

IDENTIFICATION IN RAT LIVER AND SERUM OF WATER-SOLUBLE CLASS I MHC MOLECULES POSSIBLY HOMOLOGOUS TO THE MURINE Q10 GENE PRODUCT

BY SARAH C. SPENCER AND JOHN W. FABRE

From the Blond McIndoe Centre, Queen Victoria Hospital, East Grinstead, Sussex RH19 3DZ, United Kingdom

The MHC class I gene family comprises approximately 30 genes in the mouse (1, 2) with similarly large numbers of class I genes in the rat (3) and man (4). In addition, a cluster of class I genes outside the MHC has been reported recently in man (5). An intriguing aspect that has emerged in recent years is that at least some of the class I genes give rise to water-soluble and presumably secreted molecules. The potential functions of these secreted molecules adds an interesting new dimension to the physiological role of the class I gene family.

The first water-soluble class I MHC molecule to be characterized definitively was the product of the SWR (H-2^g) allele of the C57BL/10 (H-2^b) Q10 gene (6, 7). This molecule is synthesised exclusively in the liver and secreted into the serum (8), and has been identified in many species of wild mice throughout the genus *Mus* (9). It appears to be the only, or at least the major, soluble class I molecule in the serum of mice (10).

As regards other species, a 140 bp murine Q10-specific probe failed to react with DNA from the Wistar Furth (RT1^u) strain (9). Moreover, using rabbit antisera to denatured H-2K,D and HLA-A,B,C molecules, Lew et al. (11, 12) detected soluble class I MHC molecules in the sera of some equine species, and in the rhinoceros and tapir. More than 20 other species were negative, including the rat (Wistar Furth and Sprague Dawley strains) and man (11). These authors conclude that soluble class I molecules in serum have either arisen independently twice in mammalian evolution, or that the horse and mouse have retained a Q10-like gene lost during evolution by other species (12).

The data we present in this paper question these conclusions. We have been able to purify from aqueous extracts of rat liver, and in large quantities from rat serum, a soluble non-RT1.A class I molecule with many of the characteristics of the murine Q10 class I molecule. Moreover, in conjunction with our previous work (13) it is clear that the DA rat strain expresses two distinct water-soluble class I molecules in its serum, an RT1.A class I molecule and a Q10 type class I molecule, the latter being present in much higher concentrations.

Materials and Methods

Animals. Inbred DA (RT1^{av1}), PVG (RT1^c), LEW (RT1^l), BN (RT1ⁿ), WAG (RT1^u), and SHR (RT1^k) male rats were obtained from OLAC 1976 Ltd., Bicester, United

S. C. Spencer is a Rank Foundation Scholar.

Kingdom. Normal serum from the congenic LEW.RT1^a and LEW.RT1^u strains and the parent LEW (RT1^b) strain were kind gifts of Dr. K. Wonigeit, Hannover Medical School, Hannover, Federal Republic of Germany.

Antibodies. The mouse anti-rat class I monoclonal antibodies F16-4-4 (14), MN4-91-6 (15), and MRC OX18 (16) have previously been described in detail. F16-4-4 is directed at a monomorphic determinant of rat RT1.A class I MHC antigens (13). MN4-91-6 is directed at a polymorphic determinant of the RT1.A class I molecules, the RT1.A^a and RT1.A^k strains being positive, and many other strains negative. The MRC OX18 antibody is directed at a monomorphic determinant of class I antigens, but has not been characterized in terms of locus specificity. The cell line secreting MRC OX18 was a kind gift of Dr. A. F. Williams, Cellular Immunology Unit, Oxford, United Kingdom. All three monoclonal antibodies were used as immune ascites partially purified by ion-exchange chromatography. The R2/15S, R2/10P, JY1/98, JY1/232, YR1/11, and YR1/100 rat monoclonal antibodies to RT1.A^{av1} were a kind gift of Dr. G. Butcher, Monoclonal Antibody Centre, Cambridge, United Kingdom, and have been previously described in detail (17).

LEW anti-DA spleen sera were prepared by immunizing LEW rats with DA spleen cells in CFA, as described previously (18).

Immunoabsorbent-purified rabbit F(ab')₂ anti-mouse F(ab')₂ (RAM)¹ was prepared as previously described (19), and iodinated using the chloramine T method.

A sheep anti-mouse Ig preparation and a rabbit anti-rat F(ab')₂ preparation were obtained by passing antisera down G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) gel permeation columns, and pooling the Ig fractions.

Aqueous Extracts of Liver. Livers were removed within a few minutes of death, frozen immediately, and stored at -40°C until used. On thawing, an equal volume of Tris-buffered saline (TBS) (0.025 M Tris, 0.15 M NaCl, 0.02% NaN₃, pH 7.3 at 4°C) containing proteolytic inhibitors (2.5 mM iodoacetamide, 5 mM EDTA and 2 mM PMSF) was added. The tissue was minced in a Waring blender and then mechanically homogenized using a Teflon pestle. The resulting homogenate was centrifuged at 35,000 g for 20 min, and the supernatant was removed and frozen until further use. For affinity chromatography, the aqueous extract was thawed, and centrifuged at 80,000 g for 75 min. The supernatant was then centrifuged a second time at 80,000 g for 75 min before application to monoclonal antibody affinity columns.

All of the above procedures were at 4°C or on ice.

Serum. Blood was collected into tubes without anticoagulant, allowed to clot, and the serum was separated after 1-2 h at room temperature or overnight at 4°C. The serum was then frozen at -40°C until further used. It was thawed and centrifuged at 80,000 g for 75 min before application to monoclonal antibody affinity columns.

Red Blood Cell (RBC) Suspensions. These were prepared by exsanguinating a rat into a syringe containing 300 U of heparin. The blood was washed twice in 0.15 M NaCl and then twice in PBS by centrifuging at 500 g for 10 min and removing the buffy coat and upper layer of RBC after each wash. All cell preparations were done at 4°C or on ice.

