Early Separation of B and T Lymphocyte Precursors in Chick Embryo

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Summary

Embryonic chimeras were used to demonstrate an early separation of chicken T and B cell precursors. Genetically polymorphic cell surface antigens, Bu-1 and Ov, which are expressed on cells of the B and T lineage, respectively, are useful markers in adoptive cell transfer studies. Allelic products Bu-1a and Bu-1b can be detected with monoclonal antibodies (mAbs) L22 and 11G2, respectively, and the Ov antigen with mAb 11A9. Chimeric chickens were constructed by reconstituting irradiated 14-d Ov^- H.B19 embryos with the sorted Bu-1^+ or Bu-1^- fractions of spleen cells from age-matched H.B19 Ov^+ embryos. Chimeras were analyzed, 3–4 wk after hatching, for the presence of Ov^+ cells in the bursa, thymus, spleen, and peripheral blood lymphocytes. T cell precursors giving rise to thymocytes and peripheral T cells were present only in the Bu-1^-, but not in the Bu-1^+, fraction. We previously demonstrated that, in contrast, all B cell precursors in spleen from 14-d embryos are exclusively present in the Bu-1^+ fraction. We also analyzed the immunoglobulin light chain gene rearrangement in these populations by polymerase chain reaction. We show here that VJ recombination occurs in the Bu-1^+, but not in the Bu-1^-, fraction of spleen. These data demonstrate an early commitment to the B cell lineage, which occurs before the colonization of the bursa of Fabricius. Segregation of B cell precursors from the other hemopoietic precursors, and consequently separation of T and B cell precursors, occurs before the colonization of the primary lymphoid organs.

The avian embryo provides a unique model for the analysis of the immune system development because of the accessibility of the embryo and the anatomical separation of B and T lymphocytes in the bursa of Fabricius and thymus, respectively (1–3). Sex-chromosomal or chick-quail nuclear markers were used in chimera studies to show that blood-borne hemopoietic stem cells migrate into the bursal and thymic rudiment at precise stages of embryonic development (4–8). However, colonization of the thymus and bursa follows strictly different mechanisms. While the thymus receives several waves of stem cell influx separated by nonreceptive intervals (5, 6), the bursa is colonized by a single wave of precursors between day 8 and 14 in chick embryos (7). Moreover, the colonization of the bursa of Fabricius is restricted to the embryonic life as the precursors able to seed the bursal rudiment are no longer present in the peripheral blood or in the bone marrow (BM)[1] of chickens after hatching (9–12).

Several lines of evidence now suggest an early segregation of the B cell lineage during chicken ontogeny. The cell surface alloantigen Bu-1 expressed on all B cells and on a subset of macrophages (13, 14) is also present on B cell precursors able to home to the bursa in cell transfer experiments (15). These Bu-1^+ B cell precursors appear independently of the bursa as they exist in early bursectomized chick embryos (16). We previously demonstrated, using the Bu-1a and Bu-1b allotypic markers, that the B cell precursors in 14-d embryonic (ED14) spleen and BM are exclusively present in the Bu-1^+ population (15). However, we could not follow in these experiments the origin of the precursors for T lymphocytes.

In the present studies, we used the allotypic marker Ov, an alloantigen that is present on most hemopoietic cells during embryonic life and that disappears around hatching except on T lymphocytes, thus representing a marker for T lymphocytes in post-natal chickens. Combining the use of the Bu-1 and Ov markers and of congenic lines of chickens in cell transfer experiments, we show that the T cell precursors giving rise to thymocytes and peripheral T cells are present only in the Bu-1^- population of ED14 spleen or BM, thus demonstrating an early segregation of the B and T cell lineages that occurs before the colonization of primary lymphoid organs. Furthermore, we analyzed the Ig light chain gene

[1] *Abbreviation used in this paper:* BM, bone marrow.

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rearrangement status and showed that VJ recombination occurs in the Bu-1$^+$ fraction (including B cell precursors), but not in the Bu-1$^-$ fraction (including T cell precursors), of ED14 spleen and BM. These data indicate that B cell precursors are committed before their entry into the bursal rudiment and that they have already rearranged their Ig genes.

Materials and Methods

Chickens. Chickens used in these experiments were maintained at Roche chicken farm (Gipf-Oberfrick, Switzerland). The H.B19 strain has been divided into two sublines, Ov$^+$ and Ov$^-$, homozygous for the allotypic marker Ov, and was used in cell transfer experiments. Fertile eggs were incubated at 38°C, and the embryos were staged according to the number of incubation days.

Monoclonal Antibodies. The Ov antigen, a marker for T cells in post-natal chickens, was detected by mAb 11A9 (IgM) (17). The Bu-1a and Bu-1b alloantigens were detected by mAb L22 (IgG1) (13) and mAb 11G2 (IgG1) (18), respectively. The Bu-1 antigen was previously described as a 70-kD protein present on all chicken B lymphocytes and on a subset of macrophages (14). CL-1 (IgG1) recognizes the chicken leukocyte common antigen (CD45) and reacts with all hemopoietic cells, except mature erythrocytes (19).

Immunoprecipitation. Immunoprecipitation was done according to Kaufman et al. (20). Briefly, 10^8 thymocytes or PBL were iodinated by means of the lactoperoxidase/glucoxidase method and lysed in cold NP-40 lysis buffer as described (20). 11A9 mAb was coupled to sepharose-conjugated rabbit anti-mouse Ig (Dako, Denmark), and the radiolabeled antigen was incubated with these beads for 1 h at 4°C, rotating. After washes, the samples were eluted with Laemmli sample buffer, boiled, and analyzed on a 12.5% SDS-polyacrylamide gel under reducing conditions (20). Autoradiography was on Kodak XAR-5 film at −70°C using an intensifying screen.

