

**BONE MARROW ORIGIN OF Ia-POSITIVE  
CELLS IN THE MEDULLA OF RAT THYMUS\***

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The thymus is of key importance in the development of the T cell repertoire, including the acquisition of tolerance to self antigens and the ability to recognize foreign antigens in association with their own major histocompatibility complex (MHC) antigens (1-3). The Ia antigens of the MHC have been characterized from several species, and are present on B cells, dendritic cells, and a subpopulation of macrophages in rats (4-7). In mouse and human thymus, Ia antigen has been localized on the elongated epithelial cells in the cortex with more confluent staining of the medulla (8-11). This paper describes a similar localization for Ia antigen in rat thymus. Radiation chimeras were used to show that Ia-bearing cells in the medulla were bone marrow-derived, but the majority of Ia-bearing cells in the cortex remained of host origin. This finding may help to explain conflicting results obtained on the effect of the recipient's thymus phenotype on MHC restriction in radiation chimeras (2, 3, 12).

**Materials and Methods**

*Rats.* Two inbred strains, PVG.RT1<sup>c</sup> and PVG.RT1<sup>u</sup>, and their F<sub>1</sub> hybrids were from the specific-pathogen-free unit of the Sir William Dunn School of Pathology, University of Oxford, Oxford, England (13). Chimeric rats were prepared by irradiating male PVG.RT1<sup>c</sup> rats (12-15 weeks) with 950 rad  $\gamma$ -irradiation from a <sup>137</sup>Cs source at 100 rad/min followed by reconstitution with  $3 \times 10^7$ - $10 \times 10^7$  bone marrow cells per rat from the F<sub>1</sub> hybrid.

*Antibodies.* Two mouse anti-rat Ia monoclonal antibodies, MRC OX 3 and MRC OX 4, were used (4). W6/32, a mouse monoclonal antibody that does not react significantly with rat tissues, was used as a control (7). Peroxidase-labeled purified rabbit F(ab')<sub>2</sub> anti-mouse IgG antibodies were as described elsewhere (7).

*Localization Procedure.* Lymphoid organs were removed at various times after reconstitution and 5- $\mu$ m cryostat sections cut. The staining procedure was as described previously (7), except that the peroxidase-labeled antibody was used at 40  $\mu$ g protein/ml to ensure saturation and maximum sensitivity.

**Results**

*Localization of Ia in Normal Thymus.* Ia antigens were localized on cryostat sections of rat thymus by an indirect immunoperoxidase technique. Two monoclonal mouse anti-rat Ia antibodies were used: MRC OX 3 recognizes a polymorphic determinant present on PVG.RT1<sup>u</sup> rats but not on PVG.RT1<sup>c</sup>; MRC OX 4 recognizes Ia from all

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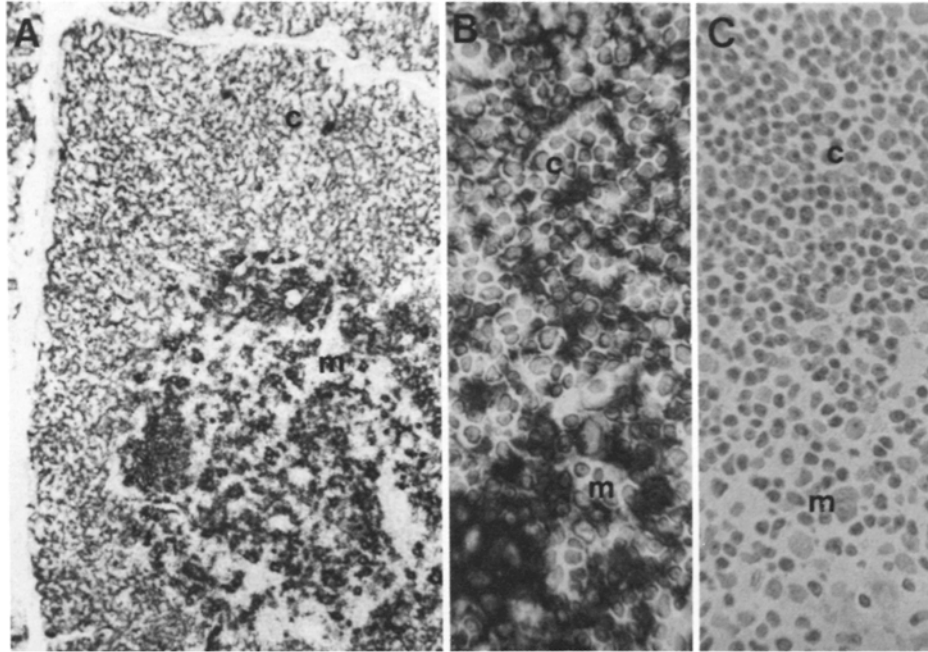


