CELLS EXPRESSING Ia ANTIGENS IN THE AVIAN THYMUS

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The thymus has a crucial role in the differentiation of T lymphocytes and the acquisition of the T cell repertoire (1–3). It is during an intrathymic differentiation step that the T cells become able to recognize foreign antigens in the context of products of genes linked to the major histocompatibility complex (MHC)[1] (4–7). Experiments based on mouse radiation chimeras have suggested that the radioresistant portion of the thymus determines T cell H-2 restriction (8, 9). Studies carried out in mouse and man have shown that thymic epithelial cells bear both class I and class II antigens on their surface (10–13). Thymic accessory cells of hemopoietic origin might also play a role in mediating MHC restriction of thymocytes. These cells include macrophages and elements analogous to the dendritic cells (DC) first described in the spleen (14, 15) and later identified also in the thymus of rodents (16) and birds (17). According to investigations carried out in mouse (18, 19), the distribution of bone marrow–derived, MHC-expressing cells is largely confined to the medulla of the thymus.

The present work addresses the problem of Ia expression in the avian thymus, where the construction of quail-chick chimeric organs allows a precise analysis of the distribution and origin of the various thymic cellular components. For this purpose the quail thymic pharyngeal primordium was grafted into the chick embryo. In this situation the thymus that develops in the graft is chimeric, with the endodermal reticuloepithelial cells and a few connective elements belonging to the quail donor while the hemopoietic cells, including lymphocytes and accessory cells, derive from the chick host. The cellular distribution of Ia antigens in these chimeras was investigated with monoclonal antibodies that we have raised which are selectively directed against quail or chick determinants. Distinct thymic compartments have been identified: the cortex, in which only epithelial cells are found to express Ia antigens; a transitional region between the cortex and medulla, where both epithelial and hemopoietic accessory cells are Ia-positive; and the medulla per se where the vast majority of the Ia-positive cells are of extrinsic hemopoietic origin. Ontogenetic studies of Ia immunoreactivity

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DC, dendritic cell; E, embryonic day; HPC, hemopoietic precursor cell; mAb, monoclonal antibody; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; RaMIg, rabbit Ig anti-mouse Ig.

have shown that in both quail and chick, thymus accessory cells of hemopoietic origin express Ia antigens in detectable amounts before reticuloepithelial cells.

Materials and Methods

Animals. Embryonic and hatched quails (*Coturnix coturnix japonica*) and chicks (*Gallus gallus*) from a commercial source were used throughout these experiments. The eggs were incubated at 38°C in a humidified atmosphere. Developmental stages were scheduled according to the duration of incubation.

Preparation of Quail-Chick Chimeric Thymuses. The technique, already described elsewhere (20, 21), consisted of grafting the third and fourth branchial pouches from quail embryos at embryonic day 3½ (E3.5) into the somatopleure of E3.5 chicks. The thymic rudiment develops from the quail pharyngeal pouch endodermal epithelium and mesenchyme and becomes seeded 2 d later (between E5 and E6) by chick hemopoietic precursor cells (HPC) that later yield the whole lymphocyte population developing in the ectopic thymus. The host embryo is sacrificed at E20 and the graft processed for chimerism analysis.

Preparation of Avian Thymic Adherent Cells. Thymic cell suspensions were prepared from 1-mo-old chicks and quails by mechanical disruption and were subsequently subjected to centrifugation on a discontinuous 10%/23% bovine serum albumin (BSA) gradient at 22,000 *g* for 30 min. The low density population was then cultured at 37°C in 7.5% CO_2 atmosphere for 1 h in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) without serum. The cultures were then rinsed by gentle pipetting to discard most lymphocytes; adherent cells, i.e., macrophages and DC, with a few associated thymocytes, remained attached to the dish. Adherent cells were then harvested by scraping the culture dishes as described (17).

Production of Monoclonal Antibodies. 10^6 to 3×10^6 thymic adherent cells, suspended in 200 μl of phosphate-buffered saline (PBS), were injected intravenously in BALB/c mice three times, at 7-d intervals. Production, selection, and maintenance of hybridomas were performed as described (22). Hybridoma supernatants were screened by indirect immunofluorescence (see below) on adherent cells and lymphocytes, isolated by the same procedure as above from either thymus or bursa of Fabricius. The cells were seeded into microtiter plates (Nunc, Intermed, Denmark) (5×10^4 cells per well) and fixed for 15 min at room temperature in 4% paraformaldehyde in phosphate buffer. Antibodies labeling adherent cells and bursal lymphocytes but not thymocytes were selected for their putative class II MHC antigen specificity. The corresponding hybridomas were cloned twice and injected into Pristane-primed mice for ascites production.

Immunoprecipitation and Electrophoresis. All the following steps were carried out at room temperature. 50×10^6 splenic cells from animals 2 wk to 1 mo old, depleted of erythrocytes by centrifugation on a discontinuous 29.5%/14.75% BSA gradient at 1,200 *g* for 15 min, were radioiodinated with 1 mCi of ^{125}I (100 mCi/ml; Amersham Corp., Arlington Heights, IL) in 500 μl PBS containing 400 μg lactoperoxidase (Sigma Chemical Co., St. Louis, MO) and 50 μl H_2O_2 (Perhydrol; Merck & Co., Inc., Rahway, NJ) at 0.03%. After 10 min incubation, the cells were washed twice in cold PBS and lysed in 250 μl PBS containing 0.5% Nonidet P-40, 10^{-3} M phenylmethylsulfonyl fluoride. The lysate was cleared of nuclei by low speed centrifugation and passed over a Sephadex G-25 column. High molecular weight radiolabeled material was collected in a volume of ~0.5 ml.

Immunoprecipitations were performed in an indirect way by using protein A–Ultrogel (IBF; LKB Produkter, Bromma, Sweden) coupled to rabbit Ig anti-mouse Ig (RaMIg) (Nordic Immunological Laboratories, Tilburg, The Netherlands) (30 μl of protein A–Ultrogel for 0.3 mg of RaMIg for 2 h). First, a 30 μl fraction of these complexes was incubated for 2 h with 30 μl of ascitic fluid obtained with unhybridized myeloma cells, and then used to clarify the labeled extract for 2 h. Other 30-μl fractions of protein A–Ultrogel-RaMIg were incubated for 2 h with ascitic fluids from specific hybridomas and then mixed for 4 h with 100-μl aliquots of labeled cell extracts.

