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Strain restricted typing sera for the use in the genetic monitoring of inbred strains of mice and rats from two danish SPF breeders

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Introduction

Even under optimal conditions in breeding facilities, it is necessary to control the inbred strains of laboratory rodents for genetic authenticity, as contamination of breeding stocks have been known to occur (*Krog* 1976, *Cramer & Silver* 1981, *Festing* 1982, *Penline et al.* 1982). Ideally the genetic monitoring method of choice should be fast, convenient, inexpensive and accurate (*Cramer* 1983).

Strain Restricted Typing Sera (SRTS) are antisera which are produced by immunizing one strain with lymphocytes from strains representing all other MHC-types in the same unit. The SRTS are described in assays combined with a complement dependent cytotoxicity test (*Festing & Totman* 1980, *Arn et al.* 1982, *Kendall & Wagner* 1985) and in an assay with hemagglutination (*Kendall & Wagner* 1985). Ideally the recipient strain should react toward all other strains in the unit.

Some of the advantages offered by the assay using SRTS are that the test is simple, reliable and repeatable (*Arn et al.* 1982). It is an inexpensive method and with some training one technician can monitor about 10 animals per day. Using this assay it is primarily the MHC haplotype which is monitored. This is an advantage, as the haplotype is important in many scientific experiments where laboratory rodents are used (*Günther* 1990). Further, it is an advantage that the antisera can be produced easily in the laboratory using the inbred strains which are bred on site (*Festing & Totman* 1980, *Kendall & Wagner* 1985). However, it must be pointed out that rodents used to produce SRTS must be genetically authentic for the SRTS produced to give valid results (*Kendall & Wagner* 1985).

The aim of this paper are to describe the production of SRTS for some of the most used inbred strains of mice and rats in Denmark, and to investigate the reaction pattern between strains having the same or different MHC haplotypes.

Materials and methods

Animals

For the production of the strain restricted typing sera (SRTS) animals from seven inbred strains of mice and five inbred strains of rats were used as recipients (sera producer). At the beginning of the experiment the age of the animals was about six weeks. As lymphocyte (spleen) donors for immunization, animals from seven inbred strains of mice and 16 inbred strains of rats were used (see Table 1 & Table 2). Age and sex were not kept constant for the donor animals. The strains were from the suppliers Bomholtgård Breeding and Research Centre Ltd. (Ry, Denmark) and Møllegaard Breeding Centre Ltd. (Ll. Skensved, Denmark), where they were bred under barrier conditions.

Antisera production

The SRTS were produced as described by *Festing & Totman* (1980). Spleens from the donors were removed and a single cell sus-

Table 1. Mice used as recipients and donors for production of SRTS.

Strain	Sex
Recipients	
A/J Bom	M
BALB/cJ Bom	F
C57BL/6J Bom	F
CBA/J Bom	M
NOD	M
NZW/N Bom	F
SJL/N Bom	M
Donor strains	
A/J Bom	
BALB/cJ Bom	
C57BL/6J Bom	
CBA/J Bom	
NOD	
NZW/N Bom	
SJL/N Bom	

Table 2. Rats used as recipients and donors for production of SRTS.

Strain	Sex
Recipients	
BB/Wor/Mol-BB	F
BB/Wor/Mol-WB	F
LEW/Mol	F
PVG/Mol	F
SHR/BM	F
Donor strains	
BB/Wor/Mol-BB	
BB/Wor/Mol-WB	
BN-Mol	
BUF/Mol	
Dahl-R/Mol	
Dahl-S/Mol	
LE/Mol	
LEW/Mol	
NEDH/Mol	
PVG/Mol	
SHR/BM	
SHR/Mol	
Sprd/Mol (Inbred)	
Stroke-Prone/Mol	
WF/Mol	
WKY/Mol	

pension was prepared in PBS (phosphate buffered saline) by cutting the spleens followed by filtration through gauze. Splenic lymphocytes were pooled from all the donor strains and injected intraperitoneally (i.p.) in the recipient animals.

The recipient groups consisted of seven mice or five rats of each strain. For each immunization, the recipients were injcted with pooled lymphocytes from the donor strains. For each rat immunization one spleen from all the donor strains was used per ten recipients, whereas for the mice, one spleen from all of the donor strains was used for all the recipient (altogether 49 mice).

The recipients received an injection of pooled lymphocytes once a week for five weeks. A week after the last injection, the animal were bled, the serum pooled within each strain and frozen at –20°C.

