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Tissue distribution of the non-polymorphic major histocompatibility complex class I-like molecule, CD1d

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SUMMARY

The CD1 gene family is composed of five distinct molecules: CD1a, b, c, d and e. CD1a, b and c are primarily expressed thymically with limited extrathymic expression. Preliminary studies have shown that CD1d is primarily expressed extrathymically in gastrointestinal epithelial cells, renal tubular epithelial cells and B cells. This report characterizes the expression of CD1d in a variety of human tissues by immunohistochemistry using two anti-human CD1d monoclonal antibodies (mAb). CD1d was found in a wide range of tissues including the intestine, liver, pancreas, skin, kidney, uterus, conjunctiva, epididymis, thymus and tonsil. Within those tissues CD1d was mainly present in epithelial cells, vascular smooth muscle cells and parenchymal cells. Therefore, the tissue distribution of CD1d is distinct from CD1a–c and classical major histocompatibility complex (MHC) proteins implicating a unique role for CD1d in the immune system.

INTRODUCTION

CD1 is a group of cell-surface proteins that are non-polymorphic major histocompatibility complex (MHC) class 1-like molecules.[1–7] Similar to classical MHC class I molecules, CD1 consists of a heavy chain of approximately 43,000–49,000 MW in a non-covalent association with β_2-microglobulin. CD1 is, however, distinguished from classical MHC class I molecules on the basis of three criteria: (1) CD1 does not map to the HLA locus on chromosome six; (2) CD1 displays a restricted tissue distribution; and (3) CD1 lacks polymorphism in the α1 and α2 domains which, in the classical class I molecules, are involved in peptide presentation to antigen-specific T cells. The function of CD1 remains unknown although it may play a role as a novel alternative ligand for unique subpopulations of T cells.

Five CD1 genes have been identified (CD1a, CD1b, CD1c, CD1d, and CD1e)[3,5] which fall into two classes primarily on the basis of deduced amino acid sequence and tissue localization of the protein products. The protein products of four of these genes, CD1a–d, have been identified. CD1a, b and c share 50% sequence homology and are primarily expressed by cortical thymocytes[8–14] and to a limited extent extrathymically. Outside the thymus, CD1a is expressed by epidermal Langerhans' cells[15] and CD1c by dermal dendritic cells[16,17] and a subpopulation of B lymphocytes.[18,19] We have previously reported on the expression of CD1d by human intestinal epithelial cells, renal tubular epithelial cells and B cells.[20,21]

In the present report, using an immunoperoxidase technique, we describe a more complete analysis of CD1d expression in other human tissues using two CD1d-specific monoclonal antibodies (mAb). These studies show that CD1d is broadly distributed on a wide variety of tissues. However, within these tissues, CD1d is restricted to certain cell types, notably epithelial cells, smooth muscle cells and certain parenchymal cells. This suggests that CD1d is unique from CD1a–c, and the classical MHC class I and II molecules and, therefore, likely plays a unique role in immune function of multiple tissues.

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MATERIALS AND METHODS

Tissues samples

Histologically normal tissues were obtained from organ donors and surgical resections submitted to pathology.

Monoclonal antibodies

The 1H1 and 3C11 antibodies are rat anti-CD1d mAb of the IgM class.[21] We have previously shown that the 1H1 mAb, which was originally raised against murine CD1.1,[22] recognized CD1d on the basis of its ability to discriminate between CD1d and CD1a–c on a transfected B-cell line by flow cytometry.[21] The 3C11 mAb has also been previously reported.[21,22] It is able to immunoprecipitate CD1d differentially from the cell surface of radiolabelled transfected, but not untransfected, recipient cell lines indicating its specificity for CD1d (R. S. Blumberg and S. P. Balk, manuscript in preparation).