Samples were diluted in electrophoresis buffer to a final concentration of 2% sodium dodecyl sulfate (SDS) and 5% 2-mercaptoethanol, and either boiled for 3 min (dissociating conditions) or incubated for 15 min at room temperature (nondissociating conditions). One-dimensional electrophoresis was performed according to Laemmli (23) in 10% polyacrylamide gels containing 10% sucrose and 0.1% SDS.

Immunohistochemistry. Quail, chicken, and chimeric thymuses were fixed for 4 h in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), rinsed in the same buffer, dehydrated in alcohol, and embedded in polyethylene glycol 1000 (24). Free-floating 5-μm sections were labeled by indirect immunofluorescence through successive incubations, for 30 min at room temperature, first in undiluted hybridoma supernatants, then in goat Ig anti-mouse Ig coupled with fluorescein isothiocyanate (Nordic). Control assays were performed by replacing the first antibody with either PBS or diluted ascitic fluid obtained from myeloma-injected mice. Double labeling experiments were carried out by further incubating the sections with a rabbit antiserum raised to human skin cytokeratin (Clinisciences, Paris). This reagent was itself indirectly labeled, in a fourth step, with rhodamine-coupled goat Ig anti-rabbit Ig (Nordic). Anti-MB1 monoclonal antibody (mAb) prepared in our laboratory was also used in these experiments (25).

Electron Microscopy. The chick host embryos were perfused at E18 with 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. Slices from the fixed grafts were incubated first with an anti-Ia antibody for 30 min and then rinsed and treated with a goat anti-mouse antibody conjugated with peroxidase (Nordic) for 30 min.

Graft slices incubated only with the second antibody served as controls. The specimens were then fixed in buffered 2% glutaraldehyde for an additional 15 min and the peroxidase reaction was carried out in 10 ml of a 0.1 M Tris (pH 7.6) solution containing 10 mg of diaminobenzidine and 0.01% H_2O_2 for 10–15 min. The samples were then osmicated, en bloc stained, dehydrated, and embedded in epoxy resin.

Results

Production of Monoclonal Antibodies to Avian Class II MHC Antigens. mAb were raised in mice against thymic DC and macrophages, from 1-mo-old quails or chickens. In each case, after the usual procedure for hybridoma production, 400 supernatants were screened by indirect immunofluorescence on thymic and bursal lymphocytes and thymic adherent cells. 19 and 33 supernatants from hybridomas respectively derived from quail cell– and chicken cell–immunized mice were found to label bursal lymphocytes and thymic adherent cells, in the species used for immunogen preparation. Assaying each antibody towards both quail and chicken target cells revealed diversely species-restricted reagents. Three of these were used throughout this work: TaC1 and TaC2, derived from quail cell immunization, and TaP1, obtained in response to chicken cells. TaP1 and TaC1 labeled, respectively, chicken and quail cells, while TaC2 reacted with cells of both species.

The anti-Ia specificity of these mAb, strongly suggested by their immunofluorescent staining properties, was further confirmed by immunoprecipitation of iodinated spleen cell membrane extracts (Fig. 1). All the reagents recognized polypeptide chains which, in dissociating conditions, exhibited apparent molecular weights close to 30,000. In addition, a co-migrating band at 35,000 mol wt, although weaker, was immunoprecipitated from chick cell extracts. Under nondissociating conditions, immunoprecipitated products migrated as a major band in the range of 55,000 mol wt. These results indicate that the antigens recognized are likely expressed on the cell surface as dimers of noncovalently linked 30,000

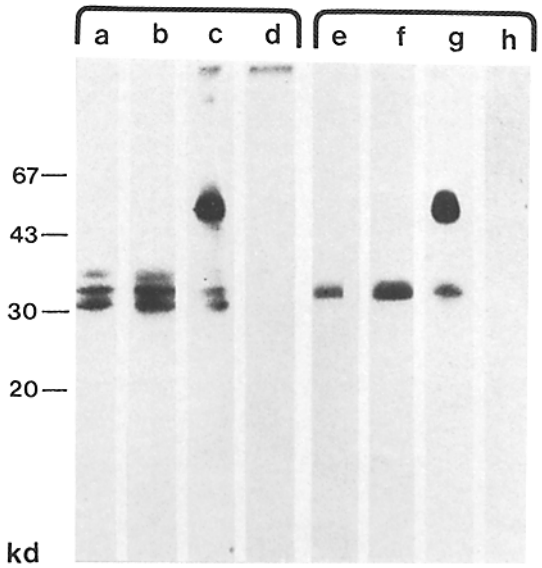

FIGURE 1. Immunoprecipitation of radioiodinated splenic leukocyte membrane extracts from chicken and quail with anti-Ia mAb. (*a, b, c, d*) In the chicken, dissociated surface antigens, precipitated with either TaP1 (chicken specific) (*a*) or TaC2 (reacting in quail and chicken) (*b*) antibodies migrated as two major bands at about 31 and 33 K mol wt and a weaker one at 35 K mol wt. In nondissociating conditions, the extracted material appeared as a major band at 55 K mol wt (*c*). (*e, f, g, h*) In the quail species, dissociated Ia products extracted by TaC1 (quail specific) (*e*) or TaC2 (*f*) migrated apparently as a single band at 34 K mol wt, although in nondissociating conditions they also exhibited an apparent molecular weight of 55 K (*g*). TaC1, restricted to the quail and TaP1, reacting only in the chick, did not precipitate any material from chicken (*d*) and quail cells (*h*).

mol wt units in both chicken and quail species, although dissociated products from quail cells seem to migrate as a single band. Thus, TaC1, TaC2, and TaP1 bind cell surface antigens that possess known structural properties of mammalian, including human, class II MHC products (26). It is also worth mentioning that the species restriction of TaC1 and TaP1 mAb could be confirmed by showing that they do not precipitate material from chicken and quail cells, respectively. Therefore, TaC1 and TaP1 will be subsequently referred to respectively as anti-quail and anti-chick Ia antibodies, while TaC2 will be considered as an anti-quail/chick Ia reagent.