Preparation of test lymphocyte suspension

The animals were killed by cervical dislocation or in a carbon dioxide gas chamber. One or more lymph nodes were removed and placed in approximately 1 ml of cell media (RPMI 1640, hepes buffered (Sigma, St. Louis, USA), with 1 % glutamine and 0,3 % bovine serum albumin or 10 % fetal calf serum). The lymph nodes were teased apart with fine scissors and the cell suspension filtrated through cotton wool. The lymphocytes were washed twice in cell media, and then the cell suspension was adjusted to $2–3\times10^6$ cells per ml.

Complement dependent cytotoxicity assay

Two different types of assays were used in the cytotoxicity test. The one performed in U bottom microtiter plates and the other performed in Terasaki plates. In each assay all animals were tested with all the SRTS for the species tested. In each assay all animals were tested with all the SRTS for the species tested. In each assay, there was a positive and a negative control for the SRTS and for the complement (guinea pig serum).

Assay performed in microtiter plates. The assay was with some modifications performed as previously described (*Nielsen &*

Holst 1983, *Günther* 1990). Twenty µl of antisera diluted in PBS was pipetted into each well. Twenty µl of lymphocyte suspension was added, the suspension mixed well with a multipipette, then the microtiter plate was covered with tinfoil to avoid evaporation and incubated at 37°C for 30 min. Fifty µl of undiluted guinea pig serum was added, the suspension mixed well with a multipipette and then the microtiter plate was incubated at 37°C for 60 min. Twentyfive µl of 0.5 % trypanblue (Sigma, St. Louis, USA), in 0.9 % saline was added to about 10 wells at a time and mixed carefully with a pipette. After a couple of minutes the supernatant was carefully sucked up with a pasteur pipette to leave the cells laying on the bottom of the wells. Immediately after the removal of the supernatant, the proportion of dead cells (blue) among live cells (colourless) was determined by estimation, using an inverted microscope with 100 × magnification.

Assay performed in Terasaki plates. The assay in Terasaki plates (Life Technologies A/S, Roskilde, Denmark) was performed as described (*Terasaki* 1964, *Festing & Totman* 1980, *Günther* 1990) with some modifications dependent on the laboratory facilities. Two µl of antisera diluted in PBS was pipetted into each well. Two µl of lymphocyte suspension was added, the suspension was mixed by carefully shaking the plate and incubated at 37°C for 30 min. To

Table 3. Results of testing seven mouse strain restricted typing sera against lymphocytes from seven strains. Major histocompatibility haplotype in brackets. Obtained by testing in microtiter and Terasaki plates. The degree of cytotoxic activity is proportional to the pluses, where 0 is no cytotoxic activity and +++ is maximal cytotoxic activity. The quotation beneath the degree of cytotixic activity indicates the final dilution giving results.

	Strains in which SRTS were produced						
Lymphocyte donor	A/J (a)	BALB/cJ (d)	C57BL/6J (b)	CBA/J (k)	NOD (g)	NZW/N (z)	SJL/N (s)
A/J (a)	0 1:4	+++ 1:4	+++ 1:4	+++ 1:8	+++ 1:32	+++ 1:4	+++ 1:16
BALB/cJ (d)	++-+++ 1:4	0 1:4	+++ 1:16	+++ 1:8	+++ 1:32	+++ 1:8	+++ 1:16
C57BL/6J (b)	++-+++ 1:4	+++ 1:4	0 1:4	+++ 1:8	+++ 1:32	+++ 1:4	+++ 1:16
CBA/J (k)	+-++ 1:4	+++ 1:8	+++ 1:4	0 1:4	+++ 1:32	++ 1:4	+++ 1:8
NOD (g)	++-+++ 1:4	++ 1:4	+++ 1:4	++-+++ 1:8	0 1:4	+++ 1:4	+++ 1:8
NZW/N (z)	++ 1:4	+++ 1:4	+++ 1:8	+++ 1:4	+++ 1:32	0 1:4	+++ 1:16
SJL/N (s)	++-+++ 1:4	++ 1:4	++ 1:4	+++ 1:8	+++ 1:32	+++ 1:8	0 1:4
BALB/cA* (d)	++ 1:4	0 1:4	+++ 1:4	++ 1:8	+++ 1:32	+++ 1:4	+++ 1:8
C3H/HeJ* (k)	0-+ 1:4	+++ 1:4	++-+++ 1:4	0 1:4	+++ 1:32	+ 1:4	+++ 1:8
C57BL/10ScJ* (b)	+++ 1:4	+++ 1:8	0 1:4	+++ 1:8	+++ 1:32	+++ 1:4	+++ 1:16
DBA/2J* (d)	+++ 1:4	0 1:4	+++ 1:8	+++ 1:16	+++ 1:32	+++ 1:8	+++ 1:16

* Only 1–2 animals per strain tested. The recipient animals are not immunized against these strains.

avoid evaporation from the wells, water was placed in the bottom of the plate. Then 5 µl of undiluted guinea pig serum was added to each well, the plate was carefully shaken and incubated for 60 min. Two µl of 7 % Eosin Y (Sigma, St. Louis, USA), was then added and the plate shaken. Within half an hour, the reading of the assays began, using an inverted microscope with phase contrast at 100 × magnification. In each well, the proportion of dead cells (dark) among live cells (bright and smaller) was determined by estimation.

