MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGEN EXPRESSION ON THE EPITHELIUM OF THE DEVELOPING THYMUS IN NORMAL AND NUDE MICE*

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Recently, attention has been refocused on cellular events within the thymus by which an effective T lymphocyte system is generated. In large part this renewed interest has been stimulated by the demonstration that the haplotype specificity of major histocompatibility complex (MHC)[1] -restricted cytotoxic effector cells and the requirement for appropriate MHC matching for cellular cooperation in the immune response are influenced by MHC antigens experienced by T cells during intrathymic lymphopoiesis (reviewed in references 1–3). In this context, the distribution and repertoire of MHC antigens present on thymic stromal cells assume special significance. Analysis of thymus MHC antigen expression is, however, rendered difficult by the complex cellular composition of this organ. Thus, although antigens defined by three MHC regions (K, D, and I) have now been demonstrated in the thymus (4, 5), the identity and degree of heterogeneity of stromal cells responsible for MHC antigen expression are not yet defined.

Developmentally, the thymus consists of cells from several sources. Of the stromal components, the epithelium is thought to have a dual derivation, receiving contributions from the ectoderm and endoderm of the third pharyngeal cleft and pouch, respectively (6, 7). The mesenchymal elements also are, at least in part, derived from the pharyngeal arch mesenchyme, which, in turn, is probably of neural crest origin (8). In contrast the lymphoid and, probably, the macrophage components are of extrinsic origin, arising from migrant blood-born stem cells (9). Early in thymus development these components assume a relatively simple architecture with an inner epithelial mass surrounded by a capsule of mesenchyme. This structure is more easily analyzed histologically than is the adult thymus and is also amenable to in vitro manipulation allowing separation of the epithelial and mesenchymal elements for short-term culture and the reconstruction of rudiments from tissues of different haplotypes.

Defects of development having a direct effect on only one element of the thymus also offer the opportunity to dissect the role of thymic components in lymphopoieses.

* Supported by a project grant from the Medical Research Council, United Kingdom.

[1] *Abbreviations used in this paper:* DPBS, Dulbecco's phosphate-buffered saline; FCS, fetal calf serum; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; RaMIg$_2$Fl, fluorescein-conjugated rabbit anti-mouse immunoglobulin 2; RaMIg-HRP, peroxidase-conjugated anti-mouse immunoglobulin.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/81/02/0280/13 $1.00
Volume 153 February 1981 280–292

In man, thymic aplasia is found in association with ataxia telangiectasia and in the Nezelof and Di George syndromes, although interpretation is often complicated by the generalized nature of the defect in these conditions and by the absence of carefully timed embryonic material. In the mouse, however, the nude mutation gives rise to a more specific defect in which, of the pharyngeal derivatives, only the thymus is involved (10), the stromal elements being unable to interact with the lymphoid stem cells, which these animals possess (11). In addition, the nude gene is now available on a variety of known genetic backgrounds, and it is feasible to produce staged embryonic material from homozygous matings.

In this study we compare and contrast the expression of MHC antigens on components of the developing normal and nude mouse thymus in vivo and after in vitro manipulation and culture. Information obtained in this way may help to answer questions relating to (*a*) the identity of H-2K and H-2I region expressing elements in the thymus, and (*b*) the nature of the stromal defect in nude mice with the implications this may have for the T cell function that can be induced in these animals (12, 13). The results demonstrate the development of H-2K and H-2I region expression on epithelial elements in the normal thymus and the selective absence of H-2I antigen development in the nude thymic rudiment. The findings are discussed in relation to the role of the various thymic components in lymphopoiesis and the development of MHC restriction.

Materials and Methods

Embryonic Material. Embryonic material was obtained from matings within the inbred strains CBA nu/nu (H-2^k) (20 backcross generations; a generous gift from Dr. D. Dresser), CBA/Ca (H-2^k), and C57BL/10 (H-2^b). Males and females maintained on an 8-h dark, 16-h light schedule were caged together overnight and checked for vaginal plugs the next morning. The day of finding a vaginal plug was designated as day 0.

Mice were sacrificed by cervical dislocation and embryos dissected from the uterus, freed of membranes, and immersed in Dulbecco's phosphate-buffered saline (DPBS). Using fine watchmakers forceps, embryos were decapitated, an incision made along the ventral aspect of the thorax, and the thoracic tree consisting of heart, lungs, esophagus, trachea, and thymic lobes, pulled out. Individual thymic lobes were dissected free under a dissecting microscope for subsequent culture. For immunohistological labeling, the entire thoracic tree was processed to facilitate handling.

Antisera. Monoclonal (IgG_2), anti H-$2K^k$ (clone number 11-4.1) and anti-I-A^k (clone numbers 10-3.6 and 10-2.6) were culture supernates kindly provided by Dr. P. Beverley, University College, London, or produced from the hydrid cell lines in the author's laboratory. Details of the specificity of these antisera are given elsewhere (14).