Distribution of Class II Antigens in Normal and Chimeric Avian Thymuses. Indirect immunofluorescent staining of tissue sections revealed an identical pattern of distribution of Ia antigens in the thymus of the newly hatched normal chick with anti-chick Ia (TaP1) and anti–quail-chick Ia (TaC2) antibodies. The same observation was made in the quail thymus when anti-quail Ia (TaC1) and anti–quail-chick Ia antibodies were used. In the cortex, small dispersed cell bodies exhibited a strong granular cytoplasmic labeling. These cells have long processes that were more faintly labeled and which build up a loose Ia-positive network. In the medulla, large areas of densely packed cells showed a strong and confluent staining (Fig. 2).

To further characterize the cell lineage and origin of the thymic Ia-positive

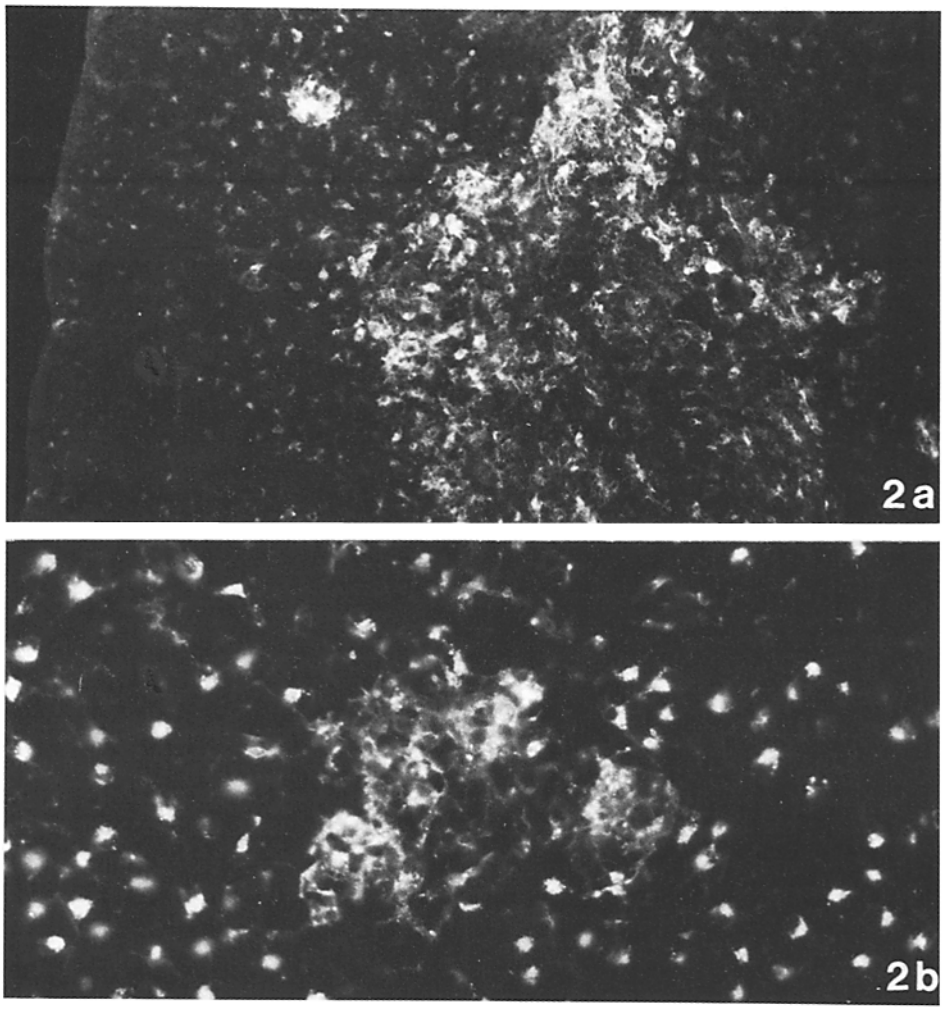

FIGURE 2. Distribution of Ia-positive cells in the avian thymus. (*a*) Normal thymus section from a 10-d quail labeled with anti-quail-chick Ia (TaC2). In the cortex, scattered cells with long processes are labeled whereas in the medulla the antibody stains very brightly compact cell aggregates. × 170. (*b*) A higher magnification of TaC2 (anti-quail-chick Ia) staining on the normal late embryonic quail thymus reveals the network of processes joining the Ia-positive cortical cells and shows the densely packed cells of the medulla. × 530.

cells, we took advantage of the species specificity of our various anti-class II mAb to examine the distribution of donor and host Ia-positive cells in quail-chick chimeric thymuses constructed as described in Materials and Methods. The thymuses were observed when they had reached 20–21 d of age, i.e., 3½ d at grafting time plus 17 d in graft.

As demonstrated previously by our group (21), the first seeding of the thymus rudiment by lymphocyte precursor cells takes place in the quail between E5 and E6 and yields a first generation of lymphocytes which is replaced by the progeny of a second influx of precursors that invade the thymus at E11 to E12. We have also shown that the first wave of incoming HPC contains precursors of accessory

