Development and characterization of the IOR/Hab inbred mouse strain

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Summary

The IOR/Hab inbred mouse strain, developed and maintained in the National Institute of Oncology and Radiobiology, is the first inbred strain to be developed in Cuba. It has a high fertility in this tropical climate. A number of physiological characteristics such as longevity, haematological characteristics, and growth are reported. Other experiments and results concerning spontaneous tumour frequency suggest that the strain is well adapted to standard environmental conditions, and could be useful for biomedical research.

Keywords: New inbred mouse strain; High fertility; Genetic quality control; Spontaneous tumour frequency; Eye malformations

Inbred mouse strains have been widely employed for studies of cancer pathogenesis, the development of new and improved anti-tumour drugs, and host–tumour interactions (Martin *et al.*, 1984; Kallman *et al.*, 1985). Usually, inbred mouse and rat strains are very susceptible to poor environmental conditions, resulting in a poor reproductive performance (Festing, 1979). Several experiments, mainly with laboratory animals, have been carried out to improve fecundity by selecting for large litter size at birth (Falconer, 1971; Eklund & Bradford, 1977; Wallinga & Bakker, 1978).

The development of a new inbred mouse strain at the National Institute of Oncology and Radiobiology (INOR) was started several years ago, in order to obtain a strain which would be better adapted to the local environment and which demanded minimal maintenance and breeding requirements.

The result is an inbred mouse strain, named IOR/Hab after the Institute of Oncology and Radiobiology, with the subline symbol Hab (Castillo, 1982).

The strain has been characterized according to its genetic quality, fertility, and normal ranges for a variety of the most common spontaneous diseases.

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Materials and methods

Animals

Mice used in these studies were IOR/Hab inbred mice, both male and female, raised and maintained from a conventional closed mouse colony in the Tumoral and Experimental Animal Department. The IOR/Hab mouse has been inbred since 1970 by strict sister–brother matings and with selection of mice for large litter size. At present, this strain has had 65 generations of brother–sister mating and is descended from a random-bred population of albino mice that had been maintained for some years in our Institute. All selected breeding mice had a body weight of between 18–22 g (6–8 weeks) and were in apparent good health when the studies were initiated. All mice were housed in standard plastic cages with autoclaved wood shaving and stainless steel wire tops. They were fed with commercial mouse diet, and drinking water, acidificated with HCl to pH 2·5–2·8, was

provided *ad libitum* in glass or plastic bottles with metal canulas.

Genetic quality control
Two methods were used to characterize the strain: skin-grafts and coat colour where the frequency of pigmentation genes was studied using several IOR/Hab × DBA/2Hab matings and IOR/Hab × YT matings. The latter mating was performed and analysed in the Yurlavo Laboratory of Experimental Animals, Moscow.

DBA/2Hab and YT were used for these studies due to the presence of recessive coat colour genes *a* and *b* in DBA/2 and *p* in YT. Strain DBA/2 was originally obtained several years ago from Institute of Experimental and Clinical Oncology, Moscow.

Potential and actual fertility
Thirty pregnant females of about 18 days gestation were autopsied and the numbers of corpora lutea (CL), living embryos (LE) and dead embryos (DE) were determined. Mortality before (MB) and after (MA) zygote implantation (ZI), as well as general mortality (GM), were calculated from the following formula:

$$MB = \frac{CL-ZI}{CL} \quad MA = \frac{DE}{ZI} \quad GM = \frac{CL-LE}{CL}$$

The breeding performance of 43 females was determined by means of the litter size of each female during the first 7 births. The weight of post-weaning mice was noted periodically until they reached 1 year of age.

Haematological determinations
Individual blood samples were collected by cardiac puncture and exsanguination under ether anaesthesia. Blood was centrifuged at 2°C for 15 min at 2500 rpm.

All haematological and clinical chemistry determinations were done by micromethod tests, used routinely in the clinical laboratory.

Spontaneous tumours and other diseases
A total of 86 breeding females (BC), 77 virgin females (VF) and 52 males were kept until just before death, then autopsied under anaesthesia.

Statistical analysis
Differences in body weight, haematology values, clinical chemistry values and life-span were evaluated with Student's *t*-tests.

Results and discussion

Genetic quality control
Five separate skin-grafting experiments have been conducted. No differences were detected for the histocompatibility genes except in the first genetic control in 1977.

Since 1979 (generation 33–34) all 31 mice have accepted a total of 74 grafts. All 50 hybrids with DBA/2 had agouti colour and it was concluded that the IOR/Hab strain is homozygous for the ABcD genes. However, the eyes were an intense red, therefore F1 offspring from IOR/Hab × YT matings were analysed and it was concluded that IOR/Hab is genetically homogeneous, with coat colour genes ABcDp.