Fluorescein-conjugated rabbit anti-mouse IgG_2(RaMIg_2FL) was obtained from Nordic Laboratories (Tilburg, N.L.), and peroxidase-conjugated anti-mouse Ig(RaMIg-HRP) was prepared by a two-step conjugation method (15) and purified using gel filtration (16). In some experiments a purified, fluorescein-conjugated Fab preparation of rabbit anti-mouse IgG_2 (a generous gift from Dr. Max Cooper) was also employed as a second-step reagent.

In Vitro Techniques

SHORT-TERM CULTURES. Separation of epithelial and mesenchymal components of the embryonic thymus was achieved by incubating isolated 14- to 16-d rudiments in 2.5% pancreatin in normal saline (GIBO UK, Ltd.), for 15–20 min at 4°C. After transfer to DPBS containing 10% fetal calf serum (FCS) rudiments were drawn in and out of a fine glass pipette to separate the outer mesenchyme from the inner epithelium. After separation, small explants of tissue were placed in the individual wells of immunofluorescence trays (Sterlin Ltd., U.K.) in 40 μl of medium RPMI containing 10% FCS (RF-10) and allowed to produce outgrowths overnight

(5). Immunofluorescence labeling of these cultures was carried out by sequential incubation with monoclonal antibodies (1/4) and RaMIg$_2$FL (1/15) diluted in RF-10 with Hepes buffer, with intervening washes in the same medium as described previously (5).

ORGAN CULTURE AND THE PREPARATION OF RECONSTRUCTED RUDIMENTS. For organ culture, intact isolated nude or normal thymic rudiments were placed on the surface of nucleopore filters (3-μ pore size, boiled before use) resting on strips of gelatin foam (Sterispong no. 2; Allen and Hanbury, London, United Kingdom) in 90-mm petri dishes containing 4 ml of RF-10 (17). Reconstructed organ rudiments consisting of 13-d epithelium and mesenchyme from different stains were also produced in organ culture by placing isolated epithelial and mesenchymal fragments side by side on the surface of nucleopore filters using a finely drawn glass pipette. These reassociated to form morphologically normal rudiments during the subsequent 1–2 d of culture.

Frozen Sections. Freshly isolated thoracic trees were rinsed in phosphate-buffered saline (PBS), placed in a pool of mountant (Tissue-Tek) on a microtome stub, orientated, and frozen by standing the stub in solid CO_2. Frozen sections were cut at 6 μ, mounted on gelatin-subbed slides, fixed by dipping in acetone, and stored at 4°C until required. For labeling, slides were washed in DPBS, and the sections were overlayed with 10 μl of a 1/10 dilution of monoclonal antibody for 30 min at 4°C after washing in DPBS and incubation for a further 30 min at 4°C with a 1/15 dilution of RaMIg-HRP or RaMIg$_2$FL.

After washing fluorescein conjugate-labeled sections were mounted directly in 30% glycerol in PBS, while peroxidase conjugate-labeled sections were first treated with diaminobenzidine to visualize conjugate binding (18).

Wax Sections. To improve the histological quality of sectioned material, wax embedding was employed using low melting point (37°C), alcohol-miscible wax (Polyester Wax, BDH Chemicals Ltd., Poole, Dorset, United Kingdom) to minimize processing and consequent antigen degradation. Isolated thoracic trees were fixed for 4 h in neutral buffered formol saline, rinsed in distilled water, and dehydrated by passage through 70, 96, and absolute ethanol (10 min each). After a 30-min incubation in absolute ethanol:polyester wax, 1:1, and two 30-min changes of polyester wax, tissues were embedded and sections cut at 4 or 7 μ for mounting on gelatin-subbed slides. Slides were allowed to dry at 4°C overnight, dewaxed in absolute ethanol, and passed down a series of graded ethanols to PBS. Labeling with antisera was carried out as described for frozen sections. After examination of fluorescent labeling, in some instances cover slips were removed, and the slides were washed and stained with Giemsa for histological examination.

Microscopy. Both cultures and sections were examined for fluorescence under 40 or 60 times objectives on a Zeiss Universal microscope with epi-illuminator III/RS, lamp HBO 50 and standard filter set 9. Photography was carried out using Ilford HP5 film with 2-min exposure and push-processing to ASA 3,200.

Results

Short-Term Culture of Separated Thymic Components

NORMAL THYMUS. Enzyme separation of the two primary tissues of the thymus, although probably not absolute, allows the production of short-term cultures enriched for either epithelial or mesenchymal components. Separated tissues cultured overnight in the wells of immunofluorescence trays, produced outgrowths of cells surrounding the central explant. Indirect immunofluorescence labeling of such living cultures gave a pattern of reactivity with anti-MHC antibodies as summarized in Table I. In the case of CBA (H-2^k) epithelium cultures, labeling was observed on the majority of cells with both monoclonal anti-H-2K^k (Fig. 1) and monoclonal anti-Ia^k (Fig. 2). CBA mesenchyme cultures, on the other hand, showed variable degrees of labeling with anti-H-2K and no specific labeling with anti-Ia, except on small rounded foci of cells identified as epithelial rests, remaining trapped within the mesenchyme at the time of separation.