Cell Suspensions and Immunofluorescence Analysis. ED14 spleen cells were prepared by passing the organs through a stainless steel mesh. 30–60 embryos were used for each experiment. Suspensions of ED14 BM cells were prepared from the femurs by flushing with cold EBSS containing heparin (1 IU/ml). Both cell populations were further purified by centrifugation on a Ficoll-Hypaque density gradient (Pharmacia Fine Chemicals, Uppsala, Sweden). Cell suspensions were also prepared from bursa, thymus, spleen, and yolk sac by teasing the minced organs through a stainless steel mesh. PBL were isolated by low speed centrifugation of heparinized blood.

Indirect immunofluorescence staining was performed by incubation of cells with supernatant of the relevant hybridoma cells followed by FITC-conjugated sheep anti-mouse Ig diluted 1:100 (Silenus Laboratories, Hawthorn, Australia). For two-color analysis, indirect immunofluorescence staining was carried out using an unlabeled primary mAb and a biotinylated mAb detected with PE-streptavidin (Amersham, Bucks, UK) as the secondary reagent. Relative immunofluorescence intensities were measured by flow cytometry using a FACScan® instrument (Becton Dickinson & Co., Mountain View, CA).

Immunoperoxidase Tissue Analysis. Fresh tissues were embedded in OCT medium (Tissue-Tek II; Miles Laboratories, Naperville, IL) and frozen in liquid nitrogen–cooled isopentane. Sections (4 μm) were air-dried, fixed in acetone, rehydrated in PBS, and stained with mAb 11A9. Immunoperoxidase staining was carried out by using a DAB enhancement kit (Amersham Int., Bucks, UK). Sections were counterstained with Mayer's hematoxylin and photographed using a dark blue filter.

Preparation of Chimeras. ED14 spleen and BM cells from Ov$^+$ H.B19 embryos were stained by L22 + 11G2 mAbs followed by FITC-conjugated sheep anti-mouse Ig. Stained cells were then sorted into Bu-1$^+$ and Bu-1$^-$ fractions using FACS® 440 equipment (Becton Dickinson & Co.), and they were injected in 0.15 ml of EBSS into a chorioallantoic vein of ED14 Ov$^-$ B19-irradiated embryos (2.5×10^5 to 2×10^6 cells/embryo, depending on the experiment). The eggs were irradiated 6 h before cell transfer by giving them 620 rad (140 rad/min) from a ^{137}Cs source (Gamma cell 40; Atomic Energy of Canada Ltd., Kanata, Canada).

Analysis of the Chimeras. We searched for donor-derived T lymphocytes, stained by 11A9 mAb, 1–4 wk after hatching of the chimeras. Cell suspensions prepared from PBL, thymus, bursa, and spleen were examined by immunofluorescence staining with mAb 11A9 using flow cytometric analysis. Tissue sections of thymus, bursa, spleen, and cecal tonsils of the chimeras were analyzed for Ov$^+$ cells by immunoperoxidase staining.

Polymerase Chain Reaction. The genomic DNA was prepared from total ED14, sorted Bu-1$^+$ and Bu-1$^-$ spleen cell populations (0.5×10^6 to 3×10^6 cells) by using standard protocols (21). PCR amplification (22) was carried out as described previously (23) using four nested primers in three successive rounds of amplification (20 cycles each). The following primers that define a 400-bp DNA segment spanning the entire variable (V) and joining (J) region of the rearranged light chain gene locus were used (24–26). Primer L1: 5′-CG GAA TTC AGC CTG CCG CCA AGT CC-3′; L2: 5′-CC GAA TTC TCA GGT TCC CTG CAG GCA-3′; L3: 5′-CC TCT AGA GGA AGA AAG ACC GAG ACG-3′; L4: 5′-CC TCT AGA CGA CAA AAT GTC ACA ATT TCA CG-3′.

DNA Sequencing. The PCR products were separated on a 1.5% low-melting agarose gel (Sigma Chemical Co., St. Louis, MO). The specific product having an expected molecular size was cut out and immersed in 1 × TBE buffer and melted at 65°C for 30 min. For subsequent sequence analysis, the specific amplification product was purified by repeated phenol chloroform extractions and ethanol precipitation, and was subcloned into M13mp19 vector. The dideoxynucleotide sequencing reactions were performed using a Sequenase Kit Version 2.0 (United States Biochemical Corporation, Cleveland, OH) according to the supplier's instructions.

Results

Biochemical Characterization of the Ov Antigen. 11A9 mAb precipitates from lysates of iodinated thymocytes and PBL a molecule with an approximate molecular mass of 38–40 kD under reducing conditions (Fig. 1). Under nonreducing conditions, this molecule seems to form dimers or even trimers (data not shown).

During Embryonic Life, the Ov Antigen Is Expressed on Most Hemopoietic Cells. We examined the appearance of cells stained by 11A9 mAb during embryonic development of the Ov$^+$ subline of H.B19 chickens. Immunoperoxidase staining of tissue sections revealed that at day 3 of the embryonic life, Ov$^+$ cells were present in the blood islands of the yolk sac as well as in the intra-embryonic area, particularly in the blood vessels.

