ABSENCE OF QUANTITATIVE EFFECTS OF HETEROZYGOSITY OF THE "NUDE" GENE ON LYMPHOCYTE POPULATIONS IN THE MOUSE

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A comparison of the heterozygous (+/nu) with the normal (+/+) and "nude" (nu/nu) mouse indicates a normal T-lymphocyte population and an intact cellular immunity. Observations consisted of complete leukocyte and differential cell counts on 100 mice (50 males and 50 females) from each genotype. Average leukocyte counts of 7.6, 6.0, and $4.5 \times 10^3/mm^3$ and differential lymphocyte counts of 76, 78, and 54% were shown for the normal, heterozygous, and "nude" mice, respectively. A brief histological examination of thymus, spleen, and lymph nodes exhibited depletion of thymus-dependent lymphoid cells in the "nude" but not in the normal or heterozygous mice.

INTRODUCTION

The mutant mouse "nude" was discovered by N. R. Gist of the Virus Laboratory in Glasgow, Scotland, because of its noticeable hairlessness. But, the lack of hair on the "nude" and its other abnormalities could not be explained by existing hairless mutants. The mouse experienced low fertility, inability to gain weight, general body weakness, and death within 3 to 14 weeks (1).

The "nude" phenotype is due to a single autosomal recessive gene (1). The *nu* gene is associated with rex and trembler on Linkage Group VII. The order of the three loci and their recombination frequencies are

Re - 13% - nu - 9% - Tr

Flanagan (1) showed that infertility in the "nude" was due to small ovaries and low egg counts in the females and a high proportion of nonmotile sperm in the males. The hairlessness reflected an absence of free sulfhydryl groups in the midfollicle region which caused coiling of the incomplete hair shafts in the dermis.

Further investigation (2) revealed congenital thymic aplasia, which accounts for the short survival of "nudes". The thymus remains rudimentary after the 14th day of development, and does not produce or acquire lymphoid-like cells (3). Leukopenia and lymphopenia are exhibited in the "nude", as well as depletion of thymus-dependent lymphoid cells in the paracortex of lymph nodes and periarteriolar regions of spleen (4, 5). The percentage of lymphocytes in lymph nodes and spleen positive for the theta antigen is also reduced (6). However, agammaglobulinemia is generally not observed (7).

The presence of gammaglobulins and the absence of T-lymphocytes indicate that the stem cells for lymphopoiesis are present in the "nude", but that the T-precursors are specifically arrested (8). The abnormal thymic epithelium in the "nude" is incapable of inducing normal differentiation of T-lymphocytes (9), hence cell-mediated immunity is nearly absent in the mutant "nude".

Because the "nude" lacks cell-mediated immunity, it will tolerate transplantation of foreign tissue. Evidence of the heterotransplantation of foreign tumors in the "nude" is extensive (10-13). Solid tumors have been serially transplanted in "nudes" and karyotype analysis agrees with that of original tumor specimen. The "nude" has been valuable in determining the sensitivity of individual tumors to drugs, studying human tumor antigenicity and related immune responses, and comparing metabolic differences among tumor types (14).

There is interest concerning whether the mouse heterozygous for the *nu* gene experiences a depression of cellular immunity. A depression may exist if the normal gene is incompletely dominant over the mutant gene. If this does occur, characteristics such as leucopenia, lymphopenia, and depletion of thymus-dependent lymphoid tissue should occur to some degree in the heterozygous mouse.

MATERIALS AND METHODS

Mice and Husbandry

Three hundred white Swiss inbred mice were used in this study: 100 dominant homozygous (+/+), 100 heterozygous (+/nu), and 100 homozygous recessive (nu/nu). Each group contained 50 males and 50 females. Only healthy mice of at least two months of age were chosen, to assure sexual maturity. Breeding of mice for the *nu* gene was according to Giovanella and Stehlin (11).

Since "nude" mice will succumb to bacterial, viral, and fungal infections which only produce latent infections in normal mice, all mice were reared and sustained in strict pathogen-free conditions (9). Cages, bedding, food, and drinking water were autoclaved to reduce sources of contamination. Since excessive loss of vitamins occurs with sterilization of food, drinking water was supplemented with multivitamin infusion. Animals were kept on antibiotic treatment until two weeks prior to blood sampling. Each drinking bottle received 6 g/liter potassium penicillin and 0.1 g/liter polymyxin B sulfate. Antibiotics were chosen for their poor absorption through the intestine in order to create a high concentration in the intestinal tract. A combination of antibiotics was used to increase their effect.

To provide maximal environmental control and minimize introduction or transmission of disease-producing agents during handling, mice were kept in a laminar air flow room such as described by Gullino (9). Sterile techniques were employed during handling of mice.

Blood Sampling and Cell Counts

All blood samples were obtained by retroorbital bleeding (9), which supplied freeflowing drops without coagulation.

A blood smear was made for the differential white blood cell count for each animal. Preparations were fixed in methanol for 2 min and stained for 25 min in May-Grunwald-Giemsa stain. This consisted of one drop Giemsa per milliliter McJunkin-Haden buffer at pH 6.4. One hundred leukocytes were counted under oil immersion and designated as either lymphocytes, monocytes, neutrophils, or eosinophils, according to nuclear morphology described by Rygaard and Povlsen (5). Basophils were not found in the peripheral blood of the mice.

