Coexpression of H2-Mb and H2-Ab genes during fetal and postnatal development

P. GEORGIADES,*† J. KIESZKIEWICZ,* M. ROZYCKA,* P. M. BRICKELL† & T. LUND* *The Medical Molecular Biology Unit, Department of Immunology, Division of Pathology, University College London Medical School, London, and †Leukaemia Research Fund Centre for Childhood Leukaemia, Molecular Haematology Unit, Institute of Child Health, London, UK*

SUMMARY

The major histocompatibility complex (MHC) class II-like molecules, H2-M, have an essential role in processing and presentation of antigens by the MHC class II molecules, because functional inactivation of these genes lead to surface expression of MHC class II molecules devoid of associated foreign peptides. We have used *in situ* hybridization to examine the expression of MHC class II and H2-Mb genes in embryonic and neonatal mice and show here that expression of H2-Ab and H2-Mb mRNA is absent in 13·5-day-old mice. However, mRNA for both genes could be detected in the thymus of 14·5- and 15·5-day-old embryos, and the patterns of hybridization suggested that the two genes were expressed in the same cells. In spleen and thymus of neonatal mice the H2-Ab and H2-Mb genes were also coexpressed, with expression being localized to the white pulp of the spleen and to the thymic medulla, which are rich in antigen-presenting cells. The steady-state levels of H2-Ab mRNA appeared to be approximately 10 to 14 times greater that the level of H2-Mb mRNA molecules, irrespective of the tissue and age, reflecting the different functions of the two molecules.

INTRODUCTION

The major histocompatibility complex (MHC) class II antigen receptors are heterodimeric molecules that bind exogenous foreign peptides and present these to CD4^{+} T cells. The $\alpha\beta$ heterodimers assemble in the endoplasmic reticulum, where they form a complex with the invariant chain, Ii, which functions partly as a signal for routing the MHC class II/Ii complex to the MHC class II compartment (MIIC)[1] and partly to block binding of peptides or denatured polypeptides to the class II molecules during this transport.[2,3] The Ii chain is degraded in the MIIC, and the class II-associated invariant peptide (CLIP), corresponding to residues 81–104 of Ii, is replaced by foreign processed peptides. The MHC class II–peptide complexes are then transported to the cell surface.

Recently, a number of class II-restricted antigen-processing mutant cell lines have been described which fail to process and present foreign peptides to CD4^{+} T cells, although they express normal levels of MHC class II molecules on the cell surface.[4] These surface-expressed class II molecules have not been stabilized with foreign peptides, however, and appear as being sensitive to sodium dodecyl sulphate (SDS). A large proportion of the surface-expressed class II molecules in the mutant cell

lines are associated with the invariant chain-derived CLIP.[5] The defects in these mutants can be restored by transfection with cDNA clones coding for the new MHC class II region genes HLA-DMA/DMB or the murine equivalent H2-Ma/Mb.[5-7] The HLA-DM/H2-M molecules are heterodimeric molecules that have sequence homology with the MHC class I and class II molecules.[8,9] They appear not to be expressed on the cell surface of antigen-presenting cells but are located in the MIIC compartment.[10] The HLA-DM/H2-M molecules function by catalysing the removal of CLIP but could also assist in loading the antigenic peptides into the class II binding groove.[11-13]

Although the function of the H2-M/HLA-DM molecules as presently understood is closely associated with the function of MHC class II molecules, it is not known whether these MHC-like molecules have other functions in addition to assisting peptide loading during antigen processing and presentation. To shed more light on this we have examined the expression of the MHC class II Ab and H2-M genes during mouse development and report here that there are co-ordinated steady-state levels of mRNA in both H2-M and MHC class II proteins from early embryonic to postnatal stages.

MATERIALS AND METHODS

Probes

A 330 base pair (bp) *Sac*II fragment containing exon 2 of the Ab[b] gene[14] was subcloned into pBluscript SK + and used as template for RNA probe synthesis by T7 RNA polymerase

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following linearization with *Bam*HI. The Mb probe was obtained by reverse transcription polymerase chain reaction (RT-PCR) of splenic RNA from the non-obese diabetic mouse using the sense primer atcac^{180}GTGGAAAGCACGTGC and the antisense primer cagtc^{886}GACTGAGCACGGTCTGG. The PCR product was subcloned into pBluescript SK+ and the plasmid was linearized with *Xba*I before *in vitro* transcription with the T7 RNA polymerase. The mouse β-actin probes were obtained from Ambion (Austin, TX). ^{35}S-labelled probe and ^{32}P-labelled probes were prepared as previously described.[15]

