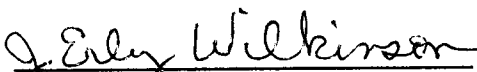


To the Graduate Council:

I am submitting herewith a dissertation written by Virginia Lee Godfrey entitled " The Scurfy Mouse: A Potential Model of Thymic Education." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Comparative and Experimental Medicine.


J. E. Wilkinson, Major Professor

We have read this dissertation
and recommend its acceptance:







Accepted for the Council:


Vice Provost
and Dean of the Graduate School

THE SCURFY MOUSE: A POTENTIAL
MODEL OF THYMIC EDUCATION

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Virginia Lee Godfrey

May, 1990

DEDICATION

I dedicate this work to my parents, who encouraged me to seek excellence in any endeavor, and to my loving husband, Craig, without whom any achievement would be meaningless.

ACKNOWLEDGEMENTS

I gratefully acknowledge the assistance of my committee members: Dr. J.E. Wilkinson, Dr. T. P. McDonald, Dr. R.A. Griesemer and Dr. E.M. Rinchik in the completion of this work. Dr. Raymond Popp, Dianna Popp and Sarah Shinpock provided advice and expertise concerning immunologic assays and surgical manipulations. I also wish to thank June Whitaker for her excellent assistance in the laboratory, and particularly for her unfailing cheerfulness and companionship throughout the course of this study. Finally, I wish to express my gratitude to Dr. W.L. Russell and Dr. L.B. Russell for the example of their dedicated scholarship and for providing me with the opportunity to investigate the fascinating scurfy mouse.

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ABSTRACT

Gene mutations in laboratory mice have provided numerous model systems for the study of disease processes. The initial description of the X-linked recessive mutation, scurfy (*sf*), noted severe runting, exfoliative dermatitis, and early death at roughly three to four weeks of age. We sought to clarify the pathogenesis of the scurfy syndrome via complete morphologic evaluation followed by in-depth analysis of the most consistently affected organ system(s). In addition to the previously described scaly skin and reddened genital papilla, gross morphologic lesions included marked splenomegaly, hepatomegaly, enlarged lymph nodes, and variable thickening of the ears. The characteristic microscopic lesion was a lymphohistiocytic proliferation that effaced lymph node architecture, thickened the dermis, expanded the splenic white pulp, and formed nodular portal infiltrates in the liver. Scurfy males had a consistently severe, Coombs'-positive anemia, polyclonal increases in serum IgG, and leukocytosis with atypical mononuclear cells. Since the morphologic lesions suggested a disease primarily affecting the lymphoid system, we attempted to modify the scurfy phenotype by *in vivo* immunologic manipulations. Neonatal thymectomy of *sf/Y* mice increased lifespan and ameliorated the histologic lesions, but did not prevent death. Mice bred to be scurfy (*sf/Y*) and nude (*nu/nu*; athymic) were viable, fertile, and free of lesions associated with the scurfy phenotype. Bone marrow from scurfy males could reconstitute lethally-irradiated, H-2 compatible animals but did not transmit disease. Morphologic lesions similar to those of *sf/Y* mice were seen in H-2 compatible nude mice that received *sf/Y* thymic grafts. These lesions resembled those described in subacute graft versus host (GVH) disease. No lesions resulted from transplantation of *sf/Y* thymuses into H-2 incompatible

nude mice. Our data indicates that the scurfy mutation causes a lymphoproliferative disease mediated by T cells, and that these T cells can be generated in a *sf/Y* thymic environment, but not in a normal (+/Y) thymus. Coupled with the observation that *sf/Y* T cells produce disease only in H-2 compatible hosts, these findings suggest that scurfy disease results from a defective thymic education of T cells.

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LIST OF ABBREVIATIONS

ABC, avidin-biotin-complex

ATCC, American Type Culture Collection

B220, cell surface glycoprotein on B lymphocytes

CD4, cell surface protein on T lymphocytes that is an indicator of the T helper subset

CD8, cell surface protein on T lymphocytes that is an indicator of the T suppressor/
cytotoxic subset

CRL, cell repository lines

DAB, 3, 3'-diaminobenzidine tetrahydrochloride

EDTA, Ethylenediaminetetraacetic acid

FBS, fetal bovine serum

HBSS, Hank's balanced salt solution

IgG, immunoglobulin G

kV, kilovolts

mA, milliamperes

OTC, embedding compound

PALS, periaarteriolar lymphatic sheath

PBS, phosphate buffered saline

R/min, Rads per minute

RFLP, restriction length polymorphism

Thy-1, cell surface protein on T lymphocytes

TIB, Tumor immunology bank

Tris, Tris(hydroxymethyl)aminomethane

PART 1. GENERAL INTRODUCTION AND OVERVIEW

CHAPTER 1.

INTRODUCTION

The Oak Ridge National Laboratory, Mammalian Genetics Section, possesses a rich collection of mice bearing spontaneous and/or induced gene mutations. As part of on-going projects to evaluate these animals for models of human disease, we chose to examine mice expressing the X-linked recessive mutation, scurfy (*sf*). Scurfy is a spontaneous mutation first recognized by Dr. W. L. Russell in 1949 and published in 1959. (1). The phenotype is only expressed in hemizygous (*sf/Y*) males; female carriers of the trait ($X^{sf}X$) are clinically normal. Initial descriptions of the scurfy mouse were sketchy, concentrating on its short lifespan (about 3 wks) and external manifestations of runting, scaly "tight" skin, and delayed opening of the eyelids. A complete histopathologic examination was not included in the initial report. Several authors have compared these limited descriptions of scurfy mice to features found in dermatologic diseases in humans, in particular, X-linked ichthyosis (2,3,4). These comparisons were based solely on the initial description of scurfy mice and were not supported by necropsy and/or histopathologic examination. We felt that the scurfy mouse might prove to be a valuable animal model because it exhibited severe disease (juvenile death) yet lived long enough to be experimentally manipulated. We also wished to substantiate, if possible, the claims that scurfy was identical to X-linked ichthyosis in man.

We approached the scurfy project with an initial plan of complete morphologic analysis followed by in depth investigation of the organ system(s) showing the most consistent pathologic changes. In this way, we hoped to identify particular cell types and

cell functions primarily affected by the scurfy mutation. These cells might then be targeted for molecular analysis of gene products, DNA structure, etc.

Initial histopathologic evaluation suggested some similarity between scurfy and the Histiocytosis X complex in humans. The most severe form of this disease, also known by several eponyms including Letter-Siwe disease, affects young children (<3 yrs), is highly fatal, and results from a proliferation of bizarre histiocytes in the skin and viscera (5). Diagnosis depends on the presence of certain cell antigens (CD4, CD6) on the proliferating cells and on the identification of a particular granule by electron microscopy (Birbeck's granules). Birbeck's granules are present in normal skin dendritic cells (Langerhan's cells) in humans and mice, but no Birbeck's granules have been seen in *sf* lesions. Histochemical staining could not be assessed since the monoclonal antibodies to human CD6 do not react with normal or abnormal mouse Langerhan's cells (personal communication, Dr. R.J.M. Fry). Also, Histiocytosis X characteristically involves bone (*sf* does not) and has a sporadic, not X-linked inheritance pattern. We, therefore, rejected scurfy as a potential model for Histiocytosis X.

Complete morphologic analysis of scurfy males indicated a primary effect on the lymphoreticular and possibly the hematopoietic systems (Part 2). The standard immunologic manipulations of neonatal thymectomy, tissue transplantation and the breeding of mice with multiple immunologic dysfunctions identified the thymus (and T lymphocytes) as the critical mediators of scurfy disease (Part 3). Finally, transplantation of scurfy thymuses into *H-2* compatible and *H-2* incompatible mice suggested restriction of scurfy T cells' actions to cells/tissues of its own, or similar, *H-2* type (Part 4). If scurfy T cells are reacting aberrantly to "self" antigens, and failing to react to "foreign" *H-2* antigens, scurfy could represent a failure in the normal processes by which T cells acquire self-tolerance in the thymus. Such

a model system could play a pivotal role in dissecting the processes of normal and abnormal thymic education of lymphocytes.

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PART 2. X-LINKED LYMPHOPROLIFERATIVE DISEASE IN THE SCURFY (*sf*)
MUTANT MOUSE

CHAPTER 1.

ABSTRACT

Scurfy (*sf*) is a spontaneous, sex-linked, recessive mutation that maps to the extreme proximal portion of the X chromosome, about 2 cM from sparse fur (*spf*). Hemizygotes for *sf* manifest several clinical disorders, evident at 14 days of age, including scaliness and crusting of the eyelids, ears and tail, runting, reddening and swelling of the genital papilla, anemia, cachexia, and early death (avg. 24 days). Our studies indicate that the phenotype of hemizygous scurfy is not, as has been suggested, a model for human X-linked ichthyosis, but appears to be a disease primarily affecting the lymphoreticular, and possibly the hematopoietic systems. Gross lesions include marked splenomegaly, hepatomegaly, enlarged lymph nodes, and variable thickening of the ears. The characteristic histologic lesion is a lymphohistiocytic proliferation and infiltration of peripheral lymph nodes, spleen, liver, and skin. In routine hematoxylin and eosin-stained sections, these lesions efface lymph node architecture, thicken the dermis and form nodular portal infiltrates in the liver. Scurfy lesions characteristically contain a population of large blast-like cells with round to oval nuclei, a vesicular chromatin pattern, and prominent single nucleoli. Mixed perivascular infiltrates of lymphocytes, macrophages, and granulocytes are sometimes found in kidney, heart, pancreas, lung, and mesenteries. There is excessive hematopoiesis in the liver and spleen. Cells expressing B220 or Thy-1 antigens localize to appropriate areas in the lymph nodes and spleen, but are rare in the portal infiltrates and are absent from the skin. The blast-cell population lacks both of these antigens. There is a marked, polyclonal increase in serum IgG, severe Coombs'-positive anemia, and leukocytosis with atypical mononuclear

cells. Scurfy mice are negative for antinuclear antibodies. Despite their morphologically aberrant lymphoreticular system, scurfy mice can exist in a conventional environment without evidence of opportunistic infection. Raising scurfy mice in a specific-pathogen-free environment does not alter disease expression. Thus, while our findings indicate that scurfy disease may be the result of immune dysfunction, it is not a classic immunodeficiency.

CHAPTER 2.

INTRODUCTION

Gene mutations affecting the murine immune system have greatly expanded our understanding of immune regulation and development. Mutations affecting particular cell types such as nude (*nu*; T cells), beige (*bg*; NK cells), or X-linked immunodeficiency (*xid*; B cell subsets) provide information on the functions of these cells and their interactions with other portions of the immune system. Immune dysfunctional mutants furnish animal models for human disease, particularly for those syndromes involving autoimmune phenomena. As part of an ongoing program that evaluates mutant mouse stocks derived and maintained at the Oak Ridge National Laboratory as potential animal models, we have examined the X-linked recessive mutation scurfy (*sf*). The morphologic lesions suggest an aggressive, rapidly fatal immune dysfunction involving lymphohistiocystic lesions in lymphoid organs, liver, and skin. Investigating the pathophysiology of these mice could provide valuable insights into atypical lymphoproliferative diseases in humans and understanding of putative lymphocyte-controlling regions on the X chromosome. In this paper, we present data on the clinical and pathologic effects of the *sf* mutation.

CHAPTER 3.

MATERIALS AND METHODS

Mice

The original scurfy (*sf*) mutation occurred spontaneously in the partially inbred MR stock at the Oak Ridge National Laboratory in 1949, and was found to be a sex-linked recessive, with the phenotype expressed only in $X^{sf}Y$ males and $X^{sf}O$ females (1). It was made congenic with the segregating inbred strain 129/Rl - $p\ c^{ch}/p\ c$ by repeated backcrosses with 129/Rl - $p\ c^{ch}/p\ c^{ch}$ males. *Sf* is also maintained on a noninbred stock (DG) by mating *sf*/+ carrier females to (C3Hf/Rl x 101/Rl) F_1 or (101/Rl x C3Hf/Rl) F_1 males. Mice are housed in a conventional environment with a standard pelleted diet (Purina 5001) and chlorinated water fed *ab libitum*. Breeding stock from both backgrounds was also cesarian derived into a barrier facility and maintained free of adventitious murine pathogens.

Histopathology

Necropsy was performed on 206 scurfy mice and 119 normal male siblings. Tissues for histologic examination were fixed in 10% neutral-buffered formalin, Bouin's or Telly's fixative, routinely processed, and embedded in paraffin. Five-micron sections were stained with hematoxylin and eosin.

Immunohistochemistry

Commercial reagents employed include biotinylated rat anti-mouse Thy-1.2 (Becton/Dickinson Mountain View, CA), rat anti-mouse L3T4 (B/D), Vectastain* ABC-

peroxidase and ABC-alkaline phosphatase kits (Vector Labs Burlingame, CA). Ascites fluid was prepared in pristane-treated BALB/c nude mice from hybridomas 7D4 (anti-IL-2 receptor, ATCC CRL-1698) and RA3-3A1 (anti-B220, ATCC TIB-146). Antisera and ascites fluids were used at 5-10 μ g of immunoglobulin/ml.