Tube Binding Assay. This was performed essentially as described by Morris and Williams (20). All procedures were at 4°C or on ice. Duplicate 25 µl samples to be assayed were transferred to LP3 tubes (Luckam Ltd., Sussex, United Kingdom). 25 µl of target DA RBC at 10⁹ cells/ml in 0.5% BSA in PBS was added to each tube, and this was incubated for 1 h on ice. The cells were then washed twice in 0.1% BSA/PBS, and 100 µl of ¹²⁵I-labelled RAM in 0.5% BSA/PBS (~300,000 cpm/tube) was added to the pellet of the second wash. This was resuspended and incubated for 1 h on ice, and the cells were washed twice as above. The pellet of the second wash was resuspended in 0.5 ml of PBS, transferred to fresh LP3 tubes, and the target cell-bound radioactivity was measured in a gamma counter (LKB Produkter, Bromma, Sweden).

Plate Binding Assay. 25 µl of sample to be assayed was added in duplicate to wells in a 96-well polyvinylchloride microtiter plate (Dynatech, Sussex, United Kingdom). This was incubated overnight to allow any class I MHC antigens present in the sample to adsorb to

¹ Abbreviations used in this paper: RAM, rabbit anti-mouse F(ab')₂; TBS, Tris-buffered saline.

the plastic. The sample was then removed, and the remaining protein binding sites were blocked by incubating the wells with 200 μ l of 5% BSA/PBS for 30 min. This was removed, and the wells washed three times with 0.1% BSA/PBS. The wells were then incubated for 1 h with 25 μ l of an appropriate dilution of antibody in 0.5% BSA/PBS. The antibody was removed, the wells washed three times in 0.1% BSA/PBS, and 50 μ l of 125 I-labelled RAM containing 300,000 cpm was added. After a further 1 h on ice, the wells were washed three times, individual wells cut out, and the bound radioactivity measured.

Quantitative Absorption Analyses. These were performed as previously described (13). Absorptions using normal rat sera or soluble antigen preparations were centrifuged at 6,000 *g* for 5 min before assay. Again, all procedures were on ice or at 4°C.

Chromatography. Monoclonal antibody affinity, lentil lectin affinity, and gel permeation chromatography were performed as previously described (13). After monoclonal antibody affinity chromatography, class I preparations were dialyzed against TBS and then passed down 5-ml columns of Sepharose 4 B to which sheep anti-mouse Ig was covalently coupled. This was to remove any trace of mouse immunoglobulin that might have been eluted from the affinity column. The class I antigens prepared from serum were contaminated with rat immunoglobulin, presumably because of the high concentration of immunoglobulin in the starting serum. These were removed by passing the antigen down a 5-ml column to which rabbit anti-rat F(ab')₂ had been coupled.

SDS-PAGE. Samples were analyzed on slab gels, essentially as described by Laemmli (21).

For preparative elution of class I heavy chains for sequencing, several precautions were necessary in performing the electrophoresis to avoid blocking of the NH₂ terminus. The gels were cast using freshly prepared reagents and left to gel overnight. They were then preelectrophoresed (with loading buffer containing bromophenol blue as marker) until the dye front had passed through the gel. In addition, 7 mg of the sodium salt of thioglycolic acid was added to the 600 ml of cathode buffer to act as a scavenger. Finally, recrystallized SDS was used for casting gels, making up loading buffers and eluting the gel slices.

Fluorescein-labelled molecular mass markers were electrophoresed in adjacent wells to localize the heavy chains of the class I molecules on the slab gel. The appropriate section of the gel was excised, macerated, covered with eluting solution (0.1% recrystallized SDS) and left at 4°C for 24 h with occasional mixing. The eluting solution was harvested, and the eluting procedure repeated. The pooled eluted sample was dialyzed extensively against 0.02% SDS at room temperature, lyophilized, and then processed for sequencing.

Amino Acid Sequence Determination. The amino acid sequencing was kindly performed by Dr. Bryan Dunbar and Professor John Fothergill at the Department of Biochemistry, University of Aberdeen, United Kingdom, using a gas-phase sequencer (Applied Biosystems, Cheshire, United Kingdom) essentially as previously described (22). The thiohydantoin derivatives of the amino acids were identified by HPLC on Apex Cyano (25 \times 0.5 cm) columns (Jones Chromatography, Mid-Glamorgan, United Kingdom) using acetate buffer (pH 5.0)/acetonitrile isocratically with a 254-nm detector.

Protein Estimations. These were done essentially as described by Lowry et al. (23) using crystalline BSA as a standard.

Results

Our strategy for trying to identify the rat homologue of the Q10 gene product contained three elements. First, we relied on our previous demonstration that the monomorphic mouse anti-rat class I mAb F16-4-4 and the polymorphic mouse anti-rat class I mAb MN4-91-6 both react only with RT1.A class I antigens and not with other class I antigens (13, 15). This meant that both F16-4-4 and MN4-91-6 affinity columns could be used to deplete preparations of RT1.A class I molecules, but that any non-RT1.A class I molecules present in the preparations should be left behind.

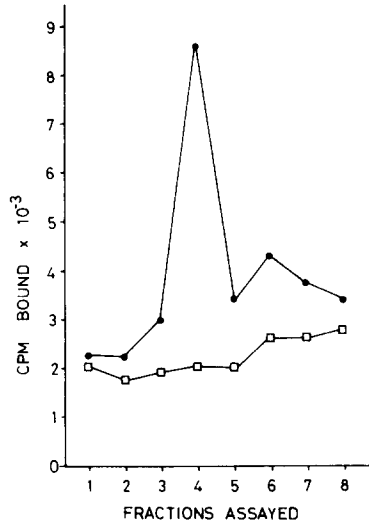


FIGURE 1. Putative water-soluble non-RT1.A class I antigens in rat liver. Ultracentrifuged aqueous extracts of DA liver were depleted of water-soluble RT1.A class I MHC antigens by passage down MN4-91-6 mAb affinity columns, and were then loaded on to MRC OX18 mAb affinity columns. The MRC OX18 column was then eluted at high pH, and the eluted fractions were tested for class I antigens using the MRC OX18 (●) and F16-4-4 (□) antibodies in plate-binding assays. Ordinate (cpm bound) refers to ¹²⁵I RAM bound to the wells of the polyvinylchloride plate.