RNA extraction and Northern blot analysis

Spleen, thymus, kidney and smooth muscles were taken from male CBA/Ca mice (OLAC) and immediately snap-frozen in liquid nitrogen. Total RNA was extracted as described by Chomczynski & Sacchi,[16] and approximately 20 μg of RNA were fractionated on a 1% (w/v) agarose gel containing 3-(*N*-morpholino)propane sulphonic acid (MOPS)–formaldehyde. The RNA were blotted on to Hybond-N (Amersham, Amersham, UK) after staining of the gel with ethidium bromide. The filters were prehybridized in 25 ml hybridization solution (5× SSC, 0·02 M sodium phosphate buffer pH 6·8, 5× Denhardt's solution, 1% (v/v) deionized formamide, 100 μg denatured sonicated herring sperm DNA, 100 μg yeast tRNA and 10 μg poly A RNA), and then hybridized with a ^{32}P-labelled RNA probe for 16 hr at 65°. The most stringent post-hybridization wash was for 30 min at 80° in 0·1× SSC, 0·1% (w/v) SDS. Blots were initially hybridized with the Ab or Mb probes and were then stripped and rehybridized with the β-actin probe as a control for RNA loading and blotting. The amounts of radioactivity in the Ab, Mb and β-actin bands were determined using a phosphorimager (GS-250 Molecular Imager system, Bio-Rad, Richmond, CA). Values for Ab and Mb were divided by the length, in bases, of the Ab and Mb probes, respectively. The ratios Ab/β-actin and Mb/β-actin were then calculated.

In situ *hybridization*

Mouse embryos were embedded in wax as described by Davidson *et al.*[17] Five-micrometre sections were cut, collected on slides coated with 3-aminopropyltriethyloxysiliane (TESPA, Sigma, St Louis, MO) and baked for 6–16 hr at 60°. *In situ* hybridization was performed essentially as described by Hogan *et al.*[18] using ^{35}S-labelled antisense RNA antisense probes for Ab or Mb or a negative control sense RNA probe. Hybridizations were performed at 55°. After post-hybridization washes and treatment with 40 μg/ml RNAse A, the slides were autoradiographed[18] and stained with 0·05% (w/v) malachite green.

RESULTS

Expression of Ab and Mb in neonatal expression

The expression of H2-Ab and H2-Mb was analysed in different tissues of a 5-week-old CBA/Ca mouse. As shown in Fig. 1(a),
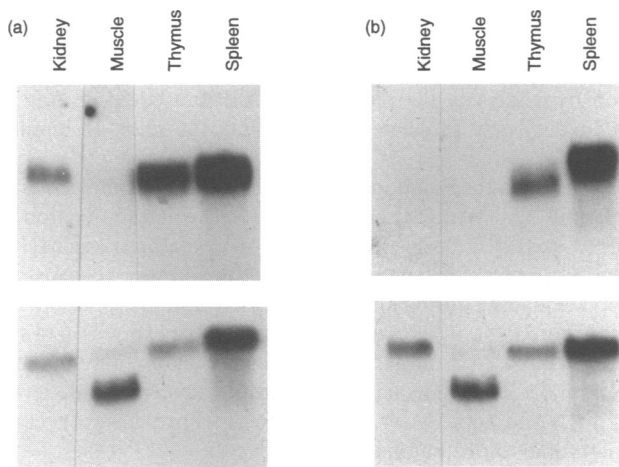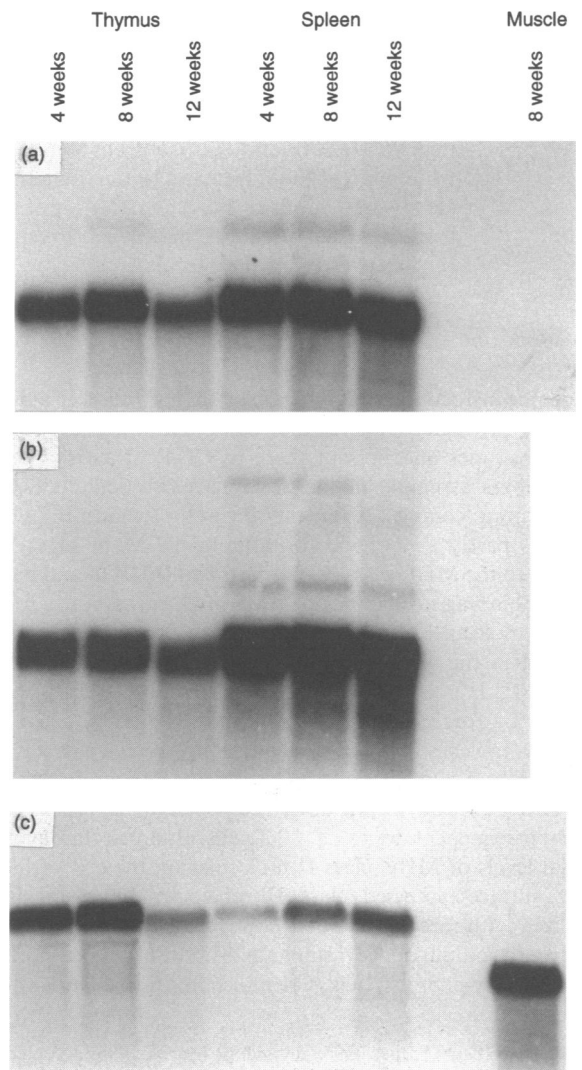