Sections of liver, spleen, lymph node, thymus, and skin were frozen in OCT compound in liquid nitrogen. Cryostat sections were cut at 5 μ m and air dried overnight. After fixation in acetone at room-temperature (10 min), sections were rehydrated in Tris-buffered saline (pH 7.6) for 5 min, then blocked with 2% dilute normal rabbit serum for 20 min. Sections were labeled with primary antibody for 30 min, biotinylated secondary antibody (30 min), and Vectastain[®] ABC reagent (30-60 min). Antibody localization was visualized with 3,3'-diaminobenzidine (DAB) substrate for peroxidase or Vector Red/Black[®] alkaline phosphatase substrate kits (Vector Labs Burlingame, CA).

Hematology

Heparinized or EDTA-treated blood was obtained via cardiac puncture in anesthetized mice. Hematocrits were determined by centrifugation using microhematocrit capillary tubes. Blood films were air dried and stained with Wright's stain. Erythrocyte and leukocyte counts, hemoglobin concentrations, and red blood cell indices were evaluated by the model ELT 15/ws Ortho blood analyzer. This automated hematology system has been modified and calibrated for mouse blood samples. Samples of blood leukocytes were prepared by Dextran sedimentation of erythrocytes as follows: (i) mix heparinized whole blood 1:1 with 2% Dextran 250 in PBS, pH 7.4; (ii) allow mixture to stand 20-30 minutes at room temperature or until erythrocytes have settled to the bottom of the tube; (iii) remove supernatant, dilute 3:1 with Hanks' Balanced Salt Solution (HBSS); (iv) pellet

leukocytes, wash twice in HBSS; (v) dilute to 5×10^5 nucleated cells/ml in HBSS plus 5% Fetal Bovine Serum (FBS), cytocentrifuge on clean glass slides; (vi) air dry and fix according to histochemistry protocols. Histochemical staining for Sudan Black B and α -Naphthol butyrate esterase (α -NBE) was assayed per manufacturer's instructions (Sigma Diagnostics procedures 380 and 180-1 St. Louis, MO). The staining procedure for acetylcholinesterase was provided by Dr. T. P. McDonald, University of Tennessee.

Immunology

Total serum IgG and IgM were quantified by use of radial immunodiffusion kits (The Binding Site, Ltd San Diego, CA). Direct and indirect Coombs' tests were performed as a modification of the previously described procedure (2). Rabbit antiserum to mouse immunoglobulins (Cedarlane Laboratories Ltd Westbury, NY) was used as the Coombs' reagent. Rheumastrip® assays for antinuclear antibodies (ANA) were graciously provided by Dr. Craig Dees, LipoGen, Inc. Knoxville, TN. ANA-positive control serum from viable motheaten mice (*me^u/me^u*) was the kind gift of Dr. Leonard Schultz, the Jackson Laboratory, Bar Harbour, ME.

Statistical Analyses

All measures of variance are provided as standard errors of the mean. Tests of significance for differences between independent means were performed by the Student *t* test.

CHAPTER 4.

RESULTS

Lifespan

Thirty-five *sf/Y* mice were monitored daily until natural death. The mean lifespan was 24 ± 0.76 days. While a few *sf/Y* mice survived to 30-39 days of age, no scurfy males lived longer than 40 days. There was no significant difference in mean lifespan between scurfy males of the 129 congenic stock and scurfy males of the noninbred DG stock.

Genetics

The genetic distance between *sf* and *spf* (sparse fur) (3) was measured in repulsion and coupling crosses. Heterozygous scurfy females (*+sf*) were mated to sparse-fur males (*spf/Y*). G_2 progenies were collected from those daughters that yielded both *sf/Y* and *spf/Y* and were thus identified as being *sf+/+ spf*. The male progeny produced in this repulsion cross consisted of 129 *sf/Y*, 146 *spf/Y*, 8 *+ +/Y*; there were no *sf spf/Y*. Males that died prior to 6 days postnatally were not classifiable for either *sf* or *spf*; those that died between 6 and 14 days were classifiable for *spf* but not *sf*. Of the 146 males classified as *spf/Y*, 10 died during the latter interval.

Genetic testing of G_2 females identified one of genotype *sf spf/+ +*. This animal and her *sf spf/+ +* descendants were crossed to *+ +/Y* males. The male progeny of this coupling cross consisted of 117 *sf spf/Y*, 151 *+ +/Y*, 1 *sf +/Y*, and 2 *+ spf/Y*. Thus, by about 14 days of age, the viability of *sf spf/Y* males appears to be 77% that of wild-type

males. The absence of *sf spf/Y* in the repulsion cross is thus presumably not due to complete lethality of this genotype.

Taking the results at face value, the recombination frequency is calculated to be 11/554, or $2.0 \pm 0.6\%$. Because of the viability complications, this value may be inaccurate. An upper estimate can perhaps be based on the two most viable genotypes of the repulsion cross, namely *spf/Y* (non recombinant) and *+ +/Y* (recombinant); this frequency is 8/146, or 5.5%.

Morphology

The first clinical sign of the scurfy syndrome is a transient swelling and reddening of the genital papilla at 12-14 days of age. By 14-15 days, there is scaliness and crusting of the ears, feet, tail, and eyelids that seals the palpebral fissure (Fig. 1). Scales on the base of the tail may form thick circumferential rings. The ears are small, thickened and sometimes folded. Affected mice are runted and exhibit a hunched posture, swollen abdomen, coarse facies, and wasted appearance. Mean body weights from ten *sf/Y* and ten *+ /Y* mice, aged 20-24 days, were 6.84 ± 0.50 g and 11.22 ± 0.56 g respectively. Visceral lesions include a marked splenomegaly, mild to moderate hepatomegaly, and prominent enlargement of lymph nodes (Fig. 2, 3). Subcutaneous lymph nodes such as inguinal, axillary, and cervical nodes are consistently involved, often measuring 4-7 mm in diameter. Enlargement of visceral lymph nodes is slight to moderate. Most scurfy mice have pale kidneys and thin watery blood (anemia). Severely anemic animals may exhibit cardiomegaly, pleural effusion, and slight icterus. Many scurfy mice have a thin, sharply demarcated rim of necrosis along the margins of the liver (Fig. 3). A bilobed thymus is present but is extremely small. The testicles are small and retained in the abdominal cavity.

Histopathology

Characteristic microscopic lesions of scurfy mice are found in the skin, liver, lymph nodes, and spleen. There is a diffuse lymphohistiocytic infiltration of the entire dermis that is most severe in the prepuce, ears, eyelids, and facial skin (Fig. 4). The infiltrate is not centered on blood vessels, hair follicles, or epithelium, although patchy infiltration of the basal layer is noted. Basal layer infiltrates can efface portions of the dermoepidermal junction. The infiltrate is densest in the deeper portions of the dermis, and occasionally extends into underlying fat and muscle. The infiltrating mononuclear cells are highly pleomorphic. Lymphocytes vary from medium-sized cells with coarsely clumped chromatin to large cells with dispersed chromatin, 1-2 small nucleoli, and moderate amounts of cytoplasm. Histiocytes have abundant cytoplasm, indistinct borders, irregularly oval nuclei, and stippled chromatin. Granulocytes and plasma cells are present in varying, and sometimes significant, amounts. Epidermal changes include moderate hyperplasia, moderate to severe orthokeratotic hyperkeratosis, multifocal parakeratosis, and occasional intraepidermal pustules. Cutaneous lesions, focally apparent at 7 days, are the earliest microscopic evidence of the syndrome (Fig. 5).

Extensive leukocytic infiltrations are present in the portal areas of the liver. These infiltrates include B & T lymphocytes (see immunohistochemistry, below), macrophages, granulocytes, and large blast-like cells (Figs. 6A and B). Blast-like cells have a large round-to-oval nucleus, a vesicular chromatin pattern, and a prominent single nucleolus. Mitotic figures are common (3-5/40x field). There is abundant hematopoiesis in the hepatic sinusoids of affected males that is not observed in livers of normal siblings. Centrolobular hepatic cords are atrophied, and adjacent sinusoids are dilated. Hepatocytes in the atrophic cords may be pale staining and finely vacuolated, but frank fatty change is absent. Areas

of acute coagulative necrosis without inflammation are present at the tips of the liver lobes. Occasionally, livers from scurfy mice have marked erythrophagocytosis and/or hemosiderin deposits in Kupffer cells.

Lymph nodes in scurfy mice are large (4-7 mm in diameter) yet appear hypocellular in histologic section. There is a complete loss of normal architecture with a lack of discernable follicles, no distinct paracortex, and thickening of medullary cords. Small lymphocytes are replaced by a randomly distributed mixture of lymphoblasts, large mononuclear cells with vesicular nuclei, macrophages and granulocytes. Plasma cells may be abundant in the medullary cords. Lymph nodes from older scurfy mice (> 20 days) may contain multinucleated giant cells and/or small eosinophilic granulomas in the deep paracortex and medullary cords. There is a mild to moderate sinus histiocytosis.

The spleen red pulp is massively expanded by hematopoietic cells, leading to spleen weights that are 2-4 times those of normal siblings (Fig. 7). The white pulp may be enlarged or shrunken, but, in either case, is composed of large mononuclear cells with vesicular nuclei, lymphoblasts, and variable numbers of plasma cells. Follicles and distinct marginal zones are lacking, and small lymphocytes are absent. Occasional erythrophagocytic macrophages and plasma cells with Russell's bodies can be seen at the margins of the white pulp.

The thymus in young scurfy mice (14-17 days) is small (Fig. 7) but densely populated with lymphocytes. A distinct cortex and medulla are present. As the disease progresses, the thymic cortex is rapidly depleted of lymphocytes. In older scurfy mice (> 25 days), the thymus is a shrunken stromal remnant lacking any corticomedullary distinction.

Scattered perivascular infiltrates of mixed mononuclear cells and granulocytes may be seen in heart, pancreas, lung, salivary gland, kidney, and mesenteries. Acute right atrial

thrombosis is found in small numbers of affected mice. The bone marrow of scurfy mice is densely cellular; cells in the erythroid, granulocytic, and megakaryocytic lines are readily distinguishable.

Immunohistochemistry

Thy-1-positive paracortical cells and B220-positive primary follicles are found in appropriate areas of *sf/Y* lymph nodes, though such distinctions are not apparent in regular histologic sections. The staining with either antibody is less intense and less organized than it is in lymph node sections from sibling controls. Scurfy lymph nodes contain many cells that stain for the IL-2 receptor (IL-2R), a marker that is expressed on activated T and B lymphocytes. Moderate numbers of L3T4 (CD4) positive cells are observed in the paracortex. The staining patterns for Thy-1, B220, IL-2R, and L3T4 in the spleen white pulp are similar to those seen in the lymph nodes: B and T cells in appropriate areas, decreased staining intensity, and increased IL-2R positive cells. Although small clusters of B220⁺ and Thy-1⁺ lymphocytes can be found in the hepatic portal infiltrates, most cells are negative for any of the antibodies used. The scurfy thymus has a thin cortex with an increased concentration of IL-2R positive cells. The skin contains occasional B220⁺ cells, but the remainder of the dermal infiltrate is negative for Thy-1, L3T4 (CD4), and IL-2R.

Hematology

Anemia is a consistent feature of scurfy mice, with mean hematocrit and hemoglobin values that are roughly one half those of sibling controls (Table 1). There is an increase in mean corpuscular volume (MCV), marked anisocytosis, poikilocytosis, and polychromasia. Autoagglutination was present in some blood films.

Mean white blood cell counts from the scurfy and normal mice in Table 1 were $4390/\text{mm}^3 \pm 101/\text{mm}^3$ and $1500/\text{mm}^3 \pm 45/\text{mm}^3$, respectively. White blood cell counts of $1500/\text{mm}^3$, or less, are normal in juvenile mice of the DG and 129 strains. Accurate differential counts of scurfy blood leukocytes could not be obtained using an automated blood analyzer. Examination of Wright's stained blood films revealed atypical mononuclear cells in the scurfy sample. These cells are characterized by large size (1.5 - 5x diameter of a neutrophil), moderate to abundant cytoplasm with dark blue to amphophilic mottled staining, fine cytoplasmic vacuoles, large nuclei with clefts and folds, and a dispersed chromatin pattern. Some cells have one to two small nucleoli. These atypical mononuclear cells represent roughly 10-20% of the peripheral blood leukocytes in the average scurfy mouse but can reach levels of 80-90% in those few *sf/Y* mice that survive beyond 30 days. Occasional multinucleated cells and mitotic figures are seen in scurfy blood films. The atypical mononuclear leukocytes do not stain with either Sudan Black B (granulocytes) or with acetylcholinesterase (megakaryocytes). They may exhibit patchy to diffuse cytoplasmic staining with α -naphthol butyrate esterase (α -NBE) or may fail to stain at all. (The α -NBE staining technique produces a pattern of 1-3 small marginal dots in up to 50% of blood lymphocytes in our control animals.)

Immunology

Total serum IgG is markedly elevated in scurfy mice. Mean total serum IgG for 12 scurfy males and 10 normal males (age 10-27d) was $1074 \text{ mg/dl} \pm 118.4 \text{ mg/dl}$ and $295 \text{ mg/dl} \pm 24.2 \text{ mg/dl}$, respectively. Elevated serum IgG is apparent as early as 10 days of age and ranges from 2-10 times the concentration of serum IgG in normal siblings. Serum IgM is also increased, and IgA synthesis is precocious (detectable in sera from scurfy mice at 21

days but not in normal siblings). Each of 12 scurfy mice tested was positive in the direct Coombs' test at dilutions of 1:512 or greater of the anti-mouse immunoglobulin reagent. Indirect Coombs' tests were negative. Tests for antinuclear antibodies (ANA) were done using the Rheumastrip® dipstick system for ribonuclear proteins (RNP), double-stranded DNA (dsDNA) and the small ribonuclear protein antigens SM, Ro, and La. Scurfy sera were negative in all categories, whereas control sera from the autoimmune mutant, viable motheaten (*me*^v), were strongly positive for antibodies to RNP and dsDNA and weakly reactive with La and Ro.