Immunohistochemistry

Blocks of human tissues were snap frozen in OCT compound, frozen in liquid nitrogen or in a cryostat and stored at −70°. Frozen tissue sections, 4 μm thick, were fixed in acetone for 10 min, air dried and stained with mAb detected by the avidin–biotin complex (ABC) method.[23] In brief, sections were first incubated with normal horse serum [4 drops of serum in 10 ml of phosphate-buffered saline (PBS), pH 7·3] for 20 min followed by incubation with mAb. The mAb were applied as undiluted hybridoma culture supernatants after washing the sections with PBS. Subsequently, the sections were incubated with a 1:600 dilution of biotinylated rabbit anti-rat IgM for 30 min followed by a 1:20 dilution of peroxidase-labelled avidin complexes for 45 min. Each incubation was followed by three washes in PBS. Because liver and kidney contain high levels of endogenous biotin activity, blocking of this activity was achieved by incubating sections with avidin [2 drops of avidin (100 μg/ml) were added to the horse serum solution during the initial incubation] and with a solution containing biotin (100 μg/ml) and 0·3% hydrogen peroxide. Finally, the sections were stained by incubating in a solution of 3-amino-9-ethylcarbazole, hydrogen peroxide and *n-n*-dimethylformamide. The sections were post-fixed in 4% formaldehyde for 10 min, counterstained with haematoxylin and mounted in glycergel.

RESULTS

In preliminary studies (data not shown), we found that the CD1d-specific mAb, 1H1 and 3C11, reacted with CD1d in fresh frozen tissues. As the 1H1 mAb, but not the 3C11 mAb, recognized CD1d in formalin-fixed tissues, only fresh frozen tissues were examined for the purposes of this study (Table 1).

In the gastrointestinal tract, subtle differences existed between the staining patterns of the 1H1 and 3C11 mAb, most notably in intensity (Fig. 1). In the small bowel, the 1H1 epitope was expressed along the entire crypt–villous axis as previously described.[21] In contrast, the 3C11 epitope (Fig. 1a) was primarily expressed in the crypts with weaker staining at the tips of the villi. The muscularis mucosa was strongly positive with the 3C11 but weak with the 1H1 mAb. In the colon, the 1H1 epitope was more lightly expressed than the 3C11 epitope. As noted in the small intestine, the 1H1 mAb uniformly stained the entire length of the crypt–villous axis while the 3C11 mAb staining was more prominent in the crypt and the muscularis mucosa (Fig. 1b). In both the colon and small intestine, the 3C11 mAb stained smooth muscle cells surrounding large and small blood vessels while the 1H1 mAb only stained small blood vessels.

Previous studies with the homologous protein in mice, CD1.1,[22] suggested that CD1d might also be expressed by hepatocytes. Indeed, within the liver the 1H1 epitope was uniformly expressed by hepatocytes and some bile ducts (Fig. 2a). The 3C11 epitope was not expressed by hepatocytes and bile ducts but was found in the smooth muscle cells around blood vessels in the portal tracts (Fig. 2b).

As noted previously within the kidney,[21] the 1H1 epitope was present in glomeruli and some, but not all, tubules. The 3C11 epitope was also expressed by proximal tubules as well as smooth muscle cells surrounding the blood vessels (Fig. 3). A variety of other tissues were also found to express CD1d (Table 1). Within the pancreas, the 1H1 and, to a lesser extent, the 3C11 mAb stained the ductular epithelium. The 3C11 mAb also stained vascular smooth muscle cells and the 1H1 mAb stained pancreatic acinar cells. The epithelial cells and stromal cells of the endometrium were stained with the 1H1 mAb with lesser staining of the endometrial epithelium by the 3C11 mAb. The 3C11 and 1H1 epitopes were both expressed in smooth muscle cells of the uterus. The 1H1 but not the 3C11 mAb stained the epithelium and smooth muscle cells of the testis. The 1H1 mAb and, to a lesser extent, the 3C11 mAb stained epithelial cells within the epididymis. As we have seen in other organs, the 3C11 epitope and, to a lesser degree the 1H1 epitope was expressed by the muscle cells surrounding the blood vessels of the epididymis. The epididymal stroma stained more with the 1H1 than the 3C11 mAb. In the breast, epithelial cells stained with the 1H1 mAb whereas vascular smooth muscle cells stained with the 3C11 mAb.