Figure 1. Northern blot of total RNA from different tissues of a 5-week-old mouse hybridized with antisense probes for, in the top panels, H2-Ab (a) and H2-Mb (b) and, in the lower panel, mouse β-actin. The autoradiograms for H2-Ab hybridization to spleen and thymus RNA were developed after 2 days of exposure whilst the rest were exposed for 6 days.

Figure 2. Northern blots of total RNA from thymus, spleen and smooth muscle hybridized with antisense RNA probes for H2-Ab (a), H2-Mb (b) and β-actin (c).
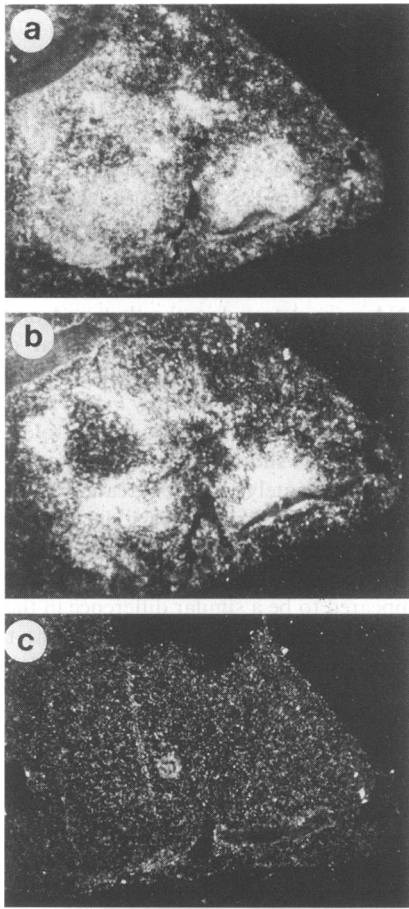
Figure 3. *In situ* hybridization of adjacent sections of a spleen from a 5-week-old mouse hybridized with an antisense RNA probe for H2-Ab (a), H2-Mb (b) or a sense control RNA probe (c); magnification ×240.

hybridization with a 330-base antisense RNA probe containing exon 2 of the Ab gene detected appreciable Ab mRNA levels in the spleen and thymus. The probe also hybridized to specific bands in the RNA isolated from kidney, albeit at significantly lower levels. Of the tissues analysed, only RNA from smooth

muscle did not contain any detectable bands following hybridization with the class II probe. When a similar Northern blot was hybridized with an 850-base antisense RNA probe for Mb1 mRNA, specific bands were detected in the RNA from spleen and thymus (Fig. 1b). Prolonged exposure of the autoradiogram also showed a very weak band in the RNA extracted from the kidney, although this was too faint to be reproduced in the photograph shown in Fig. 1(a). We have analysed spleen and thymus RNA from ten other mice with similar results (data not shown).

The amount of radioactivity in each hybridizing band was then quantified using a phosphorimager. As a control for RNA loading, blots were stripped and rehybridized with an antisense RNA probe for β-actin mRNA. Taking into account the difference in the size of the Ab and Mb probes the ratio Ab/β-actin was approximately 10–14 times greater than the Mb/β-actin for all tissues. This suggests that there are approximately 10 times more Ab mRNA molecules than Mb mRNA molecules, irrespective of the tissue expressing the MHC molecules.