Second, we wished to identify an mAb that might be broadly crossreactive with the class I gene products of the rat. In the mouse, two such antibodies have been described that react with both H-2K,D and Qa antigens (24). On reviewing the literature, we were particularly interested in the transfection experiments of Diamond et al. (25) which suggested that the MRC OX18 antibody might react with non-RT1.A membrane-bound class I molecules, and was therefore worth evaluating from the point of view of crossreactivity with putative rat Qa antigens and the rat Q10 gene product. As we shall show, this crossreactivity does exist.

Finally, because the Qa antigens of the mouse appear to be expressed predominantly as membrane-bound molecules on T cells, and because our studies would concentrate on serum and aqueous extracts of liver, we felt that we would be concentrating on a narrow range of non-RT1.A class I antigens, with a high probability of detecting a Q10-like homologue, if such existed, in the rat.

Demonstration of Putative Water-soluble, Non-RT1.A Class I Antigens in Rat Liver. In the initial experiments, ultracentrifuged aqueous extracts of DA liver, which had been depleted of water-soluble RT1.A class I antigens by MN4-91-6 antibody affinity columns, were passed down MRC OX18 affinity columns. As can be seen in Fig. 1, substantial amounts of antigen could be eluted from the MRC OX18 column, and this reacted with the MRC OX18 antibody, but not the RT1.A-specific F16-4-4 antibody. This was consistent with the presence in the initial preparation of two different class I molecules: a class I molecule that carries both the MRC OX18 and the MN4-91-6 determinants and which has been characterized previously as a water-soluble form of RT1.A antigens in liver (13); and a second class I molecule, which carries the MRC OX18 determinant only, and is a putative, soluble, non-RT1.A class I molecule in liver.

A more detailed analysis was made by passing ultracentrifuged aqueous extracts of DA liver down an MRC OX18 affinity column, eluting the bound class I antigens (which should include both RT1.A and non-RT1.A molecules), and then passing these class I antigens down an MN4-91-6 column. The results of quantitative absorption analyses are given in Fig. 2, and demonstrate clearly the

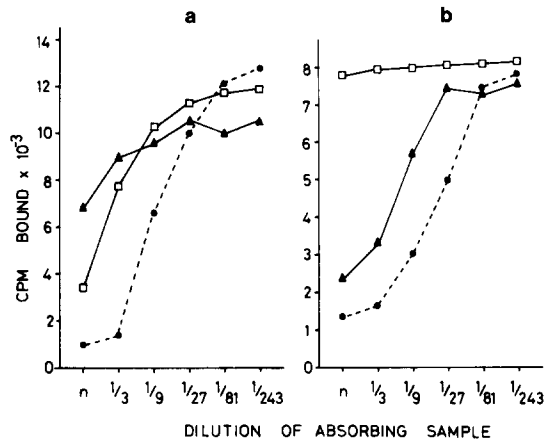


FIGURE 2. RT1.A and putative non-RT1.A class I antigens in aqueous extracts of rat liver. Ultracentrifuged aqueous extracts of DA liver were passed down MRC OX18 mAb affinity columns, and the bound class I antigens eluted. The eluted class I antigens (\bullet), the same preparation after depletion by passage down MN4-91-6 monoclonal affinity columns (\square), and the class I antigens eluted from the MN4-91-6 affinity columns (\blacktriangle) were analyzed by quantitative absorption analyses. In *a*, the absorptions were with the MRC OX18 antibody, and in *b* with the MN4-91-6 antibody. The absorbed antibodies were assayed in binding assays with fresh DA RBC as targets. Ordinate (cpm bound) refers to ^{125}I RAM bound to the target RBC.

presence of antigens unreactive with the RT1.A-specific MN4-91-6 antibody, but still strongly reactive with the MRC OX18 antibody. Fig. 2*a* shows that passage through the MN4-91-6 column resulted in a three- to fourfold reduction in the absorptive capacity of the initial class I preparation for the MRC OX18 determinant. It is difficult to make precise statements, but this result suggests that the extract contained more soluble RT1.A class I antigens than putative non-RT1.A class I antigens.

Demonstration of a Water-soluble Putative Non-RT1.A Class I Antigen in Rat Serum. 30 ml of ultracentrifuged DA serum was passed sequentially down MN4-91-6 and MRC OX18 columns. Very large amounts of class I antigen activity could be eluted from the MRC OX18 column, the amounts being much larger than could be eluted from the MN4-91-6 column that preceded it. Because the class I preparations from serum contained significant amounts of rat Ig, these preparations were passed down rabbit anti-rat $\text{F}(\text{ab}')_2$ affinity columns to remove this contaminant. Fig. 3 gives the results of assays on fractions of the class I preparations from the MN4-91-6 (Fig. 3*a*) and MRC OX18 (Fig. 3*b*) columns after they had passed through the anti-rat $\text{F}(\text{ab}')_2$ column. The data in Fig. 3 suggest that the water-soluble RT1.A class I antigens described in serum in a preceding paper (13) form only a minor component of the class I antigens in rat serum, the major component being the putative non-RT1.A class I molecule.

Purification and Biochemical Characterization of Putative Water-soluble Non-RT1.A Class I Antigens from Liver and Serum. The water-soluble RT1.A class I antigen from liver, and the putative non-RT1.A class I antigen from both liver and serum were further purified by lentil lectin affinity and gel permeation chromatography. The bulk of the RT1.A class I preparation from liver bound to lentil lectin as reported previously (13), and most of both the liver and serum putative non-RT1.A class I preparations did so (data not shown). On Sepharose CL-6 B gel permeation chromatography, (Pharmacia Fine Chemicals, Uppsala, Sweden) the putative non-RT1.A class I antigen from both liver and serum eluted late, giving a discrete peak virtually identical to and at the same position as the water-soluble RT1.A class I antigens described previously (13).