TABLE I

Expression of MHC Antigens on Separated Thymic Epithelium and Mesenchyme of Normal and Nude Mouse Embryos

Experiment no.	Age	Tissue	Labeling	
			Anti-H-2K	Anti-Ia
	d			
1	15	CBA epithelium	+	+
		CBA mesenchyme	+	−*
2	15	CBA epithelium	+	+
		CBA mesenchyme	−*	−*
		C57 epithelium	−	−
		C57 mesenchyme	−	−
3	14	CBA epithelium	+	+
		CBA nu/nu epithelium	+	−
		CBA nu/nu mesenchyme	−	−
4	15	CBA epithelium	+	+
		CBA mesenchyme	+	−*
		CBA nu/nu epithelium	+	−
		CBA nu/nu mesenchyme	−*	−
		CBA nu/nu adult spleen cells	NT	+
5	16	CBA epithelium	+	+
		CBA mesenchyme	+	−*
		CBA nu/nu epithelium	+	−
		CBA nu/nu mesenchyme	−*	−

+, labeled; −, not labeled; NT, not tested.

* Occasional rests of epithelial cells forming rounded foci showing surface labeling.

Control CBA cultures exposed only to $RaMIg_2FL$ and C57BL/10 ($H\text{-}2^b$) cultures exposed to anti-MHC reagents and $RaMIg_2FL$ showed no specific labeling. Occasional phagocytic cells, presumably macrophages, accumulating the conjugates within phagocytic vesicles were, however, noted in some epithelial cultures and were also present in greater numbers in mesenchyme cultures. Because their nonselective uptake of conjugates, the MHC status of these cells could not be determined.

NUDE THYMUS. Separated cultures of CBA nude thymic epithelium and mesenchyme produced a pattern of labeling with anti-$H\text{-}2K^k$ similar to that seen for the normal CBA. With anti-Ia^k, however, specific labeling was not observed on either epithelium or mesenchyme at any of the stages examined (Table I). The ability of the anti-Ia antibody to recognize determinants present on the CBA nude mice employed in this study was verified by labeling of suspensions of adult spleen cells taken from a pregnant female supplying embryos (data not shown).

Immunohistology

NORMAL THYMUS IN VIVO. Examination of labeling with anti-$H\text{-}2K^k$ and anti-Ia^k revealed a pattern of labeling illustrated in Figs. 3–8. Similar results were obtained on frozen sections with both immunoperoxidase and immunofluorescence techniques. Wax-embedded material showed superior histological preservation and gave results similar to the other two methods with anti-Ia labeling although labeling with anti-H-2K in this material was very weak or absent. Labeling with anti-H-2K in frozen sections was not detected at days 13 and 14, but appeared by day 16. At day 18, H-

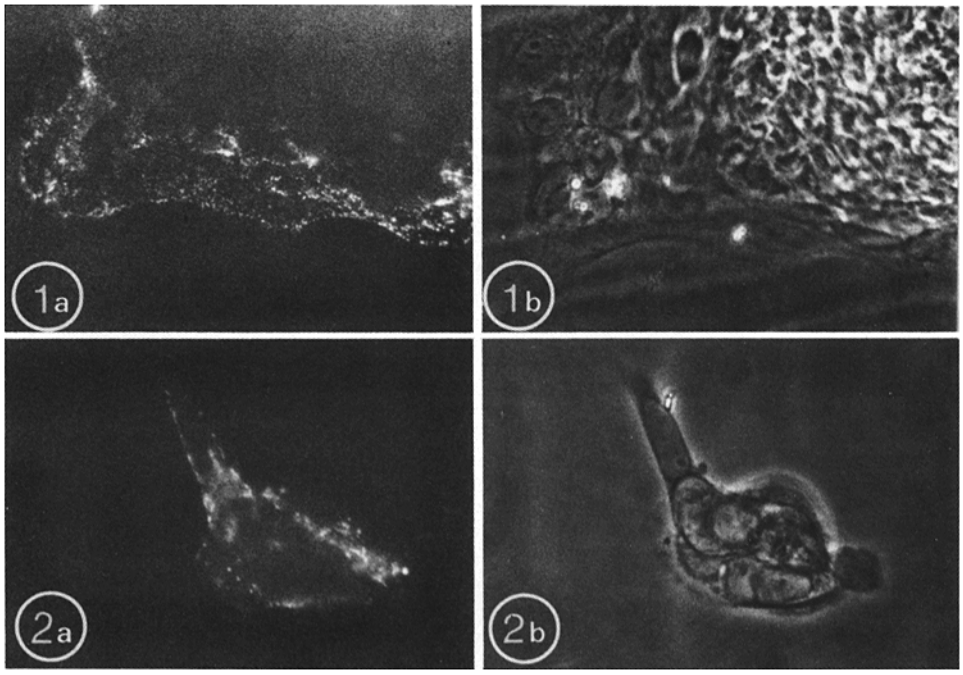

FIG. 1. (a) Short-term culture of separated normal CBA thymic epithelium treated with anti H-2K^k and RaMIg$_2$FL. Note punctate labeling on the skirt of cells growing out from the main explant. Labeling is also present over the central area of the explant, but is in a different plane of focus. × 600. (b) Phase-contrast of (a). Note confluent sheet of cells beginning to grow out from explant. Approximately 20 h in culture. × 600.