The epidermal cells and eccrine glands of the skin stained with the 1H1 mAb but not the 3C11 mAb (Fig. 4a). The 3C11 mAb only stained the vascular muscle cells in the skin (Fig. 4b). In the thymus, there was scattered faint staining in the cortical and medullary areas with both the 1H1 and 3C11 mAb. The 3C11 epitope was also expressed by vascular smooth muscle cells in the thymus. We have also found expression of CD1d in other organs such as stomach, esophagus and gall bladder (data not shown).

DISCUSSION

These results demonstrate that the tissue-specific expression of CD1d is different from other members of the human CD1 gene family as well as classical MHC class I and II molecules. In contrast to CD1a–c which are primarily expressed thymically and to a limited extent extrathymically on cells of the haematopoietic lineage (Langerhans' cells, B cells and dendritic dermal cells), CD1d is expressed weakly in the thymus and primarily extrathymically by a wide number of tissues. Of the extralymphatic tissues examined, these include the small and large intestine, liver, pancreas, kidney, uterus, male reproductive organs (testis and epididymis), conjunctiva, breast, skin, gall bladder, stomach and esophagus. Within each tissue, however,

Figure 1. Small and large intestinal CD1d staining with the 3C11 mAb. Staining of the human intestine with the 1H1 mAb has been previously reported.[21] The 3C11 staining of human small intestine (a) and colon (b) demonstrate strong staining of crypt cells and smooth muscle cells in both tissues (magnification × 13·75). **Figure 2.** Human liver distribution of CD1d by staining with the 1H1 and 3C11 mAb. The 1H1 mAb stained normal human hepatocytes uniformly (a) whereas the 3C11 mAb stained human liver (b) exclusively in smooth muscle cells surrounding blood vessels in the portal tracts (arrowhead) (magnification × 13·75). **Figure 3.** CD1d staining of human kidney with the 3C11 mAb. The 1H1 mAb has previously been demonstrated to stain glomeruli and tubules.[21] However, only staining of the proximal tubules and the smooth muscle surrounding blood vessels can be demonstrated with the 3C11 mAb (magnification × 13·75). **Figure 4.** Human skin expression of CD1d determined with the 1H1 and 3C11 mAb. 1H1 staining of skin (a) was present in epidermal cells and eccrine glands. 3C11 staining (b) was exclusive to the vascular smooth muscle cells (arrowhead) (magnification × 13·75).