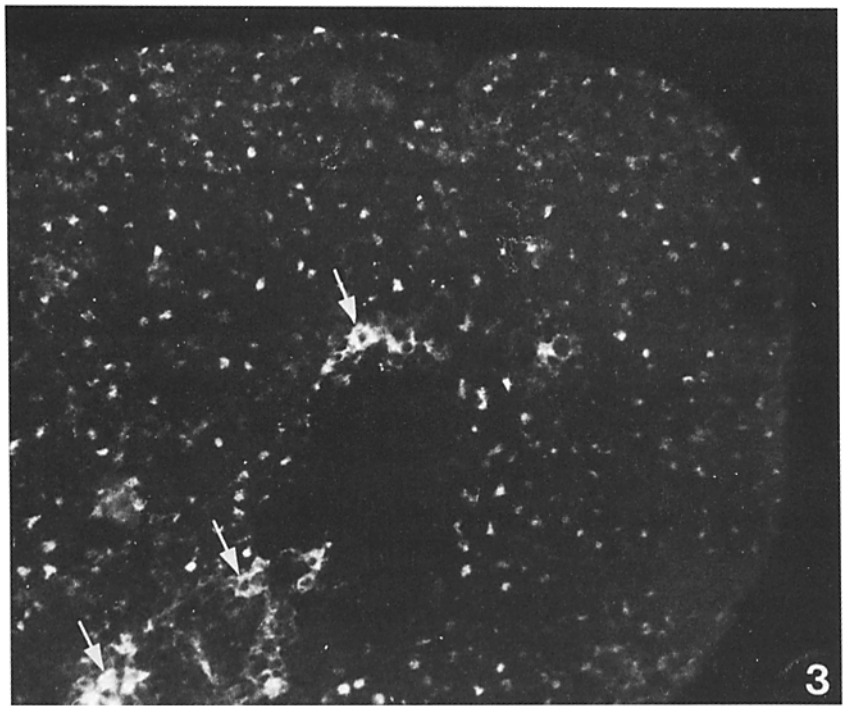

FIGURE 3. Chimeric thymus obtained by grafting the E3.5 quail pharyngeal pouches into a chick embryo for 17 d. TaC1 mAb, specific for quail Ia antigen, reacted only with the reticuloepithelial cells of the cortex and of the corticomedullary margin (arrows). Note that the center of the medulla is practically devoid of immunoreaction. × 170.

cells (17). Since the grafted quail thymus resides in the chick host during both the first and the second HPC influxes, all the hemopoietic cells that it contains at the time of observation belong to the chick species. This was controlled on sections of the grafted thymuses stained with the Feulgen-Rossenbeck procedure: the epithelial and connective tissue cells possessed the quail nuclear marker while the HPC-derived cells were of the chick type. Moreover, anti-MB1 mAb that binds exclusively with cells of the hemopoietic-endothelial lineage of the quail species (25) was applied to these chimeric thymuses. It was found that neither lymphocytes nor macrophages and DC were immunoreactive with anti-MB1 in these thymuses, where only a few quail endothelial cells taken with the branchial pouches bound the antibody. This fully confirmed that no quail HPC had contributed to the grafted thymuses in which all the cell population of hemopoietic origin was of the chick type. This rules out the possibility that precursors of lymphocytes or of macrophages and DC were taken along with the donor branchial pouches.

Application of TaC1 mAb, the specificity of which is restricted to a quail Ia determinant, labeled the epithelial cells in the cortex and at the corticomedullary boundary. Large areas remained unstained in the medulla (Fig. 3). In contrast, in control quail thymuses, Ia-positive cells were densely packed in the medullary area (Fig. 2 *b*). In the cortex itself, the antibody labeled cells with long intricated

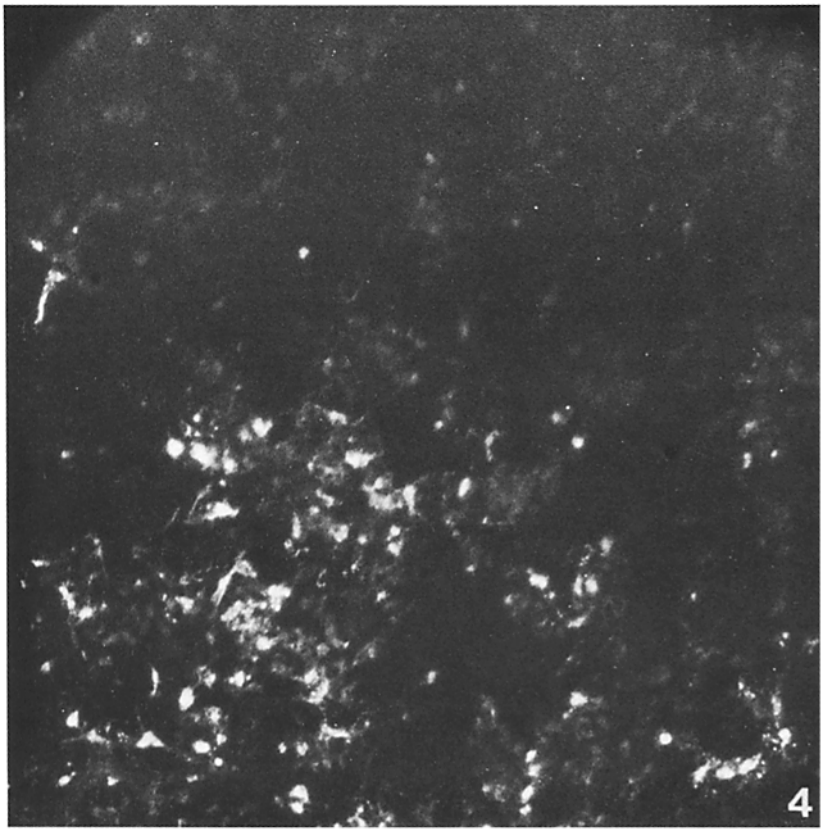

FIGURE 4. The same chimeric thymus as in Fig. 3, on which TaP1 (anti-chick Ia) mAb has been applied. Now, the medullary Ia-positive cells are brightly stained while the epithelial cells of quail endodermal origin are negative. × 230.

processes that formed the typical network observed in the normal thymus. At the limit between cortex and medulla, the epithelial cells had a different morphology and showed a more compact arrangement.

The anti-chicken Ia mAb (TaP1), applied to the same thymuses, exhibited a complementary labeling: Ia-positive cells were not found in the cortex, and the clusters of epithelial cells at the corticomedullary boundary did not show up. In contrast, the large medullary areas unstained with TaC1 now appeared brightly positive with the anti-chicken Ia reagent (Fig. 4). The superimposition of the two pictures provided, respectively, by anti-chick and anti-quail Ia reagents reconstituted the Ia-staining pattern observed on control quail or chick thymuses stained with the appropriate species-specific anti-Ia mAb. TaC2 mAb, which recognizes quail and chick Ia, yielded the same staining on quail, chick, or on chimeric thymuses in which cortical (epithelial) and medullary (HPC-derived) Ia-positive cells were labeled (Fig. 2 and 5).