FIG. 2. (a) Fragment of isolated normal CBA epithelium treated with anti Ia^k and RaMIg$_2$FL. Punctate labeling outlines the explant. × 600. (b) Phase-contrast of (a). × 600.

2K labeling showed a focal pattern with considerable areas being devoid of reactivity (Fig. 3). It was difficult to relate the distribution of foci to a cortical/medullary pattern, and it was not clear to what extent absence of labeling reflected absence of antigen or masking in regions of dense lymphocyte packing.

Labeling for Ia, also showing a focal pattern, first became detectable at day 13 in peroxidase-labeled frozen sections (Fig. 4). In contrast with H-2K, however, in older rudiments (16–18 d), labeling for Ia was evident throughout the thymus (Fig. 5). High-power examination of slides prepared for immunofluorescence showed that groups of brightly staining cells were particularly prominent in areas of low lymphocyte density, and these were found to have the appearance of epithelial cells after restaining with Giemsa (Fig. 7). The widespread distribution of such cells forming a regular pattern throughout the thymus was clearly seen in rudiments disaggregated to reduce the number of lymphoid cells and allowed to reaggregate in organ culture (Fig. 8). Cells forming the outer mesenchymal capsule of the rudiments, and blood vessels with associated mesenchyme cells in older rudiments did not label with anti-Ia antibodies.

Sections of C57BL/10 material used as haplotype controls were always negative with both anti-H-2K^k and anti-Ia^k (Fig. 6), and nonspecific binding of antisera by macrophages was not seen.