Pathogen-free Scurfy Mice

The initial signs of the scurfy syndrome are recognizable at the same age (12-14d) in barrier-reared animals and in mice maintained in a conventional facility. Gross morphology and histopathology are unchanged, including the areas of marginal necrosis in the liver. Liver lesions are present despite continued seronegativity for antibodies to mouse hepatitis virus (MHV) in the scurfy stocks and in all other stocks within the barrier (data not shown). Pathogen-free scurfy mice are likewise anemic and have elevated total serum IgG (>500 mg/dl). Initial observations of lifespan in barrier-reared scurfy mice are similar to those from the conventionally housed animals (21,25,28 days).

CHAPTER 5.

DISCUSSION

The X-linked scurfy (*sf*) mutation provided early information on the sex-determining mechanism in mice. Observation of scurfy disease in occasional female pups led Russell *et al.* (1959) to investigate the inheritance pattern of scurfy (1). Scurfy females were shown to have an $X^{sf}O$ genotype. Conclusions drawn from these studies suggested the Y chromosome to be the male determining factor in mice (4).

The initial descriptions of the *sf* syndrome were limited to external lesions and lifespan. Several authors have pointed out similarities between the described cutaneous manifestations of *sf* and those of X-linked ichthyosis in humans (5,6,7). Though the syndromes share some features (X-linked inheritance, hyperkeratosis, epidermal hyperplasia with a distinct granular layer), there are dramatic differences in pathophysiology and clinical outcome. In postnatal life, X-linked ichthyosis is a relatively benign cutaneous disease with heterogeneous expression. Dermal infiltration of leukocytes is not a prominent histologic feature. X-linked ichthyosis is caused by a deficiency of steroid sulfatase activity and may be accompanied by hypogonadism (8). By contrast, scurfy is a uniformly lethal trait in males (and in $X^{sf}O$ females) combining atypical lymphoproliferative lesions with anemia, cachexia, and exfoliative dermatitis. Hypogonadism cannot be assessed because *sf/Y* males do not live to sexual maturity. Steroid sulfatase activity is normal in scurfy mice (9), as might be expected since the *Sts* and *sf* loci map to opposite ends of the acrocentric mouse X chromosome (7). Based on these data, we emphasize that scurfy should not be considered

a model for X-linked ichthyosis in man and point out the pitfalls of drawing such conclusions from extremely limited examinations of the mutant animals.

The morphologic lesions in scurfy animals indicate a disease primarily affecting the lymphoreticular, and possibly the hematopoietic systems. The lesions are remarkable for their severity, consistent expression regardless of genetic background, mixed cell types, and presence of large blast cells. Though scurfy lesions are severe and proliferative, they lack evidence of frank neoplasia such as lymph node extracapsular invasion, solid-tumor formation, bone marrow effacement, or lymphomatous infiltration of other viscera. The combination of nonneoplastic lymphoproliferation, mixed cell types in the lesions, and hypergammaglobulinemia is more suggestive of an immune dysfunction. This dysfunction is not a classic immunodeficiency since scurfy mice can exist in a conventional environment without opportunistic infection, but may instead be a hyper-reactivity to some stimulus. The stimulus could be exposure to exogenous antigen(s), or an inherent defect in one or more cell types producing autonomous hyperactivity. Additional analyses of scurfy lesions by FACS and immunohistochemistry are under way. These should delineate the different cell types and lymphocyte subsets present in scurfy lesions and may also identify the lineage of blast cells in lymphoid organs and atypical mononuclear cells in peripheral blood. Knowledge of the cell types involved in scurfy disease should allow us to investigate their underlying functional abnormalities.

The scurfy mutation may also affect the erythropoietic system either directly or indirectly via lymphocyte-derived mediators. Scurfy mice have a positive direct Coombs' tests and severe anemia, yet lack evidence of continued gross hemolysis such as icterus or hemosiderosis. There is abundant hematopoietic activity in liver, spleen, and bone marrow. This hematopoiesis may be ineffective due to cytokine-mediated maturation failure in

addition to autoimmune erythrocyte destruction. The negative indirect Coombs' test also suggests that the erythrocyte destruction is not truly autoimmune (that is, not due to antibodies specifically directed against erythrocyte epitopes) but rather the result of passive absorption of increased serum immunoglobulins to erythrocytes. We plan to study the hematopoietic potential of scurfy bone marrow cells using *in vitro* colony forming assays and *in vivo* bone marrow transplants. These experiments should help assess the relative contribution of erythrocyte destruction and maturation failure to the anemia observed in affected mice.

The lesions of scurfy are dramatic, yet none appears to affect vital structures in such a way as to cause the animal's early death. The ultimate cause of death in scurfy mice seems to be wasting similar to that seen with neoplasia, severe parasitism, or other debilitating diseases. This sort of wasting syndrome is thought to be mediated by tumor necrosis factor (TNF) and possibly other cytokines. Administration of exogenous TNF to laboratory animals elicits some lesions similar to those observed in scurfy males, including cachexia, decreased bone marrow erythroid cell production, and dermal leukocyte infiltration (10,11,12). Scurfy disease could therefore be the result of an immune dysfunction producing lymphoproliferation and cytokine toxicity with, or without, antibody- or cell-mediated damage. Identification of the cellular and/or molecular abnormalities in scurfy mice should, therefore, provide considerable additional insights into the working mechanisms of the immune system.

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APPENDIX

Figure 1.

Scurfy (*sf/Y*) mouse exhibiting typical features of squinted eyes and small crusted ears (top).

Figure 2.

Massive splenomegaly in a scurfy mouse (bottom, left)

Figure 3.

Enlarged lymph nodes and acute coagulative necrosis on the margins of liver lobes in *sf/Y* mouse (bottom, right).



Figure 4.

Cutaneous lesions in scurfy mice include hyperkeratosis, hyperplasia, and a diffuse dermal mononuclear cell infiltrate that can efface portions of the dermoepidermal junction.

(hematoxylin and eosin, X 132)

Figure 5.

Focal dermal infiltration of mononuclear cells and epidermal hyperplasia in a 7 day old *sf/Y* mouse. (hematoxylin and eosin, X 132)

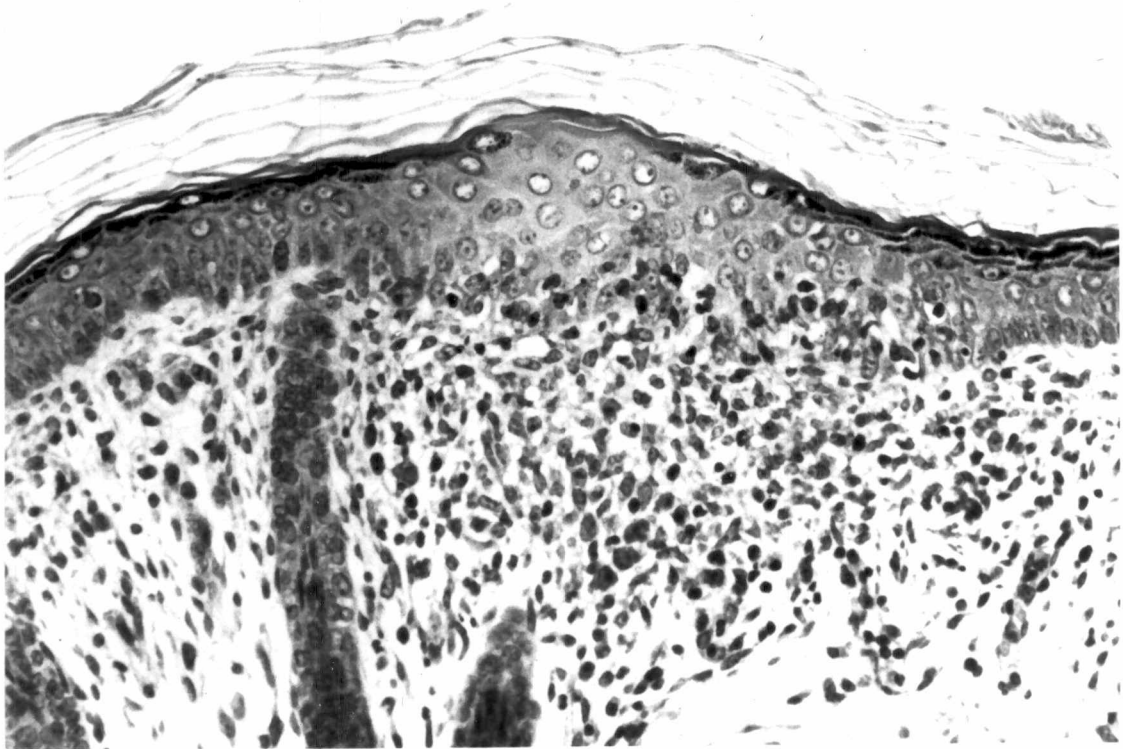
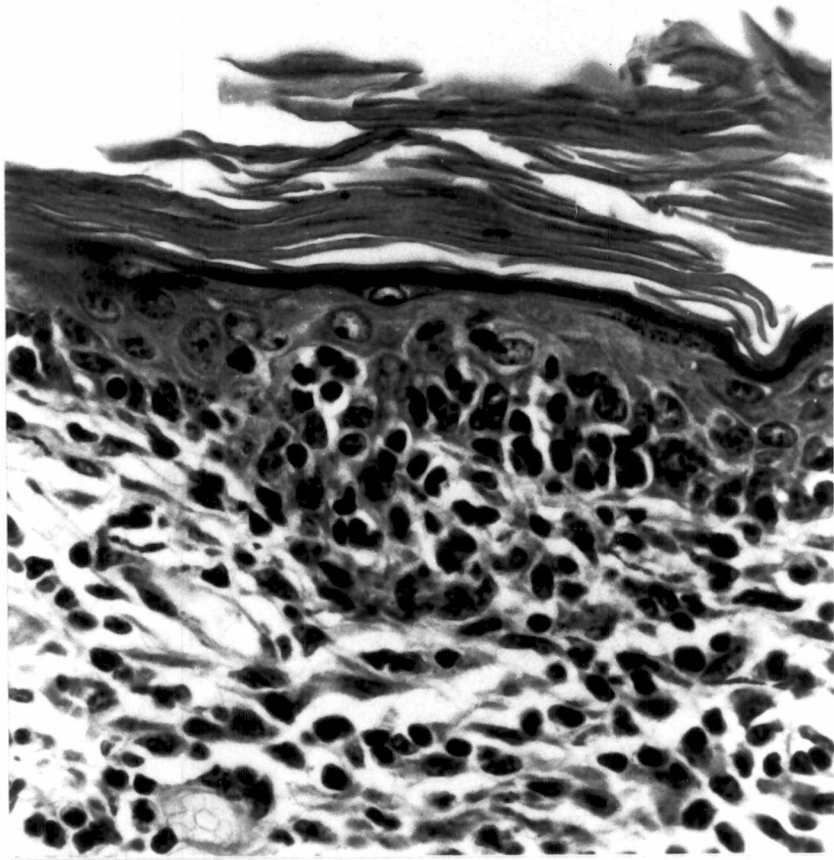


Figure 6.

