Mycobacterial Diseases

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Research Article

Inbred Mouse Strain Susceptibility to Tuberculosis Infection Vary with Phenotype, the Dose of Infection, Obesity and Composition of the Intestinal Microbiome

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ABSTRACT

In this work, we conducted comparative studies of inbred mice with different sensitivity to tuberculosis infection, I/St susceptible, C57BL/6 resistant. Two other strains C3H.JK (H2j), C3H/HeDiSn (H2k) began to grow fat by 4-5 months of age and changed their susceptibility depending on the dose of infection. Also, they were made obese or not in dependence on the dose of infection.

Keywords: *Mouse genetics; TB susceptibility; M. tuberculosis in mice; Obesity*

INTRODUCTION

In our laboratory, we have characterized more than forty strains of inbred mice for susceptibility to infection with *Mycobacterium tuberculosis* (*Mtb*). The mice demonstrated a wide range of tubercular susceptibility from highly susceptible I/St Strain to resistant A/Sn and C57BL6 [1-7]. These inter strain differences were reproducible both at high doses of intravenous infection up to 10^6 CFU [2,3], and at low doses of aerosol infection (100 CFU/lung) [8]. Another hyper-susceptible mouse strain C3HeB/FeJ was described be Kramnik's team [9]

In this case, the genes whose alleles determine the highest susceptibility to tuberculosis infection are localized in different parts of the mouse genome: H2Aβ gene in I/St mice in the 17th chromosome, and *Ipr1* gene was identified in chromosome 1 in C3HeB/FeJ [10]. As a rule, F1 hybrids show increased resistance in comparison with each of their parents [7].

We recently discovered in our mouse collection two strains with C3H genetic background demonstrating some unusual properties. Herein, we present the results of these studies with C3H.JK (H2j), C3H/DiHeSn (H2k) in comparison with I/St and C57BL/6 mice. Mice of the C3H.JK ($H2^j$), C3H/HeDiSn ($H2^k$) strains became obese prior to 3-4 months of age, whereas I/St and C57BL/6 mice strains did not do this even though they were on the same diet. In addition, C3H.JK ($H2^j$), C3H/DiHeSn ($H2^k$) mice changed the phenotype of susceptibility to tuberculosis infection and obesity depending on the dose of infection.

MATERIALS AND METHODS

M. tuberculosis strain H37Rv was originally obtained from the Institute Pasteur, Paris, France. Mycobacteria were passage through C57BL/6 mice to increase virulence. The final mycobacterial culture was washed in phosphate-buffered saline (PBS) with 0.05% Tween 80, resuspended in PBS with 0.01% BSA and 0.05% Tween 80, dispensed in aliquots into polypropylene vials, and frozen at -80°C. CFU of the frozen aliquots were determined after thawing and plating serial 10-fold dilutions on 7H10 agar plates [5].

Animals

Female inbread mouse strains I/St, C57BL/6, C3H.JK, C3H/DiHeSn were maintained in animal facility of CIT and (I/St x C3H.JK) F1, (I/St x C3H/DiHeSn) F1 and (C3H.JKx C3H/DiHeSn) F1 female were bred in the facility. Mice with initial body weight of 22-23 g were used. Mice were bred under conventional conditions at the Animal Facility of the Central Institute for Tuberculosis (CIT), Moscow, Russia in accordance with the guidelines from the Russian

Ministry of Health #755, INH Office of Laboratory Animal Welfare (OLAW). Water and food were provided at libitum. All experimental procedures were approved by CIT animal care committee (IACUC protocols #12, 13, 15).