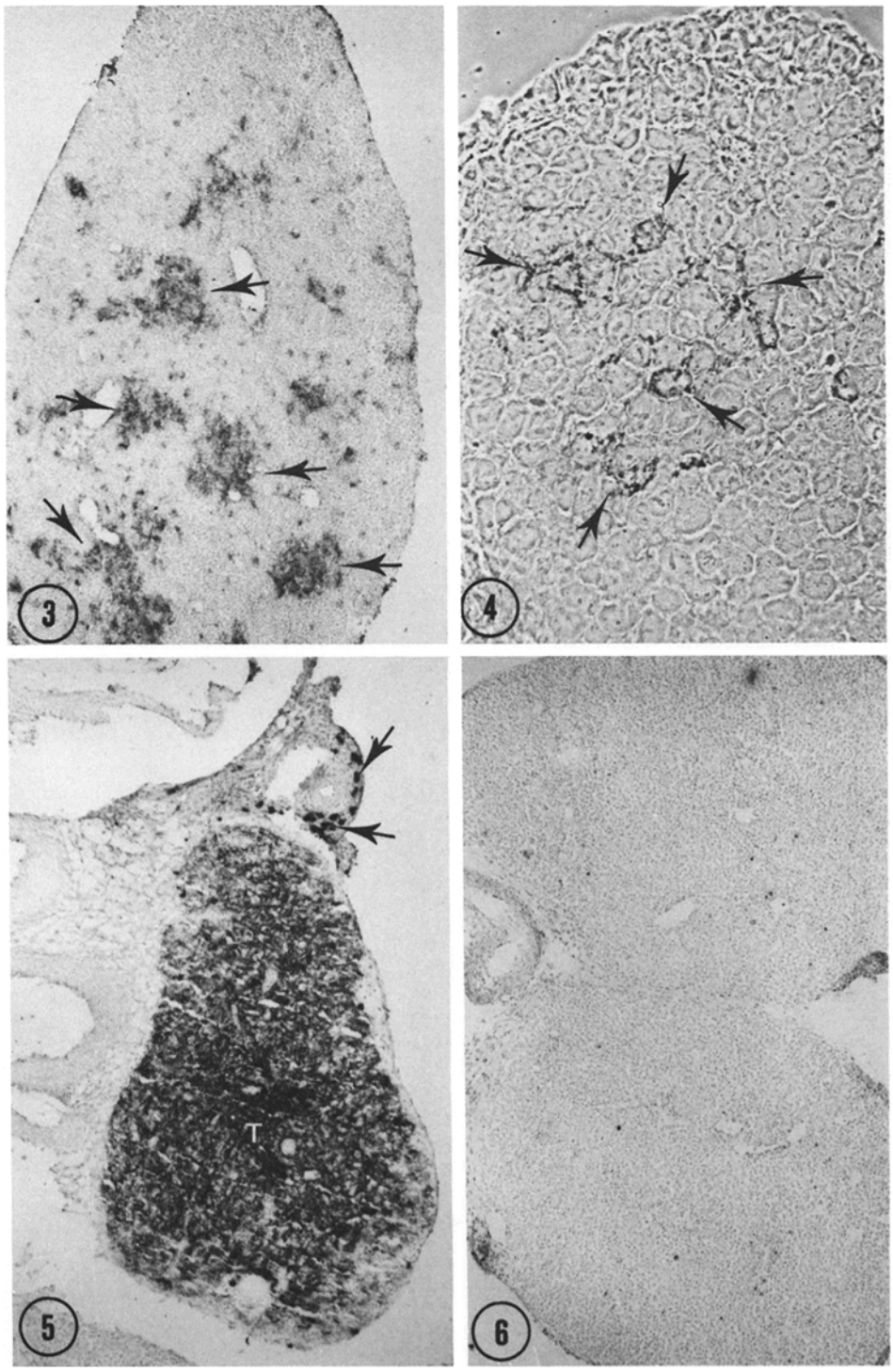

FIG. 3. Frozen section of 18-d normal CBA thymus rudiment treated with anti H-2K^k and RaMIg-HRP. Foci of labeling cells (arrowed) are set within a matrix of nonlabeled cells. Bright-field; × 75.

FIG. 4. Frozen section of 13-d CBA rudiment treated with anti-Ia^k and RaMIg-HRP. Small foci of labeling cells (arrowed) are beginning to appear. Bright-field with superimposed phase-contrast. × 690.

FIG. 5. Frozen section of 16-d normal CBA rudiment treated with anti-Ia^k and RaMIg-HRP. Labeling is present throughout the thymus lobe (T). Other thoracic tissues are not labeled except for strongly peroxidase-positive unidentified cells (arrowed) surrounding a vessel near one pole of the thymus. Bright-field; × 75.

FIG. 6. Frozen section of 16-d C57BL thymus treated with the same reagents as in Fig. 5. Both thymus rudiments are visible and in contrast with Fig. 5 show an absence of labeling. Bright-field; × 75.

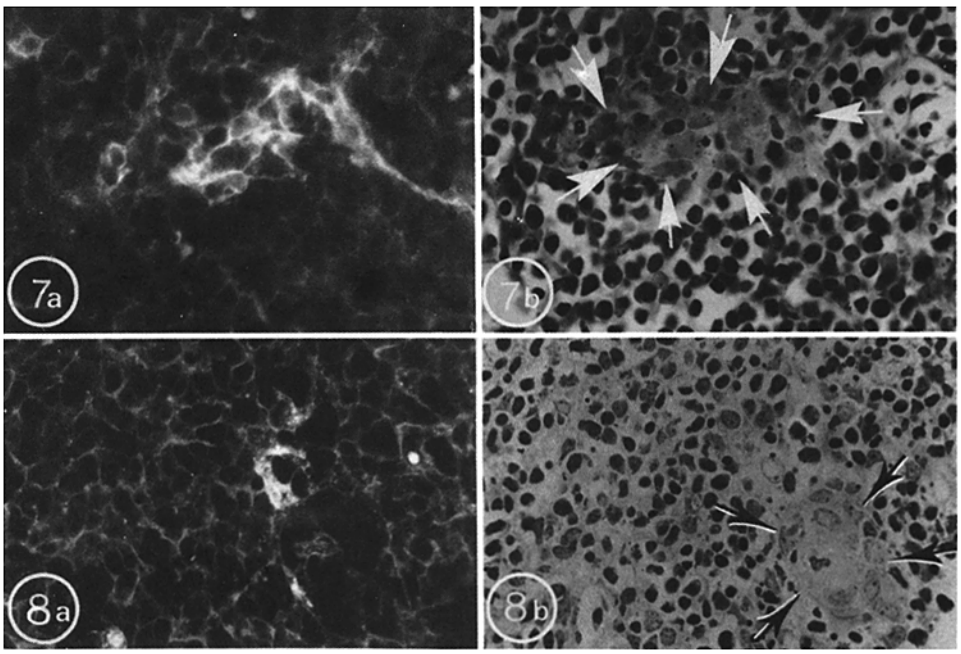

FIG. 7. (a) Wax section of 17-d normal CBA thymus treated with anti-Ia^k and $RaMIg_2FL$. A network of labeling is visible throughout the rudiment but is particularly prominent around a nest of epithelial cells not masked by lymphoid cells (compare with b). × 400. (b) Same field as Fig. 7a after removal of cover slip and histological staining. The darkly staining nuclei and scant cytoplasm of the basophilic lymphoid elements are in marked contrast with the much paler epithelial cells forming a nest in the centre of the figure (arrowed). Giemsa stain; × 400.