In Fig. 1 there is a small difference in the size of Mb mRNA in thymus and spleen. However, this was not found to be reproducible. The probes were Northern blots containing RNA from thymus and spleen of 4-, 8- and 12-week-old CBA/Ca mice (Fig. 2). Quantification of the Mb and Ab bands relative to the β-actin mRNA showed that there were approximately 10 times fewer Mb than Ab mRNA molecules in both thymus and spleen cells irrespective of the age of the animals. It is interesting to note that the relative amounts of Ab and Mb RNA in the spleen, but not the thymus, decreased with age.

To examine the distribution of the Mb- and Ab-expressing cells, adjacent sections of spleen and thymus from a 5-week-old mouse were hybridized with the antisense Ab and Mb probes and, as control, a sense RNA probe. In spleen (Fig. 3), Ab and Mb mRNA were co-localized in the central areas of the white pulp, corresponding to MHC class II-rich secondary follicles surrounded by a rim containing fewer class II-expressing cells. Cells expressing both Ab and Mb were also co-localized in the thymus (Fig. 4), where hybridization was strongest in the medullary area, which is rich in stroma and antigen-presenting cells.
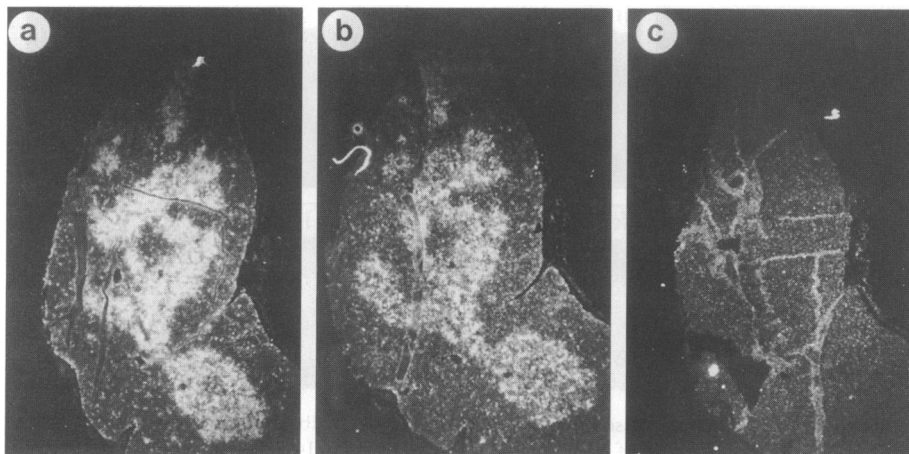

Figure 4. *In situ* hybridization of H2-Ab (a) and H2-Mb (b) antisense RNA probes and a sense control RNA probe (c) to adjacent sections of a thymus from a 5-week-old mouse; magnification ×80.

Expression of Ab and Mb in mouse embryos

Having established that Ab and Mb are co-expressed in adult life we determined where and when the genes are switched on during fetal development by using *in situ* hybridization of radiolabelled antisense cRNA probes to adjacent sections of mouse embryos. Sections from embryos of 13·5 days or younger did not show any detectable hybridization above background with either the Ab or the Mb probe (Fig. 5a, b, c). At day 14·5, hybridization was detected in the developing thymus with the Ab probe and, to a lesser degree, with the Mb probe (Fig. 5d, e, f). No hybridization was seen in any other tissues, with either probe. With both probes, hybridizing cells were distributed throughout the developing thymus, but there was a concentration of grains in the central part. Both probes hybridized more strongly to sections of the thymus from a 15·5-day-old embryo (Fig. 5g, h, i), suggesting either an increase in the levels of mRNA per cell or, more likely, an increase in the number of class II and Mb-positive cells. The cells detected with both probes were evenly distributed over the whole area of the developing thymus (Fig. 5g–l), again suggesting that both genes are expressed in the same cells. As seen in the earlier embryos, no other tissues contained detectable levels of Ab or Mb mRNA.