Infection, Cfu Counts and Survival of Infected Mice

Mice were infected with different doses (see results) of *M. tuberculosis* H37Rv intravenously (0.2 ml via tail vein) or aerosol in Glas-Col aerosol camera (USA). Mortality was monitored daily starting at week 1 post-infection. To assess CFU counts, lungs from individual mouse were homogenized in 2.0 ml of sterile saline, and 10-fold serial dilutions were plated on Dubos agar (Difco) and incubated at 37°C for 20–22 days. Samples of intestinal flora were collected directly from the large intestine of two C3H.JK mice groups (5 per group): first with body weight of 20-22 g, and second with body weight 39-45 g. Samples were placed in Trizol, and the flora was analyzed using gastroflor diagnostic system based on RT-PCR

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RESULTS AND DISCUSSION

Mice of I/St (highly susceptible to *Mtb*) and C57BL/6 (resistant) strains reached body weight of 20-23 g and retained it for a long time while C3H.JK (H2*j*) and C3H/DiHeSn (H2^k) mice became obese prior to 3-4 months of age and with body weight reaching 35-45 g (Figure 1). After infection with low aerosol dose of *Mtb* (600 CFU/lung) resistant C57BL/6 mice maintained initial body weight till 250 days, while C3H/DiHeSn (H2k) mice reached about 40 g and highly susceptible I/St mice began to lose body weight at day 150 of infection and died by day of 250. C3H.JK (H2^j) mice started body weight decrease at day 200 and at day 275 all these mice were alive with starting average body weight of 25 g (Figure 2).

Mice of all these strains were tested at i.v. infection with 3 gradual high doses of *Mtb*: 5x10^4, 10^5 and 5x10^5 CFU. Survival curves presented in Figures 3-5 and Table 1 demonstrate that after infection with different doses of mycobacteria mice changed their relative susceptibilities. Thus, at highest dose 5x105 CFU mice of C3H/DiHeSn demonstrated highest susceptibility, median survival time equal 46±5 days. Both I/St and C3H.JK strains demonstrated similar but slightly higher level of survival (86±23 and 80±32 days respectively). At a dose of 5x10^{4}CFU I/St mice became the most susceptible, and C3H.JK (H2^j) and C3H/DiHeSn (H2^k) mice became more resistant and survived longer (Table 1). C57BL/6 mice remained resistant with all doses of infection and did not die at all. So, at relatively low dose of infection I/St mice have demonstrated highest TB susceptibility, C57BL/6 mice were tuberculosis resistant, and mice with obesity were significantly more resistant than I/St mice.

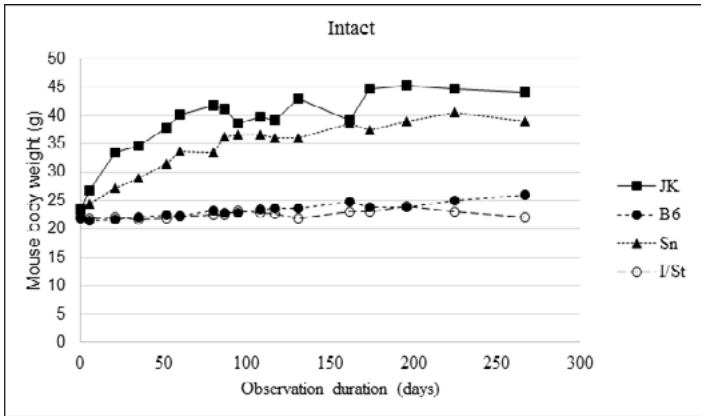

Figure1: Dynamics of body weight of intact mice. Differences between normal and obesity mice beginning day 50 was significant, p<0.001, ANOVA.

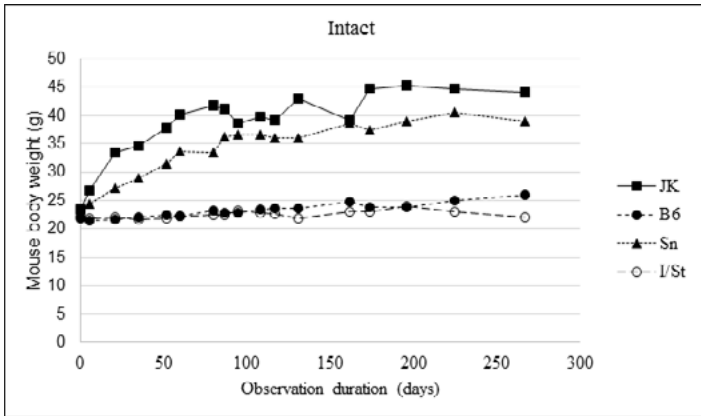

Figure2: Dynamics of body weight of mice aerosol infected with 600 CFU/lung. Differences between normal and obesity mice in interval 50 -200 days was significant, p<0.001, ANOVA.

