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

By A. NEIL BARCLAY and GRAHAM MAYRHOFER‡

From the MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, England

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.RT1c and PVG.RT1b, and their F1 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.RT1c rats (12–15 weeks) with 950 rad γ-irradiation from a 137Cs source at 100 rad/min followed by reconstitution with 3 × 10^7–10 × 10^7 bone marrow cells per rat from the F1 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')2 anti-mouse IgG antibodies were as described elsewhere (7).

Localization Procedure. Lymphoid organs were removed at various times after reconstitution and 5-μm cryostat sections cut. The staining procedure was as described previously (7), except that the peroxidase-labeled antibody was used at 40 μg 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.RT1c rats but not on PVG.RT1b; MRC OX 4 recognizes Ia from all

* Supported by the Princess Margaret Children's Medical Research Foundation, Inc.
‡ Present address: Clinical Immunology Research Unit; Princess Margaret Children's Medical Research Foundation, Inc.; G.P.O. Box D 184; Perth; Western Australia 6001; Australia.
Localization of Ia antigen in normal rat thymus (PVG.RT1\(^c\)). 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) × 100; (B and C) × 400.

Fig. 1. Localization of Ia antigen in normal rat thymus (PVG.RT1\(^c\)). 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) × 100; (B and C) × 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\(^c\) rat stained with MRC OX 4. In the 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\(^c\) tissue is illustrated in Fig. 1C.

Localization of Ia Antigens in the Thymus of Irradiated Rats Reconstituted with F1 Bone Marrow. Chimeric rats were prepared by irradiation of PVG.RT1\(^c\) (MRC OX 3 negative) rats and reconstitution with bone marrow from (PVG.RT1\(^u\) × PVG.RT1\(^c\))F\(_1\) 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
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 re-populated 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. 4 A), and some of the staining may be a result of internal antigen. In all the chimeras examined (≤12 wk), there was no staining of the epithelial network in the cortex with MRC OX 3 (Fig. 4 B), although this stained well with MRC OX 4. MRC OX 3 gives weaker staining of Ia-positive cells in PVG.RT1\textsuperscript{a} rats than MRC OX 4 (4), but it is still clearly detectable in the F\textsubscript{1} hybrid (Fig. 4 C).

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 F1 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. × 100.

Fig. 4. Localization of Ia with MRC OX 3 in thymus of chimeric rats and F1 hybrid rats. (A and B) localization with MRC OX 3 in thymus 8 wk after reconstitution with F1 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 F1 hybrid stained with MRC OX 3. (A) × 700; (B and C) × 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 F1 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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References
1. Cantor, H., and I. Weissman. 1976. Development and function of subpopulations of thymocytes and T lymphocytes. Prog. Allergy 20:1.
2. Waldeman, H. 1978. The influence of the major histocompatibility complex on the function of T-helper cells in antibody formation. Immunol. Rev. 42:202.
3. Zinkernagel, R. M., A. Althage, E. Waterfield, B. Kindred, R. M. Welsh, G. Callahan, and P. Pinchtl. 1980. Restriction specificities, alloreactivity, and allotolerance expressed by T cells from nude mice reconstituted with H-2-compatible or -incompatible thymus grafts. J. Exp. Med. 151:376.
4. McMaster, W. R., and A. F. Williams. 1979. Identification of Ia glycoproteins in rat thymus and purification from rat spleen. *Eur. J. Immunol.* 9:426.
5. Thorbecke, G. J., I. Silberberg-Sinakin, and T. J. Flotte. 1980. Langerhans cells as macrophages in skin and lymphoid organs. *J. Invest. Dermatol.* 75:32.
6. Beller, D. I., and E. R. Unanue. 1980. Ia antigens and antigen-presenting function of thymic macrophages. *J. Immunol.* 124:1433.
7. Barclay, A. N. 1981. The localization of populations of lymphocytes defined by monoclonal antibodies in rat lymphoid tissues. *Immunology.* 42:593.
8. Hoffmann-Fezer, G., D. Götze, H. Rodt, and S. Thierfelder. 1978. Immunohistochemical localization of xenogeneic antibodies against Ia<sup>+</sup> lymphocytes on B cells and reticular cells. *Immunogenetics.* 6:367.
9. Rouse, R. V., W. von Ewijk, P. P. Jones, and I. L. Weissman. 1979. Expression of MHC antigens by mouse thymic dendritic cells. *J. Immunol.* 122:2508.
10. Janossy, G., J. A. Thomas, F. J. Bollum, S. Granger, G. Pizzolo, K. F. Brandstock, L. Wong, A. McMichael, K. Ganeshaguru, and A. V. Hoffbrand. 1980. The human thymic microenvironment: an immunohistologic study. *J. Immunol.* 125:202.
11. Bhan, A. K., E. L. Reinherz, S. Poppema, R. T. McCluskey, and S. F. Schlossman. 1980. Location of T cell and major histocompatibility complex antigens in the human thymus. *J. Exp. Med.* 152:771.
12. Longo, D. L., and R. H. Schwartz. 1980. T-cell specificity for H-2 and Ir gene phenotype correlates with phenotype of thymic antigen-presenting cells. *Nature (Lond.)* 287:44.
13. Mason, D. W., and G. G. Gallico. 1978. Tissue distribution and quantitation of Ia-like antigens in the rat. *Eur. J. Immunol.* 8:741.
14. Steinman, R. M., J. C. Adams, and Z. A. Cohn. 1975. Identification of a novel cell type in peripheral lymphoid organs of mice. IV. Identification and distribution in mouse spleen. *J. Exp. Med.* 141:804.
15. Rannie, G. H., and K. J. Donald. 1977. Estimation of the migration of thoracic duct lymphocytes to non-lymphoid tissues. *Cell Tissue Kinet.* 10:523.
16. Raviola, E., and M. J. Karnovsky. 1972. Evidence for a blood-thymus barrier using electron-opaque tracers. *J. Exp. Med.* 136:466.