Spleen, thymus, and bursa from ED10 embryos were stained, and the distribution of Ov$^+$ cells was compared with that of CD45$^+$ cells stained by CL-1 mAb (Fig. 2). The distribution of Ov$^+$ cells and CD45$^+$ cells was very similar. Positive cells were numerous in the spleen with both antibodies

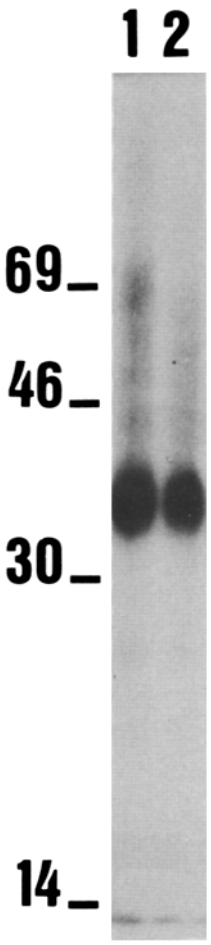

Figure 1. Gel electrophoretic analysis of the Ov antigen. Cell lysates of ^{125}I-labeled thymocytes (lane *1*) and blood mononuclear cells (lane *2*) were immunoprecipitated with 11A9 antibody and separated under reducing conditions by 12.5% SDS-PAGE. The M_r is indicated on the left.

(Fig. 2, *C* and *D*). In thymic rudiment, thymocytes appeared strongly labeled, and some hemopoietic cells located outside the thymus were also positive for the Ov antigen (Fig. 2, *E* and *F*). In the bursa, Ov$^+$ cells, as well as CD45$^+$ cells, appeared located in the mesenchyme (Fig. 2, *A* and *B*). No positive cells were found in the bursal epithelium, thus confirming our previous observations about the colonization of the bursal rudiment by hemopoietic precursors (8). The Ov marker appeared restricted to the hemopoietic lineage, as cells of other origin, endothelial, or mesenchymal cells, did not react with 11A9 mAb.

We also examined the distribution of Ov$^+$ cells during ontogeny by immunofluorescence screening of cells in suspension prepared by pooling the organs from 10 embryos (Fig. 3). In ED7 yolk sac, 35% of cells were Ov$^+$ and they appeared brightly stained. Such suspensions are very heterogenous as they contain both hemopoietic cells and cells from the three germ layers. In spleen, bursa, and bone marrow from ED14 embryos, nearly all hemopoietic cells carried the Ov antigen (77%, 82%, and 84%, respectively). Double labeling with L22 and 11A9 mAbs indicated that the Bu-1$^+$ cells found in ED14 spleen and bursa were Ov$^+$ (Fig. 4). Among the leukocytes, different intensities of staining, from brightly to weakly stained cells, were observed. Granulocytes were clearly positive while mature erythrocytes appeared negative. It thus appears that the Ov antigen is present on most hemopoietic cells during the embryonic life.

In Post-natal Chickens, the Ov Antigen Is a Marker for the T Cell Compartment. The Ov alloantigen disappears around hatching, except on T cells where it remains throughout adult life. We could not detect Ov$^+$ cells in bursa of Fabricius from 5-, 9-, or 20-d newborn chicks. In adult chickens (6–10 wk old), 11A9 mAb reacted with 50–80% of PBL, 50% of splenic lymphocytes, whereas <3% of bursa cells were positive. The majority of thymocytes (85–95%) were positive although they were weakly stained compared with PBL. In a previous study (17), we showed that all cells positive with mAb 11A9 were also stained by a rabbit anti-T cell serum and vice versa, indicating T cell specificity of the Ov antigen.

The H.B19 line of chickens has been divided into Ov$^+$ and Ov$^-$ sublines (27). Transfer of Ov$^+$ ED14 spleen or BM cells into Ov$^-$ age-matched irradiated recipients (2–7 × 10^6 cells/embryo) resulted in the presence of Ov$^+$ cells, stained by 11A9 mAb, in the thymus and in the peripheral T cell compartment of the recipient after hatching (Table 1, Exps. 1 and 2). Depending on the chimera tested, up to 33% of 11A9$^+$ cells could be detected in the thymus, 25% in spleen, and 19% among PBL. In contrast, only 1 or 2% of 11A9$^+$ cells were found in the bursa. They correspond to the few mature T cells that we previously described in bursal follicles, located exclusively in the cortex (28). Irradiated H.B19 Ov$^-$ control chickens of the same age were also tested with 11A9 mAb. Only a low level of background staining could be observed with spleen and bursa cells (Table 1, Exp. 3). These experiments show that by transferring ED14 spleen or BM cells, most probably representing all kinds of hemopoietic precursors, only the progeny of T cell precursors can be followed, due to the disappearance of the Ov antigen after hatching, except on T cells.

T Cell Precursors Are Exclusively Present in the Bu-1$^-$ Population of ED14 Spleen Cells. In a fourth and fifth series of experiments, density gradient isolated ED14 Ov$^+$ H.B19 spleen cells were stained with mAbs L22 (anti-Bu-1a) and 11G2 (anti-Bu-1b) and sorted into Bu-1$^+$ and Bu-1$^-$ fractions with a cell sorter (Fig. 5). ED14 Ov$^-$–irradiated H.B19 embryos were reconstituted either with these Bu-1$^+$ or Bu-1$^-$ populations. 7 d after reconstitution, i.e., the day of hatching of the recipient, a small Ov$^+$ population (~2% of positive cells) was observed in the outer part of the cortex of the Ov$^-$ host thymus (Fig. 6 *A*). Chimeras were analyzed 3–4 wk after hatching for the presence of Ov$^+$ cells in the thymus, bursa, spleen, and PBL by flow cytometry. As shown in Table 1, the Bu-1$^-$ population (Exp. 5) repopulated the thymus as well as the peripheral T cell compartment in the spleen and PBL with up to 15%, 23%, and 19% of 11A9$^+$ cells, respectively. In contrast, the Bu-1$^+$ population accounted for no T cells in the thymus or periphery, the low percentage of 11A9$^+$ cells counted in bursa and spleen corresponding to the background observed in irradiated non-reconstituted H.B19 Ov$^-$ controls (Exp. 3). We could not determine the origin of the B cell compartment (bursa cells and peripheral B cell) in this series of experiments, as the