It is therefore clear that the class II antigens are expressed by two cell types of different embryonic origin in the thymus, the epithelial cells localized in the cortical area and the HPC-derived cells whose distribution is restricted to the

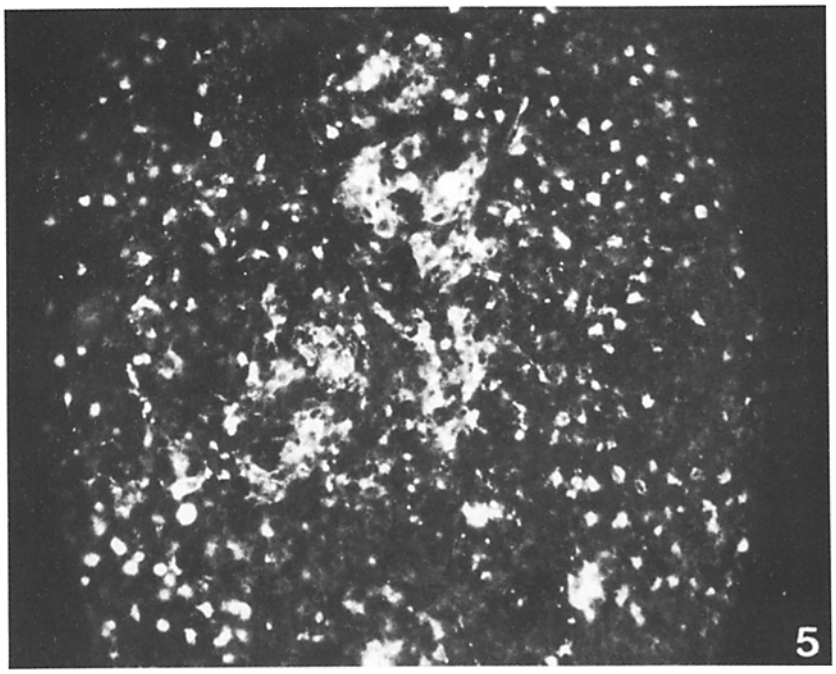

FIGURE 5. The same chimeric thymus as in Figs. 3 and 4 has been stained with TaC2 mAb endowed with quail and chick specificities. The picture is similar to that of normal thymuses of either quail or chick. × 275.

medullary compartment. At the boundary between these two regions, densely packed epithelial cells are closely associated with the Ia-positive thymic cells of extrinsic origin.

The chimeric thymuses were further analyzed in immunoelectron microscopy after processing with anti-quail Ia (TaC1) and anti-chicken Ia (TaP1) antibodies and a second antibody conjugated with peroxidase. The presence of desmosomes, tonofilaments, and basal lamina were used as criteria to identify epithelial cells. The exclusive labeling of the epithelial cells in the cortex and corticomedullary boundary by TaC1 and not by TaP1 was confirmed. Reaction product was found not only on the cell membrane but also in cytoplasmic vesicles, as expected from the granular pattern exhibited in the epithelial cell bodies in fluorescence microscopy (Fig. 6). Staining with anti-chick Ia antibody (TaP1) did not reveal a positive reaction in the cortical area at all but labeled the nonepithelial cells of the medullary region (of chick host origin).

To further illustrate the distribution of Ia antigens on epithelial vs. hemopoietically derived cells, the anti-quail Ia mAb (TaC1) was applied in conjunction with an antibody directed against cytokeratin. In the thymus of 1-mo-old quail, two distinct zones were present. The cortex showed a typical reticular network when viewed after keratin staining. The epithelial component of the medulla consisted of more widely dispersed single cells, except in Hassall's corpuscles. In addition, the densely packed epithelial cells visible at the corticomedullary boundary in the younger thymus as described above (4 d after hatching) are not visible in 1-

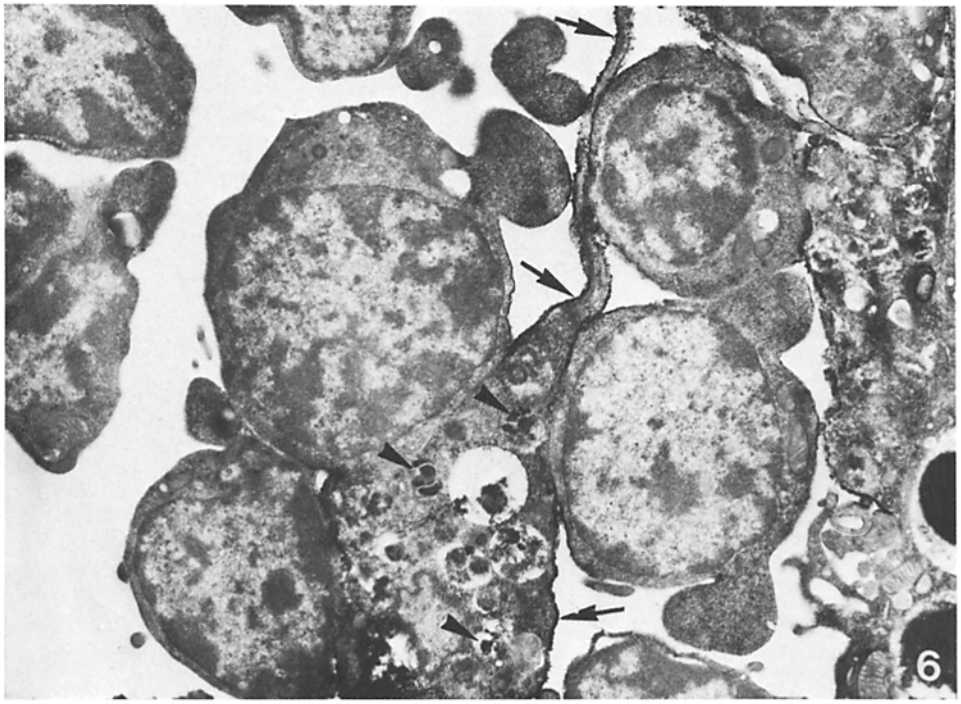

FIGURE 6. Immunoelectron microscopy of a chimeric thymus obtained by grafting an E3.5 quail thymic primordium into a chick host for 15 d. Sections were incubated with TaC1, the anti-quail Ia antibody, then with a second peroxidase-conjugated antibody. A positive quail cell with a long process is shown in the cortical area. Immunoreactivity concerns both the cell membrane (arrows) and intracytoplasmic vesicles (arrowheads). Note that thymic lymphocytes show patches of peroxidase activity, in the membrane area close to the nonlymphoid Ia-positive cell. × 9000.

