NIH:rnu; are they all created equal? A comparison of Taconic and Charles River rat strains

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INTRODUCTION

History

The first observation of the athymic hairless mutation was in 1953 in a colony of outbred hooded rats at the Rowett Research Institute in England and was designated rnu. The colony died out in the 60s but the mutant gene was not lost and homozygous mutants were recovered in the mid 1970s. Independently, a similar mutant arose in a colony of albino rats in New Zealand and was named New Zealand nude rats with the assignment nznu. The National Institutes of Health (NIH) nude rat was developed in 1979-80 through a series of crosses in which the rnu gene was added and backcrossed into eight inbred rat strains: BN/SsN, NR/N, BUF/N, WN/N, ACI/N, WKY/N, M520/N and F344/N. The Taconic source rats were obtained from the NIH in 1981 and were derived by hysterectomy in 1987 and again in 1998, designation NTac: NIH-Whn. The Charles River source rats were obtained from the NIH in 2001 and cesarean rederived, designation Crl:NIH-Foxn1rnu. Animals are outbred, rnu/rnu males crossed with rnu/+ females as the homozygous females do not successfully produce/rear offspring. The nude rat is athymic and T cell deficient; it does have a normal complement of B and NK cells. There are reports that nude rats develop "T-like" cells as they age; status and function of these cells is not clear as there is variability in the available references. Nude rats have been used as models for tumor studies and for investigations of immune mechanism particularly as applicable to the field of organ transplantation. (1 - 3)

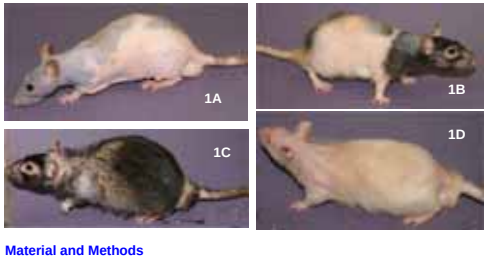
Nude Rats in Pre-clinical Models

We have been using nude rats here at Pfizer for about six years, primarily for Tumor xenograft studies. Our studies have included implantation of xenografts not only in the subcutaneous compartment but also in the primary (prostate, breast, liver) and metastatic (bone, liver) compartments. Over the course of our work with nude rats we have observed a high degree of inconsistency in "take-rate" of xenografts regardless of the compartment or vendor source. We have used rats from both Taconic and Charles River and have observed a marked difference in the appearance (hair coat and pattern) between the two sources and also a qualitative difference in take-rate between sources.

Objectives

The goal of these studies was to characterize the phenotype of the immune status of the Taconic and Charles River source nude rat in order to more fully understand the variability that we have observed and set up the best environment for productive application of the nude rat to our research goals.

Figures 1A-1D. Showing different appearance of Nude rats. 1A Charles River, 1B Charles River, 1C Taconic, 1D Taconic



Material and Methods

Animals

Nude male rats, 6 – 8 weeks old were obtained from Charles River (Fall River, MA) designation (Crl:NIH-Foxn1rnu) or from Taconic (Germantown, NY) designation (NTac:NIH-Whn) and were maintained according to the NIH standards established in the "Guide for the Care and use of Laboratory Animals". Normal CD rats, designation (Crl:CD(SD)) from Charles River were included for comparison in immunopheotyping experiments. The Internal Animal Care and Use Committee (IACUC) approved all experimental protocols. Rats were pair housed in polycarbonate micro-isolator cages lined with autoclaved bedding. Autoclaved reverse osmosis (RO) water and autoclaved standard rat chow were provided *ad libitum*.

Immunophenotyping

Sample Preparation:

Blood was collected in EDTA by jugular bleed and kept at room temperature until labeled with appropriate antibodies. A multi-cocktail four-color immunophenotyping strategy was used to enumerate the relative percentage of lymphocyte subpopulations of mononuclear leukocytes. Peripheral blood leukocytes were labeled using a whole blood lysis method. Briefly, 100ul of whole blood was aliquoted per tube per test and incubated for 30 minutes at room temperature protected from light. Following incubation, the red blood Cells were lysed using BD FACSTM Lysing Solution (BD Biosciences) and washed with phosphate buffered saline. Samples were immediately acquired using a BD Biosciences FACSCalibur flow cytometer.