(A) Nodular leukocyte accumulations in the portal areas of scurfy liver sections.
(hematoxylin and eosin, X 66)

(B) Portal area leukocytes in liver of an *sf/Y* mouse. There is extreme variation in nuclear size; blast cells with vesicular chromatin patterns are evident; mitotic figures are common.
(hematoxylin and eosin, X 132)

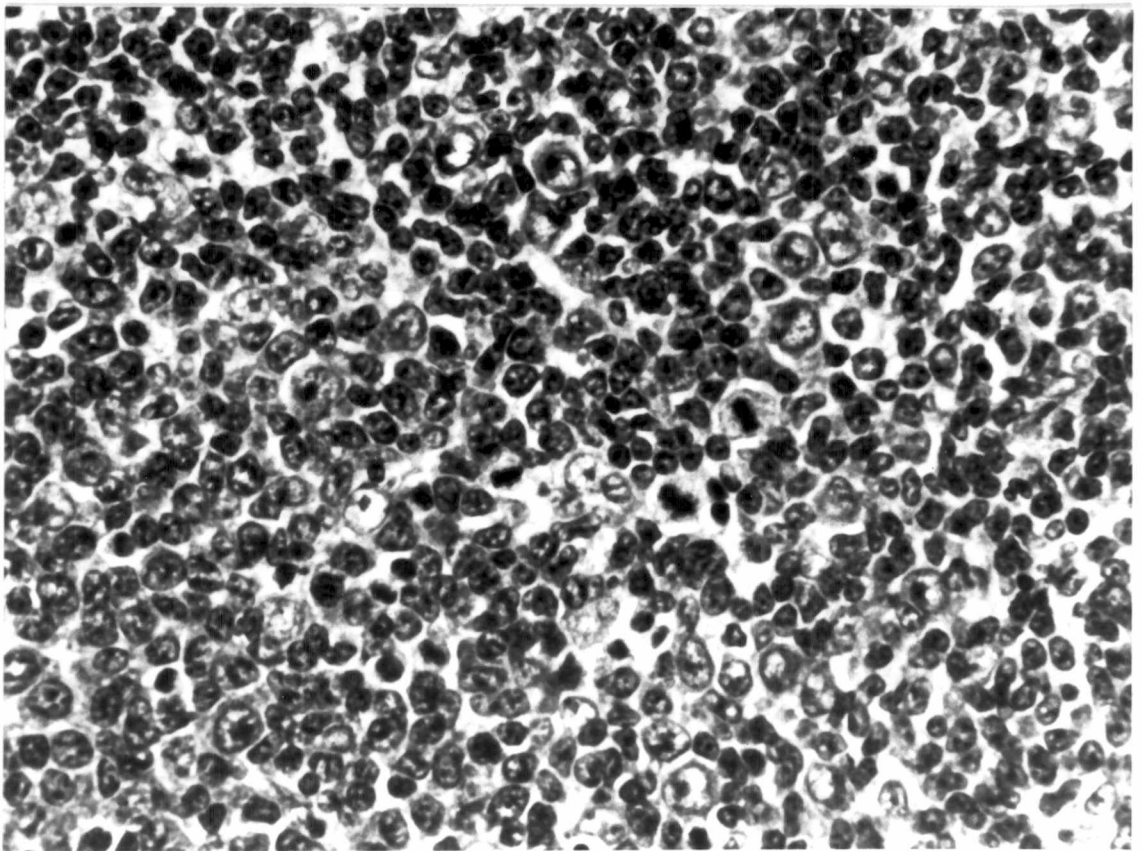
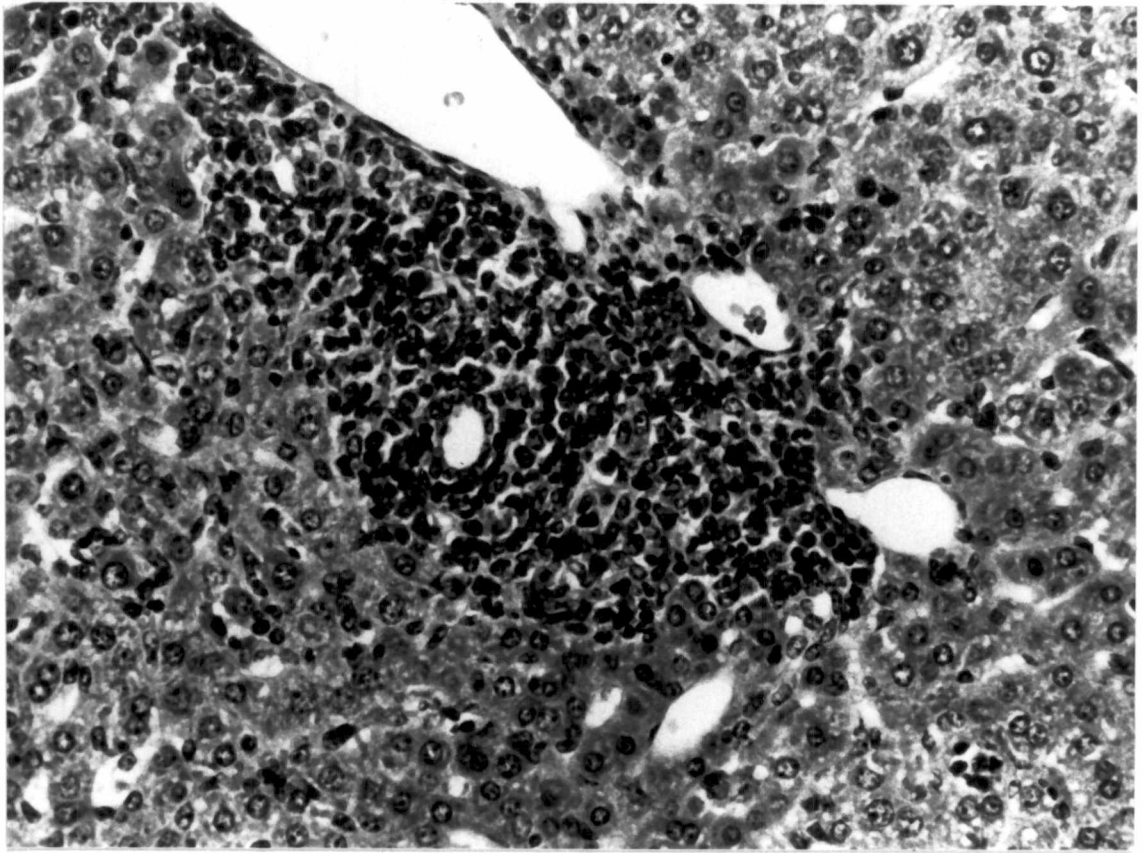


Figure 7.

Lymphoid organ to body weight ratios (milligrams per gram of body weight) in *sf/Y* (○—○) and *+/Y* (●—●) littermates. Each point represents the mean and standard error of values from 5-7 mice.

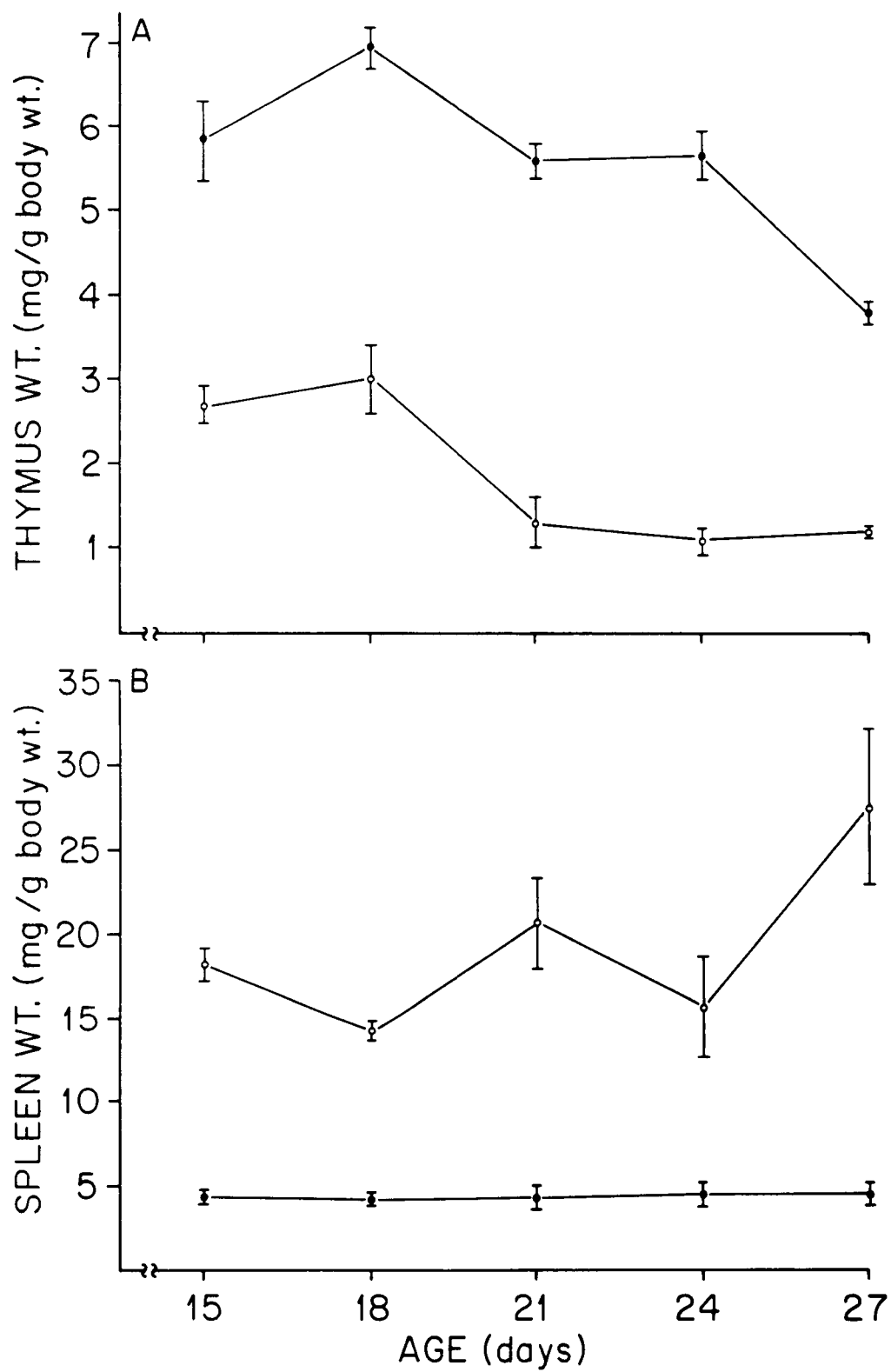


Table 1. *Macrocytic, normochromic anemia in scurfy mice.*^a

	RBC x 10 ⁶	Hgb(g/dl)	Hct(%)	MCV(fl)	MCH(pg)	MCHC(g/dl) ^b
Scurfy	2.71 (± 0.39)	5.7 (± 1.06)	17.8 (± 1.89)	70 (± 3.14)	26.1 (± 2.85)	36.8 (± 4.78)
Normal	6.47 (± 0.40)	11.9 (± 0.61)	35.5 (± 2.03)	55 (± 1.39)	18.4 (± 0.08)	33.4 (± 0.42)

^aResults are mean values (± standard error of the mean) derived from ten *sf/Y* mice and nine *+ / Y* siblings aged 15-25 days.

^bAbbreviations: RBC, red blood cells; Hgb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

PART 3. FATAL LYMPHOPROLIFERATIVE DISEASE IN THE SCURFY (*sf*) MOUSE
REQUIRES T CELLS THAT MATURE IN A *sf* THYMIC ENVIRONMENT:
POTENTIAL MODEL FOR THYMIC EDUCATION

CHAPTER 1.

ABSTRACT

The role of the thymus in the development of fatal lymphoproliferative disease in the scurfy mouse was investigated. Characteristic lesions of the X-linked mutation, scurfy (*sf*), include lymphohistiocytic proliferation in the skin and lymphoid organs, Coombs' positive anemia, hypergammaglobulinemia, and death by 24 days of age. Neonatal thymectomy doubled the lifespan of scurfy mice, moderated the histologic lesions, and prevented anemia, despite the continued presence of high levels of serum IgG. Animals bred to be scurfy (*sf/Y*) and nude (*nu/nu*) were viable, fertile, and free of scurfy lesions. Bone marrow from scurfy mice could reconstitute lethally irradiated, H-2 compatible animals but did not transmit scurfy disease. We conclude, from these data, that scurfy lesions are mediated by T lymphocytes that are required to mature in an abnormal (*sf*) thymic environment.

CHAPTER 2.

INTRODUCTION

Scurfy is a X-linked recessive mutation that produces lymphoproliferative disease and early death in hemizygous (*sf/Y*) males (1). Gross morphologic lesions of the scurfy syndrome include runting, scaly skin, squinted eyes, hepatosplenomegaly, enlarged lymph nodes and variable thickening of the ears. The characteristic histologic finding is a lymphohistiocytic proliferation and infiltration that effaces lymph node architecture, thickens the dermis, and forms nodular accumulations in portal areas of the liver. In addition to these morphologic lesions, scurfy males evidence a severe Coombs'-positive anemia and a marked polyclonal increase in serum IgG. All of the described lesions in the scurfy mouse suggest a disease primarily affecting the lymphoreticular system. Since T cells play an important role in other genetically determined lymphoproliferative diseases in the mouse (2), we investigated the contribution of the thymus to the development of the scurfy syndrome. Here we present evidence that T cells cause scurfy disease but only if they are educated in a genotypically scurfy thymic environment.

CHAPTER 3.

MATERIALS AND METHODS

Mice

Scurfy (*sf*) is maintained on a noninbred background (DG) by crossing *sf/+* carrier females to (C3Hf/Rl x 101/Rl) F₁ or (101/Rl x C3Hf/Rl) F₁ males. The mutation is also maintained on a 129/Rl congenic strain by mating *sf/+* carrier females to 129/Rl - *p c^{ch} p c^{ch}* males. Both *sf* stocks are housed in a conventional environment and fed a standard laboratory chow (Purina* 5001) and chlorinated water *ab libitum*. Twelve-week-old C57BL/6 female mice were kindly provided by Dr. Raymond Popp, Biology Division, ORNL. Twelve to sixteen week old, male, BALB/c nude mice were purchased from Life Science Inc., St. Petersburg, FL. Nude mice were housed in a semi-barrier facility that is free of mouse hepatitis virus (MHV).

Thymectomy.

Neonatal thymectomy was performed on 1 day old offspring of DG carrier females with slight modification of a previously described procedure (3). Modifications included: (i) ventral midline incision of the skin over the anterior thorax, (ii) manual removal of the thymus with a cotton-tipped swab instead of a vacuum apparatus, and (iii) closure of the skin incision with 6-0 silk sutures.

Bone Marrow Transplants.