All three samples were analyzed by SDS-PAGE and the results are given in

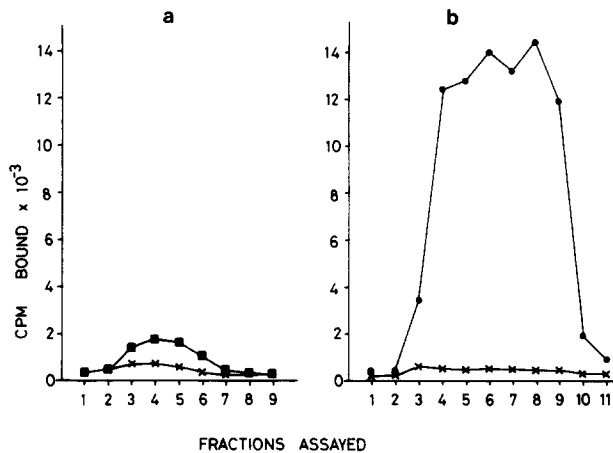


FIGURE 3. RT1.A and putative non-RT1.A class I antigens in rat serum. 30 ml of ultracentrifuged DA serum was passed sequentially down an MN4-91-6 mAb affinity column and then down an MRC OX18 mAb affinity column. The bound class I antigens were eluted from each column, and each preparation was then passed down a rabbit anti-rat F(ab')₂ column to remove contaminating rat immunoglobulins. The depleted fractions were collected from the anti-rat F(ab')₂ column and analyzed for class I antigens using the plate-binding assay. In *a*, the class I antigens originally eluted from the MN4-91-6 column were analyzed, using the MN4-91-6 antibody (■) or buffer control (×). In *b*, the class I antigens originally eluted from the MRC OX18 column were analyzed, using the MRC OX18 antibody (●) or buffer control (×). Ordinate (cpm bound) refers to ¹²⁵I RAM bound to the wells of the polyvinylchloride plate.

Fig. 4. All the samples had bands at $\sim 12,000 M_r$, presumably representing β_2 microglobulin. Fig. 4*a* clearly demonstrates that the putative non-RT1.A class I preparation from liver (lane *b*) had a heavy chain distinctly larger than that of the RT1.A class I preparation from liver (lane *a*) the M_r being 41,000 and 40,000, respectively. This supports the suggestion that we are dealing with two distinct class I molecules. Fig. 4*b*, lane *a* shows the putative non-RT1.A class I antigen purified from serum. The heavy chain is very similar in size to the putative non-RT1.A class I molecule from liver (lane *b*), although a slight difference in mobility is seen.

The amount of non-RT1.A class I antigen that could be purified from 20–30 ml batches of DA serum was $\sim 20 \mu\text{g/ml}$ of starting serum, as measured by the Lowry method (22). Thus the level of this class I molecule in DA serum is probably in excess of $20 \mu\text{g/ml}$, which is similar to the serum levels that have been reported for the Q10 class I molecule in the mouse (10).

NH₂ Terminus Amino Acid Sequence of the RT1.A and Putative non-RT1.A Class I Molecules. The bulk-purified class I antigens were subjected to preparative SDS-PAGE, primarily to remove the β_2 microglobulin, and the heavy chains were submitted to NH_3 terminus sequence analysis. The results are given in Fig. 5, and there are several points to note. First, all three of our molecules gave sequences with very high homology to murine and human NH_3 -terminal MHC class I sequences (26). This strongly suggests that the putative non-RT1.A class I molecules described in this paper are coded for within the rat MHC.

Second and most important, there are two amino acid substitutions when the putative non-RT1.A class I molecule from serum is compared to the RT1.A

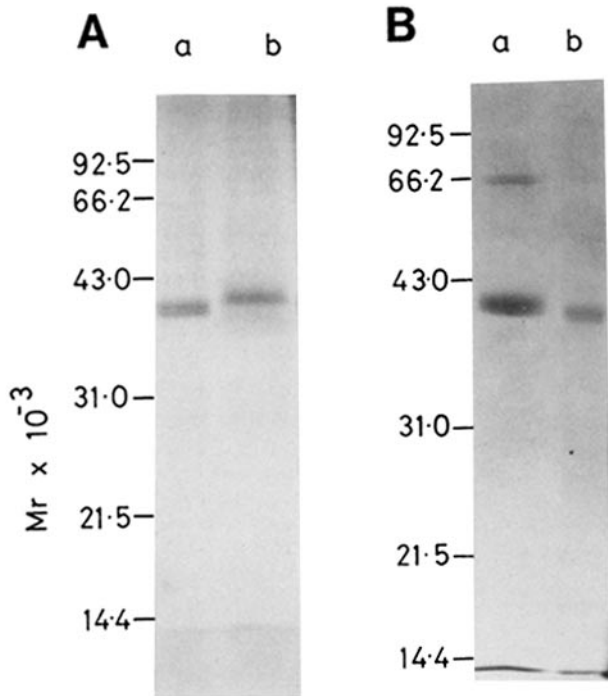


FIGURE 4. SDS-PAGE analysis of water-soluble RT1.A and non-RT1.A class I antigens from liver and serum. RT1.A class I antigens eluted from MN4-91-6 affinity columns, and putative non-RT1.A class I antigens (from preparations depleted of RT1.A class I antigens by passage down MN4-91-6 columns) eluted from MRC OX18 affinity columns were further purified by lentil lectin and gel permeation chromatography. In A, the RT1.A class I antigens (lane a) and the putative non-RT1.A class I antigen (lane b) from ultracentrifuged aqueous extracts of DA liver were analyzed on 12.5% polyacrylamide gels under reducing conditions. In B, the non-RT1.A class I antigens from ultracentrifuged extracts of DA liver (lane b) and from ultracentrifuged serum (lane a) were analyzed on a 10% polyacrylamide gel under reducing conditions. The gels were stained with Coomassie Blue. The molecular weight standards were: phosphorylase b (92,500); bovine serum albumin (66,200); ovalbumin (43,000); carbonic anhydrase (31,000); soybean trypsin inhibitor (21,500); and lysozyme (14,400).

