Unusual expression pattern of the MHC class I Q5^k gene in the AKR mouse: Possible role in embryo development

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Summary

Analysis of the tissue-specific expression of the Q5^k gene in the AKR mouse reveals an unusual expression pattern. The Q5^k mRNA is present in embryos from day 12, but expression is switched off in most tissues except thymus and testis shortly after birth. Late in pregnancy the gene is again transcribed in females. Analysis at the epitope level, with a Qa-2 specific monoclonal antibody revealed that in most cases the Q5^k product is confined to the cytoplasm. These results suggest that Q5^k has a most unusual tissue distribution and timing of expression among all the H-2 class I and Q genes so far described.

Key words: major histocompatibility complex (MHC); mouse embryo development; Qa products

Introduction

The major histocompatibility complex (MHC) region of the mouse encodes three groups of class I genes, the classical H-2K, D and L loci, a
cluster of genes encoded by the H-2Q/TL region and a recently described subfamily known as H-2M genes (Singer et al., 1988; Klein et al., 1990). The most extensively characterized antigen of the Q series is the Qa-2 antigen, which is expressed on haematopoietic cells and in a variety of adult tissues (Harris et al., 1984), as well as at specific stages of embryonic development (Fahrner et al., 1985; Warner et al., 1987). Although the Q antigens share a similar structure with the classical H-2 class I molecules including their association with B2M, there is no evidence for peptide binding in MHC-restricted antigen presentation to effector T cells bearing the alpha-beta TCR (Watts et al., 1989). However, a peptide specific response mediated by gamma-delta TCR-positive lymphocytes via Qa-1 has been documented (Vidovic et al., 1989).

A number of biological functions in addition to peptide binding have been attributed to class I molecules. These include interactions with hormone receptors (Fehlmann et al., 1985), secretion into the urine and mating control (Singh et al., 1987), stage-specific cell differentiation (Harris et al., 1986) and signal transduction (Dasgupta et al., 1990). Although in most cases the detailed mechanisms are not yet well understood, the possibility exists that Q products share some of these functions on embryonic or adult tissue.

The Q subregion of the AKR mouse strain has been cloned (Weiss et al., 1989) and it differs from the corresponding regions of the H-2b and H-2d haplotypes in that only six Q genes are present (Q1, Q2, Q3, Q4, Q5 and Q10), but it is similar to the Q subregion of the C3H mouse (H-2K). The Q5 gene is polymorphic. The Q5\(^k\) allele has a transmembrane coding sequence similar to that of H-2D\(^d\) (Weiss et al., 1989) and thus should encode an integral membrane class I heavy chain. It is expressed in adult AKR-derived spontaneous thymomas and other tumour cell lines of the H-2K haplotype (Labetta et al., 1989; Schwemmle et al., 1991).

Here we report the expression of the AKR-Q5 gene both at the mRNA and epitope level in the adult mouse and in post-implantation embryonic development.

Materials and Methods

Animals and cells

AKR/J (H-2k) mice were bred and maintained at the London Hospital Medical College and Essex University animal facilities. Spleen, lymph node and thymus cells were prepared by dissection of the organs followed by lysis of contaminating red blood cells by brief incubation in Tris–NH\(_4\)Cl (pH 7.2). The leukaemic cell lines 369, 365, 424 and K36.16 derived spontaneously from AKR mice have been described previously (Old et al., 1965).
Monoclonal antibodies

The monoclonal antibodies (mAbs) H100.5 (anti-H-2K^k) (Lemke et al., 1979), 15.5.5. (anti-H-2D^k) (Ozato et al., 1980) and D3.262 (Qa-2 specific) (Forman et al., 1982), were generous gifts from Dr. H. Lemke, Dr. D.S. Sachs and Dr. L. Flaherty, respectively. The mAbs H100.5 and 15.5.5. were purified by protein A-Sepharose 4B (Pharmacia Fine Chemicals, UK) affinity chromatography (Ey et al., 1978). The mAb D3.262 (IgM) was used as diluted ascites fluid in parallel with an IgM irrelevant mouse ascites. All the above mAbs have been assayed previously for haplotype specificity by cell binding radioimmunoassay (Labela et al., 1989).