Eleven normal male offspring of 129/RJ - *sf/+* carriers, aged 12-16 weeks, and eight twelve-week- old C57BL/6 female mice received bone marrow transplants from 17-23 day old 129/RJ - scurfy (*sf/Y*) and normal (+/Y) mice. The recipients were given lethal X-irradiation (750 R at 70 R/min; 300 kV; 12 mA; 1 mm aluminum filter) were injected with $1-2 \times 10^6$ bone marrow cells via the caudal vein. Bone marrow recipients were monitored at least twice weekly for clinical signs of scurfy disease. Periodic blood samples were obtained from tail veins for hematocrit determination, serum IgG quantitation, and/or hemoglobin analyses (C57BL/6 only). One control C57BL/6 female received lethal irradiation, but no bone marrow replacement, and died at 10 days post-irradiation.

Blood Analysis.

Hematocrits were determined by centrifugation of whole blood in heparinized microhematocrit tubes for five minutes in a Clay-Adams® Micro-Hematocrit centrifuge. Total serum IgG was quantified using radial immunodiffusion kits (The Binding Site, Inc., San Diego, CA). Hemoglobin electrophoretic patterns were generated on cellulose acetate plates using cystamine treatment (4). Direct Coombs' tests were performed as described (5).

Histopathology.

Tissues were fixed in 10% neutral buffered formalin, routinely processed, sectioned at 5 μ m, and stained with hematoxylin and eosin.

Southern Blots.

DNA was prepared from the spleen, lymph nodes, bone marrow, thymus, and ear (negative control) of the C57BL/6 mice that received bone marrow transplants.

The procedure for DNA for preparation was a slight modification of standard methods for the distal tail-tip biopsies (6,7). Southern blot analysis of genomic DNA was performed as described (8). A 0.8 kb BamHI-Hind III fragment, obtained from a locus closely linked to the pink-eyed dilution (p) locus was kindly provided by Dr. E.M. Rinchik.

Karyotypes.

Metaphase chromosomes were examined in C-banded preparations from the spleen and bone marrow cells of scurfy female mice (X^sX^s) (9). At least twenty-five metaphases per animal were examined.

CHAPTER 4.

RESULTS

Seventeen male and ten female DG neonates were subjected to thymectomy but only three males and one female survived until weaning age (3 wks). The causes of death in this group were hemorrhage during surgery and cannibalism by the dam. The three surviving males showed signs of the scurfy syndrome at the expected age of 14-15 days, including squinted eyes, scaly skin, swollen and reddened genital papilla, alopecia, and runting. One thymectomized *sf/Y* died at 37 days of age and the other two were euthanized, when moribund, at 48 days and 38 days respectively. These lifespans were considerably longer than the 24 day mean lifespan of euthymic scurfy males. Thymectomized *sf/Y* mice had histopathologic lesions similar to, but less severe than, nonthymectomized scurfy males. These characteristic microscopic lesions included leukocyte infiltrates in the portal areas of the liver, epidermal hyperplasia and hyperkeratosis, and diffuse dermal infiltration by mononuclear cells (Fig. 1A,B). Lymph nodes from thymectomized *sf/Y* mice were smaller than those seen in euthymic *sf/Y* males but had similar histologic lesions of increased lymphoblasts, no distinct cortical follicles, and expanded paracortical regions. The splenic white pulp in sections from thymectomized *sf/Y* mice was pale-staining and poorly organized.

Because elevated amounts of serum IgG, anemia, and a positive direct Coombs' test are characteristic of the scurfy syndrome, we tested the three thymectomized scurfy mice for these features. Hematocrits from thymectomized *sf/Y* males were normal despite elevated mean serum IgG levels of >1300 mg/dl compared to mean serum IgG values of

300 mg/dl in samples from age-matched, euthymic, normal mice. One of the three thymectomized *sf/Y* males was tested and found to be positive on the direct Coombs' test. The thymectomized female mouse, a non-scurfy control, had normal hematocrit and serum IgG values and normal microscopic anatomy when sacrificed at 36 days.

Neonatal thymectomy ameliorated the histologic lesions of scurfy, prevented anemia, and increased the lifespan of scurfy males. It did not, however, completely cure the disease nor did it prevent death. Since these data suggested that scurfy, at least in part, was mediated by T cells, some of which were already present at birth, we decided to create scurfy mice that were congenitally athymic. This was done by a breeding scheme employing homozygotes for the autosomal recessive mutation, nude (*nu*), that causes a lack of pelage and thymic hypoplasia.

Scurfy-nude mice were generated in offspring of (DG x BALB/c)-*nu/+*, *sf/+* carrier females mated to BALB/c-*nu/nu* males. They had no cutaneous lesions typical of haired scurfy mice, were not anemic, and survived far beyond the average lifespan of euthymic scurfy males (5.5-8.5 months vs 24 days, respectively). Two scurfy-nude mice died from opportunistic bacterial infections at 5.5 and 6.3 months of age. Complete necropsy of these scurfy-nude mice revealed no gross or microscopic lesions associated with the *sf/Y* genotype, but cystic hypoplasia of the thymus and hypocellular T cell regions in secondary lymphoid organs, typical of the nude phenotype, were found. Genetic proof of the *sf/Y*, *nu/nu* genotype in scurfy-nude mice was obtained by test crosses with DG carrier females (*sf/+*, *+/+*) that resulted in female scurfy (*sf/sf*, *nu/+*) progeny. Female *sf/sf* mice have gross, microscopic, and hematologic lesions identical to those of euthymic *sf/Y* mice and lifespans of less than 30 days. The female scurfy mice had normal microscopic anatomy in the reproductive tract and normal diploid karyotypes, indicating that they were not the result

of an $X^{sf}O$ genotype (1) and, therefore, must have received an X^{sf} chromosome from their scurfy-nude parent. Lack of scurfy disease in athymic sf/Y mice showed the absolute requirement for thymus-derived cells in this syndrome. We questioned whether the defect in the T cells of scurfy mice was inherent to the T cell lineage, *i.e.*, a stem-cell defect, or a functional abnormality acquired in transit through the thymus. Bone marrow transplants from mice with hematopoietic stem-cell defects can effect the mutant phenotype in lethally irradiated normal recipients. Examples include the dominant spotting mutation (W) and x-linked immunodeficiency (xid) (2). We, therefore, attempted to transmit scurfy disease via bone marrow transplants into H-2 compatible animals.

Bone marrow from 129/Rl- sf/Y mice was able to reconstitute lethally irradiated congenic animals without transmission of scurfy disease. All recipients were alive at 200 days post transplantation and had no visible cutaneous lesions. There were no significant differences in mean hematocrits or serum IgG levels in samples drawn from sf/Y and normal bone marrow recipients at 4, 8, and 20 weeks post transplantation. No histologic lesions indicative of the scurfy syndrome were seen in tissues from a sf/Y bone marrow recipient necropsied at 28.5 weeks.

The initial bone marrow transplant experiment suggested that scurfy disease is not an inherent defect in bone marrow stem cells. We could not, however, prove that the bone marrow-derived cells in those animals receiving sf/Y bone marrow were of the donor or of the recipient genotype. It was possible that donor sf/Y bone marrow provided temporary hematopoietic function to the lethally-irradiated recipient while a few remaining recipient stem cells eventually reconstituted the animal. To test this hypothesis, we transplanted 129/Rl- sf/Y bone marrow into C57BL/6 mice. These strains are H-2 compatible (both H-2^b) but differed at the locus for the beta chain of hemoglobin (Hbb) [129, Hbb^d ;

C57BL/6, *Hbb*'] and at the pink-eyed dilution (*p*) locus. Genetic differences at the *Hbb* locus produce variations in the electrophoretic mobility of the hemoglobin molecule. These variations can be readily detected in blood samples. Differences at the *p* locus between homozygous recessive (129/Rl,*p*) and wild-type (C57BL/6, +) DNA can be detected by Southern blot analysis. The efficacy of the bone marrow transplantation from 129/Rl-*sf*/Y mice to C57BL/6 recipients could therefore be monitored via assays for the *Hbb* and *p* loci in tissues of the transplant recipients.

C57BL/6 mice receiving transplants of 129/Rl - *sf*/Y or 129/Rl - +/Y bone marrow had no evidence of disease at 180 days post transplantation. Blood samples from these mice were serially assayed for hemoglobin type. At thirty days post-transplantation, both hemoglobin types were present in samples from all recipients (Fig. 2A) while at ninety days after transplantation, three of three animals receiving *sf*/Y marrow and three of four recipients of +/Y marrow expressed only the donor hemoglobin type (Fig. 2B). Recipients of *sf*/Y marrow continued to express only the 129/Rl hemoglobin type at 150 days (Fig. 2C). These results indicated complete ablation of endogenous erythroid stem cells in the recipients of *sf*/Y bone marrow, and suggested probable replacement of recipient leukocyte lines, *i.e.* lymphocytes, with donor cells. Using a 0.8-kb Bam HI-Hind III fragment that detects a restriction fragment length polymorphism (RFLP) between 129/Rl and C57BL/6 DNA, we assessed the genotype of cells constituting the bone marrow, spleen, lymph nodes, and thymus of C57BL/6 mice receiving 129/Rl - *sf*/Y or 129/Rl - +/Y bone marrow transplants. This was done using Southern blot analysis on DNA from these tissues. The bone marrow and lymphoid organs of mice that had received either *sf*/Y or +/Y bone marrow revealed mostly the 129/Rl (donor) type fragment while DNA from the pinna of the ear, a non-leukocyte rich control organ, showed largely the C57BL/6 RFLP (Fig. 3).

Based on these results, it appears that the recipients of scurfy bone marrow transplants have bone marrow and lymphoid organs repopulated by cells of the *sf/Y* genotype yet lack any evidence of scurfy lymphoproliferative disease.

CHAPTER 5.

DISCUSSION

We have previously described a novel, X-linked, lymphoproliferative disease in mice characterized by hypergammaglobulinemia, lymphadenomegaly, Coombs'-positive anemia, runting and early death. The critical role of T lymphocytes in this disease was suggested by limited studies of neonatally thymectomized *sf/Y* mice which had increased lifespans compared to euthymic *sf/Y* controls. Thymectomy moderated the lesions of scurfy but did not entirely prevent death. Failure to eliminate scurfy disease completely by neonatal thymectomy could have resulted from thymic remnants that remained after surgery, yet no thymic structures could be identified in any thymectomized scurfy male at necropsy. Alternatively, disease-producing T cells could have been generated in the fetal thymus, have exited to other parts of the body prenatally, and effected disease despite antenatal thymectomy. If scurfy-producing T cells are generated *in utero*, an animal that is scurfy and congenitally athymic (*nude*) (10) should be free of disease in postnatal life. We have shown that scurfy-nude mice are indeed free of scurfy disease though they may exhibit lesions normally associated with the immune-deficient, athymic state. These data indicate that mature T cells, the leukocyte lineage that is missing in nude mice (10), must be the critical mediators of scurfy disease.

We questioned whether the scurfy defect was inherent to the T cell lineage itself or the result of some environmental influence in the scurfy mouse. Lymphocytes in other genetically-determined immune dysfunctions of mice, such as *xid*, Y-linked autoimmune accelerator (BXS*B-*yaa**), and viable motheaten (*me^v/me^v*), continue to express the mutant

phenotype when transplanted into H-2 compatible, normal recipients (11, 12). We have shown that *sf/Y* bone marrow is capable of reconstituting lethally-irradiated, congenic or H-2 compatible animals, including the lymphoid compartment, but does not transmit scurfy disease.

Failure of disease expression in recipients of *sf/Y* bone marrow could be the result of a radio-resistant suppressor cell population capable of inhibiting the actions of scurfy T cells. Anti-scurfy suppressor activity in normal animals would be consistent with the observations that female carriers of scurfy (*sf/+*) have no disease and that grafts of *sf/Y* bone marrow, spleen cells, or thymus into nonirradiated, euthymic, congenic mice produce no clinical effect.

Alternatively, the microenvironment in an H-2 compatible, normal thymus might prevent the development of *sf/Y* T cells capable of mediating scurfy disease. The thymus provides a microenvironment where T cell precursors proliferate, differentiate, and mature into functional T lymphocytes that show major histocompatibility complex (MHC)-restriction and tolerance to self-antigens (13). Many recent reports have elegantly demonstrated acquisition of self-tolerance by intrathymic clonal deletion of self-reactive T cells (14, 15, 16, 17). This selection process is thought to be based on the affinity of MHC-restricted interactions between immature thymocytes and thymic epithelial and dendritic cells (18). It is possible that subsets of *sf/Y* T cells have an aberrant affinity for self-MHC antigens that induces a normal thymus to clonally delete them. This would explain the lack of scurfy disease in normal recipients of *sf/Y* bone marrow. It would also suggest that the scurfy thymic environment is abnormal and does not properly delete self-reactive T cells. In addition, nondeletional mechanisms may contribute to the acquisition of self-tolerance. Rammensee *et al.* have demonstrated that induced unresponsiveness in self-reactive T cells,

in vivo, can result from clonal anergy, not clonal deletion (19). The anergy does not appear to be caused by a classic suppressor cell activity. It could be that normal recipients of *sf/Y* bone marrow inhibit the actions of *sf/Y* T cells, via induced anergy, instead of deleting them in the thymus. This would be consistent with the Southern blot analysis of the recipients' lymphoid organs showing the majority of cells to be of the donor, *sf/Y* genotype. These bone marrow transplant experiments also prove that *sf/Y* bone marrow does contain cells capable of producing normal erythroid, myeloid, and lymphoid progeny in an appropriate environment.