	1				5					10
RT1.A class I	Gly	Ser	His	Ser	Leu	Arg	Tyr	Phe	Tyr	Thr
Q10-like (serum)	Gly	Ser	His	Ser	Leu	Arg	Tyr	Phe	His	Thr
Q10-like (liver)	Gly	Ser	His	Ser	Leu	X	Tyr	Phe	His	Thr
	11				15					20
RT1.A class I	Ala	Val	Ser	Arg	Pro	Gly	Leu	Gly	Glu	X
Q10-like (serum)	Ala	Val	Ser	Arg	Pro	Gly	Leu	Gly	Glu	Pro
Q10-like (liver)	Ala	Val	X	X	X	Gly	Leu	Gly	X	X
	21				25					
RT1.A class I	X	X	X	Ala	Val					
Q10-like (serum)	Arg	Phe	Ile	Ser	Val					
Q10-like (liver)	X	X	X	X	X					

FIGURE 5. NH₃-terminal amino acid sequence of RT1.A class I molecules and water-soluble putative non-RT1.A class I molecules from serum and liver of the DA rat strain. Some serines were identified mainly by their dehydroalanine derivatives.

class I molecule. There is a Tyr → His substitution at position 9, and an Ala → Ser substitution at position 24. This result clearly demonstrates that this class I molecule is the product of a non-RT1.A class I gene.

The putative non-RT1.A class I antigen from liver also has the Tyr → His substitution at position 9. The sequence for the non-RT1.A class I molecule

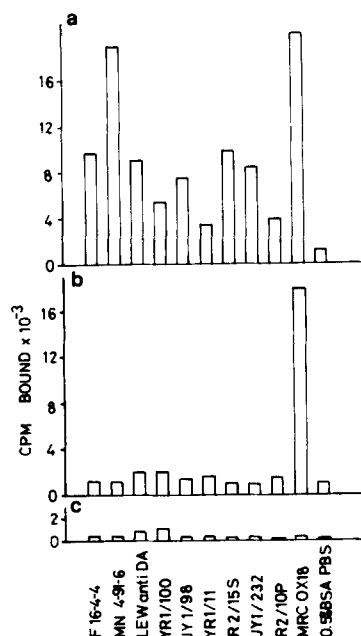


FIGURE 6. Antigenic determinants on RT1.A and non-RT1.A class I antigens. Water-soluble RT1.A class I antigens from ultracentrifuged extracts of DA liver (*a*) and non-RT1.A class I antigens from ultracentrifuged DA serum (*b*) were analyzed by plate-binding assays with saturating concentrations of the antibodies indicated. LEW anti-DA indicates a LEW anti-DA spleen alloserum. Results with control wells incubated with buffer instead of antigen are shown in *c*. Ordinate (cpm bound) refers to ¹²⁵I RAM bound to each well of the polyvinylchloride plate.

from liver did not extend as far into the molecule as was possible for the molecule from serum because of the smaller quantities available.

All three preparations gave single sequences consistent with the amount of protein loaded onto the sequencer. There was no evidence for heterogeneity, even with the non-RT1.A molecule from serum, where it was possible to load large amounts of protein. This result suggests that the large amounts of non-RT1.A class I molecules in serum are the products of a single gene.

In the class I sequences of the mouse, Leu at position 12 and Ala-Ile-Ser at positions 16–18 are characteristic of T1a sequences, but these were not seen in our class I sequences (26).

Finally, it is worth noting that the data presented in Fig. 5 is the first sequence available for the NH₃ terminus of rat class I antigens, apart from early and incomplete radiosequencing of radiolabelled RT1^a class I antigens precipitated by immune allosera (27). This latter approach assigned residues to 11 of the first 25 positions, all of which are in agreement with our RT1.A sequence.

Serological Characterization of the Non-RT1.A Class I Antigens. We wished to characterize the non-RT1.A class I molecules from the point of view of a large number of antigenic determinants known to be present on RT1.A class I molecules of the DA strain. The results given in Fig. 6*a* demonstrate that the RT1.A class I molecules, as expected, react with the MRC OX18 antibody, the RT1.A-specific mouse anti-rat antibodies F16-4-4 and MN4-91-6, LEW anti-DA spleen alloserum, and with all six monoclonal alloantibodies directed at determinants of the RT1.A^{av1} molecule. However, the non-RT1.A class I molecules from serum (Fig. 6*b*) react only with the MRC OX18 antibody.

The results for the non-RT1.A class I molecule from liver were virtually identical to those in Fig. 6*b*, and are not presented.