FIG. 1. Localization of Ia antigen in normal rat thymus (PVG.RT1<sup>c</sup>). The dark reaction product of the immunoperoxidase technique contrasts with the pale counterstain of the nuclei. (A and B) MRC OX 4 gives a lattice-like staining pattern in the cortex (c) with large cells and more confluent staining in the medulla (m). (C) Similar area to B reacted with MRC OX 3 shows only the counterstain with no peroxidase reaction product. (A)  $\times 100$ ; (B and C)  $\times 400$ .

rat strains tested. Both monoclonals cross-react with Ia antigens from mouse strains and map to the I-A region (4). Fig. 1 (A and B) shows the strong labeling obtained in the thymic cortex and medulla of a PVG.RT1<sup>c</sup> rat stained with MRC OX 4. In the thymic cortex, the staining has a lattice-like pattern, suggesting that it is present on the thymic epithelium as has been described in the human and the mouse (8-11). In the medulla the staining was strong and more confluent, but could be associated with large irregular cells in some areas (Fig. 1A and B). Areas containing unlabeled lymphocytes were also visible. The specificity of the method and the failure of MRC OX 3 to recognize Ia antigen in PVG.RT1<sup>c</sup> tissue is illustrated in Fig. 1C.

*Localization of Ia Antigens in the Thymus of Irradiated Rats Reconstituted with F<sub>1</sub> Bone Marrow.* Chimeric rats were prepared by irradiation of PVG.RT1<sup>c</sup> (MRC OX 3 negative) rats and reconstitution with bone marrow from (PVG.RT1<sup>u</sup>  $\times$  PVG.RT1<sup>c</sup>)F<sub>1</sub> hybrids (MRC OX 3 positive). Localization of Ia antigen by MRC OX 3 in the thymus 2 wk after reconstitution (Fig. 2A) showed antigen of donor-bone marrow origin mainly on cells in the medulla, with a few positive cells scattered in the cortex. When both donor-derived and host Ia antigens were demonstrated using MRC OX 4 (Fig. 2B), staining was widespread throughout the cortex and the medulla. Thus the majority of the Ia antigen in the thymic cortex of the chimera remained of recipient type. After 2 wk, there was considerable variation within the thymus; some parts resembled normal thymus whereas other parts that showed virtually no restoration with lymphocytes gave heavy confluent staining with MRC

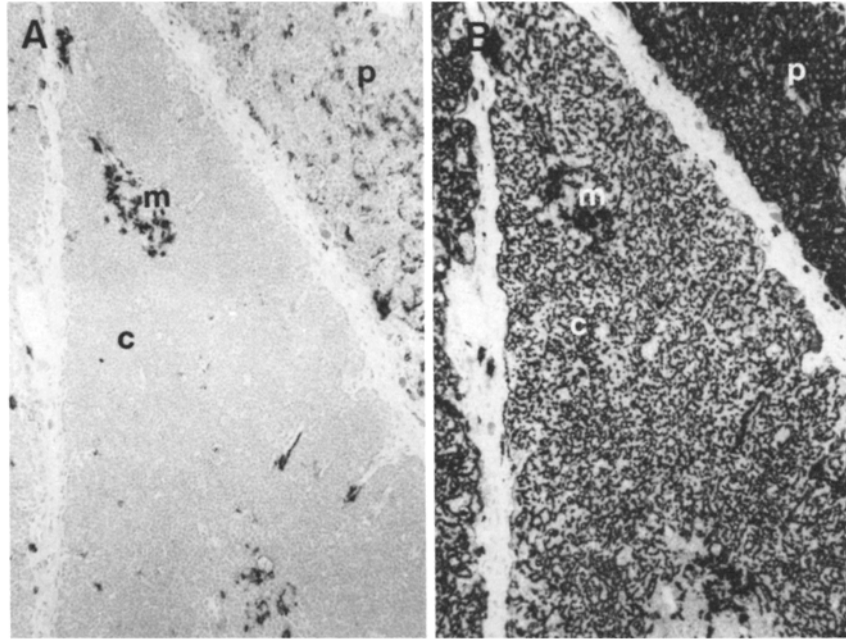


FIG. 2. Localization of Ia antigen in thymus 2 wk after irradiation and reconstitution with F<sub>1</sub> hybrid bone marrow. (A) MRC OX 3 stains large cells in the medulla (m) and an area poorly reconstituted with lymphoid cells (p). (B) Serial section from A; MRC OX 4 stains cells in the medulla and also the network in the cortex (c). Strong amorphous staining was present in the poorly reconstituted region (p). X 100.