The fact that scurfy disease requires an intact thymus in *sf/Y* mice, but is not transmissible by T cell precursors, is obviously intriguing. These observations suggest that scurfy may be a defect in thymic education of lymphocytes, and not an inherent abnormality in the T cell lineage. Education of scurfy T cells in a normal thymic environment can inhibit (or delete) cells producing the diseased phenotype. Despite the limitations of its short lifespan, the scurfy mouse could provide a useful study system for understanding the mechanisms of thymic education and how they relate to abnormal T cell function.

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APPENDIX

Figure 1.

Comparison of skin histopathology of thymectomized and euthymic scurfy mice. Sections from thymectomized scurfy mice (A) have dermal mononuclear cell infiltrates similar to those in the skin of euthymic scurfy mice (B). (hematoxylin and eosin X 132)

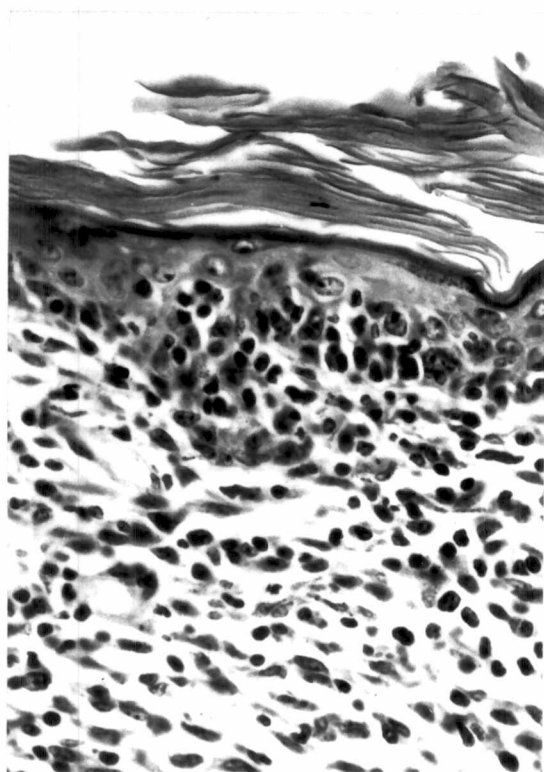
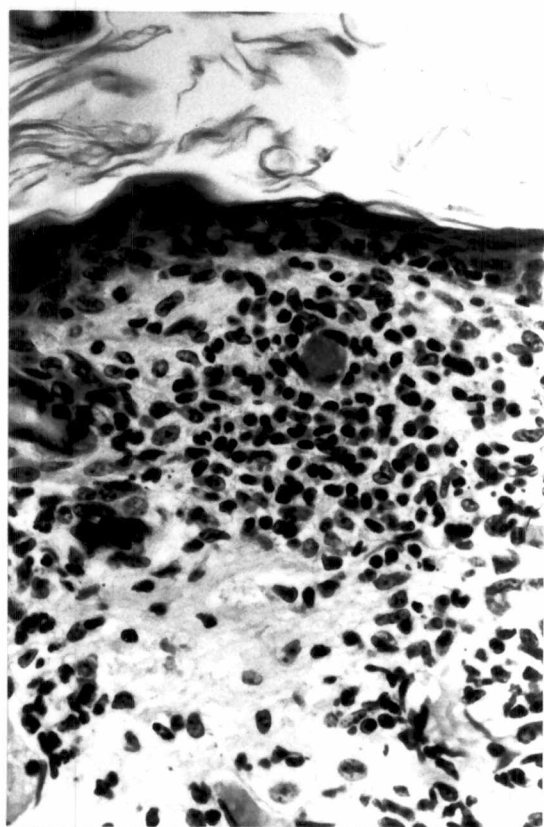


Figure 2.

Hemoglobin electrophoretic patterns in C57BL/6 mice receiving bone marrow transplants from 129/Rl - *sf*/Y (numbers 1-4) or 129/Rl - +/Y mice (numbers 5-8). Hemoglobin from C57BL/6 produces a single band (*Hbb*^s) while hemoglobin from 129/Rl yields two bands for the diffuse pattern (*Hbb*^d). Note the presence of only 129/Rl - type diffuse hemoglobin bands in recipients of 129/Rl - *sf*/Y bone marrow at 90 and 150 days. *Animal #3 died at 30 days post-transplantation from an overdose of anesthetic.

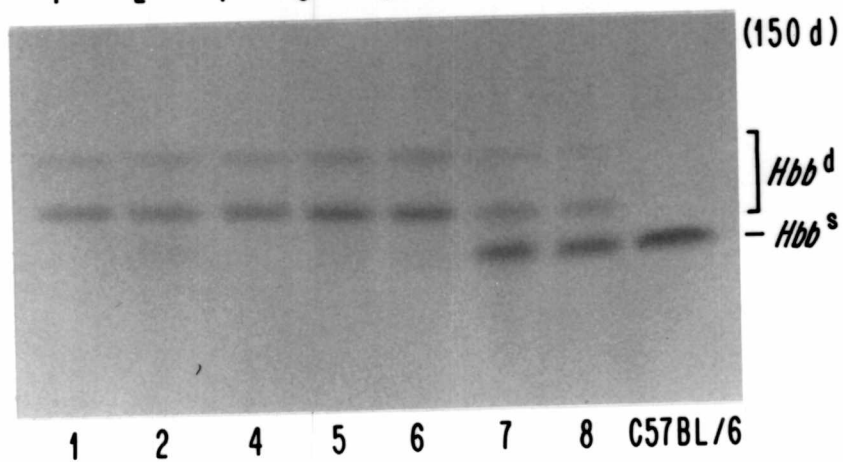
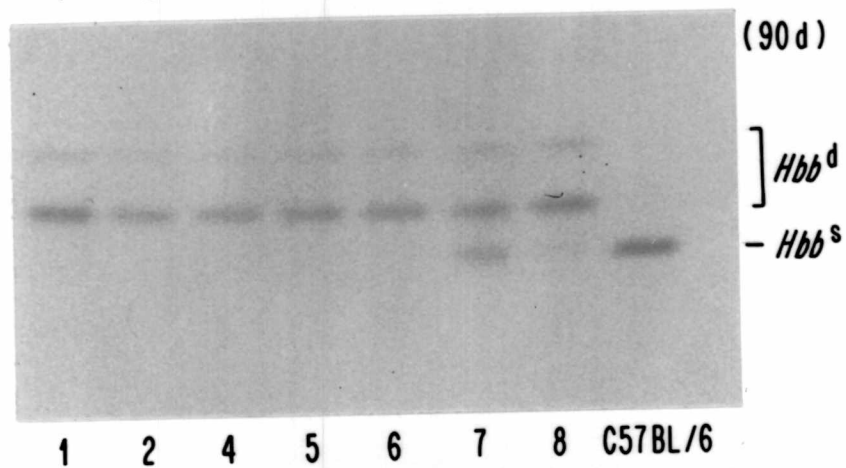
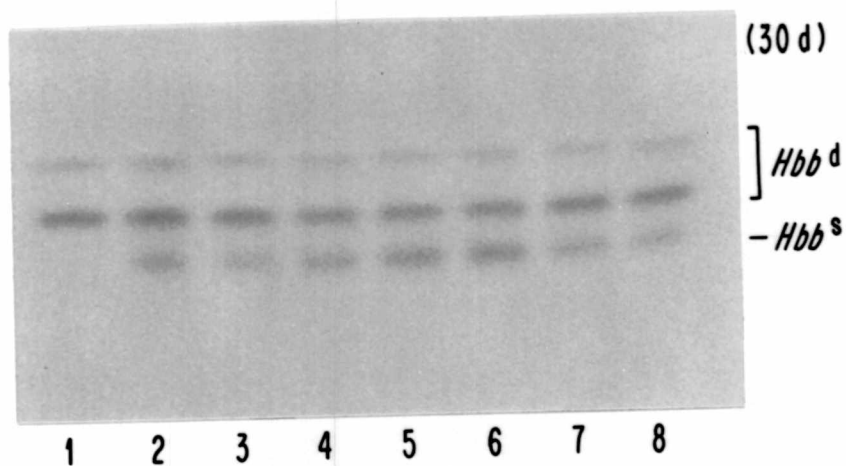
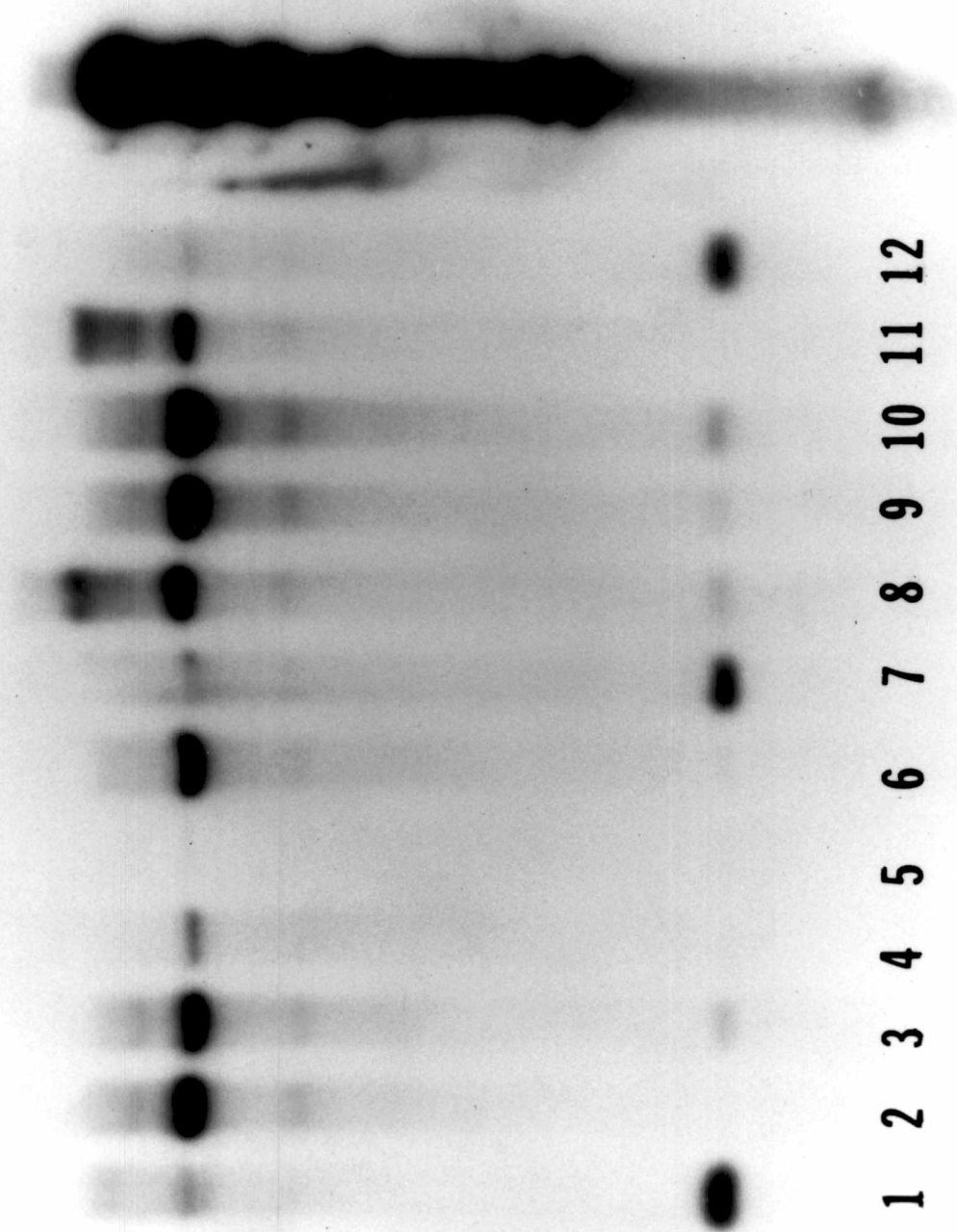


Figure 3.

Genotype of cells constituting the bone marrow and lymphoid organs of C57BL/6 mice receiving 129/Rl - *sf*/Y or - +/Y bone marrow transplants. Southern blot analysis of DNA treated with Bam-HI. Strain 129/Rl is homozygous recessive for pinkeye (*p*) and strain C57BL/6 is homozygous wild-type (+). DNA from the lymph node, thymus, spleen, and bone marrow of mice receiving 129/Rl - +/Y bone marrow (lanes 3-6) and - *sf*/Y recipients (lanes 8-11) reveals large amounts of the 10 kb 129/Rl-type Bam-HI fragment. DNA from the ear (lanes 7 and 12) shows an abundant 1.5 kb C57BL/6-type fragment. Control DNA from strain C57BL/6 and strain 129/Rl are in lanes 1 and 2, respectively.



1 2 3 4 5 6 7 8 9 10 11 12

PART 4. TRANSMISSION OF SCURFY LYMPHORETICULAR DISEASE BY
THYMIC TRANSPLANTS IS *H-2* RESTRICTED

CHAPTER 1.