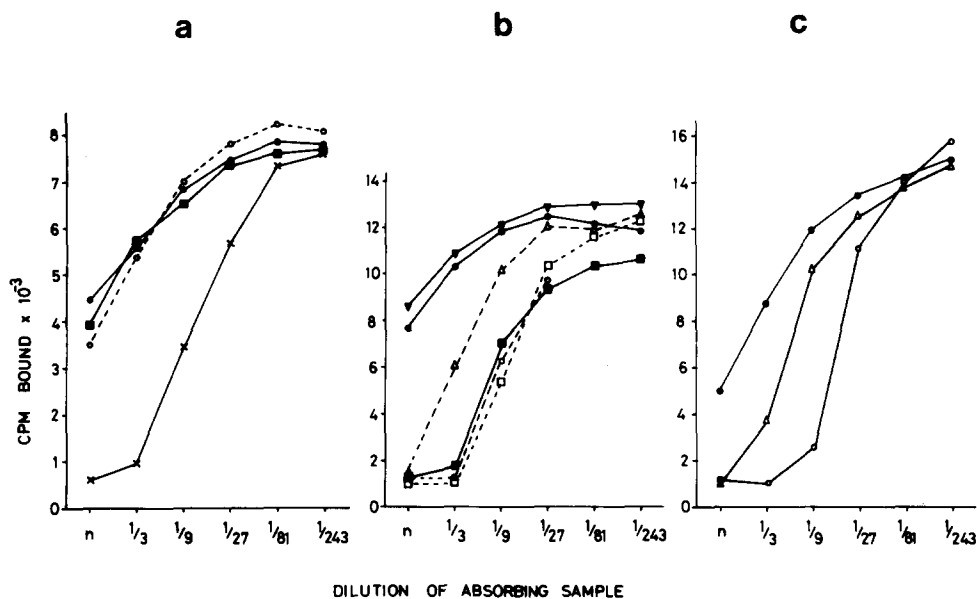


FIGURE 7. RT1.A and non-RT1.A class I MHC antigens in the serum of various rat strains. Sera were analyzed by quantitative absorption analysis with the RT1.A-specific mAb F16-4-4 (*a*) and with the MRC OX18 mAb (*b* and *c*). Tripling dilutions of sera from the following strains were used: DA (○), PVG (■), LEW (●), BN (□), SHR (▼) and WAG (△). Sera from LEW.RT1^a (○) and LEW.RT1^u (△) congenic rats were reanalyzed in *c*. In addition, a suspension of DA RBC at an initial concentration of 3×10^9 cells/ml was analyzed in *a* (×). The absorbed preparations were assayed in tube-binding assays using fresh DA RBC targets. Ordinate (cpm bound) indicates ¹²⁵I RAM bound to target RBC.

Non-RT1.A Class I Molecules Are Not Present on Liver Membranes. Liver homogenate was solubilized in the detergent Brij as described previously (13), and passed sequentially down F16-4-4 and MRC OX18 columns. Large amounts of RT1.A class I antigens could be eluted from the F16-4-4 column, but no class I antigens could be eluted from the MRC OX18 column (data not shown). This demonstrates that the water-soluble non-RT1.A class I molecules present in the aqueous extract of liver exist purely as a secreted form and do not have a membrane-bound counterpart.

Strain Variation and Possible MHC Control of Non-RT1.A Class I MHC Antigens in Serum. We have previously demonstrated that the rat has water-soluble RT1.A class I antigens in its serum (13), in addition to the non-RT1.A class I molecules identified in this paper. Fig. 7*a* gives the results of absorptions with the RT1.A-specific antibody F16-4-4, and it can be seen that sera from all three strains shown (RT1^a, RT1^c, RT1^l) had roughly similar absorptive capacities. The absorptive capacities were weak in relation to DA RBC. Similar results were obtained with the sera of WAG (RT1^u), SHR (RT1^k), and BN (RT1ⁿ).

Fig. 7*b* shows the absorptions with the MRC OX18 antibody. The RT1^a, RT1^c, and RT1ⁿ strains absorbed very strongly, while the RT1^l and RT1^k had only about one-twentieth this absorptive capacity. The RT1^u strain had intermediate absorptive capacity, about two- to threefold less than that of the best strains. Given that these strains all absorb the RT1.A-specific F16-4-4 antibody

approximately equally well, and only weakly, the strain differences seen in Fig. 7*b* almost certainly reflect differences in the serum levels of the non-RT1.A class I molecule described in this paper. Thus it is very likely that the RT1^l and RT1^k strains are weak expressors or nonexpressors of the putative rat homologue of the Q10 gene product, while the RT1^a, RT1^c, and RT1ⁿ strains are high expressors, and the RT1^u strain is an intermediate expressor.

We also wished to examine the sera of LEW, LEW.RT1^u, and LEW.RT1^a rats (kindly provided by Dr. K. Wonigeit, Hannover Medical School). The results are given in Fig. 7*c*, and show that the LEW.RT1^a was similar to the DA (RT1^a) strain, and the LEW.RT1^u was very similar to the WAG (RT1^u) strain. This suggests that the key factors controlling the expression of the non-RT1.A class I molecule in the serum of the rat strains we have studied lie within the MHC.

Discussion

In this paper, we demonstrate the presence of small, water-soluble, non-RT1.A class I molecules in the liver and serum of the DA strain rat. These class I molecules are distinct from the previously described water-soluble RT1.A class I MHC molecules (13) in terms of molecular mass, NH₂-terminal amino acid sequence, and the expression of polymorphic and monomorphic antigenic determinants. The non-RT1.A class I molecules are highly homologous to HLA and H-2 class I molecules, and differ from the RT1.A class I molecules at only two of the first 25 NH₂-terminal amino acid residues. There can be little doubt that these molecules are the product of a non-RT1.A class I gene within the rat MHC. Moreover, the presence of this molecule in large amounts (~20 µg/ml) in the serum of the DA and some other rat strains, the strain variation in its level of expression in serum, and the finding of what appears to be a similar molecule in aqueous extracts of DA liver (but not in detergent extracts of liver membranes) suggest that we have identified a liver-secreted class I molecule that is perhaps homologous to the murine Q10 gene product. However, identity of the liver and serum product remains to be formally established, and identification of Q10-type changes at the COOH terminal of our class I molecules will be necessary before definitive comments as regards homology with Q10 can be made.