Northern and dot blot hybridisation analysis

Total RNA was extracted from mouse tissues and tumour cells using the guanidium isothiocyanate method (Chirgwin et al., 1979). Total RNA (20 g) was separated on denaturing 1% agarose gels containing 2.2 M formaldehyde (Maniatis et al., 1982) and transferred to a Gene Screen Plus filter (New England Nuclear, Dupont Ltd, Hertfordshire, UK). The quality and quantity of the RNA samples loaded was monitored by checking the 28S and 18S ribosomal RNA in the agarose gel stained with ethidium bromide (Figs. 1–3) and checked for RNA degradation with a probe specific for all class I transcripts. A Q5^k specific (Weiss et al., 1989) oligonucleotide (5'-GGT CAC TTT TGC CTC TGG TGG-3'; derived from exon 4 (aa 184-190) of the Q5^k gene from the AKR strain was used to probe the RNA samples. The oligonucleotide was labeled with 32P with T4 polynucleotide kinase and

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<th>AKR LYMPHOID CELLS</th>
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<tr>
<td>THYMOMAS</td>
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<td>K36,16</td>
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-28 s

Fig. 1. Northern blot analysis of AKR spontaneous thymomas and adult AKR spleen cells. Total RNA was separated on denaturing 1% agarose gels, transferred and probed with the Q5^k oligonucleotide (5'-GGT CAC TTT TGC CTC TGG TGG-3') (Weiss et al., 1989). The Q5^k hybridisation bands are shown below the 28S ribosomal RNA which was used to control the amount of RNA applied to the gels.
Fig. 2. Northern blot analysis of RNA isolated from AKR embryos, newborn and adult tissues. Total RNA was isolated and probed as described in Fig. 1 and Materials and Methods. Embryos were harvested at days 7, 9, 12 and 15 of development. LV = total liver preparation; L = total lymph nodes preparation; T = thymocytes; S = total spleen preparation. 1 w, 2 w, 3 w and 4 w indicates age of mice in weeks.

hybridised to northern and dot blot (Mellor, 1987). The filters were exposed to X-ray film in cassettes equipped with intensifying screens (Kodak, Rochester, NY, USA) for 24 h at -70°C.

**Semi-quantitative flow-cytometry**

Aliquots of $2 \times 10^5$ cells were incubated at 4°C with mAbs; bound antibody was detected with fluorescent mouse immunoglobulin-specific goat antibody (Dakopatts, Denmark). For cytoplasmic staining, the cells were incubated for 1 h at 4°C in 70% ethanol. After 3 washes, indirect

Fig. 3. Northern blot analysis of lymph node RNA isolated from pregnant and postpartum AKR donors as well as AKR testis. Total RNA was isolated and probed as described in Fig. 1 and Materials and Methods. Lymph nodes were isolated from day 7 (7 d), day 14 (14 d) and day 18 (18 d) of pregnant mice. Postpartum lymph nodes were isolated from day 2 (2 d), day 4 (4 d) and day 7 (7 d). Total testis RNA extraction was carried out from 5 weeks (5 w), 6 weeks (6 w) and 7 weeks (7 w) old mice. 24 weeks (24 w) old mice were used to obtain lymph node RNA as control.
immunofluorescence was carried out (Labetz et al., 1989). Staining was measured on an EPICS ‘C’ flow cytometer (Coulter Co., UK).