mo thymuses (Fig. 7 *a*). When the cellular Ia distribution is superimposed on this image (Fig. 7 *b*), one can see that the epithelial cells, as revealed by their content in cytokeratin, regularly express the Ia antigen. In the medulla the situation is more complex: the cytokeratin-containing cells are more dispersed than in the cortex, except in the Hassall's corpuscles, where most cells do not express Ia. The isolated epithelial cells were generally labeled by the anti-Ia reagent. It appears that large zones containing Ia-expressing cells are actually devoid of epithelial cells, as already perceived above in the chimeric thymuses.

Ontogeny of Class II MHC Antigens in Quail and Chick Thymuses. The emergence of Ia immunoreactivity in the thymic cell components was investigated both on the adherent cells prepared from thymus cell suspensions and on tissue sections. The first Ia-positive cells were detected among the adherent cells from suspensions of thymic cells made at E9 in the quail and E11 in the chick. At this stage, however, it was not regularly possible to detect those cells in tissue section, probably because of the low level of expression of the antigen. Labeling with anti-MB1 of the adherent cells from 9–10-d quail thymuses confirmed the hematopoietic (nonepithelial) nature of the Ia-positive cells recovered in these conditions.

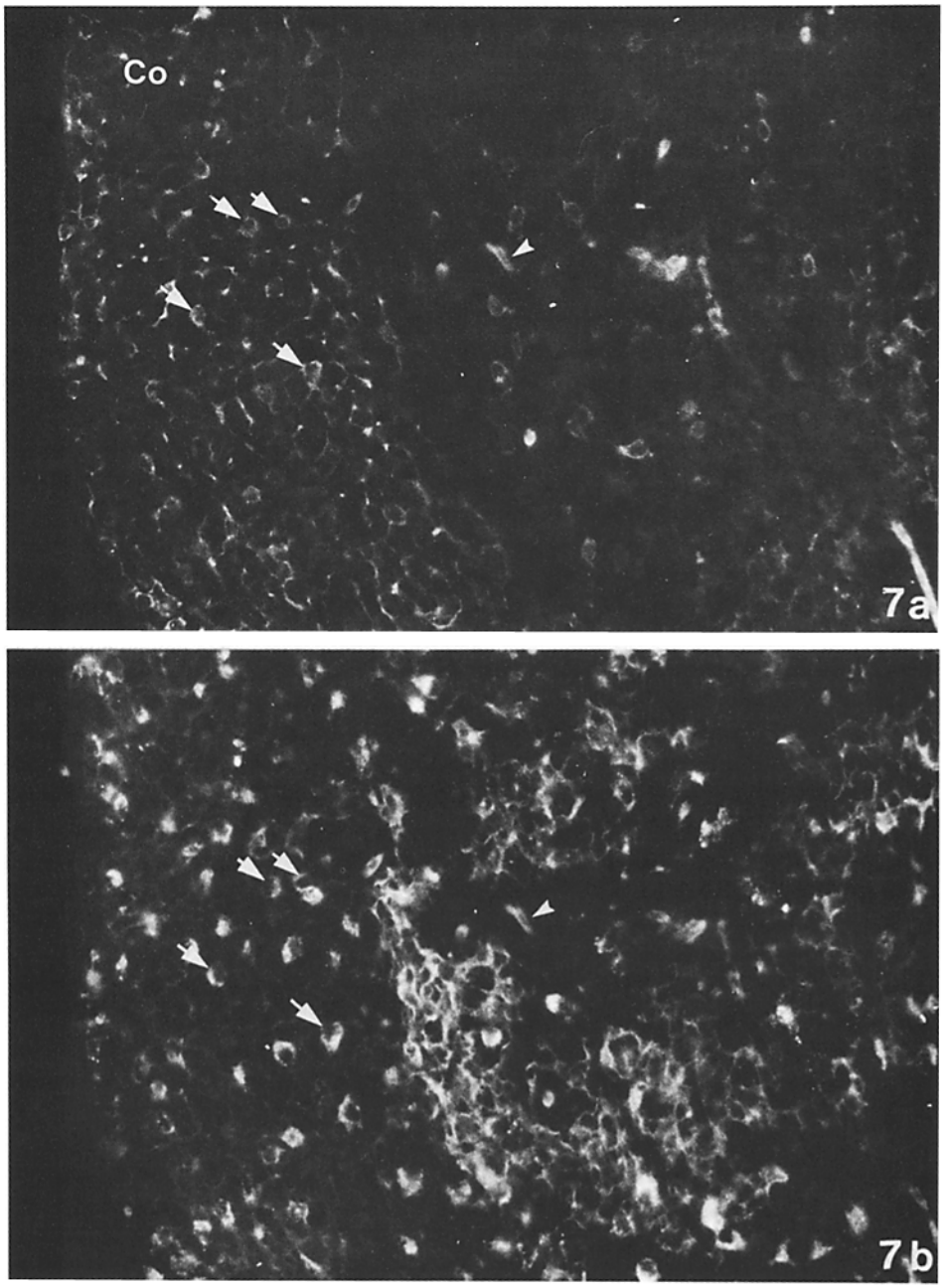

FIGURE 7. Double labeling of a section of a normal 1-mo quail thymus with an anticytokeratin serum (*a*) and the anti-quail Ia reagent (TaC1) (*b*). (*a*) Labeling with the anticytokeratin reagent shows a distinct epithelial reticulum in the cortex (*Co*), whereas in the medulla the epithelial cells are more dispersed and do not seem to be interconnected by processes (arrowhead). (*b*) The same section labeled with the anti-quail Ia antibody (TaC1) shows the cellular distribution of Ia determinants. In the cortex, the Ia-positive cells appear to be the same as those labeled with the anticytokeratin reagent (arrows). In the medulla, both epithelial cells (arrowhead) and accessory cells carry the Ia determinants. × 500.

