cooper. 1989. Tissue localization and CD8 accessory molecule expression of T $\gamma\delta$ cells in human. *J. Immunol.* 142:3045.
27. Trejdosiewicz, L.K., C.J. Smart, D.J. Oakes, P.D. Howdle, G. Malizia, and D. Campana. 1989. Expression of T-cell receptors TcR1 (γ/δ) and TcR2 (α/β) in the human intestinal mucosa. *Immunology.* 68:7.
28. Coltey, M., F.V. Jotereau, and N.M. Le Douarin. 1987. Evidence for a cyclic renewal of lymphocyte precursor cells in the embryonic chick thymus. *Cell Differ.* 22:71.
29. Bucy, R.P., C.H. Chen, and M.D. Cooper. 1990. Development of cytoplasmic CD3⁺/T cell receptor negative cells in the peripheral lymphoid tissues of chickens. *Eur. J. Immunol.* 20:1345.
30. Klärre, K., M. Hansson, and R. Kiessling. 1991. Multiple interactions at the natural killer workshop. *Immunol. Today.* 12:343.
31. Bucy, R.P., M. Coltey, C.H. Chen, D. Char, N.M. Le Douarin, and M.D. Cooper. 1989. Cytoplasmic CD3⁺ surface CD8⁺ lymphocytes develop as a thymus-dependent lineage in chick-quail chimeras. *Eur. J. Immunol.* 19:1449.
32. Ibrahim, B., L.A. Gartland, T. Kishimoto, R. Dzialo, H. Kubagawa, R.P. Bucy, and M.D. Cooper. 1991. Analysis of T cell development in xenopus. *Fed. Proc.* 5:7651.
33. Pardoll, D., B. Fowlkes, A. Lew, L. Maloy, M. Weston, J. Bluestone, R. Schwartz, J. Coligan, and A. Kruisbeck. 1988. Thymus-dependent and thymus-independent developmental pathways for peripheral T cell receptor γ/δ -bearing lymphocytes. *J. Immunol.* 140:4091.
34. Palacios, R., and J. Samaridis. 1991. Rearrangement patterns of T-cell receptor genes in the spleen of athymic (*nu/nu*) young mice. *Immunogenetics.* 33:90.
35. Ota, Y., T. Kobata, M. Seki, H. Yagita, S. Shimada, Y.Y. Huang, Y. Takagaki, and K. Okumura. 1992. Extrathymic origin of V γ 1/V δ 6 T cells in the skin. *Eur. J. Immunol.* 22:595.
36. Ikehara, S., J. Shimizu, R. Yasumizu, T. Nakamura, M. Inaba, S. Inoue, N. Oyaizu, K. Sugiura, M.M. Oo, Y. Hamashima, and R.A. Good. 1987. Thymic rudiments are responsible for induction of functional T cells in *nu/nu* mice. *Thymus.* 10:193.
37. Carding, S.R., S. Kyes, E.J. Jenkinson, K. Bottomly, J.J.T. Owen, and A.C. Hayday. 1990. Developmentally regulated fetal thymic and extrathymic T-cell receptor γ/δ gene expression. *Genes & Dev.* 4:1304.
38. Kyes, S., W. Pao, and A. Hayday. 1991. Influence of site expression on the fetal γ/δ T-cell receptor repertoire. *Proc. Natl. Acad. Sci. USA.* 88:7830.
39. Hein, W.R., L. Dudler, and B. Morris. 1990. Differential peripheral expansion and *in vivo* antigen reactivity of α/β and γ/δ T cells emigrating from the early lamb thymus. *Eur. J. Immunol.* 20:1805.