Evaluation of the results.

In the microtiter plates the negative control was accepted when the proportion of dead cells was less than 15 %. In the Terasaki plates, 20–25 % dead cells were accepted.

The degree of cytotoxic activity was evaluated as 0 = cell death equivalent to the cell death observed in the negative control, + = 40–60 % cell death, ++ = 60–80 % cell death and +++ = 80–100 % cell death.

Results

Most of the results given in Table 3 and Table 4 were tested in microtiter plates, as there were some technical problems with the use of the Terasaki plates. In the Terasaki plates, negative control for SRTS was 15–50 % dead cells, where it generally was less than 10 % in the microtiter plates. The tests performed in Terasaki plates, which reacted satisfactory, gave a cell death similar to the cell death in microtiter plates about the same dilution of antisera.

The results presented in Table 3 and Table 4 were obtained by testing at least two and usually four animals from each strain with each of the SRTS for the species.

As seen in Table 3, within the seven strains of mice used for the immunization (all with different H-2 haplotype), the serum tested against target cells from the same strain gave a negative result in all cases, as predicted. Among the four extra strains tested which were not used in the immunizing of the recipient animals, it can be seen that except

Table 4. Results of testing five strain restricted typing sera against lymphocytes from five strains obtained by testing in microtiter and Terasaki plates. Major histocompatibility haplotype in brackets. The degree of cytotoxic activity is proportional to the pluses, where 0 is no cytotoxic activity and +++ is maximal cytotoxic activity. The quotation beneath the degree of cytotoxic activity indicates the final dilution giving results.

	Strains in which SRTS were produced				
Lymphocyte donors	BB/BB (u)	BB/WB (u)	LEW (l)	PVG (c)	SHR/BM (k)
BB/BB (u)	0 1:4	0 1:4	+++ 1:32	++-+++ 1:8	+++ 1:8
BB/WB (u)	0 1:4	0 1:4	+++ 1:32	++ 1:4	++ 1:8
LEW (l)	++ 1:4	+++ 1:16	0 1:4	+++ 1:16	+++ 1:16
PVG (c)	+++ 1:8	+++ 1:32	+++ 1:64	0 1:4	+++ 1:16
SHR/BM (k)	+++ 1:4	+++ 1:32	+++ 1:64	+++ 1:16	0 1:4
LE* (u)	0 1:4	0 1:32	+++ 1:32	++ 1:8	++-+++ 1:16

* Only one animal of the strain tested.

for C3H/HeJ, the SRTS reacted with lymphocytes from strains with another H-2 haplotype than the haplotype from the immunized strain.

From Table 4, it can be seen that the same pattern of reaction occur within the rat strains as for the mice. The serum tested against target cells from the same strain gave a negative result, and serum tested against target cells from a strain with the same haplotype as the test serum also gave a negative result. Another interesting thing in Table 4 is that the c MHC-haplotype (PVG) especially but also the k MHC-haplotype (SHR/BM) had difficulties in reacting with the u MHC-haplotype, but that there were no problems the other way around as the BB/WB is one of the best antisera producers among the five strains immunized.

As seen in Table 3 and Table 4, the sera concentration needed depended on which strain the antisera were produced in and which strain was tested. It is seen that among the mouse strains, A/J, BALB/cJ and NZW/N were not too good in producing SRTS, whereas NOD was much better than the other strains. Among the rats BB/BB was a poor producer of SRTS, which was expected as the strain is immunodeficient. Especially LEW but also BB/WB were good in producing SRTS.

Discussion

In the experiments reported in this paper, it was not possible to distinguish strains which have the same MHC-haplotype, while *Festing & Totman* (1980) and *Kendall & Wagner* (1985) could partly distinguish within the same haplotype. If a genetic contamination was detected it would not have been possible to find the source of contamination, as the test lymphocytes would have been killed by all the test sera if the contaminant had been a F_1-hybrid.

Two different assays were used for the cytotoxicity test. The one carried out in microtiter plates gave the most stable results, but was more laborious than the assay carried out in the Terasaki plates. Moreover, the assay carried out in the Terasaki plates did not use so much SRTS and other reagents, and it was faster to carry out. But the assay did not work optimally, the background death of lymphocytes being 15–50 %, which is unacceptably high. One reason could be that the quantities when using Terasaki plates are very low, resulting in either lack of nutrients, pH changes or a general toxicity of one of the reagents.