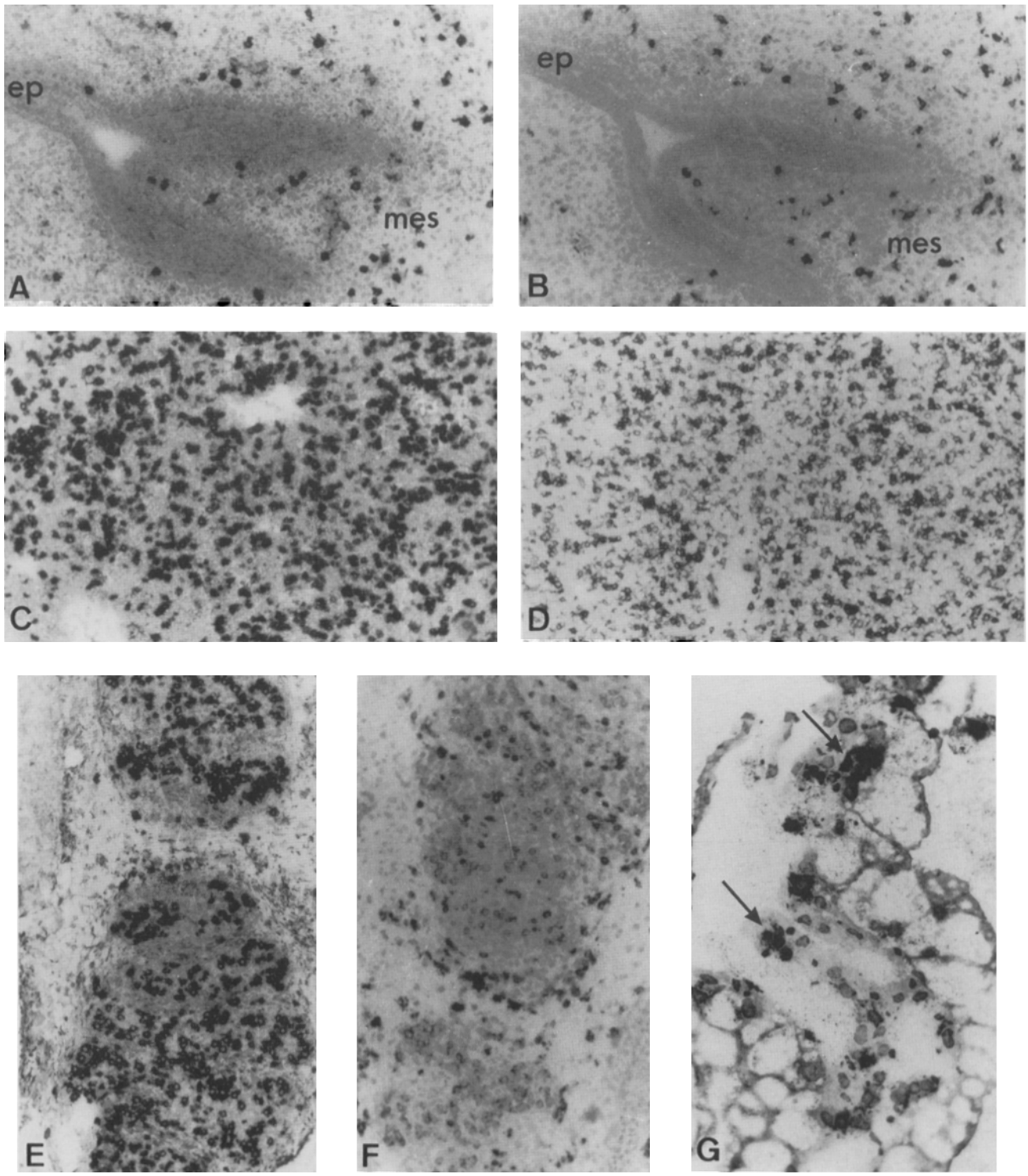

Figure 2. Comparative distribution of the Ov antigen detected by 11A9 mAb (*A*, *C*, *E*, and *G*) and of the CD45 detected by CL-1 mAb (*B*, *D*, and *F*). Cells stained by the two different mAbs have about the same tissue distribution. ED10 bursa (*A* and *B*): Ov^+ and $CD45^+$ cells are located in the bursal mesenchyme (mes) but not in the epithelium (ep). ED10 spleen (*C* and *D*): numerous cells are Ov^+ and $CD45^+$. ED10 thymus (*E* and *F*): numerous cells of the thymic lobes are strongly labeled by 11A9 mAb (*E*) and more weakly by CL-1 mAb. ED7 yolk sac (*G*), where Ov^+ cells are detected (*arrows*). Immunoperoxidase staining and Mayers's hematoxylin counterstain (×400).