OX 4 with only scattered cells stained with MRC OX 3. The staining with MRC OX 4 was probably a result of Ia antigen on the collapsed cortical epithelial network after irradiation and the staining with MRC OX 3 may be analogous to the medullary cells in the better-reconstituted areas (Fig. 2). Thymuses examined at 4, 8, and 12 wk after reconstitution had increasingly normal morphology as the organ became repopulated with lymphoid cells. However, cells with Ia of donor origin were abundant and remained confined mainly to the medulla (Fig. 3). These stained cells were large with irregular outlines (Fig. 4A), and some of the staining may be a result of internal antigen. In all the chimeras examined ( $\leq 12$  wk), there was no staining of the epithelial network in the cortex with MRC OX 3 (Fig. 4B), although this stained well with MRC OX 4. MRC OX 3 gives weaker staining of Ia-positive cells in PVG.RT1<sup>u</sup> rats than MRC OX 4 (4), but it is still clearly detectable in the F<sub>1</sub> hybrid (Fig. 4C).

#### Discussion

In the thymuses of normal and chimeric rats, Ia antigen was present on two cell types of different origin. In the cortex, Ia antigen was distributed in a lattice-like pattern on epithelial cells (Figs. 1-3) and remained of host origin in radiation chimeras. This suggests that it is produced by these cells, and not acquired from bone marrow-derived cells. The medulla contained large, irregular cells that were strongly stained for Ia antigen that was of donor bone marrow origin (Figs. 2 and 3). Although Ia antigen has been detected on ~20% of rat thymocytes by analysis on the fluores-

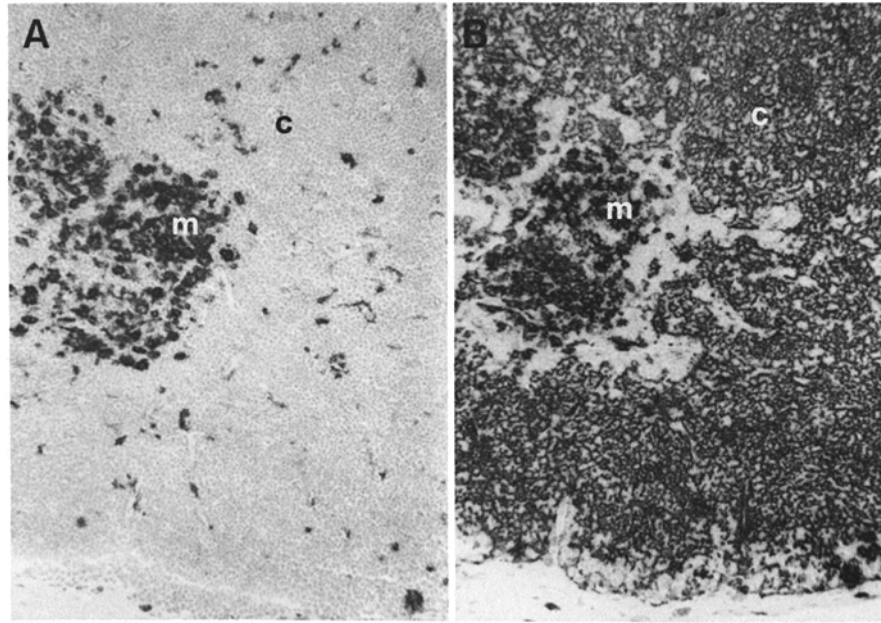


FIG. 3. Localization of Ia antigen in thymus 4 wk after irradiation and reconstitution with  $F_1$  hybrid bone marrow. (A) MRC OX 3 stains many cells in the medulla (m), but only rare large cells scattered in the cortex (c). (B) MRC OX 4 stains cells in the medulla and also the fine network in the cortex.  $\times 100$ .