The conclusion (11, 12) that the rat and many other species lack serum class I molecules was based on studies using rabbit antisera to denatured HLA-A,B,C and H-2K,D molecules. Such sera reacted with membrane-bound class I molecules of many species but did not react with normal sera from more than 20 species, except for strong reactions with some equine species. We could readily detect class I molecules in the sera of several rat strains, including WAG (RT1^u), whereas Lew et al. (11) obtained negative results with outbred Sprague Dawley and inbred Wistar Furth (RT1^u). It is possible that the WAG and Wistar Furth strains might differ in the Q region, although both are designated RT1^u. It also is possible that the assumption that species crossreactivity of the rabbit anti-HLA-A,B,C and -H-2K,D sera for the membrane-bound H-2K,D-type class I molecules should extend to Q10 homologues in serum could be wrong. For example, although there is nothing to distinguish the primary structures of Q region from H-2K,D region class I molecules, it remains possible that evolutionary constraints within the H-2K,D type class I genes are sufficient to allow rabbit anti-H-2K,D

sera to react with homologous, membrane-bound class I molecules in other species. However, for reactions with Q10 homologues in other species one requires evolutionary conservation of determinants in an additional dimension, i.e., within the putative homologues of the Q10 molecules in other species. This might not occur.

To date, three different types of water-soluble class I MHC molecules have been described. First, studies establishing the existence of water-soluble forms of the classical membrane-bound transplantation antigen have been done in man (28) and in the rat (13). Krangel (28) has identified mRNA species, for HLA-A24, lacking exon 5 (coding for the transmembrane domain), the clear inference being that this unusually spliced mRNA coded for the small HLA-A24 molecules. Second, there is the Q10 gene product, produced exclusively by the liver and found in large quantities in the serum of some mouse strains. The Q10 gene product exists purely as a secreted molecule because anomalies in exon 5 (coding for the transmembrane region) preclude membrane insertion. Finally, *in vitro* studies suggest that activated (but not resting) T cells in the mouse produce a water-soluble form of the Qa-2 molecule (29).

As regards other possible soluble class I molecules, it is interesting to consider the only class I DNA sequences available in the rat. Kastern (30) has sequenced the 3' end of two class I cDNAs from an insulin-secreting tumour, one of which is transcribed from a gene with a defective splice donor site at the exon 4/intron 4 junction. This has a premature stop codon 10 codons on the 3' side of the defective exon/intron boundary. If translated, this mRNA would result in a truncated and presumably secreted class I molecule with a hydrophilic stretch of nine amino acids immediately after the third external domain. This mRNA was found in the original insulin-secreting tumour, and in normal testis, but not in liver or kidney. However, Kastern was unable to detect a polyadenylation signal at the 3' end of the cDNA or the corresponding genomic clone, and it is therefore possible that this gene is transcribed into mRNA but not translated into protein (30).

Water-soluble class I molecules might also be produced by Q-region genes, such as the 27.1 gene of the BALB/c mouse, with premature stop codons in the regions coding for the transmembrane domain (31). The 27.1 gene is transcribed in spleen and kidney, as well as liver, but a polypeptide corresponding to this gene has not been identified (32).

Our results demonstrate that a Q10-like class I molecule does exist in the rat, and that one should perhaps reconsider the question as regards man and other species. In addition, given that we have found both RT1.A class I as well as Q10-type class I molecules in rat serum (13 and this paper) it is necessary to exclude the possibility that the equine serum class I molecules (11, 12) are products of alternate splicing of mRNA for the normal equine H-2K,D-type transplantation antigens.

Finally, it is worth noting that the MRC OX18 antibody has been used as a monomorphic anti-class I antibody, almost invariably with the assumption that its reactivity reflects that of the RT1.A class I transplantation antigens. However, the fact that it is broadly crossreactive with both RT1.A and non-RT1.A class I antigens, and that the tissue distribution and control of expression of these two

types of class I antigens will be different means that interpreting the results of serological, histological, and immunochemical studies with this antibody could be complicated.

Summary

We have identified large quantities of a water-soluble, non-RT1.A class I MHC molecule in the serum of the DA rat strain, with a similar molecule being found in aqueous extracts of DA liver. The non-RT1.A class I molecules have heavy chains of 41 kD, which is smaller than RT1.A class I molecules isolated from liver membranes (45 kD) but larger than water-soluble RT1.A class I molecules previously identified in serum and aqueous extracts of liver and kidney (40 kD). NH₃-terminal amino acid sequencing of bulk-purified RT1.A class I molecules and of this novel non-RT1.A class I molecule revealed two substitutions, in the first 25 amino acids, Tyr → His at position 9, and Ala → Ser at position 24. The non-RT1.A class I molecule did not react with any of the well-characterized polymorphic and monomorphic antibodies directed against RT1.A^a class I molecules, but did react with the MRC OX18 antibody. A similar class I molecule could not be identified on liver membranes. The non-RT1.A class I molecule was found in large quantities (~20 μg/ml) in the serum of the DA rat strain, and similarly large quantities appeared to be present in the sera of BN, PVG, and LEW.RT1^a rats. WAG and LEW.RT1^u rats had readily detectable but lower amounts of this molecule in their serum, while LEW and SHR rats had little if any present. This molecule probably represents the rat homologue of the murine Q10 gene product, and is the major class I product in the serum of the DA rat strain.

We thank Dr. Bryan Dunbar and Professor John Fothergill for performing the amino acid sequencing, and in particular for the time they spent in detailed discussions on the particular requirements of our experiments.

Received for publication 24 November 1986 and in revised form 26 February 1987.