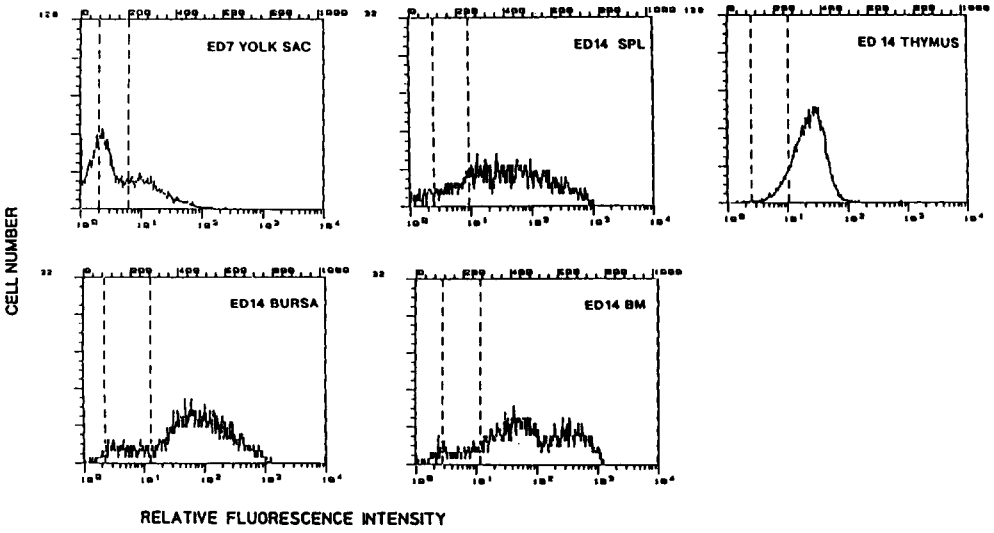

Figure 3. Expression of Ov antigen during ontogeny. Positive cells were detected in ED7 yolk sac and in spleen (SPL), thymus, bursa, and bone marrow (BM) of 14-d embryos.

Table 1. *The Bu-1⁻, but not the Bu-1⁺ Population, of ED14 Spleen Repopulates the T Cell Compartment*

Exp.	Donor cells injected: Source	Donor cells injected: No.	Age of host at analysis	Percent 11A9⁺ cells: Bursa	Thymus	Spleen	PBL
			d				
1	Unsorted spleen	5×10^6	27	1.6	0.6	4.9	7.8
		"		3.2	7.4	13.6	9.5
		7×10^6		1.0	14.9	18.8	19.1
		"		1.7	11.0	17.0	11.2
2	Unsorted BM	2.8×10^6	27	2.8	29.3	25.6	15.6
		2.2×10^6	20	1.4	5.2	8.7	4.5
		"		1.7	20.7	21.5	16.2
		"		1.8	5.7	6.8	5.0
		"		1.8	33.6	15.4	11.3
3	Irradiated B19⁻		27	1.6	0	1.7	0
	controls (not			0.7	0	1.0	0
	reconstituted)		20	1.8	0	0.6	0.5
				0.7	0	2.3	0.1
4	Spleen sorted	5.5×10^5	27	2.0	0	1.9	0
	Bu-1⁺ population	"		3.3	0.1	4.8	0
		2.5×10^5	20	2.5	0	2.2	0
		"		1.3	0	0.1	0
5	Spleen sorted	2.1×10^6	27	0.9	4.6	11.0	4.5
	Bu-1⁻ population	"		2.0	1.7	13.1	9.6
		"		0.7	0.2	5.7	4.6
		1.6×10^6	20	2.5	15.2	23.4	19.2

Irradiated ED14 Ov⁻ H.B19 embryos were reconstituted with spleen or BM cells from ED14 Ov⁺ H.B19 donors. The percent of Ov⁺ cells stained by 11A9 mAb in the host is shown for individual animals.

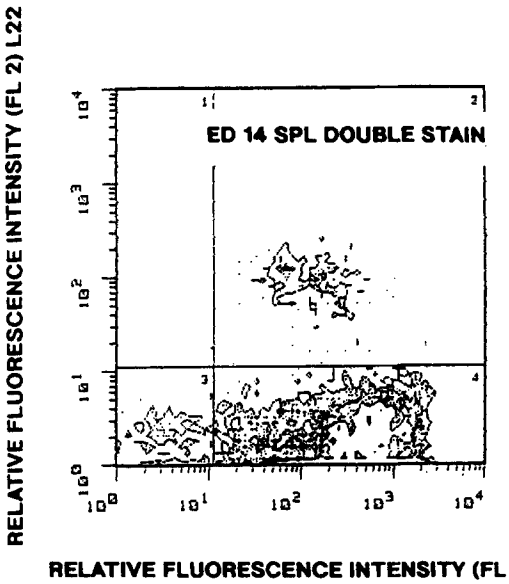

Figure 4. Two-color immunofluorescence analysis of Ov and Bu-1 expression on ED14 spleen cells. Cells were stained with the 11A9 antibody and FITC-conjugated goat anti-mouse IgG, and then with biotinylated L22 mAb followed by PE-streptavidin. All L22$^+$ cells also carried the Ov antigen.

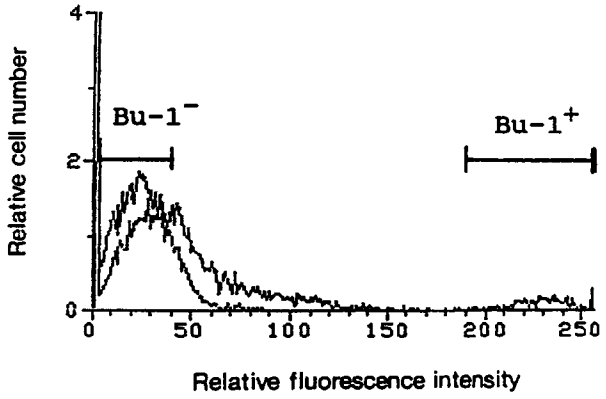

Figure 5. Selection of Bu-1$^+$ and Bu-1$^-$ fractions of spleen cells from ED14 Ov$^+$ embryos after staining with L22 and 11G2 mAbs. Background staining was done with FITC-conjugated sheep anti-mouse Ig alone. The negative and positive fractions were selected such that no contamination by the other fraction could occur.