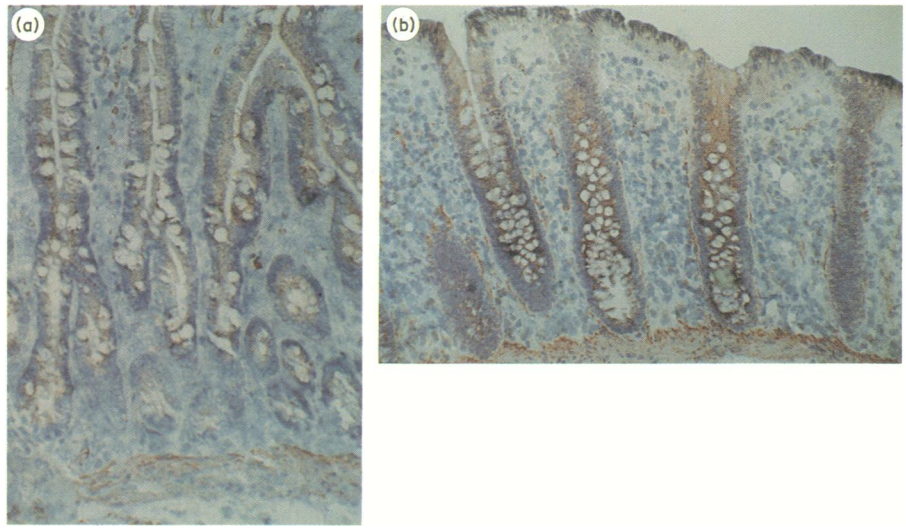

Figure 1

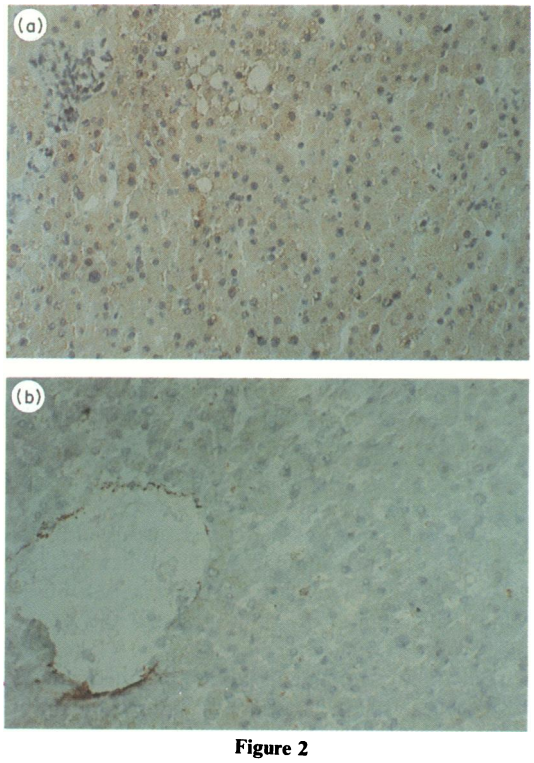

Figure 2

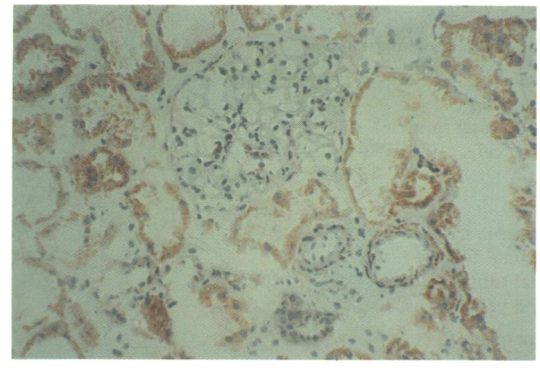

Figure 3

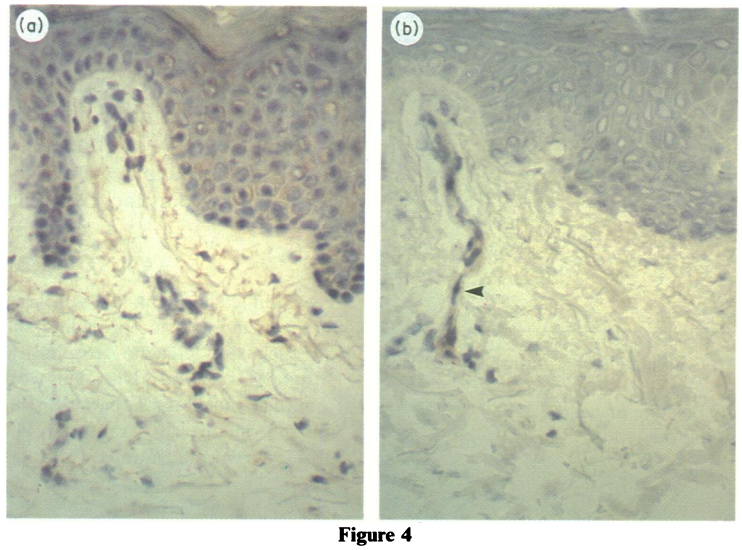

Figure 4

Table 1. Tissue distribution of CD1d

Tissue/cells	Monoclonal antibodies 3C11	1H1
Small intestine		
Epithelial cells (villi)	++*	++
Crypts	++	++
Blood vessel (muscle)	++	+
Smooth muscle	++	+
Colon		
Epithelial cells	+	++
Crypts	+	++
Blood vessel (muscle)	+	−
Smooth muscle	+	+
Liver		
Hepatocytes	−	++
Sinusoidal cells	−	−
Bile duct	−	+
Blood vessel (portal areas)	+	−
Pancreas		
Epithelium	+/−	+/−
Blood vessel (muscle)	+	−
Acinar cells	−	+
Kidney		
Glomeruli	−	+
Glomeruli capsule	−	+
Proximal tubule	++	++
Distal tubule	−	++
Blood vessel (muscle)	++	+
Endometrium		
Epithelial cells (glands)	+/−	+
Stromal cells	−	+
Muscle	++	++
Testis		
Seminiferous tubules	−	+
Muscle	−	+
Epididymis		
Epithelial cells	+/−	+
Blood vessel (muscle)	++	+/−
Stroma	+/−	++
Conjunctiva		
Epithelial cells	+/−	++
Breast		
Epithelial cells (ducts)	−	+†
Blood vessel (muscle)	++	−
Skin		
Epidermis (keratinocytes)	−	+
Sweat glands/ducts	−	+†
Blood vessel (muscle)	+	−
Thymus		
Cortical (thymocytes)	+/−‡	+/−‡
Medullary (thymocytes)	+/−‡	+/−‡
Hassal's corpuscles	−	−
Blood vessel (muscle)	+/−	−
Tonsil		
Epithelium	−	+/−
Blood vessel (muscle)	+	−
Germinal centre	+/−	+/−
Interfollicular	−	−

(++) Very positive; (+) positive; (+/−) faintly positive; (−) negative.

* Lower half, upper half faintly positive or negative.

† Some are negative, fibres around ducts.

‡ Scattered.

CD1d seems to be expressed preferentially by certain cell types. These primarily included epithelial cells, vascular smooth muscle cells and certain parenchymal cells of the liver (hepatocytes), pancreas (acinar cells), endometrium (stromal cells) and skin (eccrine glands).

Within the lymphoid lineage, CD1d is expressed weakly by thymocytes, B cells[21] and certain subpopulations of T cells, such as a subset of intestinal intraepithelial lymphocytes (IEL) (R. S. Blumberg and S. P. Balk, unpublished results). Taken together, the expression of human CD1d on particular cell types in many tissues is distinct from the ubiquitous expression of the HLA-A, B, C gene products. The expression of CD1d also differs from the murine non-polymorphic class I-like molecules, thymus leukaemia antigen (TL)[24,25] and Qa,[26] which are primarily restricted in their expression to cells within the haematopoietic lineage as well as gastrointestinal epithelial cells in the case of TL.[24,25] Similar to CD1d, TL is also expressed on intestinal IEL.[24]