FIG. 8. (a) Wax section of normal CBA thymus partially disaggregated and pipetted to reduce the number of lymphoid cells then maintained in organ culture for several days to allow reintegration. Antibody treatment as in Fig. 7a. A network of labeled cell processes is readily visible throughout the field except in certain areas corresponding with the arrowed regions in (b). × 400. (b) Histological staining of same field as in (a). Note reduced number of lymphoid cells. Arrowed region of epithelial-like cells corresponds with the nonlabeling area in (a) and may indicate the existence of a non-Ia-bearing as well as an Ia-bearing epithelial population (cf., Fig. 11 and see text). Giemsa stain; × 400.

NUDE THYMUS IN VIVO. Wax-embedded CBA nude material treated with anti-Ia, in accordance with observations made on living cultures, showed no evidence of antibody binding up to day 16 by which time it was readily detectable on normal CBA rudiments by the same technique.

ORGAN CULTURE OF NUDE AND NORMAL THYMUS RUDIMENTS. To examine the possibility that Ia expression in the nude is retarded rather than totally absent, both normal and nude CBA rudiments were explanted into organ culture at day 16 when the nude rudiment is still sufficiently discrete to be easily recognized and dissected. After 6-d culture, individual lobes were processed for immunohistology. Under these conditions Ia expression did not develop in the nude rudiments (Fig. 9) but was strongly developed in the normal (Fig. 10). Some non-Ia-expressing epithelial cells, usually forming cysts set within the matrix of Ia-positive tissue, were, however, noted in normal cultured rudiments (Figs. 8 and 11). These structures resembled the embryonic nude thymus in appearance and may reflect the contribution of two different types of epithelium to the thymus, one of which, responsible for Ia expression,

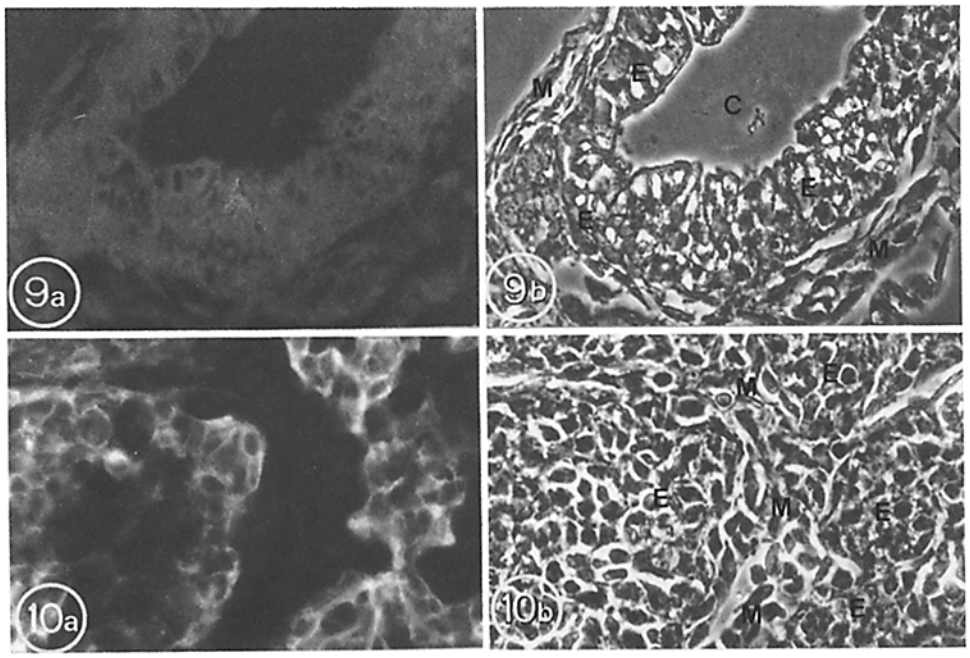

FIG. 9. (a) 16-d CBA nude thymus rudiment organ cultured for 6 d. Wax section treated with anti-Ia^k and RaMIg$_2$FL. Complete absence of labeling. × 400. (b) Phase-contrast of (a). Mesenchymal capsule (M). Epithelial cells (E) surrounding a persistent central cleft (C). × 400.

FIG. 10. (a) 16-d normal CBA thymus organ cultured for 6 d. Wax section, antibody treatment as in Fig. 9a. In contrast with the nude thymus (Fig. 9a), there is a distinct labeling of the epithelial regions. Comparison with Fig. 9b shows nonlabeling areas to be mesenchymal septae or blood vessels. × 400. (b) Phase-contrast of (a). During the 6-d culture period mesenchymal septae (M), some with small blood vessels, have invaded the epithelium (E) dividing it into lobules. × 400.

is absent in the nude (see Discussion). Sections of adult nude thymus containing characteristic cystic structures were also found not to show Ia expression.