The Unopette system was utilized to prepare blood for complete white cell counts. A 1:100 dilution was counted by hemacytometer under 450 power. A total of 10 1-mm squares were counted from two separate chambers. Counts from separate chambers were compared for precision. Concentrations are reported as "cells/mm^3 of blood".

Histology

One mouse from each genotype was sacrificed and autopsied by a midline incision. Thymus, spleen, and axillary lymph nodes were isolated. Aplastic tissue from the superior mediastinum of the "nude" was substituted for thymus. Tissues were fixed in formalin and embedded in paraffin, and 7-μm sections were stained with hematoxylin and eosin. Sections were screened for lymphoid depletion in the thymus cortex, periarteriolar reticulum of the spleen, and the paracortical region of the lymph nodes.

RESULTS

Outward Appearance

All "nude" mice displayed absence of hair with thick, wrinkly skin. The heterozygous and normal mice were phenotypically identical, having normal amounts of hair.

Cell Counts

The complete white cell count averages for the three genotypes are given in Table 1. The "nude" mice were leukopenic, having an average count 40% lower than the normal mice. The heterozygous mice displayed a count approximately 20% lower than the normal mice. Statistical analysis of T-values and F-values showed the means and variances of the "nude" and heterozygous genotypes to be significantly lower than the normal mice at the 95% confidence level, with the "nudes" showing the most significance.

TABLE 1. *Complete white blood cell counts* ($\times 10^3/mm^3$)

Genotype	$\overline{X}$	S.D.	Range
(+/+)	7.64	2.65	2.4-14.7
(+/nu)	6.08	1.93	2.5-11.1
(nu/nu)	4.57	1.61	1.8-9.8

TABLE 2. *Differential counts of peripheral blood cells.*

Genotype	% Neutrophils			% Lymphocytes			% Monocytes			% Eosinophils		
	X̄	S.D.	Range	X̄	S.D.	Range	X̄	S.D.	Range	X̄	S.D.	Range
(+/+)	13.9	6.30	4-30	76.7	6.90	60-93	6.4	3.55	1-24	3.0	2.39	0-12
(+/nu)	12.6	4.51	3-24	78.9	6.67	63-92	5.7	3.02	1-16	2.8	2.26	0-11
(nu/nu)	28.7	7.74	6-45	54.2	8.90	34-75	13.4	5.38	0.26	3.7	2.71	0-12

Differential cell counts showed no qualitative difference of leukocytes among the genotypes. The differential counting of 100 nucleated cells from each animal gave the distribution in Table 2. The "nude" mice displayed a lower percentage of lymphocytes, approximately 30% lower, than the other two genotypes and an increased percentage of monocytes and granulocytes. Analysis of T-values showed that the percentage of lymphocytes in "nudes" were significantly lower than the normal mice at the 95% confidence level, while the heterozygous were not.

Further, a correlation analysis proved that no specific cell type was significantly responsible for fluctuations of complete white cell counts among the genotypes.

Histology

The thymus appeared typical in both the normal and heterozygous mice, having fine nodular structure with normal proportions of cortex and medulla. Tissue taken from the superior mediastinum of the "nude" mouse exhibited fat, connective tissue, and a cystic structure - interpreted to be the rudimentary thymus epithelium. No Hassall bodies were seen in the organized cell masses from the "nude" specimen.

Axillary lymph nodes from the normal and heterozygous mice were similar with typical proportions of cells in the outer cortex, paracortex, and medullary cords. Nodes from the "nude" exhibited depletion of lymphoid cells in the paracortex.

No qualitative differences could be detected between the spleen of the three genotypes in morphology of red and white pulp. However, the Malpighian bodies differed in the "nude" in that the periarteriolar reticular cell structures were lacking lymphoid cells.

DISCUSSION

Observations of the complete white cell count, differential count, and morphology of thymus-dependent lymphoid tissue were made on three genotypes.

The average complete leukocyte count of the heterozygous mice was between those of the normal and the "nude" mice. Factors other than the *nu* gene may have depressed the leukocyte concentration. Decreased production of thymosin, the hormone secreted by thymus epithelium, depresses the number of circulating leukocytes (15). However, only a general reduction of immune response is indicated by a lowering of leukocyte concentration, and not a specific reduction in cellular immunity.

The heterozygous mice did not display a reduction in the percentage of lymphocytes, which is a characteristic of the expression of the *nu* gene. Since 50% of the leukocytes in the peripheral blood of the mouse are T-lymphocytes (16), then even a slight reduction of T-lymphocytes should be evident. In the lymphopenic "nude", the small percentage of lymphocytes represents a relatively stable population of B-lymphocytes (5).

The brief histological study showed a normal thymus in the heterozygous mice, and no evidence of depletion of thymus-dependent lymphoid tissue.

Depression of cellular immunity from an incomplete dominance of the normal gene over the *nu* gene was not detected by the methods used in this study.

Although differential counts show a normal percentage of lymphocytes in the heterozygous mouse, only quantitation of the theta antigen can identify the actual proportion of T and B-lymphocytes. Further investigation is indicated to clarify the expression of the *nu* gene on lymphocyte populations in the heterozygous mouse.

ACKNOWLEDGMENTS

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