Results

The Q5\(^k\)-specific oligonucleotide probe derived from exon 4 (aa 184–190) of the Q5\(^k\) gene from the AKR mouse strain (Weiss et al., 1989) was used to probe total RNA samples obtained from tissues of AKR origin. Thymoma cell lines K36.16, 369, 424 and 365 yielded a 1.6-kb Q5K-specific mRNA band (Fig. 1). However, adult AKR spleen RNA consistently failed to show the presence of any Q5\(^k\) transcripts (Fig. 1). The Q5\(^k\) transcript also appeared from day 12 of embryo development and persisted in the liver,

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<th>Monoclonal antibodies</th>
<th>H100.5 (H-2K)</th>
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<th>D3.262 (Qa)</th>
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<td>365</td>
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<td>424</td>
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<tr>
<td>K36.16</td>
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<td>2 Weeks</td>
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<td>3 Weeks</td>
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<td>+++</td>
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<td>Spleen cells</td>
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<tr>
<td>Spleen cells</td>
<td>+++</td>
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TABLE 1

Major histocompatibility class I and Qa-like expression on lymphoid cells of the AKR mouse.

The table shows the expression of H-2 class I structures as detected with monoclonal antibodies specific for H-2K (H100.5), H2-D (15.5.5) and Q-encodes (D3.262) determinants. The reactivity of D3.262 was examined against membrane-bound (mb) and cytoplasmic (cyt) forms of the antigens. The intensity of fluorescence was scored 0–30 (–); 30–50 (+); 50–100 (++) and 100–250 (+++). NT: not tested.
lymph nodes and thymocytes of the newborn mice up to 1 week of age (Fig. 2). Lymph nodes, liver and spleen did not express Q5\(^k\) transcripts from week 2 onwards (Fig. 2). The transcript was also expressed by 2-week-old thymocytes (Fig. 2) and persisted with varying levels of expression throughout adult life.

The expression of the Q5\(^k\) transcript was further examined on lymph nodes isolated from pregnant and postpartum donors (Fig. 3). The transcript was detectable from day 18 post-conception of pregnant females and persisted up to day 4 after parturition. The Q5\(^k\) was not detectable in lymph nodes during the early stages of pregnancy (i.e., days 7 and 14) nor on day 7 postpartum or on 24-week-old normal adult AKR lymph nodes (Fig. 3). Q5\(^k\) transcripts were also found in mRNA isolated from whole testis preparations commencing from week 5 after birth and throughout weeks 6 and 7 (Fig. 3) as well as in testis isolated from 24-week-old mice (data not shown).

To relate transcription to protein expression, as since the Q5\(^k\) gene has the potential to encode a transmembrane product, a series of binding experiments were carried out (Table 1). As no Q5\(^k\) specific antibody is currently available, we utilized the mAb D3.262, which recognizes an epitope located on the alpha 3 domain of the Qa-2 molecule (Stroynowski et al., 1987). D3.262 has previously been shown to react specifically with epitopes located on several other Q and T region encoded heterodimers (Forman et al., 1982; Sherman et al., 1984; Fernandez et al., 1987; Oudshoorn-Snoek and Demant, 1990). The AKR-derived thymoma cell line K36.16 showed staining with D3.262 in both the cytoplasm and cell-membrane. The related sub-lines 369, 365 and 424 did not react with D3.262. A Qa-like antigen was also detected in the cytoplasm of neonatal thymocytes up until 2 weeks of age and in the lymph nodes and spleen cells of peri-partum females. In all cases staining was only seen in ethanol-permeabilised cells and not on the cell membrane. Thymocytes, lymph nodes and spleen cells from adult non-pregnant AKR mice did not react with D3.262. All of the various cell types tested reacted with the class I specific mAb H100.5 (H-2K\(^k\)) and 15.5.5 (H-2D\(^k\)).