ORGAN CULTURE OF RECONSTRUCTED THYMUS RUDIMENTS. Although epithelial cells appear to express Ia antigens, it remains possible that these are synthesized by another cell type (e.g., thymic macrophages that have been reported to express Ia antigen [19]) and are passively acquired by the epithelial cells. To investigate this possibility, reconstructed rudiments were prepared by reassociating fragments of epithelium and mesenchyme isolated from CBA and C57BL/10 thymus rudiments at day 13 when detectable levels of Ia antigen are very low. These reassociated to form normal rudiments within the first few days of culture, and after a total of 7 d of culture were wax embedded and processed for immunohistology. When treated with anti-Ia^k reconstructed rudiments were found to have developed strong Ia antigen expression resulting in labeling according to the haplotype of the epithelium i.e., rudiments containing CBA epithelium and C57 mesenchyme showed labeling with anti-Ia^k (Fig. 11), whereas the reverse combination did not (Fig. 12). It is unlikely, therefore, that Ia expression on the epithelium is caused by antigen passively acquired from cells in the mesenchyme component.

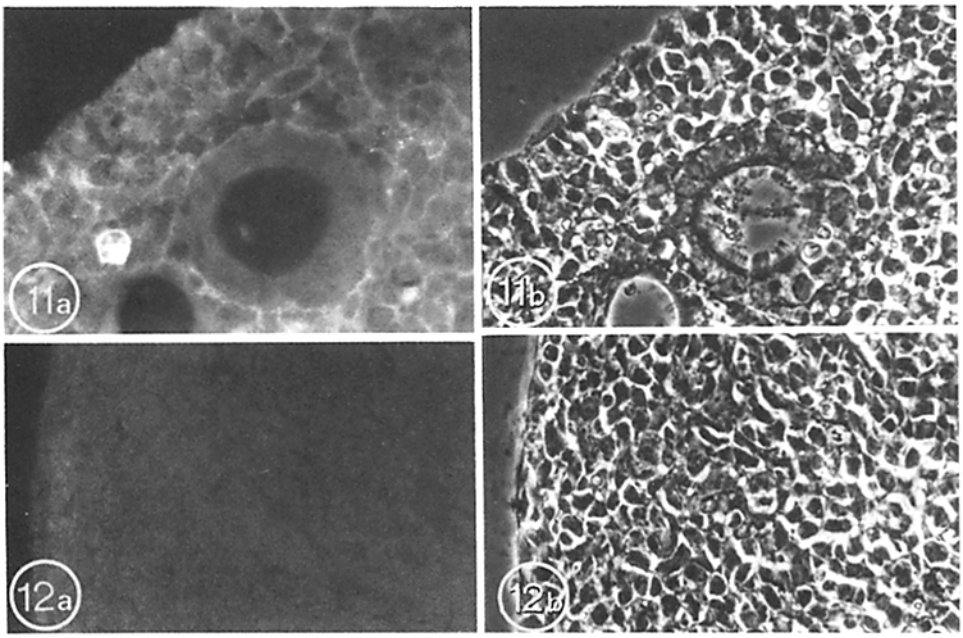

FIG. 11. (a) Normal CBA thymus epithelium recombined with C57BL mesenchyme. Wax section, anti-Ia^k and $RaMIg_2FL$. Labeling is present on most epithelial cells except for those forming cysts. × 400. (b) Phase-contrast of (a). Note similarity of the cystic structures, which appear to bear cilia on their inner surface, to cultured nude thymic rudiment (Fig. 9b). × 400.

FIG. 12. (a) C57BL thymus epithelium recombined with normal CBA mesenchyme. Wax section, antibody treatment as in Fig. 11a. Note complete absence of labeling. × 400. (b) Phase-contrast of (a). × 400.

Discussion

Evidence is now available to suggest thymic involvement in the tuition of lymphocyte populations in self-MHC antigen recognition (1–3) although the extent of this involvement is still a matter of debate (20). In support of a role for the thymus in the selection of the T cell receptor repertoire for self-MHC products, it has been shown that K, D, and I region antigens are all present on thymic stromal cells (4) appearing early in thymus development (5). Further definition of the cell types constituting the stroma is, however, required for an understanding of the way in which the thymic environment may direct T cell selection and differentiation. In this study, by immunohistological examination of the predominantly epithelial embryonic thymic rudiment and in vitro examination of separated thymic components, we present evidence that epithelial derivatives of the pharyngeal region make a major contribution to the expression of I as well as K region antigens in the thymus. The absence of Ia expression in the abnormal embryonic nude thymus, where the defect appears to be primarily epithelial (see below), adds further support to this view. Similarly, immunoelectron microscopical studies on the intact thymus have now confirmed the presence of Ia antigen on cells with the ultrastructural characteristics of epithelium in the adult (21), and these antigens have also been reported on separated adult thymus cells showing epithelial morphology (22).