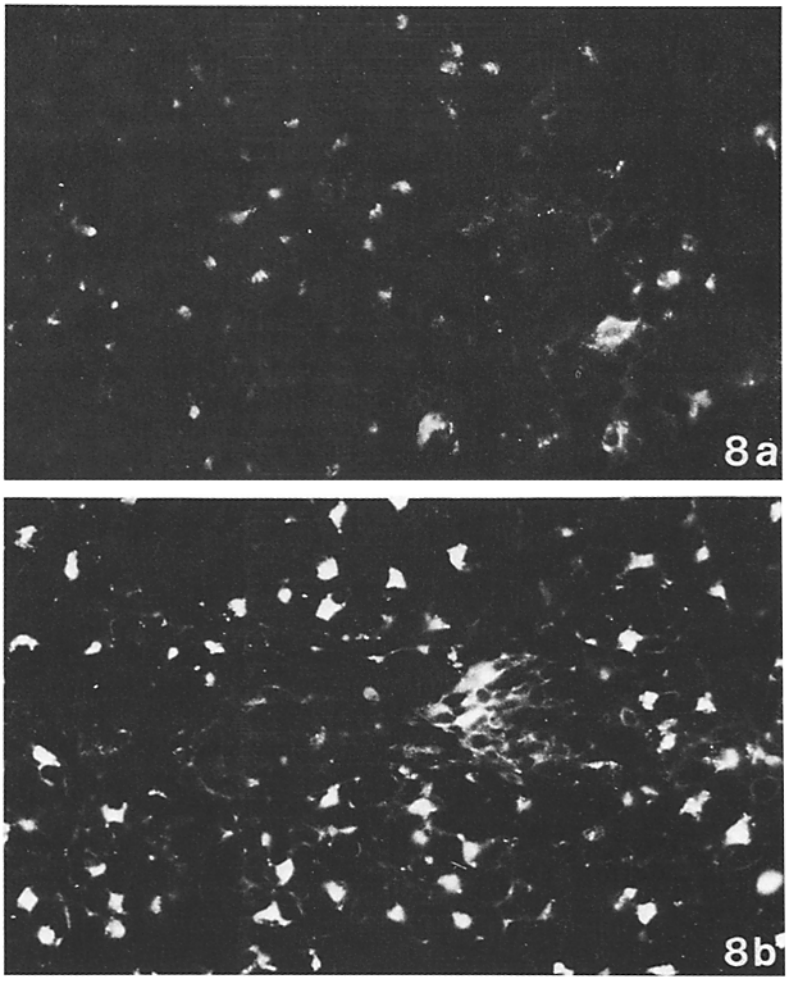

FIGURE 8. Ontogeny of Ia expression on quail thymus cells. (*a*, *b*) Thymus sections from (*a*) 11-d and (*b*) 14-d quail embryos labeled with anti-quail-chick Ia antibody (TaC2). At 11 d, only scarce Ia-positive cells were seen whereas clusters of Ia-positive cells appeared at 14 d. × 500.

From E10 in the quail and E14 in the chick the cortical epithelial cells bodies became faintly reactive while no immunoreactive cell processes were yet detectable. It is at 2–3 d before hatching that the immunostaining pattern of the differentiated thymus could be observed in the two species, with both the epitheliocortical and the medullary hemopoietic Ia-positive cells clearly visible (Fig. 8).

Discussion

The avian MHC, although not documented as well as its mammalian counterpart, has been studied in the chicken (27–30), where the B complex determines products homologous to the class I and II antigens of mammals. B-F antigens,

expressed in practically all cells, belong to class I. A closely linked region, B-G, encodes antigens that are produced by erythrocytes only and which have no mammalian homologue (31). Immune response associated antigens, corresponding to the murine Ia antigens, are encoded by the B-L region of the complex (32–34). Alloantisera and mAb with anti-chicken B-L specificities have been produced before (33, 35–39). The available biochemical data indicate that the B-L chicken antigen is a glycoprotein consisting of an α and a β chain of ~30,000 mol wt, uncovalently bound (39).

We undertook in this work the preparation of mAb directed against both the quail and chicken Ia determinants. We used as the immunogen an enriched preparation of quail and chick thymic adherent cells (17) from 1-mo post-hatch birds.

The antibodies have been selected on the basis of their recognition of an antigen expressed by bursal lymphocytes and thymus adherent cells and absent from nonstimulated T cells. Three differently species-restricted reagents were obtained, which were specific for chick, quail, or both species.

The biochemical characteristics of the molecules recognized by these antibodies have been analyzed by immunoprecipitation. They are slightly different according to the species considered. Although in both quail and chick, undissociated immunoprecipitated molecules migrate as a major band in the range of 55,000 (55 K) mol wt, the same materials under dissociating conditions exhibit different migration patterns in these two species. In the chick, two bands at 31 and 33 K mol wt likely represent the Ia β and α subunits, previously described in the chicken (39). The nature of a minor band at 35 K is under investigation. It could be homologous to the invariant chain, associated to the α-β complex, found in mammals (40). In the quail, the immunoprecipitated material appears as a single large band at 34 K mol wt. Preliminary experiments suggest that the quail Ia antigen is a dimer of co-migrating chains. Another alternative is that only one chain of the class II antigens is radiolabeled in these conditions. In addition to the species-specific anti-Ia reagents, we have also used an mAb strictly specific for the quail hemangioblastic cell lineage. This antibody (called anti-MB1) reacts with surface molecules expressed by endothelial and blood cells (except erythrocytes) of the quail and with no cells of any kind of the chick.