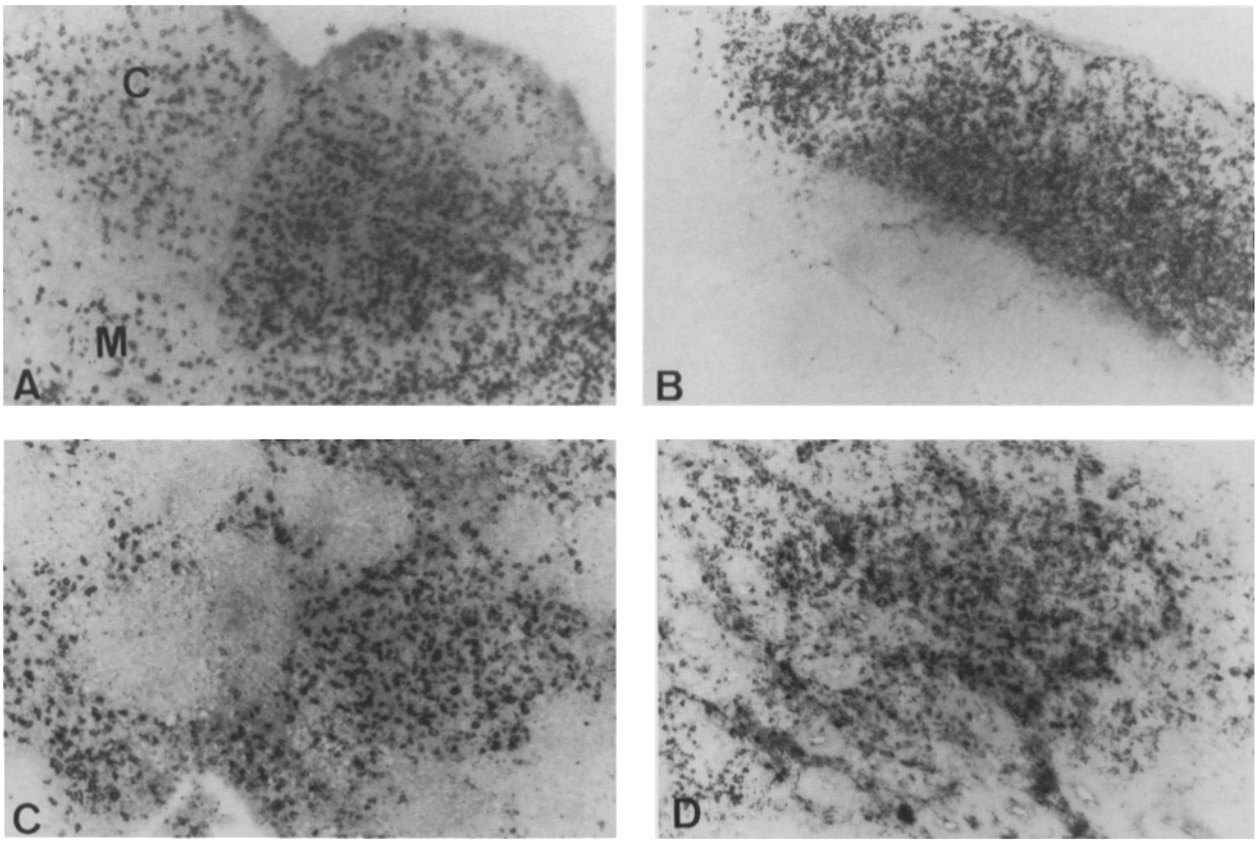

Figure 6. Immunoperoxidase staining with 11A9 mAb after transfer of the Bu-1$^-$ population of ED14 spleen from Ov$^+$ embryos into Ov$^-$ embryonic hosts (*A*): thymus from a chimera analyzed 7 d after transfer. Ov$^+$ cells are located in the outer cortex of the thymic lobe. (*B*, *C*, and *D*): thymus (*B*), spleen (*C*), and cecal tonsils (*D*) from a chimera analyzed 27 d after transfer. Ov$^+$ cells are located in the cortex (*C*) as well as in the medulla (M) of the thymus (*B*). Ov$^+$ cells were also numerous in T-dependent areas in spleen (*C*) and in cecal tonsils (*D*) (*A–D*, ×470).

