Tight Skin Mouse Subcutaneous Hypertrophy Can Occur in the Absence of αβ T Cell Receptor-Bearing Lymphocytes

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ABSTRACT. ***Objective.*** The tight skin (TSK) mouse has been proposed to be a murine model for heritable fibrotic disorders, and studies have implicated immune dysregulation in TSK pathogenesis. We evaluated the core features of TSK cutaneous pathology to ascertain whether cutaneous features were altered in TSK mice deficient in αß T cell receptor (TCR)-bearing lymphocytes.

Methods. Blinded qualitative evaluations of cutaneous and subcutaneous tissues were performed to determine the effect of the TSK mutation and whether the TCR-$\alpha^{-/-}$ mutation had any influence on TSK mediated pathology.

Results. Analysis by light microscopy revealed no difference in the thickness or any obvious changes in dermal architecture in 2 to 4-month-old TSK mice compared to age and sex matched littermate controls. The most consistent feature of TSK dermatohistopathology is the substantially expanded superficial fascia with accentuated "lamellar" architecture. We found that this reliable pathological marker and the characteristic skin tightness were not abrogated when the TSK mutation was crossed onto the TCR-$\alpha^{-/-}$ background.

Conclusion. The results dispute assertions that cutaneous and subcutaneous sclerosis in the TSK mouse are dependent upon the presence of CD4+ cells and that skin tightness can be dissociated from these pathological changes. (J Rheumatol 2001;28:1852–5)

Key Indexing Terms:

TIGHT SKIN MOUSE | MOUSE MODEL | CONNECTIVE TISSUE
AUTOIMMUNITY | FIBROSIS | FIBRILLIN-1

The tight skin (TSK) mouse was first described in 1976 as an autosomal dominant condition that occurred spontaneously in the inbred mouse strain B10.D2(58N)/Sn[1]. The mutation has recently been identified as a tandem duplication in the *fibrillin-1* gene involving 30–40 kbp of genomic DNA[2], which results in the production of a partially duplicated protein that is incorporated into ultrastructurally abnormal microfibrils[3]. Mice homozygous for the mutation die *in utero*, while heterozygotes develop a spectrum of abnormalities including pulmonary hypoplasia, right ventricular hypertrophy, bony and tendon sheath hypertrophy, and a distinctive accumulation of fibrous tissue in the superficial fascia[1]. Since Green's original description of the syndrome, many studies have implicated the immune system in mediating its cutaneous features[4-10]. On the basis of the skin thickening, enhanced collagen production, and a similar autoantibody profile, it was proposed that the TSK mouse might represent an animal model for scleroderma. Despite some similarities, very significant differences exist between the TSK mouse and patients with scleroderma[10]. This led some to question the appropriateness of this disease model[10] and others to propose that the syndrome was a better model for congenital fascial dystrophy[11]. This scepticism was amplified when it was recently determined that unmitigated skin tightness developed in Rag$^{-/-}$ TSK mice[12]. However, this latter study did not examine the effects of profound immunodeficiency on the characteristic dermatohistopathology, which is reported to arise independently of skin tightness and to be mediated by CD4+ cells[8]. Studies published since this report have implicated interleukin 4 from Th2 cells in the mediation of dermal sclerosis, with no mention of subcutaneous changes or skin tightness[9].

After reviewing these reports we were unsure of what exactly constituted the TSK cutaneous phenotype, and what features of TSK cutaneous pathology would be altered by a concurrent T cell receptor (TCR)-$\alpha^{-/-}$ mutation. We reevaluated the core features of TSK cutaneous pathology to determine whether these could be altered in the absence of αß TCR-bearing lymphocytes.

MATERIALS AND METHODS

Mice. TSK and TCR-$\alpha^{-/-}$ (deletion of the constant gene for the α TCR chain) mice on a C57BL/6 (B6) background were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Animals were maintained in microisolator cages in the animal facility of the Department of

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Supported by The Arthritis Society and The Natural Sciences and Engineering Research Council of Canada.

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Submitted November 27, 2000; revision accepted February 3, 2001.

Microbiology and Immunology, and received acidified water and laboratory rodent diet 50001 ad libitum (PMI Feeds Inc., St. Louis, MO, USA).

Genotyping of mice. TSK mice were genotyped by polymerase chain reaction (PCR) as described[9]. The PCR based genotyping strategy was always consistent with a blinded assessment of skin tightness, and with TSK-specific histopathology.

TCR-$\alpha^{-/-}$ mice were genotyped by flow cytometry (Becton Dickinson Facscan using CellQuest software) of peripheral blood leukocytes. TCR-$\alpha^{-/-}$ mice had insignificant numbers of CD4+ and CD8+ peripheral lymphocytes, and this was not influenced by a concurrent TSK mutation.