ABSTRACT

The X-linked recessive mutation, scurfy (*sf*), causes a fatal lymphoproliferative disease characterized by runting, enlarged lymph nodes and spleen, hypergammaglobulinemia, exfoliative dermatitis, Coombs'-positive anemia and death by 24 days of age (Godfrey, *et. al.* manuscript in review). T lymphocytes are absolutely required to mediate this syndrome, as shown by disease abatement with neonatal thymectomy and, total lack of disease in athymic scurfy-nude (*sf/Y; nu/nu*) mice. The disease is not transmitted by bone marrow transplants, though cells of scurfy origin do reconstitute all lymphoid organs in the recipient mouse (Part 3). These data suggest that scurfy disease results from an abnormal thymic education process, and not from an intrinsic stem cell defect. We, therefore, tested the ability of *sf/Y* thymus transplants to transmit scurfy disease to athymic (nude) and euthymic mice. Morphologic lesions similar to those seen in *sf/Y* occurred in all *H-2* compatible nude mouse recipients, yet no disease was found in nude recipients that differed from the scurfy donor at the *H-2* locus. Euthymic recipients of *sf/Y* thymic grafts remained clinically normal as did all recipients of normal thymus transplants. These data suggest that the pathogenic actions of scurfy T cells are *H-2* restricted to targets expressing self-antigens. While some lesions found in *H-2* compatible nude mice receiving *sf/Y* thymus grafts resemble those of classical graft vs. host disease (GVH), the distribution of lesions to primarily skin, lymphoid organs, and liver is more typical of the scurfy phenotype.

CHAPTER 2.

INTRODUCTION

Mutations producing immune dysfunctions in the mouse such as beige (*bg*), nude (*nu*), and X-linked immunodeficiency (*xid*), have allowed the study of particular lymphoid cell types and their respective roles in the immune system. We have recently described the phenotype of a second X-linked gene mutation, scurfy (*sf*), with primary effects on the lymphoreticular system. Preliminary studies have shown that T lymphocytes are the critical mediators of this disease, but only if they mature in an *sf/Y* thymic environment. These studies suggest that scurfy disease might result from a defect in thymic education which permits the development of abnormal T cells. Here we present evidence that *sf* disease is transmissible by thymic transplants on an *H-2* restricted basis.

CHAPTER 3.

MATERIALS AND METHODS

Mice.

Scurfy (*sf*) is maintained on a 129/R1 (*H-2^b*) congenic background by mating *sf/+* carrier females to 129/R1 - *p c^hp c^h* males. Scurfy males (*sf/Y*) and normal male siblings (*+/Y*), aged 16-23 days, were chosen as thymus donors. Female C57BL/6 (*H-2^b*) mice and BALB/c (*H-2^d*; *nu/nu*) nude females were obtained from the Biology Division breeding colonies at 6-8 weeks of age. Female, C57BL/6NTac-nude mice (*H-2^b*; *nu/nu*) were purchased from Taconic, Germantown, NY. 129/R1-*sf* stocks are housed in a conventional environment while the nude mice and all recipients of thymus grafts were maintained in the pathogen-free barrier facility.

Thymus Transplants.

Thymus grafts were inserted under the kidney capsule as previously described (1). Irradiated thymus grafts received 830 R of X-irradiation (300 kV, 12 mA) at 515 R/min, *in vitro*, immediately prior to transplantation.

Histopathology.

Tissues were fixed in 10% neutral buffered formalin or Bouin's fixative, routinely processed, sectioned at 5 μ m, and stained with hematoxylin and eosin.

CHAPTER 4.

RESULTS

Thymic Transplants.

We chose to use C57BL/6 nude mice as recipients of the 129/Rl-*sf/Y* thymus grafts for two reasons. First, the athymic (nude) mouse should lack endogenous T cell activity that might influence the behavior of the grafted cells and, second, the *H-2* compatibility of strains 129/Rl and C57BL/6 should minimize the risk of graft vs. host disease. Fourteen C57BL/6 nude mice were given scurfy (*sf/Y*) thymuses, twelve received normal (+/Y) thymuses, and two received only sham surgery. All but one recipient of a *sf/Y* thymus showed signs of disease by 22 days post-transplantation with a mean time to onset of 17.3 days. The exceptional *sf/Y* thymus recipient remained healthy until 90 days after transplantation, but it then developed the same clinical syndrome as the other 13 animals. Four *sf/Y* thymus recipients died at 4, 18, 19, and 21 days, respectively, after transplantation; all other recipients were sacrificed, for histologic examination, when they became debilitated. Though one recipient of +/Y thymus was sacrificed at 85 days post-transplantation due to a sudden onset of diarrhea and rectal prolapse, all other recipients of normal thymus grafts remained clinically healthy.

The initial lesions in C57BL/6 nude mice receiving *sf/Y* thymuses were diffuse erythema, depigmentation, and progressive scaliness over the entire body. By 7-14 days after the onset of disease, the mice exhibited severe wasting, a hunched posture, tight leathery skin with thick sheets of scales, and intermittent diarrhea (Figs. 1,2). Animals surviving longer than 14 days after the onset of clinical signs continued to have

depigmented, leathery skin, but lacked the severe scaling seen early in the disease.

At necropsy, the gross morphologic lesions included severe loss of body fat, enlarged lymph nodes, and occasional thickening of the walls of the distal colon. Characteristic microscopic lesions in C57BL/6 nude recipients of 129/RJ - *sf/Y* thymuses were identified in the skin, lymph nodes, spleen, and liver.

Early cutaneous lesions consisted of diffuse dermal infiltrates of mononuclear cells that were more concentrated in the deeper layers of the dermis. In later stages of the disease, there was abundant dermal fibrosis and sclerosis with extensive loss of epidermal appendages. The epidermis exhibited early lesions of severe hyperkeratosis, parakeratosis, occasional intraepithelial vesicles, erosions, and serocellular crusts (Fig. 3). In animals surviving longer than 30 days post-transplantation, the epidermis was severely atrophic.

Lymph nodes, sampled in the second week after *sf/Y* thymus transplantation, were edematous and contained multifocal lymphoid necrosis, fibrinous exudation, abundant activated macrophages, and swollen endothelial cells. By the third or fourth week after engraftment, recipient lymph nodes were depleted of small lymphocytes and lacked normal architecture. There was a diffuse increase in background reticulum cells and macrophages, scattered multinucleated giant cells and multifocal fibrosis. Some lymph nodes were entirely filled by fibrous tissue.

The splenic white pulp was expanded by large blast cells originating in the peripheral B cell zones. These blast cells compressed the hypocellular, periarteriolar lymphatic sheath (PALS) which remained devoid of small lymphocytes. Abundant, pleomorphic mononuclear cells were found in the hepatic sinusoids and surrounding the portal triads (Fig. 4). Those mononuclear cells seen in the sinusoids were associated with slight, scattered, single cell necrosis of hepatocytes and areas of extramedullary hematopoiesis.

Thymic grafts could be identified in sections from five recipients of *sf/Y* thymuses. The grafts were largely composed of epithelial cells with large oval nuclei, finely stippled chromatin, and indistinct borders, mixed with medium to large lymphocytes and occasional multinucleated giant cells. There was no corticomedullary distinction in *sf/Y* thymic grafts and a paucity of small lymphocytes.

Sections from the thickened portions of the colon (2 of 14 animals) revealed focally-extensive mucosal hyperplasia, scattered erosions, and subacute colitis. In the longest surviving *sf/Y* thymus recipient (101 days), the colonic mucosal hyperplasia formed polypoid masses with extensive secondary infection. Fecal cultures for *Citrobacter freundii* var 4280 were negative in these *sf/Y* thymus recipients and in the *+/Y* thymus recipient sacrificed at 85d due to diarrhea and rectal prolapse. Though the colonic lesions were typical of *Citrobacter freundii* 4280 infection, we could not explain the failure to isolate the agent from these animals. The cause of the rare colonic lesions remained undetermined.

C57BL/6 recipients of 129/RJ - *+/Y* thymus grafts had microscopic anatomy that was essentially the same as the sham surgery controls. T cell-dependent areas in lymph nodes and spleen remained depleted of lymphocytes, no skin lesions were seen, and sections from the liver were normal. Thymus grafts could be identified in four of the seven animals necropsied. In contrast to the thymus grafts from scurfy mice, those from normal siblings were well populated by small lymphocytes and had multiple lobules with distinct cortical and medullary zones.

Irradiated Thymic Transplants.

We questioned whether the lesions in nude mice receiving *sf/Y* thymuses were mediated by the donor T cells and/or by cells derived by the recipient's bone marrow and

"educated" in the *sf/Y* thymic milieu. In order to decrease the number of viable *sf/Y* T cells transferred with the graft, we subjected the thymic lobes to intense, *in vitro*, X-irradiation prior to implantation. At 22 days after transplantation, recipients of irradiated scurfy thymuses developed lesions identical to those seen in recipients of non-irradiated thymuses. The killing of large numbers of *sf/Y* thymocytes prior to engraftment did not appear to significantly inhibit the transmission of scurfy disease.

Thymic Transplants into Euthymic Recipients.

The irradiated-thymic-graft experiment suggested that very small numbers of radio-resistant, *sf/Y* T cells might be able to produce severe disease in a T-cell deficient host. Scurfy T cells might proliferate in the host's target organs, release potent soluble mediators and/or recruit normal, host-derived cells, such as B cells, macrophages, etc, to participate in the disease process. We tested whether these *sf/Y* T cells were also capable of effecting disease in a normal, euthymic host by grafting 129/RJ - *sf/Y* or *+/Y* thymuses into euthymic C57BL/6 mice. All recipients remained clinically normal until sacrifice at 30 or 60 days after engraftment. At necropsy, no gross or microscopic abnormalities were seen. Thymic grafts from both *sf/Y* and *+/Y* animals were reduced to small fibrous scars, suggesting a non-*H-2* based, allojection of the grafts (C57BL/6 anti-129). This graft rejection may have inhibited the development of scurfy disease. In order to eliminate the possibility of allograft rejection, we transplanted thymus lobes from 129/RJ-*sf/Y* mice into euthymic congenic males. No clinical disease or microscopic lesions resulted from these transplants and the grafts were replaced by scar tissue.

Thymic Transplants into BALB/c Nude Mice.

Because of reports that show many T cell functions to be restricted on the basis of "self" MHC antigens (2,3), we decided to test the ability of *sf/Y* T cells to effect disease in athymic mice that were also *H-2* incompatible. Transplantation of competent T cells into immune deficient, *H-2* incompatible hosts should provoke a graft vs. host (GVH) reaction. We might then compare the lesions produced by 129/Rl-+/Y lymphocytes in GVH disease with those induced by *sf/Y* T cells in an *H-2* compatible nude mouse. BALB/c (*H-2^d*) nude mice received thymic grafts from 129/Rl (*H-2^b*) - *sf/Y* or +/Y mice and were sacrificed at 68, 91, and 119 days post-transplantation. There were no clinical signs of disease and no gross morphologic lesions in these mice. BALB/c nude mice receiving *sf/Y* thymuses had no microscopic lesions in skin, lymph nodes, or spleen. A well-populated graft was identified in only 2 of 8 recipients; one animal had received a *sf/Y* thymus and the other a +/Y graft. Scattered areas of hepatic necrosis, consistent with mild GVH disease, were present in sections from recipients of *sf/Y* grafts and +/Y grafts. These data suggest that scurfy T cells may require an *H-2* compatible environment to effect *sf* disease.

CHAPTER 5.

DISCUSSION

Transplantation of 129/RJ - *sf/Y* thymuses into *H-2* compatible, C57BL/6 nude mice produces severe cutaneous and visceral lesions, wasting, and death. The histologic lesions in skin, liver, and lymphoid organs resemble those described in subacute GVH disease (4). Subacute GVH disease is thought to arise from differences at minor histocompatibility loci outside of the *H-2* complex. In contrast to the lesions induced by *sf/Y* thymus grafts, subacute GVH disease also produces proliferative glomerulonephritis, intestinal ulcers and lymphoplasmacytic infiltrates, myelofibrosis, and lymphoid perivascular cuffing in visceral organs and glands. These lesions of subacute GVH disease were not found in any recipient of a *sf/Y* thymus and, in fact, *sf/Y* thymus-induced lesions were confined to the same organs (skin, liver, lymph nodes, spleen) that are primarily affected by naturally occurring, scurfy disease. Also, the lesions in *sf/Y* thymus recipients could not be a result of an alloreaction to minor histocompatibility loci since transplants of normal (+/Y) thymuses invoked no disease. Therefore, it is possible that scurfy represents an aberrant, T-cell response to some self *H-2* antigen. Abnormal T-cell reactions to *H-2* compatible cells have been previously described for another mouse strain, MRL-*lpr/lpr*, that exhibits lymphoproliferative disease (5). Reaction to self *H-2* antigens would also explain the failure to transmit disease to the *H-2* incompatible, BALB/c nude mouse; the *sf/Y* T cells would fail to see the appropriate *H-2* target. The inability of *sf/Y* T cells to interact with *H-2*-incompatible host cells in MHC-restricted immune functions such as antigen presentation, cytotoxic T cell killing, etc., might also contribute to the lack of disease in the BALB/c nude recipients.

We could not determine, from these experiments, whether transplanted *sf/Y* T cells require the assistance of host-derived leukocytes to effect scurfy disease. The presence of blast-transformation in the B cell areas of the splenic white pulp suggests an influence on these cells by the *sf/Y* T cells. The host might also have contributed thymocyte-precursors to the irradiated thymic grafts that would have been "educated" in a scurfy thymic environment. Previous studies, however, have demonstrated a radio-resistant thymocyte population capable of completely repopulating an irradiated thymus in nine days, while bone marrow-derived precursors do not begin to arrive until 10-13 days after irradiation (6,7). This fact, combined with the observation that recipients of irradiated *sf/Y* thymuses developed lesions at roughly the same time as recipients of non-irradiated grafts, suggests that the lesions were mediated by *sf/Y* T cells and not host-derived cells educated by the *sf/Y* thymic environment.