Flow Cytometer Instrument Settings:

An argon-ion laser (488nm excitation) was used for FITC, PE, and PerCP fluorophores. FITC (green fluorescence) was collected in Fluorescence detector 1 (FL1530/30 bandpass filter). PE (orange fluorescence) was collected in Fluorescence detector 2 (FL2585/42 bandpass filter). A red-diode laser (635 nm excitation) was used for APC measurement in FL3 (red fluorescence; 661/16 band-pass filter). Lymphoid cells were selected by a light scatter gating (forward vs. side scatter), while myeloid cells positive for OX41 were excluded from the analysis by Boolean gating.

Statistics:

Results were analyzed using student's unpaired T-test and/or ANOVA with a confidence interval of 5%.

Antibodies:

Biotin conjugated mouse monoclonal [OX41] to SIRP-α (cat.no. ab33987, Abcam Inc., Cambridge, MA) was used in conjunction with Streptavidin PerCP (cat.no. 554064; BD Biosciences) to identify myeloid lineage cells (macrophages, monocytes, granulocytes, dendritic cells). This antibody pair was included with each lymphocyte-specific cocktail. The three lymphocyte-specific antibody cocktails used for phenotypic analysis included the Rat T/B/NK Cell Cocktail, Rat T Lymphocyte Cocktail, and Rat Activated Lymphocyte Cocktail (558495, 558493, 558494 respectively; BD Biosciences). Antibodies present in the T/B/NK Cocktail include CD3-APC (T-cells), CD45Ra-FITC (B-cells), and CD161a-PE (NK cells). The T Lymphocyte Cocktail contains CD4-PE (Helper T-cells), CD8a FITC (Cytotoxic/Supressor T-cells), and CD3-APC (all T-cells). The antibody cocktail specific for Activated T Lymphocytes consists of CD3-APC (all T cells), CD25-PE (Lymphoblasts and Dendritic cells), and RT1B-FITC (MHC Class II expressing cells). Fluorescence compensation settings were established using the Rat Compensation Set kit (558517) and the Rat Activation Compensation Set kit (558512, BD Biosciences).

Each experiment consisted of six animals per condition in addition to control cells from adult normal rats. All three antibody cocktails were used to characterize the lymphocyte subset phenotype of each animal. Variability between tubes was determined by comparing the CD3 positive cell population independently identified with each of the three antibody cocktails.

Tumor Take-Rate and Kinetics

HepG2 human hepatocarcinoma cells, 22RV1 human prostate tumor cells, Colo 205 colorectal tumor & MDA-MB-231 human breast cancer cells (American Type Culture Collection, Manassas, VA) or Huh7.5 human hepatocarcinoma cells (Apath, LLC; St. Louis, Missouri) were RAP tested, expanded under standard cell culture conditions and implanted either subcutaneous in matrigel or by direct injection in the tibia, liver or prostate in media. Endpoints included: body weight; tumor size (caliper method) and serum biomarkers (jugular bleed). The human specific biomarkers, human IL 8 and prostate specific antigen (PSA) were measured in serum by ELISA (R & D Systems, Minn., MI) and on an Immulite system (Siemans, Los Angelus, CA),respectively.

Results

Immunophenotyping

The lymphocyte subpopulations were identified by their unique antigen binding cell surface receptors which mediate the immune response by defining specificity, diversity and self/non-self recognition. Three primary populations were identified; B cells which are the antigen presenting cells, T cells of which there are two primary types, cytotoxic CD 8+and helper (CD4+) that release cytokines critical in immune response and the natural killer (NK) cells which respond to and kill tumor cells.

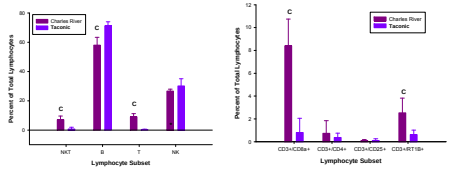
In addition, a small population of cells (NKT) that was positive for both the T and NK markers was identified; function of these is not known. The activated T cell population was further defined as CD25+ lymphoblasts and dendritic cells and RT1B+ MHC Class II expressing cells.

Data demonstrated that normal CD rats have significantly higher values for NKT, T, CD8+, CD4+ and CD25+ than either strain of homozygote nude rats while both strains of homozygote nudes have higher B and NK cell values than the normal CD rats. The two strains of nudes show differences: CR source nudes have higher NKT, T, CD8a+, and RT1B+ levels while Tac source nudes have higher B levels than CR. Experiment was repeated with a second group of rats at the same age to confirm results.