Potential and actual fertility
Table 1 shows the results for potential fertility and embryo mortality. A high percentage of living embryos and low mortality before and after implantation are evident.

Table 1. Potential fertility and embryo mortality in the IOR/Hab inbred mouse strain

Females	30
Corpora lutea (mean ± SD)	9·5 ± 2·3
Living embryos (mean ± SD)	9·2 ± 2·3
Percentage of the mortality:	
MB[a]	1·2
MA[b]	2·0
GM[c]	3·2

SD standard deviation; [a]mortality before implantation; [b]mortality after implantation; [c]general mortality.

Among 43 breeding females, mean litter size was 7·3±3 live pups with a pre-weaning mortality of 2·1%. The high percentage of living offspring and the low mortality before and after weaning are evident too.

This strain also reaches a greater weight at a younger age than other inbred mouse strains imported to this laboratory (data not showed).

These results show that the IOR/Hab strain has high fertility, low mortality and rapid growth after birth in tropical environmental condition. These characteristics were reached after about 25 generations and have been maintained until today.

Haematological findings

The total erythrocytes, leucocytes, platelets and reticulocyte counts, haemoglobin concentration and percentages and packed cells volumes are shown in Table 2. In general, there were no significant differences in the haematological values between female and male IOR/Hab mice.

Total marrow cell counts, erythroid, myeloid, and lymphoid elements are shown in Table 3. The total marrow cell counts in males were slightly lower than in females although there were no significant differences between them.

Table 2. Haematology values for female and male IOR/Hab mice

	Female	*Male*
Red blood cells ($\times 10^6$ cells/ml)	9·07±1·6[a] (32)[b]	8·97±1·5 (38)
White blood cells; ($\times 10^3$ cells/ml)	10·09±3·1 (32)	10·21±3·3 (38)
Granulocytes ($\times 10^3$ cells/ml)	1·71±0·9	1·8±0·9
Lymphocytes ($\times 10^3$ cells/ml)	8·21±2·4)	8·09±3·0
Platelets ($\times 10^5$ cells/ml)	4·95±0·7	4·95±0·8
Reticulocytes ($\times 10^4$ cells/ml)	5·04±2·3	4·46±1·9
Haemoglobin (g/dl)	14·02±1·1	13·81±1·0
Packed cell volumen (%) (haematocrit)	50·12±3·1	50·39±1·8

[a]Mean±SD; [b]number of mice.

Table 3. Percentages of bone marrow haematologic values for female and male IOR/Hab mouse strain

	Female	*Male*
Total bone marrow cells (10^6 cells/ml)	1·53±0·4[a] (47)[b]	1·29±0·34 (48)
Bone marrow cells[c] composition (%):		
Proerithroblast	0·2	0·15
Erithroblasts:		
basophils	1·5	1·3
polichromatic	3·3	4·0
orthochromatic	25·0	35·1
Myeloblast	0·5	1·0
Promyelocytes	1·7	0·5
Myelocytes	2·6	1·5
Metamyelocytes	2·5	2·0
Stabskerniger	12·0	10·0
Neutrophils	31·0	33·7
Lymphoblasts	0·7	0·3
Prolymphocytes	3·7	1·2
Lymphocytes	15·1	9·0
Myeloid/erithroid	1·6	1·2
Myeloid/lymphoid	2·5	4·6

[a]Mean±SD; [b]number of mice; [c]bone marrow cell composition is studied in 200 cells and arithmetic mean expressed in percentage.

Compared to other reports, the total leucocyte counts for both sexes was high. However, in previous studies the strains were maintained under strict pathogen-free conditions, which may be the reason for the low total leucocyte counts (Frith *et al.*, 1980). The haematocrit values in the inbred mouse strain IOR/Hab (6–8 weeks) were higher than other strains and slightly low for the haemoglobin content (Frith *et al.*, 1980). Other studies showed that haematocrit values decreased with age in both sexes and some investigators speculated that the decreased haematocrit with ageing resulted primarily from a reduced number of erythrocytes (Finch *et al.*, 1969; Frith *et al.*, 1980).