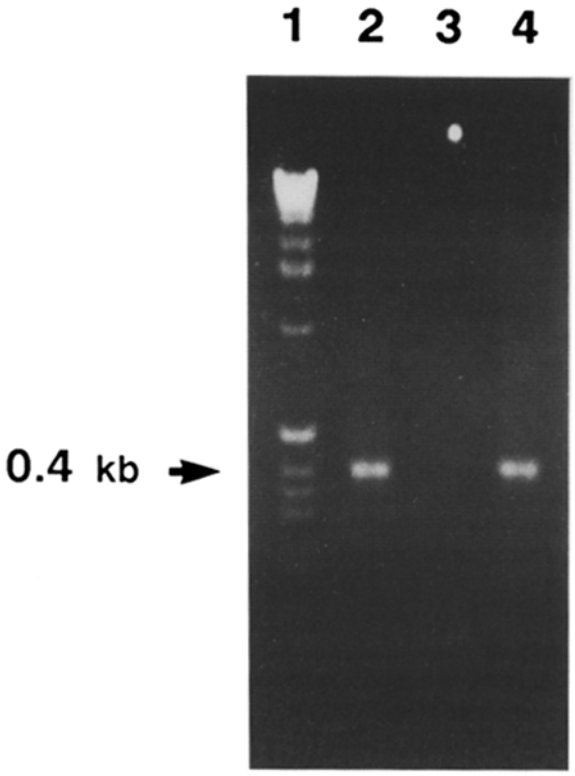

Figure 7. The DNA samples isolated from unsorted (lane *2*), sorted Bu-1$^-$ (lane *3*), and sorted Bu-1$^+$ (lane *4*) ED14 spleen cells were used to amplify a gene segment spanning the entire variable and joining region of the rearranged light chain gene locus by PCR. 10% of the reaction mixture was run on a 1% agarose gel. The 0.4-kb amplification product identified light chain rearrangement in unsorted and sorted Bu-1$^+$ ED14 spleen cells (lanes *2* and *4*) but not in the sorted Bu-1$^-$ ED14 spleen cells.

Bu-1-encoding genes are segregating in H.B19 embryos. In previous experiments, using two sublines of the H.B21 strain homozygous for the allotypic marker Bu-1, we determined that B cell precursors able to home to the bursa are exclusively present in the Bu-1$^+$ fraction of ED14 spleen and BM (15), as well as that bone marrow cells enriched for T cell precursors do not cause B cell chimerism in transplanted embryonic hosts (27).

Immunoperoxidase staining of frozen tissue sections of thymus, bursa, spleen, and cecal tonsil from the Ov chimeras with 11A9 mAb confirmed these findings (Fig. 6). Donor-derived cells were found in the thymus cortex and medulla and in spleen, in the periarteriolar lymphoid sheath. Ov$^+$ cells were also found in the lamina propria of the intestine and in cecal tonsil. In contrast, no positive cells were found in the bursa.

Immunoglobulin Light Chain Rearrangement Is Observed Only in the Bu-1$^+$ Population of ED14 Spleen Cells. To study the commitment to the B cell lineage of the ED14 spleen cells, we analyzed the Ig light chain gene rearrangement status using PCR. Oligonucleotide primers specific for the sequence upstream of the Vλ_1 gene and downstream of the Vλ_1-J region were used. In the rearranged light chain locus, use of these primers results in amplification of a 0.4-kb DNA fragment spanning the entire V and J regions of the rearranged light chain gene locus. The expected 0.4-kb amplification product indicating light chain rearrangement was obtained from DNA prepared from unsorted and sorted Bu-1$^+$ ED14 spleen cells (Fig. 7, lanes *2* and *4*, respectively). However, amplification of the DNA from sorted Bu-1$^-$ population of ED14 spleen cells did not result in product of the proper size (Fig. 7, lane *3*), indicating that cells in this fraction had not recombined their Ig light chain genes. To analyze the diversity of the rearranged Vλ genes from the Bu-1$^+$ population, we cloned the PCR products into M13mp19 vector and sequenced 10 random recombinants. All the sequences (five shown in Fig. 8) were encoded by germline sequences, except a single base pair change in the CDR1 of the sequence number one.

Discussion

We demonstrate in this study the early separation of T and B cell precursors in chick embryo by combining the use of two allotypic markers and selected lines of chickens to trace T cell precusors and their progeny. The Bu-1 antigen is a

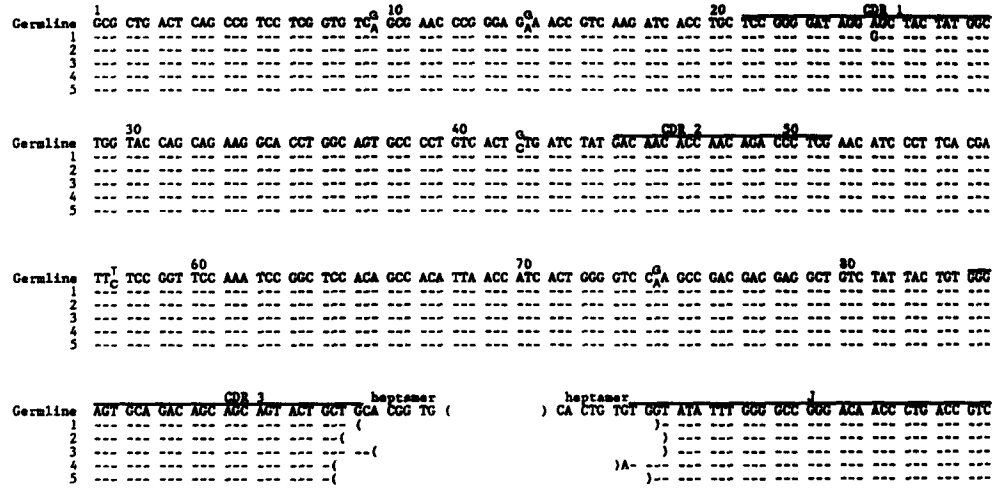

Figure 8. Ig light chain sequence from ED14 Bu-1$^+$ spleen cells, after PCR amplification. The nucleotide sequences of the VJ region are compared with the germline sequence. Identity to the top germline sequence is indicated by a dash. Positions of the three complementarity determining regions (CDRs) and of the J segment are indicated. Positions of the putative heptamer and nonamer recombination signals are marked with bars. (*1–5*) Five representative sequences from 10 sequenced PCR products. All are rearranged but have not undergone somatic diversification by gene consersion.