Figure 2. Shows lymphocyte subset results analyzed by flow cytometry

Type	NKT	B	T	NK
CR CD	2.84 ± 0.59 [A]	40.22 ± 5.28 [A]	46.63 ± 2.60 [A]	6.93 ± 2.81 [A]
CR -/-	7.10 ± 2.50 [C]	58.03 ± 5.43 [C]	9.05 ± 2.12 [C]	26.61 ± 1.39
Tac -/-	0.71 ± 1.24	71.24 ± 2.74	0.34 ± 0.28	30.12 ± 4.97
	CD8a+	**CD4+**	**CD25+**	**RT1B+**
CR CD	15.39 ± 1.05 [A]	31.13 ± 2.07 [A]	2.88 ± 0.19 [A]	1.51 ± 0.32 [C]
CR -/-	8.41 ± 2.34 [C]	0.74 ± 1.11	0.11 ± 0.08	2.54 ± 1.29 [C]
Tac -/-	0.81 ± 1.25	0.38 ± 0.37	0.11 ± 0.16	0.63 ± 0.39
	p < 0.05 vs.	**A** all -/-	**C** Tac-/-	

Figure 3. Shows lymphocyte subset results in Charles River versus Taconic source nude rats



Tumor Take-Rate and Kinetics

Take-rate percentage of several human tumor cell lines was evaluated in the rat subcutaneous xenograft model; Huh 7.5 (human hepatocarcinoma), HepG2 (human hepatocarcinoma), MDA-MB-231 (human breast cancer), 22RV1 (human prostate cancer). Take-rate of all cell types were nearly 100% and were very similar when comparing vendor source. In addition, several orthotopic models were evaluated; Huh 7.5 injected directly into the liver, MDA-MB-231 injected directly into the tibia and 22RV1 injected directly into the prostate. Take-rates in the orthotopic models varied but were similar between compared strains of rats.

Figure 4. shows take-rate of various tumor types in several models

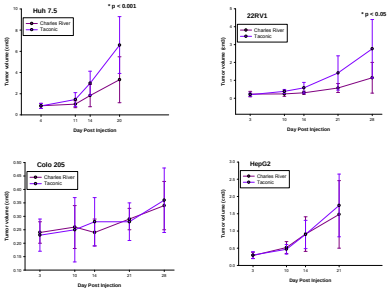
		Take-Rate %	
		Charles River	Taconic
Cell Type	**Location**		
Huh 7.5	SC	100	100
	Intra-hepatic	21.4	26.7
HepG2	SC	100	100
22RV1	SC	70	90
	Intra-prostate	70	60
MDA-MB-231	Intra-tibia	61	67
Colo 205	SC	80	70

Take-rate of the intra-tibial tumors was confirmed with Faxitron X-ray and intra-prostate take rate by prostate size and shape at necropsy. In addition human specific serum biomarkers were measured for the MDA-MB-231 intra-tibial tumors (IL-8) and for the 22RV1 intra-prostate tumors (PSA). Although take-rate was similar across strains, biomarker expression tended to be higher in the Taconic source rats than the Charles River source; IL-8 147.8 ± 137.2 vs. 30.9 ± 19.8 ng/ml; PSA 0.12 ± 0.07 vs. 0.06 ± 0.05 ng/ml.

Figure 5. Shows appearance of lytic MDA-MB-231 tumor when implanted in the tibia (Faxitron image)

Growth kinetics of subcutaneous tumors were examined by tracking tumor size measurements over a three to four week period. Of the tumor types studied, the Huh 7.5 and 22RV1 grew more rapidly and to a larger final size in the Taconic source rats. Terminal tumor weight correlated with caliper measure of tumor volume (data not shown).

Figure 6. Shows growth kinetics of subcutaneous xenografts where tumor volume was measured by caliper and calculated using the formula ((w x w)/2)*L) = TV



CONCLUSIONS

- Some differences in lymphocyte subset and activation markers were observed when comparing the Charles River to Taconic source of nude rats
- Take-rates of the cell types evaluated were very similar in rats from the two vendor sources
- Growth kinetics were different for some cell types, with more rapid growth observed in the Taconic versus Charles River source nude rats
- Biomarker expression (IL-8 & PSA) was higher in the Taconic source rats
- Vendor source may be an important consideration when using immunodeficient rats for pharmacological studies in oncology.

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