Clinical chemistry

The values of the serum glucose, urea, nitrogen, creatinine, total protein, albumin, calcium, sodium, potassium, inorganic phosphorus, and glutamic pyruvate transaminase (GPT) are shown in Table 4. Clinical chemistry values for males and females in IOR/Hab mice were not significantly different although males

Table 4. Clinical chemistry values for female and male IOR/Hab mouse strain

	Female	*Male*
Glucose (mg/dl)	135·9±21·4[a] (44)[b]	138·5±14·8 (35)
Urea nitrogen (mg/dl)	42·9± 6·8	42·8±10·2
Creatinine (mg/dl)	1·0± 0·1	1·0± 0·4
Total protein (g/dl)	5·8± 0·4	5·5± 0·4
Albumin (g/dl)	3·2± 0·2	3·1± 0·3
Calcium (mg/dl)	9·9± 2·7	8·7± 1·7
Inorganic phosphorous (mg/dl)	7·9± 1·8	12·5± 4·3
Sodium (Meq/l)	142·2±17·2	152·7±14·8
Potassium Meq/l	6·3± 1·7	7·8± 1·2
GPT (u/l)[c]	29·7± 7·2	21·3± 3·4

[a]Mean ± SD; [b]number of mice; [c]glutamic piruvic transaminase.

showed higher inorganic phosphorus levels than females.

The haematological and clinical chemistry aspects have been evaluated and described with the aim of establishing a standard reference for future experiments.

Spontaneous tumours

Table 5 shows the mean life-span in male, VF, and BF mice. No differences between mean life-span in tumour-bearing mice and tumour-free mice of the same group were found. However, the VF groups has a higher mean life-span than the male and BF groups. Overall mean life-span was 578 ± 179 days.

Table 6 shows that the spontaneous tumour frequency in IOR/Hab mice is relatively low (37·2%). A greater tumour frequency was found in female than male mice, i.e. 47·6% (BF) and 40·2% (VF) versus 14·8% for males. BF had more malignant tumours than the VF but the last group had a greater probability of acquiring benign tumours. Spontaneous tumours were observed in this strain between 400 and 650 days old, except in the males where there was greater variability. These results were compared with published data (Staats, 1985) and showed that this strain has an intermediate tendency to acquire spontaneous lymphoproliferative malignant diseases and mammary gland tumours for BF.

Other spontaneous diseases

Table 7 shows the most common diseases observed in this strain. The most important condition being amyloidosis, associated fundamentally with the malignant processes and chronic nephritis in old mice.

Eye malformations also appear in about 95% of the IOR/Hab mice over 1 year old.

Results with inbred IOR/Hab strain with their hybrids (IOR/Hab × C57BL/6Hab)F1 and these mice mating with the IOR/Hab females and C57BL/6Hab males (back-cross matings), suggest that fundamentally the eye malformations are in the lens (cataract) and that this pathology may be caused by a recessive mutation. Verrusio and Frazer (1966), described an autosomal mutant gene in the inbred A/Jax mouse strain, with intermediate

Table 5. Mean life-span of IOR/Hab mice

		Mean ± SD (days)				
Group	*n*	*With tumours*	*n*	*Without tumours*[1]	*n*	*Weighted mean*
VF	14	608±150	63	646±106	77	639±115[2]
BF	28	532±128	58	552±137	86	546±133
M	7	616±339	45	530±268	52	542±276

[1]Differences in life-span between with and without-tumour groups were not statistically significant at $P = 0{\cdot}05$.
[2]The VF group lived significantly ($P < 0{\cdot}01$) longer than the other two groups.

Table 6. Spontaneous tumour frequency depending on histologic type, the organ and where it appeared

	VF[a] *n*	*77*[b] %	*BF*[c] *n*	*86*[b] %	*Male*[d] *n*	*54*[b] %	*Total* *n*	*(215)*[b] %
Lymphoma-leukaemia	10	13	12	14	6	11·5	28	13
Mammary gland	1	1·4	13	15·1	0	0	14	8·6
Lung	2	2·6	2	2·3	1	1·5	5	2·3
Liver	1	1·4	0	0	0	0	1	0·5
Sex organs	2	2·6	3	3·5	1	1·9	6	2·8
Benign	15	19·0	11	12·7	0	0	26	12·0
Total	31	40·2	41	47·6	8	14·8	80	37·2

[a]Virgin females; [b]number of mice; [c]breeding females; [d]males.

Table 7. Spontaneous frequency of the non-tumour pathologies found in the IOR/Hab mice

	VF[a] *n*	*77*[b] %	*BF*[c] *n*	*86*[b] %	*Male*[d] *n*	*52*[b] %	*Total* *n*	*(215)* %
Amyloidosis and glomerulosclerosis	41	53·3	21	24·4	26	50	88	40·9
Pyelonephritis	23	30	17	19·8	18	34·6	58	26·9
Ovary cysts	30	39	14	16·3	–	–	44	27
Cystic hiperplasia of the endometrium	11	14·3	3	3·5	–	–	14	8·6

[a]Virgin females; [b]number of mice; [c]breeding females; [d] males.

dominance which caused degeneration of the fibres of the ocular lens followed by cataract.

The observed characteristic in spontaneous tumours and other disease frequency in the inbred mouse strain IOR/Hab should be taken into consideration in experimental cancer research and other experiments.

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