Scurfy T cells are required to mediate disease but appears to be subject to control in a normal, euthymic animal. Euthymic C57BL/6 mice rejected both *sf/Y* and *+/Y* thymus grafts without any gross or microscopic evidence of disease. Studies of thymus transplants from 129/R ℓ - *sf/Y* mice into euthymic congenics also failed to produce disease and the grafts were reduced to scar tissue. Finally, *sf/Y* thymocyte precursors that are educated in a normal, *H-2*-compatible thymus do not produce scurfy disease. The fact that *sf/Y* T cells transmit disease to *H-2* compatible hosts while *sf/Y* bone marrow does not, suggests that the defect in scurfy mice lies in the thymic education process, particularly as it applies to recognition and tolerance of self-histocompatibility antigens. The scurfy mouse should, therefore, provide a valuable *in vivo* system for investigating the mechanisms of thymic education of T cells.

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APPENDIX

Figure 1.

C57BL/6 nude mice given grafts of 129/Rl-sf/Y thymus (left) or 129/Rl-+/Y thymus (right). Mice receiving 129/Rl-sf/Y thymus exhibit diffuse erythema, severe exfoliative dermatitis, crumpled ears, leathery skin, and wasting of body fat. Recipients of 129/Rl-+/Y thymus are clinically normal.

Figure 2.

Skin of C57BL/6 nude mouse receiving 129/Rl-sf/Y thymus. There is severe scaling, loss of pigment, and thinning of the epidermis.

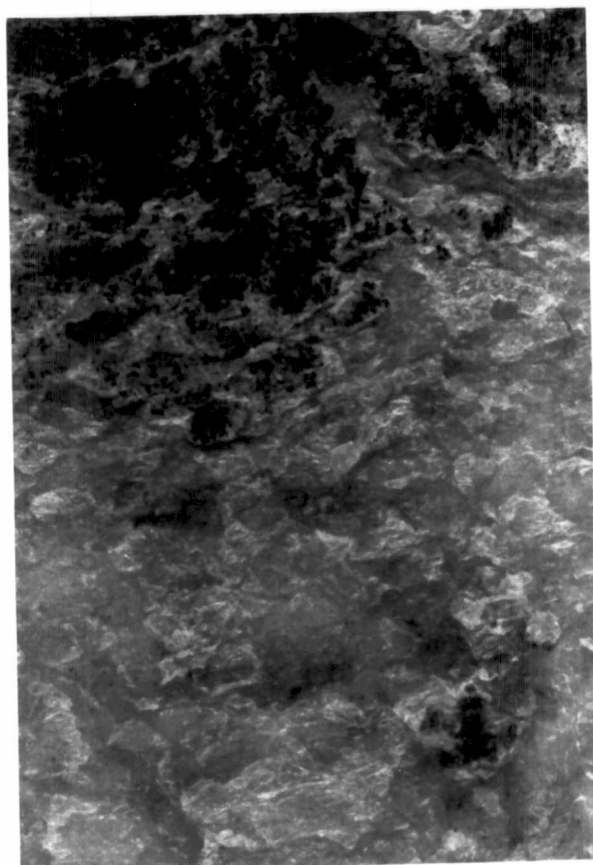
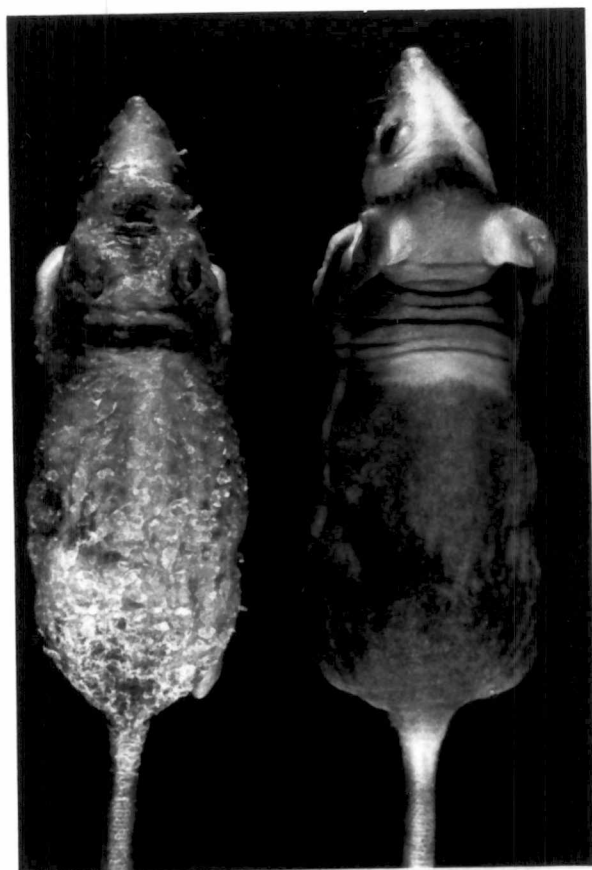
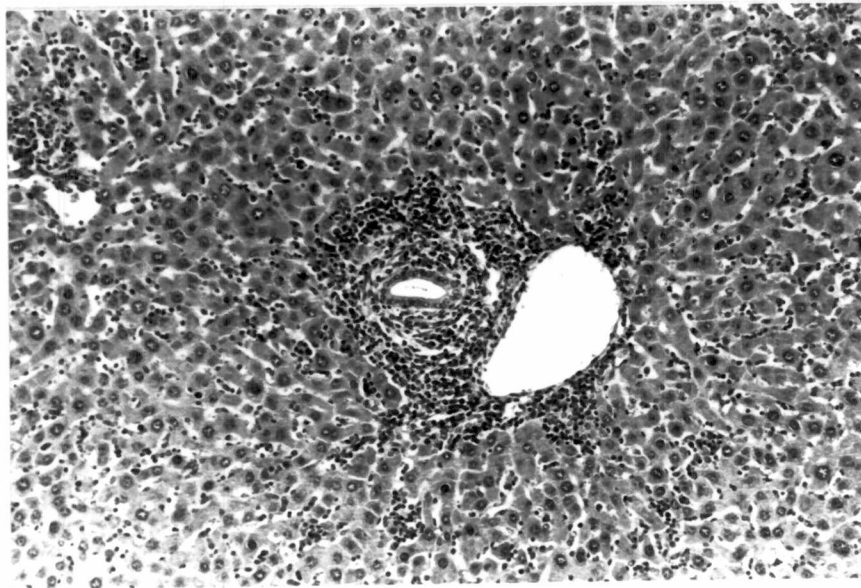


Figure 3.

Microscopic lesions in the skin of C57BL/6 nude mouse receiving 129-*sf*/Y thymus include serocellular crusts, epidermal atrophy, dermal sclerosis and a dense mononuclear cell infiltrate. (Hematoxylin and eosin, X 33)

Figure 4.

GVH-like lesions in the liver of a C57BL/6 nude mouse recipient of 129-*sf*/Y thymus. There is marked infiltration of mononuclear cells in the sinusoids and portal triads and scattered small foci of hepatocellular necrosis. (Hematoxylin and eosin, X 33).



PART 5. OVERALL SUMMARY

CHAPTER 1.

SUMMARY

Complete morphologic analysis of the scurfy mutant mouse indicated primary effects on the lymphoreticular, and possibly the hematopoietic systems. Scurfy mice consistently exhibit a lymphoproliferative disease that enlarges the spleen, infiltrates the liver and effaces the normal architecture of the lymph nodes. Mononuclear cells of a similar morphology are found throughout the dermis in association with pathologic changes in the overlying epidermis. Concomitant with these lymphoproliferative lesions, there is a severe atrophy of the thymus. Obviously, the identification of the cell types in *sf/Y* lesions would be an important first step for further investigation of the pathogenic mechanisms in scurfy disease.

Identification of mononuclear cell types by antibody-labeling of cell surface proteins (markers) is a common procedure in both research and clinical laboratories. Previous works have clearly established many correlations between the presence of certain cell surface proteins and those cells' functional capabilities, cell lineage, and maturation status, etc. (1,2,3,4,5). We attempted to identify the cells in the *sf/Y* lesions, in situ, using antibodies that recognize lineage-specific antigens on murine leukocytes. While *sf/Y* spleens and lymph nodes contain many cells with markers of mature B or T lymphocytes (B220, Thy-1, CD4, CD8), they also contain cells that lack any of these antigens. In addition, most mononuclear cells in the liver nodules and all mononuclear cells in the skin failed to stain for these common lymphoid markers. It could be that these non-staining mononuclear cells are not lymphocytes but this seems unlikely given the distribution of *sf/Y* lesions (lymphoid organs primarily) and the bland ultrastructural morphology of *sf* mononuclear cells (data

not shown). Therefore, they must be an abnormal type of lymphocyte deficient in typical murine lymphoid markers. Similar types of unusual lymphocytes have been reported in other genetically-determined, murine lymphoproliferative diseases such as those seen in MRL-*lpr/lpr* or C3H-*gld/gld* mice (6,7). Cells deficient in surface markers could mediate scurfy disease alone or in conjunction with the normally-marked B and T cells in the lymph nodes and spleen.

We next tested the functional capabilities of scurfy lymphocytes using classic in vitro assays for mitogen-induced proliferation, mixed lymphocyte reactions (MLR) and natural killer cell activity (NK). These assays were hampered by several factors. First, many of these functions were barely detectable in normal mice at the time (3-4 weeks of age) when scurfy mice were reaching the end of their lifespan. Responses in the control mice (+/Y siblings) were, therefore, difficult to compare with values from scurfy mice. Second, this is also the time period in which protection by maternal antibodies is waning. Such juvenile mice, housed in a conventional environment, would be susceptible to viral infection that might influence their lymphocyte responses during in vitro assays. Third, the spleen of a scurfy mouse contains many lymphoid cells that lack normal cell surface markers. If these cells are also functionally deficient, they might "dilute" out the response of the normally marked cells. This would make it difficult to detect true lineage-specific, functional deficiencies. Such dilution of in vitro lymphocyte responses has been reported in the MRL-*lpr/lpr* mouse (8). Finally, scurfy mice of sufficient age to respond to these assays (greater than 3 weeks) are already debilitated by their disease. This could certainly influence their in vitro lymphocyte responses.

Overall, lymphocytes from scurfy mouse performed poorly in all of the in vitro assay systems when compared to normal siblings (data not shown). The defective performance

could not be assigned to any particular lymphocyte or accessory cell population. we, therefore, decided to attempt immunomodulation of scurfy disease, in vivo, as a method of identifying the lineage of cells in scurfy lesions.

Neonatal thymectomy is a classic manipulation designed to virtually ablate T lymphocyte function in a mouse. Limited studies on thymectomized scurfy mice showed reduction of disease and increased lifespan. This suggested that T cells were important in the pathogenesis of scurfy disease. We then created mice that were scurfy and congenitally athymic (nude); these animals lacked any evidence of scurfy disease. T lymphocytes must, therefore, be the critical mediators of disease in scurfy mice, since all other leukocyte lines are present and functional in a nude mouse (8).

Defects in T cells might be inherent to a T cell precursor population or acquired by some environmental influence in the thymus. A defect inherent to the T cell precursors should be transmissible by bone marrow transplantation. Bone marrow from scurfy mice was able to reconstitute a lethally irradiated, *H-2* compatible mouse but did not transmit disease. In view of these results, it seems unlikely that scurfy is an inherent defect in all T cell precursors. Instead, it appears that the *sf/Y* thymic microenvironment causes (or allows) the development of aberrant T cells. Transit of *sf/Y* T cell precursors through a normal thymic microenvironment prevents the development of disease. This might be done by deletion of scurfy-mediating clones and/or induction of clonal anergy in these same cells.

Clonal deletion of thymocytes is thought to be a major mechanism by which the body generates T lymphocytes that are tolerant of "self" histocompatibility antigens and are restricted, in many of their functions, to interactions with only those cells that display self-antigens (9,10). The exact mechanism(s) of this clonal deletion is a subject of intense interest. Current dogma states that thymocytes are retained (positively selected) in the

thymus by interaction with Class I major histocompatibility (MHC) antigens on thymic epithelial cells (11). This positive selection might be based on the affinity of the Class I MHC recognition (12). Thymocytes are then thought to be deleted (negatively selected) by thymic dendritic cells on the basis of Class II MHC interactions (12). If an aberrant affinity for self H-2 antigens is evidenced by subsets of immature *sf/Y* T cells, a normal thymus should delete these clones. This might explain the failure of disease transmission by *sf/Y* T cell precursors in bone marrow transplants. Alternatively, the *sf/Y* T cells might have been allowed to develop in the normal thymus but rendered inactive or unable to respond to their respective target antigens. Evidence for a mechanism of tolerance by clonal anergy has been demonstrated in several systems (11, 13).

In summary, the X-linked recessive mutation, scurfy induces a fatal atypical, lymphoproliferative disease mediated by T lymphocytes. This disease is preferentially expressed in the lymphoid organs, liver and skin in an *H-2*-restricted manner. This *H-2* restriction, combined with the failure of *sf/Y* T cell precursors to effect disease in a normal host, suggests that scurfy is a defect in the mechanisms of intra-thymic acquisition of self-tolerance. The scurfy mouse should, therefore, offer a challenging model system for the study of these fundamental immunologic processes.

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