Transpl Int (1994) 7: 43–46


ORIGINAL ARTICLE

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# Does high MHC class II gene expression in normal lungs account for the strong immunogenicity of lung allografts?

Received: 14 July 1992
Reveived after revision: 19 April 1993
Accepted: 4 May 1993

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**Abstract** Clinical experience has demonstrated that lung allografts are considerably more immunogenic than liver allografts although both organs contain equivalent amounts of lymphoreticular tissue. Northern blot analysis of MHC class II gene expression in various murine organs with I-AB and I-EB gene-specific oligonucleotide probes revealed that MHC class II expression in lungs is semiquantitatively higher than in the liver and other organs with the exception of the spleen. We conclude that this high MHC class II expression strongly suggests that the lymphoreticular tissue in the lungs is in a state of activation. This may be an important reason for the strong immunogenicity of lung allografts.

**Key words** Lung transplantation, mice · MHC class II expression, lung · Rejection, lung, mice

## Introduction

The importance of the expression of class II major histocompatibility complex (MHC) antigens with respect to the immunogenicity of allografts is widely recognized [1, 9]. Class II antigens are expressed not only on cells belonging to the immune system, such as activated T cells, B cells, dendritic cells, and macrophages, but also on endothelial and epithelial cells after immunological stimulation [5, 14]. Furthermore, increased expression of MHC class II antigens is also found on tissues during rejection and graft-versus-host disease [3, 8, 15].

Of all vascularized organ allografts, the lung is probably the most immunogenic tissue: episodes of acute rejection are higher in lung transplants, and some studies indicate poor short- and long-term survival rates of lung allografts [6]. The long-term survival in lung transplantation has been relatively low due to the development of obstructive bronchiolitis, which some believe is a manifestation of chronic rejection of the lung [18].

Several studies in animal models have indicated that high amounts of lymphoreticular tissue in the lung may account for its high immunogenicity [10, 11]. However, the liver also has substantial amounts of immunologically competent cells, such as Kupffer cells, and yet liver allografts are generally less immunogenic than lung allografts and, in some combinations, can be tolerogenic [2]. These observations prompted us to study the transcription of class II genes in various organs to determine if the level of class II expression correlates with the immunogenicity of these organs.

## Materials and methods

We extracted total cellular RNA from the skin, skeletal muscle, liver, lung, heart, and brain of H-$2^d$ haplotype mice using a modification of the method originally described by Chirgwin et al. [4]. Briefly, tissues were homogenized in guanidium isothiocynate and subsequently ultracentrifuged on a cesium chloride gradient at 35000 rpm at 4 °C for 18 h. The RNA pellet thus obtained was solubilized in TE buffer

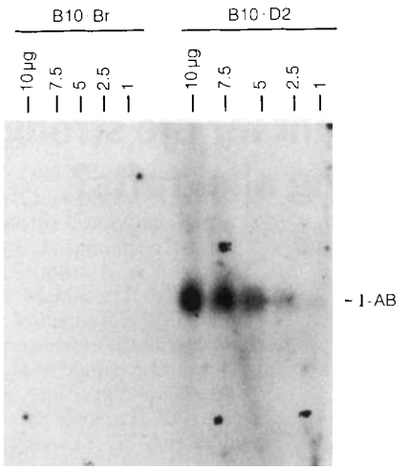


**Fig. 1** Specificity of I-AB$^d$ oligonucleotide probe. Increasing amounts (1–10 µg) of poly A mRNA, obtained from *B10.Br* (haplotype H-2$^k$) and *B10.D2* (haplotype H-2$^d$) mice, were Northern transferred, and hybridized with an I-AB$^d$-specific oligonucleotide probe. The autoradiogram obtained after 24 h indicates that the I-AB$^d$ probe specifically hybridizes to I-AB mRNA from B10.D2 mice but not to I-AB mRNA from B10.Br mice. The autoradiogram also demonstrates that the signal increases with increasing quantities of the I-AB$^d$ mRNA, allowing for quantification