DISCUSSION

The mouse MHC-like molecule, H2-M, has been linked functionally to MHC class II molecules because cell lines defective in the equivalent human HLA-DM genes fail to process foreign exogenous proteins and to present antigenic peptides to CD4^{+} T-cells, even though they have normal levels of cell surface MHC class II molecules.[5-7] We used a combination of Northern blot analysis and *in situ* hybridization to examine the expression of H2-Mb and H2-Ab RNA in mice from embryonic day 13·5 to 12 weeks postpartum. H2-Ab and H2-Mb mRNA were detected in the thymus from embryonic day 14·5. The two mRNA had a very similar distribution, suggesting that both genes were expressed in the same cells, a notion which was further supported by *in situ* hybridization to sections of spleen and thymus from a 5-week-old mouse. The Northern blot analysis showed that steady-state levels of Ab mRNA were approximately 10 times greater than those of Mb mRNA, irrespective of tissue or age in the adult mouse. Although *in situ* hybridization to tissue sections of 14·5- and 15·5-day mouse embryos may not allow accurate quantification, there appeared to be a similar difference in the number of silver grains on the sections hybridized with the two probes. Since the expression of H2-M and MHC class II genes can be
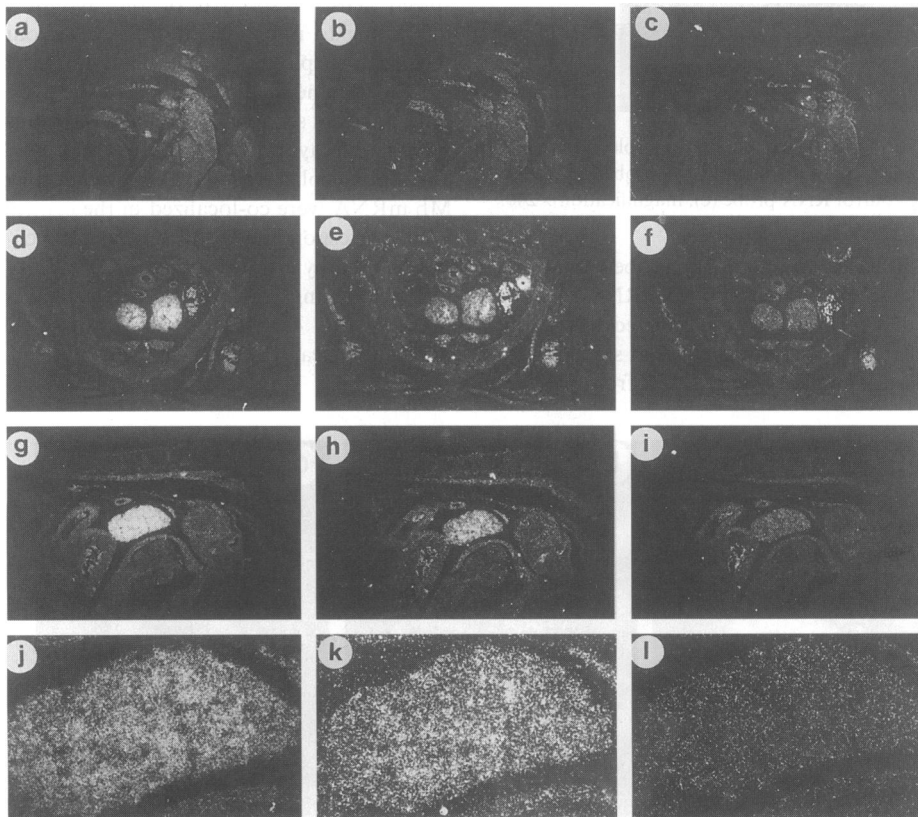

Figure 5. *In situ* hybridization of an H2-Ab antisense RNA probe (a, d, g, j), an H2-Mb antisense RNA probe (b, e, h, k), and a control sense RNA probe (c, f, i, l) to adjacent sections of a 13·5-day-old embryo (a, b, c), 14·5-day-old embryo (d, e, f) and a 15·5-day-old embryo (g, h, i). Higher magnifications of (g), (h) and (i) are shown in (j), (k) and (l); magnifications, (a)–(c) ×150, (d)–(i) ×125 and (j)–(l) ×500.

induced to similar levels with both interferon-γ and concanavalin A,[19] it seems likely that expression of H2-M and MHC class II genes is co-ordinately regulated from early in development. Assuming that the MHC class II mRNA and the Ma and Mb mRNA molecules are translated with equal efficiency, the difference in the expression levels between the MHC class II and H2-M genes reflects the differences in their function, because whereas MHC class II molecules are expressed on the cell surface and may thus have a shorter half-life, the H2-M molecules accumulate in the MIIC compartment[10] and have an extremely long half-life of 24 hr.[5] This suggests that H2-M has no other functions than to participate in the processing and presentation of exogenous antigens.

ACKNOWLEDGMENTS

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