References

1. Hood, L., M. Steinmetz, and B. Malissen. 1983. Genes of the major histocompatibility complex of the mouse. *Annu. Rev. Immunol.* 1:529.
2. Steinmetz, M., A. Winoto, K. Minard, and L. Hood. 1982. Clusters of genes encoding mouse transplantation antigens. *Cell.* 28:489.
3. Palmer, M., P. J. Wettstein, and J. A. Frelinger. 1983. Evidence for extensive polymorphism of class I genes in the rat major histocompatibility complex. *Proc. Natl. Acad. Sci. USA.* 80:7616.
4. Orr, H. T., and R. DeMars. 1983. Mapping of class I DNA sequences within the human major histocompatibility complex. *Immunogenetics.* 18:489.
5. Calabi, F., and C. Milstein. 1986. A novel family of human major histocompatibility complex-related genes not mapping to chromosome 6. *Nature (Lond.).* 323:540.
6. Cosman, D., M. Kress, G. Khoury, and G. Jay. 1982. Tissue-specific expression of an unusual H-2 (class I)-related gene. *Proc. Natl. Acad. Sci. USA.* 79:4947.
7. Mellor, A. L., E. H. Weiss, M. Kress, G. Jay, and R. A. Flavell. 1984. A nonpolymorphic class I gene in the murine major histocompatibility complex. *Cell.* 36:139.
8. Maloy, W. L., J. E. Coligan, F. Barra, and G. Jay. 1984. Detection of a secreted form

- of the murine H-2 class I antigen with an antibody against its predicted carboxyl terminus. *Proc. Natl. Acad. Sci. USA.* 81:1216.
9. Siwarski, D. F., Y. Barra, G. Jay, and M. J. Rogers. 1985. Occurrence of a unique MHC class I gene in distantly related members of the genus *Mus*. *Immunogenetics.* 21:267.
 10. Lew, A. M., W. L. Maloy, and J. E. Coligan. 1986. Characteristics of the expression of the murine soluble class I molecule (Q10). *J. Immunol.* 136:254.
 11. Lew, A. M., R. B. Valas, W. L. Maloy, and J. E. Coligan. 1986. A soluble class I molecule analogous to mouse Q10 in the horse and related species. *Immunogenetics.* 23:277.
 12. Lew, A. M., E. Bailey, R. B. Valas, and J. Coligan. 1986. The gene encoding the equine soluble class I molecule is linked to the horse MHC. *Immunogenetics.* 24:128.
 13. Spencer, S. C., and J. W. Fabre. 1987. Water-soluble form of RT1.A class I MHC molecules in the kidney and liver of the rat. *Immunogenetics.* 25:91.
 14. Hart, D. N. J., and J. W. Fabre. 1981. Major histocompatibility complex antigens in rat kidney, ureter and bladder. Localisation with monoclonal antibodies and demonstration of Ia-positive dendritic cells. *Transplantation (Baltimore).* 31:318.
 15. Milton, A. D., and J. W. Fabre. 1985. Massive induction of donor-type class I and class II major histocompatibility complex antigens in rejecting cardiac allografts in the rat. *J. Exp. Med.* 161:98.
 16. Fukumoto, T., W. R. McMaster, and A. F. Williams. 1982. Mouse monoclonal antibodies against rat major histocompatibility antigens. Two Ia antigens and expression of Ia and class I antigens in rat thymus. *Eur. J. Immunol.* 12:237.
 17. Diamond, A. G., A. P. Larkins, B. Wright, S. T. Ellis, G. W. Butcher, and J. C. Howard. 1984. The alloantigenic organisation of RT1.A^a, a class I major histocompatibility complex molecule of the rat. *Eur. J. Immunol.* 14:405.
 18. Hart, D. N. J., and J. W. Fabre. 1979. Quantitative studies on the tissue distribution of Ia and SD antigens in the DA and LEWIS rat strains. *Transplantation (Baltimore).* 27:110.
 19. Dalchau, R., and J. W. Fabre. 1979. Identification and unusual tissue distribution of the canine and human homologues of Thy-1 (θ). *J. Exp. Med.* 149:576.
 20. Morris, R. J., and A. F. Williams. 1975. Antigens on mouse and rat lymphocytes recognised by rabbit antiserum against rat brain: the quantitative analysis of a xenogeneic antiserum. *Eur. J. Immunol.* 5:274.
 21. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 227:680.
 22. Russell, G. A., B. Dunbar, and L. A. Fothergill-Gilmore. 1986. The complete amino acid sequence of chicken skeletal-muscle enolase. *Biochem. J.* 236:115.
 23. Lowry, O. H., N. J. Rosebrough, A. Farr, and A. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265.
 24. Sharrow, S. O., L. Flaherty, and D. H. Sachs. 1984. Serologic cross-reactivity between class I MHC molecules and an H-2-linked differentiation antigen as detected by monoclonal antibodies. *J. Exp. Med.* 159:21.
 25. Diamond, A. G., J. M. Windle, G. W. Butcher, A. Winoto, L. Hood, and J. C. Howard. 1985. Identification and expression of genes encoding rat class I and class II major histocompatibility complex molecules from two genomic cosmid libraries. *Transplant. Proc.* 17:1808.
 26. Klein, J., and F. Figueroa. 1986. The evolution of class I MHC genes. *Immunol. Today.* 7:41.
 27. Blankenhorn, E. P., J. M. Cecka, D. Goetze, and L. Hood. 1978. Partial N-terminal amino acid sequence of rat transplantation antigens. *Nature.* 274:90.

28. Krangel, M. S. 1986. Secretion of HLA-A and -B antigens via an alternative RNA splicing pathway. *J. Exp. Med.* 163:1173.
29. Soloski, M. J., J. Vernachio, G. Einhorn, and A. Lattimore. 1986. *Qa* gene expression: biosynthesis and secretion of *Qa-2* molecules in activated cells. *Proc. Natl. Acad. Sci. USA.* 83:2949.
30. Kastern, W. 1985. Characterisation of two class I major histocompatibility rat cDNA clones, one of which contains a premature termination codon. *Gene.* 34:227.
31. Steinmetz, M., K. W. Moore, J. G. Frelinger, B. T. Sher, F.-W. Shen, E. A. Boyse, and L. Hood. 1981. A pseudogene homologous to mouse transplantation antigens: transplantation antigens are encoded by eight exons that correlate with protein domains. *Cell.* 25:683.
32. Lalanne, J.-L., C. Transy, S. Guerin, S. Darche, P. Meulien, and P. Kourilsky. 1985. Expression of class I genes in the major histocompatibility complex: identification of eight distinct mRNAs in DBA/2 mouse liver. *Cell.* 41:469.