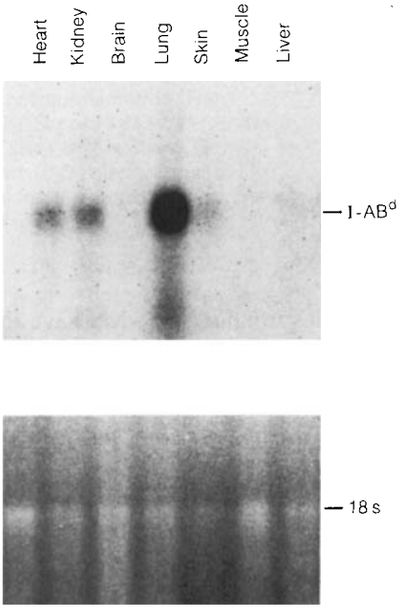


**Fig. 2** I-AB$^d$ expression in various organs of B10.D2 mice. *Upper panel* Equal amounts (5 µg) of poly A mRNA from various organs were Northern transferred and hybridized with the I-AB$^d$ specific probe. The autoradiogram obtained after 48 h reveals a significant amount of I-AB mRNA expression in the lung. The skin mRNA shows a relatively low signal, but this could be a result of less RNA loaded into that lane. This is demonstrated by the low intensity of ribosomal RNA in the corresponding lane of the lower panel. The amount of I-AB mRNA expression in the skin is therefore comparable to that in the kidney. *Lower panel* Ethidium bromide-stained pricture of the RNA gel to demonstrate the equal loading of mRNA as indicated by the ribosomal bands

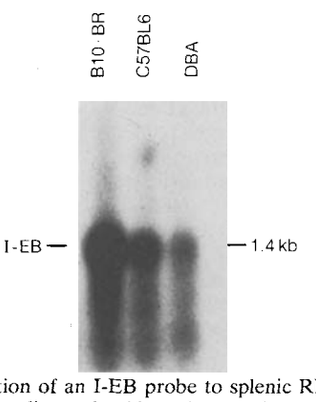


**Fig. 3** Hybridization of an I-EB probe to splenic RNA from several strains of mice. The oligonucleotide probe complementary to I-EB gene hybridizes intensely to splenic RNA from B10.BR (H-2$^k$), C57BL6 (H-2$^b$) and DBA (H-2$^d$) mice, suggesting its binding to a sequence common to the three strains of mice

[10 mM TRIS-Cl (pH 7.4); 1 mM EDTA (pH 8.0)]. Poly A RNA was then isolated by affinity chromatography using an oligo (dT) cellulose column (Pharmacia) and size-fractionated by electrophoresis in denaturing 1% agarose, formaldehyde gel. The intensity of the 18S and 28S ribosomal RNA bands on ethidium bromide-stained gels was used as an indication of the amount of RNA loaded in each lane. This was then compared with the intensity of the hybridizing band on the autoradiogram (Figs. 2, 4). The mRNA was Northern blotted to nylon membranes (Genescreen plus$^{Tm}$) and hybridized with an MHC class II gene and allele-specific oligonucleotide probe [17]. The mRNA obtained from spleens served as a standard for quantification.

## Results

The specificity of the I-AB$^d$ probe is demonstrated in Fig. 1. The I-AB$^d$ probe hybridizes specifically to I-AB mRNA from H-2$^d$ haplotype mice but not to I-AB mRNA from H-2$^k$ haplotype mice. The autoradiogram indicates that increasing quantities of I-AB$^d$ mRNA resulted in increasingly stronger signals.

The levels of I-AB mRNA in the various organs of H-2$^d$ haplotype mice are shown in Fig. 2. The highest levels of I-AB mRNA expression are seen in the lung; on a semiquantitative base they compare to approximately 50% of I-AB expression in the spleen. No I-AB mRNA expression was detected in either the brain or the muscle. The kidney and the skin contained approximately equal amounts of I-AB mRNA expression. Surprisingly, I-AB mRNA expression was detected in significant amounts in the heart in this experiment; the reason for this finding is unclear.