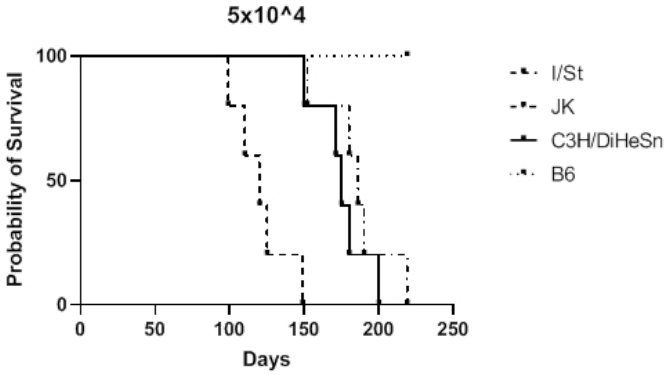

Figure3: Mice infected with 5x104 CFU

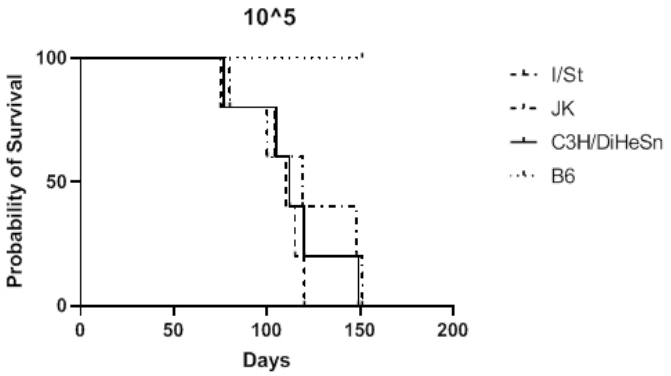

Figure4: mice infected with 10^5 CFU

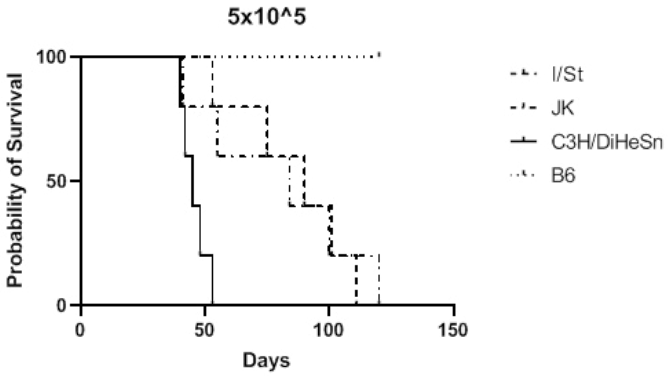

Figure5: mice infected with 5x10^5 CFU

Log-rank (Mantel-Cox) test, P	Dose of infection		
	5x10^4	10^5	5x10^5
I/St vs JK	0,0018	NS	NS
I/St vs C3H	0,0018	NS	0,0044
C3H vs JK	NS	NS	0,0269

Log-rank (Mantel-Cox) test, P	Dose of infection		
	5x10^4	10^5	5x10^5
I/St vs JK	**	NS	NS
I/St vs C3H	**	NS	**
C3H vs JK	NS	NS	*

Figure 6: Dose of infection

Strainà	I/St	C3H.JK	C3H/DiHeSn	C57BL/6
Dose of $5x10^4$	121 ± 19	185 ± 24	175 ± 18	>250
Dose of 10^5	105 ± 18	120 ± 31	113 ± 26	>250
Dose of $5x10^5$	86* ± 23	80 ± 32	46* ± 5	>250
*~ Difference between I/St and C3H/DiHeSn, p=0.0048				

Table1 : Median survival times (days) of mice infected with different doses of M. tuberculosis

Intestine Microbiome

Mice were divided on 2 groups: I/St ($H2^j$) that did not gain body weight at least for 200 days (19g, 21 g, 21 g, 22 g and 22 g) and C3H.JK ($H2^j$) that became obese prior 4 months of age (38 g, 41 g, 45 g, 47 g and 50 g) (Table 2). Intestinal contents were studied for 16 genera of bacteria. General bacterial mass was almost the same. In obese mice we found absolute absence of *Bifidobacterium spp.* while all I/St mice had 28.9 (28.4;29.4) gram-equivalents/ml. All other 15 genera were present almost in equal amounts.