marker for all B cells and their precursors (14–16), while the Ov antigen is a marker exclusively present on T cells in post-natal chickens. We previously demonstrated that all B cell precursors in 14-d-old embryonic spleen, capable of repopulating the B cell compartment of an irradiated host, are exclusively present in the Bu-1$^+$ fraction (15). Evidence from studies presented here demonstrate that all T cell precursors are, on the contrary, contained in the Bu-1$^-$ fraction of ED14 spleen. Transfer experiments indicated that cells included in the Bu-1$^-$, but not in the Bu-1$^+$, fraction could colonize the thymus and repopulate the peripheral T cell compartment of 14-d irradiated embryonic hosts.

Furthermore, we show here that only cells present in the Bu-1$^+$, but not in the Bu-1$^-$, population of ED14 spleen have rearranged Ig_L genes. The Bu-1$^+$ cells show functional rearrangement of Ig_L genes but no somatic diversification. In fact, the chicken has a unique way of generating Ig light and heavy chain diversity. The L chain locus has only one functional variable gene ($V\lambda_1$) that is expressed. During B cell development, the $V\lambda_1$ gene is diversified by gene conversion, using a pool of pseudogenes as donors of DNA segments (25, 26, 29). A similar strategy is used for diversification of the heavy chain repertoire (30). These data indicate an early determination of B cell precursors that acquire the Bu-1 B cell marker and rearrange Ig_L genes before bursa colonization. This is an accordance with data from other groups suggesting that the bursa is not required for Ig_L gene rearrangement (23, 31). Joining of the single functional V_L segment with the single J_L segment has been shown to occur in several extrabursal tissues between days 10 and 15 of development (31). Moreover, Ig_L chain DNA segments in the rearranged configuration were amplified from cells of the intraembryonic mesenchyme as early as day 7 of the embryonic life (23). Previous studies have also shown that removal of the bursa anlage at 60 h of development does not prevent the expression of normal levels of serum Ig or the appearance of sIg$^+$ B cells in peripheral lymphoid organs, though the serum Ig of such bursectomized chickens display extremely limited Ig_L diversity (32–34).

Our data indicate that the Ig_L chain rearrangement is not a random process but that it occurs only in the Bu-1$^+$ fraction of ED14 spleen where all B cell precursors, capable of migrating into the bursa, are included. It is interesting to note that we previously described the distribution of Bu-1$^+$ cells during early development of chick embryos. In 10–12-d embryos, Bu-1$^+$ cells were found mainly in bursa, spleen bone marrow, and also in liver (14), in the same tissues in fact were McCormack et al. (31) detected Ig_L gene rearrangement.

In these transfer experiments, we used ED14 spleen cells as a source of hemopoietic precursors. We are aware that ED14 spleen cells do not represent all hemopoietic precursor populations responsible for the colonization of the thymus and of the bursa. However, several arguments can be put forward in favor of this choice. (*a*) We previously described a peak of large Bu-1$^+$ cells occurring around day 14 in spleen (14). (*b*) Due to the very small number of hemopoietic precursors, it will be difficult to test the reconstitutive capacity and the differentiation status of sorted subpopulations of hemopoietic precursors before day 14 of the embryonic life. (*c*) The colonization of the primary lymphoid organs is still going on around ED14. A second wave of hemopoietic precursors enter the thymus (6) and the colonization of the bursa is probably lasting until ED18 or ED19, when Bu-1$^+$ cells were shown to disappear (14).

These arguments, taken together with the recent data of Mansikka et al. (23), lead us to propose that the determination to the B cell lineage, involving at least Ig_L chain gene rearrangement and acquisition of the Bu-1 marker, occurs early during embryonic life (probably around day 7) independently of the bursa of Fabricius. These committed precursors colonize the bursa during a limited period of the embryonic life (7, 9–12).

These data also involve that segregation of B cell precursors from the other hemopoietic precursors and, consequently, separation of T and B cell precursors occurs before the colonization of the primary lymphoid organs. Precursors for B cells are Bu-1$^+$ and have rearranged Ig_L genes, while precursors for T cells are Bu-1$^-$ and have nonrearranged Ig_L genes. However, it is important to emphasize that we do not infer from these data that precursors for T cells are determined pre-thymically. Our results only indicate an early segregation of B cell precursors, due to their early commitment. The Bu-1$^-$ population of ED14 spleen includes a wide range of hemopoietic precursors, and we do not bring evidence that T cell precursors are separated from the others. Acquisition of T cell differentiation markers and of TCR for antigen occurs a few days after thymus colonization, which will rather suggest that determination to the T cell lineage, at least for the majority of T cells, occurs in the thymus (see reference 35 for a review).

We show in this study that the Ov antigen is carried by nearly all hemopoietic cells from the very beginning of embryonic life. This antigen disappears around hatching, except on T cells, where it remains throughout adult life. mAb 11A9 reacts with a 38–40-kD molecule present on T cells. This antigen was previously described as a T cell–restricted antigen (17). This is true if we consider the post-natal period but not the embryonic life, and this underlines the necessity to screen the embryonic stages to determine the exact distribution of an antigenic determinant. An interesting feature of the Ov marker is that it is a genetically polymorphic cell surface antigen. It represents a useful marker in cell transfer studies, as sublines of the H.B19 strain of chickens expressing it or deficient in its expression are now available.

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