Moreover, in order to see if high levels of MHC class II expression in the lung is restricted to H-2$^d$ haplotype mice or is a general phenomenon, we assessed class II expression in several other species of mice. In this instance, the I-EB probe that hybridizes to class II RNA from several species (Fig. 3) was used. High levels of I-EB expression were seen

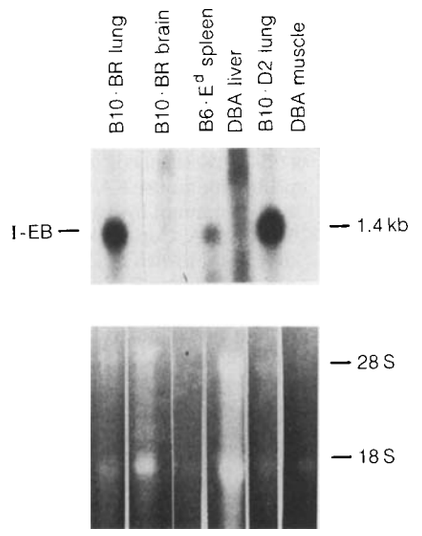


**Fig. 4** Hybridization of the I-EB probe to different tissues from several strains of mice. Similar to the results with the I-AB probe (Fig. 2), lung consistently shows high levels of MHC class II (I-EB) RNA compared to brain or liver in two additional strains of mice (B10.Br – H-2$^k$ and B10.D2 – H-2$^d$). Also note that despite the considerable amount of RNA in the liver, there is almost no I-E expression detected in this experiment. Other strains in this figure are B6.E$^d$ (C57BL6 transgenic mice for I-E$^d$ gene) and DBA – H-2$^d$. The low intensity of the signal with the spleen in this figure is due to partial degradation of the RNA

in B10.Br (H-2$^k$) and B10.D2 (H-2$^d$) mice (Fig. 4). Other organs indicated that not only the I-A but also the I-E gene is expressed at a high level in H-2$^d$ and H-2$^k$ mice.

## Discussion

The finding that the lung contains significantly more MHC class II RNA than the liver, although both organs contain comparable lymphoreticular tissue, is interesting. Since MHC class II expression is increased when lymphoid cells are activated, the explanation that immediately comes to mind is that the lymphoreticular tissue in the lung is, in contrast to other organs, constantly exposed to antigens via the lymphatics, bloodstream, and airways. This constant antigenic stimulation may activate class II expression [18]. Von Willebrand et al. have already demonstrated that infection with the cytomegalovirus can induce expression of epithelial, endothelial, and lymphocyte class II antigens [16]. Additionally, other investigators have shown that induction of MHC antigens in the rat lung allograft can be induced by aerosolized particles [12, 13]. On the other hand, the lymphoreticular tissue in the liver may not be exposed to antigenic stimulation to the same extent as the lung, thus accounting for the minimal MHC class II expression in this organ. Thus, our findings that the expression of MHC class II in mice that were otherwise healthy is higher than in other tissues raises some interesting questions.

On a different note, Hruban et al. found evidence that the expression of MHC class II antigens is not diagnostic of lung allograft rejection [7]. This finding may be explained by our finding of a high basal level of MHC class II expression in normal lungs that may not increase any further, therefore accounting for a lack of correlation between MHC class II induction and rejection of lung allografts.

In conclusion, our findings suggest that the lymphoreticular tissue in the lung is in a more activated state than that in the liver and, therefore, may be able to present alloantigens more effectively. And, although high class II expression can sometimes lead to tolerance, in general it increases immunogenicity. Therefore, we suggest that increased class II expression may be a molecular reason for the higher immunogenicity of lung allografts.

**Acknowledgement** This work was supported by grant AI 25550-02 from the U.S. Department of Health and Human Services.

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