Assays for TSK cutaneous sclerosis. Mice were analyzed for skin fibrosis at 2–4 months of age. Mice were anesthetized with avertine and perfused with 10% buffered formalin. Skin measuring about 1 cm^2 and extending down to include the body wall musculature was removed by dissection from the para-midline, lower back region. The tissue was fixed and processed routinely. Sections were carefully cut perpendicular to the skin surface and stained with Masson's trichrome. Dermal thickness was measured from the top of the papillary dermis to the top of the fibrofatty tissue situated immediately above the panniculus carnosus, using an objective micrometer and eyepiece retinaculum (Nikon, Richmond, BC, Canada). Ten random measurements were taken per section, sampling from areas containing equal densities of hair follicles, glands, and other adnexal structures. Assessment of subcutaneous thickness using the above methodology was very imprecise because of considerable artifactual changes affecting this delicate layer. Therefore comparison of the superficial fascia from TCR-$\alpha^{-/-}$ TSK and regular TSK mice involved quantitating the number of distinct fibrous lamellae, an approach that generated more precise results. Blinded qualitative evaluations of cutaneous and subcutaneous tissues were performed to determine the effect of the TSK mutation and whether the TCR-$\alpha^{-/-}$ mutation had any influence on TSK mediated pathology.

RESULTS

Cutaneous phenotype of TSK mice. The Wilcoxon signed rank sum test was used to compare the difference in dermal thickness between 18 pairs of age and sex matched TSK and B6 littermate mice aged 2 to 4 months (both females and males). There was no correlation between dermal size and the TSK mutation with rank sum values equalling 81 (T_-) and 91 (T_+). This same instrument indicated that a statistically significant difference in this variable exists between the sexes, which was not influenced by the presence of the TSK mutation (Figure 1). The consistent 2-fold difference in dermal size between the sexes was seen in all experimental mice, which ranged in age from 2–4 months (Figure 1). No obvious qualitative differences in dermal architecture were readily apparent between the sexes, with or without the TSK mutation (Figure 2). Gross tissue disorganization was conspicuously absent in the TSK dermis, and unlike what is seen in sclerodermatous skin, adnexal obliteration and vascular changes were not apparent (Figure 2). The most significant aspects of TSK cutaneous pathology are the enormous expansion and architectural alteration of the subcutaneous tissue (Figure 3E). This layer in B6 mice consisted of less organized, smaller caliber fibrous bundles, with considerably more extracellular matrix between the bundles (Figures 3C, 3G). The TSK subcutis consisted of distinctive layers of large, faintly staining fibrous bundles (Figures 3A, 3E). We found that blinded qualitative evaluations of subcutaneous tissue can definitively identify the presence of the TSK mutation, even when performed by relatively inexperienced investigators.

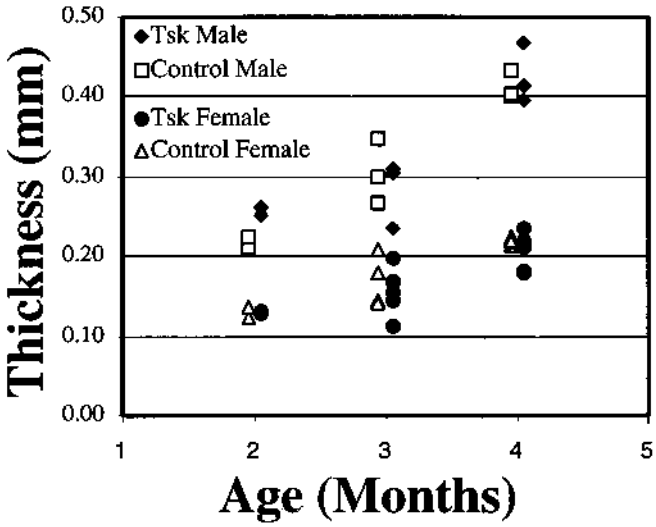

Figure 1. Dermal thickness was compared between 10 sets of age matched male and female TSK and B6 control mice (n = 40). Wilcoxon signed rank sum test was used for statistical analysis. The rank values of 81 and 91 indicate that no correlation exists between the TSK mutation and dermal thickness in mice aged 2–4 months. The same statistical instrument was used to compare this variable in age matched male and female mice aged 2–4 months, with or without the TSK mutation. The test revealed a clear relationship between male sex and increased dermal thickness ($p < 0.0001$), which is consistently 2-fold greater than that seen in female mice. This relationship was not influenced by the presence of the TSK mutation. Spacing between TSK and B6 control mice at each of the time points (2, 3, 4 months) does not reflect differences in age, but an attempt to make the scatter plot more legible.