The reason, why our assay was less succesful when performed in Terasaki plates might be due to the fact that the trays were incubated at 37° rather than at room temperature. This may account for the high background rate of cell death. Preliminary filling of the wells with paraffin oil might also have improved the results.

Reactions of e.g. the mouse strains A/J and NZW and the rat strain BB/BB are weak. These results might have been improved by testing with undiluted serum as done by *Festing et al.* (1980).

In the assay carried out in the Terasaki plates, it seems as if lymphocytes from some strains were less capable of surviving than lymphocytes from other strains (e.g. C57BL/6J, C57BL/10Sc and NOD among the mouse strains, and BB/BB and BB/WB among the rat strain did not survive well). The antisera concentration needed was about the same in the two methods.

SRTS produced in A/J and NZW/N reacted badly with lymphocytes from C3H/HeJ, but this could possibly be explained by these two sera being weak.

When comparing the concentration of SRTS needed for the cytotoxicity test, it was seen that the rat SRTS were more concentrated than the mouse SRTS. This could be because the rats were immunized with approximately five times as many lymphocytes as the mice.

In the experiments here reported, it was possible to distinguish strains with different haplotype by means of SRTS combined with a complement dependent cytotoxicity assay

primarily carried out in microtiter plates, but it was not possible to distinguish strains within the same haplotype. This means that the use of SRTS combined with a cytotoxicity assay is a valuable tool in the genetic monitoring in combination with other methods e.g. other serological and biohemical markers.

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Summary
Strain restricted typing sera (SRTS) were produced for some of the most used inbred mouse and rat strains in Denmark by injecting lymphocytes pooled from several different strains intraperitoneally into the recipients. The SRTS were combined with a complement dependent cytotoxicity assay. It was possible to distinguish animals from strains with different MHC haplotypes but not animals from strains with the same MHC haplotype. It was concluded that the SRTS combined with a complement dependent cytotoxicity assay could distinguish between some of the most commonly used inbred strains of rats and mice in Denmark.

Sammendrag
Stamme begrænset type sera (SRTS) blev produceret for nogle af de mest brugte indavlede muse- og rotte-stammer i Danmark, ved at injicere lymfocytter blandet fra forskellige stammer intraperitonealt i recipienterne. SRTS'et blev brugt i en komplement afhængig cytotoxicitetes test. Det var her muligt at adskille dyr fra stammer med forskellige MHC haplotyper, men ikke dyr fra stammer med den samme MHC haplotype. Det blev konkluderet, at SRTS kombineret med en komplement afhængig cytotoxicitets test kan skelne mellem nogle af de mest brugte rotte- og muse-indavlsstammer i Danmark.

References
Arn JS, SE Riordan, D Pearson & DH Sachs: Strain restricted typing sera (SRTS) for use in monitoring the genetic integrity of congenic strains. I. Imm. Met. 1982, *55,* 141–153.
Cramer DW & WK Silver: Workshop report. Genetic uniformity in inbred rat strains. transpl. proc. 1981, *13,* 1492–1493.
Cramer DV: Genetic monitorings techniques in rats. ILAR News 1983, *XXVI,* 15–19.
Festing MFW & P Totman: Polyvalent strain-specific alloantisera as tools for routine genetic control of inbred and congenic strains of rats and mice. Lab. Anim. 1980, *14,* 173–177.
Festing MFW: Genetic contamination of laboratory animal colonies: An increasingly serious problem. ILAR News 1982, *25,* 6–10.
Günther E: Immunological markers. In "Genetic monitoring of inbred strains of rats", HJ Hedrich, Ed., Gustav Fischer Verlag, Stuttgart, New York 1990.
Kendall C & JE Wagner: Characterization of strain specific typing antisera for genetic monitoring of inbred strains of rats. Lab. Anim. Sci. 1985, *35,* 364–369.
Krog HH: Identification of inbred strains of mice, *Mus Musculus.* I. Genetic control of inbred strains of mice using starch gel-electrophoresis. Biochem. Genet. 1976, *14,* 319–326.
Nielsen IM & H Holst: Cytotoxicity test, using monoclonal H-2 antibodies in the genetic control program for mice. GV-SOLAS XXI meeting, Münster 1983.
Pennline KP, JP Smith & H Bitter-Suermann: My kingdom for an inbred rat. Transplantation 1982, *34,* 70.
Terasaki PI & JD McClelland: Microdroplet assay of human serum cytotoxins. Nature 1964, *204,* 998–1000.