The data previously accumulated on the ontogeny of the quail and chick thymus (see 41 and 42 for reviews) and the availability of these species-specific reagents allowed the avian thymus cellular composition to be analyzed in a more detailed way than before. The hemopoietic origin of the Ia-expressing cells of the medulla, demonstrated in radiation chimeras in rat (18) and mouse (19), was extended to the avian thymus, in which no Ia-positive hemopoietic cells could in fact be detected in the cortical area. All the methods used in this study, including immunoelectron microscopy, supported the contention that in the cortex the cells expressing Ia antigens are epithelial in nature, endodermal in origin, and derived from the third and fourth branchial pouch epithelium. The fact that no cells reacted positively with anti-MB1 in the chimeric thymuses (except a few highly recognizable endothelial cells) demonstrated that no hemopoietic cells were present in the thymic rudiment at the time of grafting. This adds a complementary proof of the purely epithelial nature of the Ia-positive cells

present in the thymic cortex, However, this does not exclude that the avian thymic cortex might contain Ia-negative macrophages. Macrophages have been described in the thymic cortex of mammals (43), and the presence of Ia-negative macrophages in the chicken thymus has been reported (38).

The use of antibodies directed against cytokeratin, a marker for epithelial cells, as well as the quail-chick marker system applied to chimeric thymuses, showed that epithelial cells are scarce in the medullary area, in which the nonlymphoid cells belong mostly to the Ia-positive, HPC-derived cell population. As in the cortex, most medullary epithelial cells express Ia antigens, except for nearly all cells that enter in the constitution of the Hassall's corpuscles of the mature thymuses. In the perinatal thymus, the epithelial cells that occupy the corticomedullary boundary assume a particularly compact distribution that was not found in the older (1 mo) quail thymuses.

The Ia-positive network of the thymus appears spatially divided into two compartments of different embryonic origin. The endoderm-derived epithelial cells form the permanent cortical stroma, which is unequivocally shown here to synthesize class II antigens and not merely to absorb them from another source, since they can be detected by a quail-specific reagent in quail thymuses grafted into the chick. Sessile Ia-positive cells of extrinsic hemopoietic origin are confined to the medulla and subjected to a turnover (17), the rhythm of which has not yet been fully documented. It has been established, however, that precursors for nonlymphoid medullary cells and for lymphocytes penetrate the thymus rudiment during the first influx of HPC at E6 in the quail species. Moreover, these hemopoietically derived medullary cells appear to express Ia antigen before epithelial cells during thymic ontogeny, whereas, in the mouse, Ia expression by both epithelial and hemopoiesis-derived, antigen-presenting cells was shown to be initiated at the same stage (E14), respectively, by immunofluorescence (44) and functional assays (45).

It is still a question whether it is a common precursor, able to differentiate into both lymphocytes and accessory cells, that reaches the thymus at these early stages of development. Cloning of single quail HPC in the chick thymus rudiment is now under way in our laboratory and might provide an answer to this question. Until now these experiments (unpublished) have confirmed that a very small number of precursor cells is sufficient to provide thymic lobes with lymphocytes, as elegantly demonstrated by Ezine et al. (46) in a recent article.

The thymic microenvironment appears more and more complex as its various cell components are more precisely analyzed, regarding their embryonic lineage origins and their tissular localization. The possibilities provided by the mAb technology allowed a detailed investigation of the cellular composition of the human thymus, in which epithelial and connective cells and their respective distributions could be clearly identified (47). In birds, a battery of mAb, used in conjunction with the classical quail-chick nuclear marker on microsurgically constructed chimeric thymuses, made it possible to distinguish three sources for the thymus cell components, deriving from the initial embryonic germ layers: the endoderm for the thymic epithelial cells, the mesoderm for the endothelial cells of the blood vessels, lymphocytes, macrophages, and DC, and the ectoderm of the neural crest for perivascular interlobular and capsular connective cells.

A comparison of these data with other studies on the mammalian thymus (10, 11, 18, 19, 47) indicates a large uniformity in thymic microanatomy in higher vertebrates. These findings might be of importance in the understanding of certain aspects of the acquisition of the T cell repertoire, namely in interpreting the results obtained in radiation chimera experiments regarding the effect of the recipient's thymus phenotype on MHC restriction (3, 48, 49).

Summary

The various cell types expressing Ia antigens in the chick and quail thymus have been studied by means of monoclonal antibodies (mAb) prepared by using chick and quail thymic adherent cells (macrophages and dendritic cells) as immunogens. Three reagents were selected by the following criteria: (*a*) they react with a surface determinant carried by thymic adherent cells and bursal lymphocytes, (*b*) they can be used to immunoprecipitate from spleen cell membrane extracts molecular entities of an apparent molecular weight close to 55,000, which can be fractionated into monomers at ~30,000 mol wt in dissociating conditions. Among these three reagents, two are strictly species specific, i.e., they recognize either chick (TaP1) or quail (TaC1) Ia determinants, whereas the third, TaC2, recognizes both chick and quail Ia molecules.

Chimeric thymuses in which the epithelioconnective stroma is derived from the quail thymic primordium and the whole hemopoietic cell population (lymphocytes and accessory cells) are of chick origin were constructed as previously described by our group (20). The different mAb were applied on normal (quail and chick) and chimeric thymuses. It appears that the thymus is divided into two compartments in terms of the nature of cells expressing Ia: the cortex, in which class II antigens are exclusively expressed by endodermal epithelial cells, and the medulla, where the majority of nonlymphoid cells are Ia-positive cells of hemopoietic origin. A few epithelial cells only are present in the thymic medulla. They are closely intricated with the sessile Ia-positive cells, whose precursors penetrate the thymus along with the lymphocyte progenitors and which are renewed in the course of thymic development. In contrast, the epithelial reticulum, expressing Ia both in the cortex and medulla, contributes a stable thymic component. During early thymic ontogeny, the hemopoietic cells expressed detectable levels of Ia antigen before the epithelial cell network.

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