Apart from the epithelium, Ia antigens have also been recently demonstrated on

macrophage-like cells, obtained from the adult thymus, which have antigen-presenting capabilities (19) and have been reported to influence T cell development in vitro (23). Unlike the epithelium, with which the lymphocytes are intimately associated from the early stages of thymus development, it is not clear to what extent these cells (constituting less than 0.5% of mechanically produced thymocyte suspensions) are available to interact with developing lymphocytes in vivo. In the embryo there is some evidence to suggest that the frequency of phagocytic cells is very low at the time (13–14 d) when Ia becomes detectable on the epithelium, although this increases as development proceeds (24). It is, however, conceivable that both epithelium and macrophages play a role in the development of MHC restriction, perhaps acting at different states of T cell maturation or on different subpopulations. The possibility that different T cell subpopulations may be influenced by different stromal cell populations has also been raised by the finding of differences in the distribution of various MHC antigens in immunohistological studies of the adult thymus (4). The pattern of labeling observed in sectioned embryonic material in this study provided comparable results in that over-all labeling for Ia, and focal labeling for H-2K was observed in thymic rudiments by day 18 of development. In contrast, H-2K labeling was widespread on unfixed cells in overnight cultures of normal thymic epithelium, which is a potentially more sensitive assay given the susceptibility of H-2K antigens to fixation and processing. Thus, if these cultures are representative of all the thymic epithelium, most, if not all, epithelial cells must express both H-2K and Ia. The question of regional variation in H-2K expression, therefore, remains unresolved, and our results do not rule out the possibility of quantitative microenvironmental differences in the expression of this antigen.

Whatever the distribution of its MHC products, by virtue of its repertoire of MHC antigens, the thymus may operate in MHC restriction either to drive the proliferation of clones from colonizing lymphoid stem cells bearing receptors for various self-MHC antigens or to select for survival those cells expressing self-receptors during intrathymic proliferation occurring independently of MHC drive (25–30). In addition, a full range of MHC antigens in the developing thymus may be important in the development of self-tolerance. Conceivably the induction of tolerance, on the one hand, and the selection of self-receptor-bearing cells on the other, may be a function of different MHC antigens bearing cell types within the thymus. With regard to these functions, the status of the thymus in embryo may be particularly important in that restricted cytotoxic T lymphocyte precursors, showing considerable proliferative potential on transfer to thymectomized adults, can be found in the fetal liver by day 18 (31). This finding, together with the fact that T cell function remains relatively intact unless thymectomy is carried out within a short time of birth, implies that the periphery in normal animals may be dominated by cells derived from postthymic precursors that have been subject to intrathymic influence during fetal life.

The embryonic nude thymus although comparable with the normal in its expression of K region MHC antigens differs markedly in its lack of demonstrable Ia antigens. This failure of Ia expression might reflect (*a*) a defect in or (*b*) a total absence of those components that normally express Ia. In either case this could involve a direct effect of the mutation on the Ia-bearing cells or an indirect effect arising from the failure of inductive tissue interactions in the pharyngeal region. On the basis of histological studies, it has been suggested that the normal contribution of ectoderm from the third

pharyngeal cleft to the thymic epithelium does not occur in the nude, leaving only epithelium of endodermal origin (7). If this proves to be the case, it is likely that the Ia antigen-expressing epithelial components of the thymus are derived from the ectoderm while the endodermal components that may also be responsible for the cystic epithelial structures occurring within the normal thymus are Ia negative. Experimental investigation using isolation and recombination of normal and nude pharyngeal tissues of different haplotypes and the induction of Ia expression as a marker, is being attempted to assess these possibilities and to investigate the final distribution of ectodermal and endodermal components in the maturing thymus.

Irrespective of the cell type responsible for Ia expression, the inability of the nude thymus to accommodate T cell development suggests that either Ia expression itself or those epithelial components that normally express Ia are crucial to the development of T cell populations within the thymus. In the light of evidence that nude mice possess prethymic stem cells (11), one possibility is that at least part of the nude defect is caused by the absence of those (Ia-expressing) components that are responsible for the production of lymphoblast attractant. The absence of a major component in the embryonic nude thymus also implies that any prenatal processing of lymphoid cells through the thymus in these mice is unlikely and that even if this did occur such cells should not show thymus-imposed, I region-related, MHC restriction. In this regard it will be important to determine the pattern of restriction, if any, shown by T cells generated from these animals.

Summary

The expression and distribution of antigens coded by the K and I regions of the major histocompatibility complex in the developing thymus of normal and nude mice has been investigated using monoclonal antibodies. Both immunohistological studies of intact rudiments and in vitro labeling of cultures derived from microdissected rudiments indicate that, while K region antigens are present on epithelial and mesenchymal elements, I region antigens are only detectable on the epithelium. This view is also substantiated by the selective absence of I region antigens in the abnormal nude thymic rudiment where the defect is considered to be epithelial in nature. The findings are considered in relation to the role of the thymus in providing an environment for the differentiation and selection of developing T cells, and it is proposed that the Ia-expressing epithelial elements play a central role in these functions.

We are grateful to Mr. W. Bohgal for technical assistance, Mrs. G. Taylor for typing the manuscript, and Mrs. A. Kingman for assistance with the photography.

Received for publication 21 July 1980 and in revised form 16 September 1980.

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