	5 wk C3H.JK (19g, 21 g, 21 g, 22 g, 22 g)	5 mo C3H.JK (38 g, 41 g, 45g, 47g,50 g)
Bifidobacterium spp.	28.9 (28.4;29.4) genome-equivalents/ml	Absent
Saccharomyces cerevisiae	27.5 (27.3; 28.8) genome-equivalents/ml	Absent
Debaryomyces hansenii (C. famata) Debaryomyces hansenii (C. famata)	Absent	39.4 (39.2;39.6) genome-equivalents/ml
Results are indicated in Medians and quartiles		

Table2: Difference in composition of intestinal microbiome between I/St and C3H.JK mice

Study of fungi in mice with obesity revealed absence of *Saccharomyces cerevisiae* while I/St mice had 27.5 (27.3; 28.8) gram-equivalents and on the contrary obese mice had 39.4 (39.2;39.6) gram-equivalents of Debaryomys hansenii (*C. famata*)) while in non-obese I/St mice these fungi were not found at all. So, we can indicate some characteristic differences in microbiome: mice with obesity have significant amounts of *Debaryomyces hansenii* (*C. famata*) whereas normal mice do not have at all. Intestinal contents of mice with normal metabolism (I/St) display significant amounts of *Bifidobacterium spp.* and of *Saccharomyces cerevisiae* while in mice with obesity these bacteria are not found at all.

The results presented here show that susceptibility to TB may somehow be associated with obesity in mice. At high doses of challenge, C3H.JK ($H2^j$) and C3H/DiHeSn mice do not gain weight and show a higher susceptibility than I/St mice. With a decrease in the dose, the situation changes, they become more resistant, but they also do not gain weight and die. When infected with even lower doses, these mice gain weight and their survival time is very long. Analysis of the intestinal microbiome revealed, at least, differences in the three groups of bacteria and fungi. An attempt to infect obese and normal mice with doses of *Mtb* adequate to body weight has shown significant resilience in obese mice. We found that the critical dose of *Mtb*, which determines which path the process will take whether infected mice will gain weight or not is approximately 2,500 CFU/lung. In addition, hybrids (I/St x C3H.JK) F1, (I/St x C3H/DiHeSn) F1 and (C3H.JKx C3H/DiHeSn) showed even greater resistance (not shown). This would not be surprising for the last two hybrids, but (I/St x C3H.JK) F1, like the C3H.JK mice themselves, are represented by the H2j allele, which determines hypersensitivity to tuberculosis infection [8] and was previously considered by us as an easy reason for hypersensitivity [3,4] or the phenomenon of epistatic gene action. However, the high resistance of mice (I/St x C3H.JK) F1, in which the genes for hypothetical obesity are in a heterozygous state (I/St mice are not obese), indicates the influence of some factors associated or not associated with obesity, we do not know, which leads to compensation for the defective H2j allele. Another possibility is also possible, namely, H2j of I/St and C3H.JK mice are not identical.

CONCLUSION

Mice of obese strains change their susceptibility to TB infection depending on the dose of infection. Whereas non-fatty mice retain susceptibility in line with the genotype. At a dose of *Mtb* 2,500 CFU/lung, obese mice no longer gain weight and move into the category of susceptible to TB mice. The intestinal microbiota of C3H.JK mice in obesity loses *Bifidobacterium spp.* and *Saccharomyces cerevisiae*, and instead acquires *Debaryomyces hansenii* (*C. famata*), which were not found in animals with normal body weight.

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