**Discussion**

In view of the possible role of MHC class I in developmental processes, including embryo cleavage and maternal tolerance of the fetus, we have investigated the expression of the MHC class I gene Q5\(^k\) of the AKR mouse. Different tissues from normal AKR mice isolated before and after birth and AKR-derived spontaneous thymomas were evaluated. Our data show an unusual tissue distribution which differs from that of all other Q/TL and the classical H-2 class I genes so far known.
Class I RNA which hybridised with the Q5k-specific oligonucleotide probe was found in embryos from day 12 of development, whilst the gene was switched off in most tissues, except thymus and testis, shortly after birth. Our results are in agreement with those recently described by Schwemmle et al. (1991) showing Q5k mRNA expression in tissues of 4-week-old AKR mice. In our experiments, late in pregnancy, the Q5k gene was found to be expressed again in the spleen and lymph nodes of pregnant females and transcripts were still present up to 1 week after delivery. Thus, female H-2K mice are often Q5k positive. During embryonic development the Q5k gene has an expression pattern similar to the classical H-2 class I and other Q region genes (Ozato et al., 1985; Fahrner et al., 1985; Schwemmle et al., 1991), whereas the tissue distribution in the adult AKR mouse does not correlate with any of the presently known MHC class I genes. It is well established that the classical class I antigens have a wide tissue distribution and are present on most somatic cells. In contrast, most of the class I Q region encoded antigens are restricted to cell subpopulations mostly of haematopoietic origin (Klein, 1986). With regard to the expression in the thymus and T cell leukaemias, the Q5 allele of the AKR strain is similar to the T3 and T13c genes encoding the TL antigens (Flaherty et al., 1990). All thymus RNA preparations were Q5k positive, as were the T cell leukaemias. The Q5k expression in the same spontaneous leukaemic cell lines has been analysed previously using an oligonucleotide probe derived from exon 5 which also detects transcripts of H-2Dd (Labeta et al., 1989). The promoter regions of the TL genes appear unique among class I genes and do not contain consensus sequences homologous to the H-2 class I regulatory elements: MHC class I enhancer and interferon consensus sequence. In contrast, the Q5k promoter is highly homologous to the conserved MHC class I promoter (Watts et al., 1989; Weiss et al., 1989). Only recently a nucleotide exchange in the region I element of the Q5k gene has been implicated in the loss of constitutive expression (Schwemmle et al., 1991). The regulatory factors and mechanism that govern the expression of this gene in the testis and in female mice in the late stages of pregnancy remain to be studied. A hormonal influence on Q5k transcription could be envisaged.

Recently, Seo et al. (1992) demonstrated protein expression of a Q5k determinant cross-reactive with antibodies specific for Qa2 alpha two and alpha three domains. Similarly, in our studies, a Qa-like determinant reactive with D3.262 (Qa2 alpha three domain specific) was detected in the cytoplasm of neonatal thymocytes until approximately 2 weeks postpartum and in splenocytes and lymph node cells of peri-partum females. The Qa-like determinants were only detected in ethanol-permeabilized cells and not on the cell surface. Similar results were obtained with Saponin-permeabilised cells (data not shown). Absence of the Q5k-encoded heavy chain transport to the cell
surface, has also been seen upon transfection of the Q5\textsuperscript{k} gene into BHK cells; no cell-surface expression was detected on transfected clones using monomorphic H-2 class I specific antibodies, including DR.262 (Weiss et al., 1989). Several explanations might account for this finding. Some lines of evidence indicate that B2M and peptide ligand participate in the intracellular transport and surface expression of class I molecules and that the availability of peptides which can bind to class I molecules represents the limiting factor controlling cell surface expression (Townsend et al., 1989; Lie et al., 1991). Thus, it is conceivable that the lack of an appropriate peptide ligand might explain the observed aberrant expression of the Q5\textsuperscript{k} antigen and it remains to be determined whether available endogenous self or foreign peptides in some tissues might allow cell surface expression of Q5\textsuperscript{k}.

In conclusion, our studies show that Q5\textsuperscript{k} mRNA is detected in embryos from day 12 of development and is turned off within a few weeks after birth except in testis and thymus and it is expressed in female mice in the late stages of pregnancy. The possibility of hormonal regulation of H-2 Q expression is at present being investigated.

Acknowledgements

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References