Although the staining patterns were very similar among the tissues examined, some minor differences were observed suggesting the existence of different epitopes delineated by the two mAb. Support for epitopic differences is also supported by our original biochemical description of the 1H1 and 3C11 mAb with murine transfectants.[22] In contrast to the 1H1 mAb which co-precipitated large amounts of β_2-microglobulin with murine CD1, the 3C11 mAb only co-precipitated small amounts. In the present report, whereas the 1H1 mAb recognized intestinal epithelial cells along the entire crypt–villous axis, hepatocytes and bile ductular epithelium, the 3C11 mAb did not stain hepatocytes and bile duct epithelial cells and recognized intestinal epithelial cells within the crypt better than the villous. On the other hand, the 3C11, but not the 1H1 mAb, consistently stained vascular smooth muscle cells better than the 1H1 mAb in many tissues.

Other data also suggest that CD1d may be inducible. In primary biliary cirrhosis, for example, a disease of intrahepatic biliary radicles in which immunological mechanisms have been implicated, we have found that 3C11 reactivity is observed on canalicular membranes (R. S. Blumberg and S. P. Balk, unpublished data). Similarly, the 3C11 and 1H1 staining varies between individual samples and the 3C11 staining appears to be significantly increased in certain inflammatory conditions such as coeliac disease and ulcerative colitis (data not shown).

In summary, CD1d is a unique MHC class I-like molecule whose cell and tissue distribution is distinct from MHC class I and II molecules and other non-polymorphic MHC class I-like molecules such as murine Qa and TL. Of note, an allogeneic mixed lymphocyte reaction between peripheral blood T lymphocytes and intestinal epithelial cells can be almost completely abrogated by the 3C11 mAb.[27] Taken together, these data suggest that CD1d plays a unique role in the immune function of multiple organs distinct from class I and II MHC molecules.

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