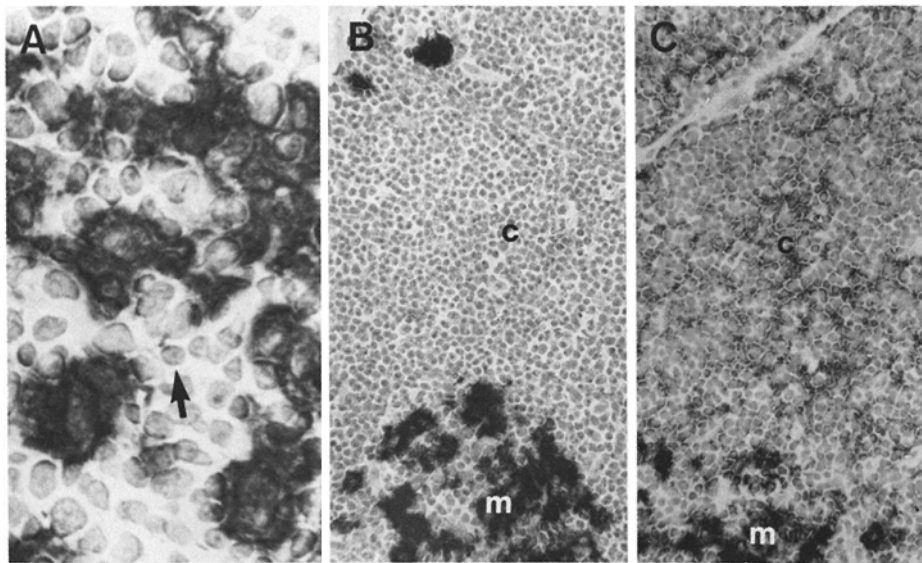


FIG. 4. Localization of Ia with MRC OX 3 in thymus of chimeric rats and  $F_1$  hybrid rats. (A and B) localization with MRC OX 3 in thymus 8 wk after reconstitution with  $F_1$  bone marrow. (A) The staining is associated with large cells with an irregular outline in the medulla, but not with the much smaller thymocytes (arrow). (B) there is no lattice-like staining in the cortex as seen in (C), a similar area of the  $F_1$  hybrid stained with MRC OX 3. (A)  $\times 700$ ; (B and C)  $\times 220$ .

cence-activated cell sorter (4, 13) the labeling was weak and would not account for the labeling observed here.

The staining of Ia-positive cells in the medulla of the thymus resembled that obtained in the T-dependent areas of spleen and lymph node ([8]; and A. N. Barclay and G. Mayrhofer, unpublished observations). This and because they are bone marrow derived suggest that these cells may be analogous to dendritic or interdigitating cells. Cells in this category, which includes Langerhans's cells, bear Ia (5, 6), and at least some can present antigen (5, 6, 12). Cells with a similar ultrastructure have been observed in the thymus (5). If the medullary Ia-bearing cells can present antigen, their function may be to produce tolerance to self antigens. The medulla is a likely place for such a process as it is more accessible to both circulating cells (15) and macromolecules (16) than the cortex.

The presence of increasing amounts of donor-bone marrow-derived Ia antigen in the thymus during reconstitution of radiation chimeras affects the interpretation of all experiments in which similar chimeras are employed to study the effect of the thymus epithelium on restriction of T cells to Ia antigen. It may help to explain some of the conflicting data obtained in mice (2, 3) as results will depend on which Ia antigen is important for restriction, i.e., the Ia on cortical epithelial cells or the Ia on medullary cells. Longo and Schwartz (12) have recently shown that T cells produced by the thymus shortly after reconstitution of radiation chimeras were restricted to host Ia antigen, but that later populations were restricted to donor Ia antigen in the mouse. This together with our results suggest that the final restriction of T cells to Ia antigen may be imposed at the level of Ia-bearing medullary cells. Whether the Ia-bearing epithelial cells in the thymus cortex have a role on restriction remains to be resolved.

### Summary

Irradiated rats were reconstituted with bone marrow from F<sub>1</sub> hybrids. Ia antigen of donor-bone marrow origin was detected by an immunoperoxidase technique on cryostat sections and found predominantly in the medulla of rat thymus 2 wk after reconstitution. These Ia-bearing cells increased in number with time after reconstitution, but the Ia on the cortical epithelial cells remained of host origin. The nature of the bone marrow-derived cells and their implication for major histocompatibility complex restriction are discussed.

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