Effect of the TCR-$\alpha^{-/-}$ mutation on TSK pathology. TCR-$\alpha^{-/-}$ TSK mice developed characteristic skin tightness that was indistinguishable from that of immunocompetent TSK littermates. Most importantly, the TCR-$\alpha^{-/-}$ mutation failed to arrest the diagnostic accumulation of faintly staining matrix bundles within the subcutis (Figure 3F). The thickness of this hypertrophied subcutis in TCR-$\alpha^{-/-}$ TSK mice, as measured by the number of fibrous lamellae present, was reduced by 12% compared to age and sex matched TSK controls (32.5 vs 37.0 lamellae, respectively); however, this difference was not statistically significant (Figure 4). Although a slight reduction in the volume of the subcutaneous tissue resulted from the TCR-$\alpha^{-/-}$ mutation, the light microscopic morphology of the TSK/TCR-$\alpha^{-/-}$ subcutis was indistinguishable from that of an immunocompetent TSK mouse (Figures 3A, 3B, 3E, 3F). The TCR-$\alpha^{-/-}$ mutation had no effect on the size or the morphology of the dermis or superficial fascia in mice possessing wild-type *Fibn-1* (Figures 3D, 3H).

DISCUSSION

The purpose of this study was to address some of the striking inconsistencies in the TSK literature. Our first goal

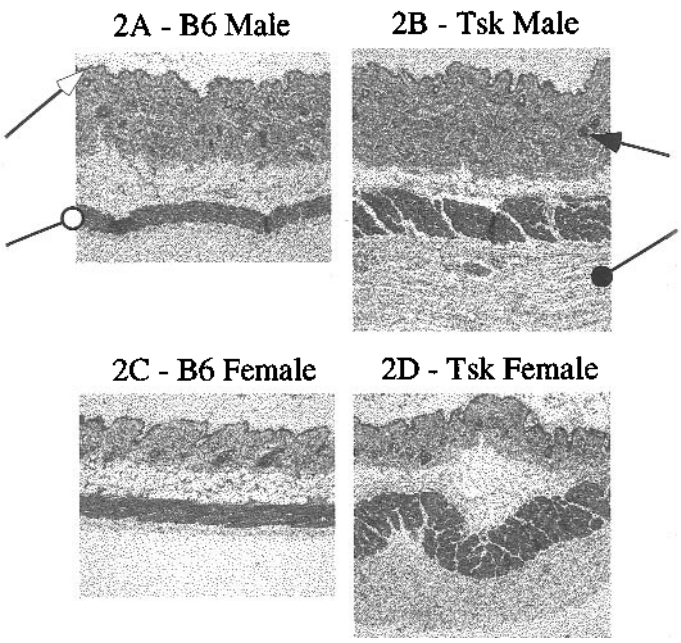

Figure 2. Cutaneous histology from 4-month-old animals is shown at 5× objective magnification. White arrow points to the epidermis from a male B6 mouse. No difference existed in the size or structure of this layer between the 4 animal types shown. White circle indicates the panniculus carnosus muscle, which appeared slightly enlarged in TSK animals (panels B, D). Black arrow points to an apparently normal sebaceous gland situated within the dermis. No obvious changes in dermal architecture were imparted by the TSK mutation, and adnexal obliteration and vascular changes were not apparent. Comparison of panel A with B, and C with D, reveals that the TSK mutation does not lead to a significant increase in dermal thickness in mice of this age. Black circle indicates the superficial fascia (i.e., subcutaneous tissue, hypodermis, subcutis). The subcutis from TSK animals is greatly hypertrophied (panels B, D), relative to age and sex matched B6 controls (panels A, C); however, the sections shown do not extend down to underlying musculature and thus are not appropriate for hypodermal comparison.

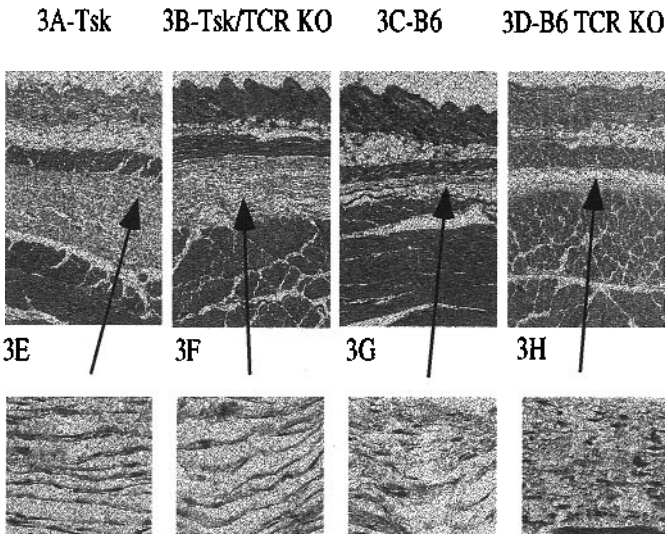

Figure 3. Cutaneous histology from 2-month-old female animals is shown at 5× objective magnification. Arrows from lower panels delineate locations where 40× objective magnification images were acquired. Both TSK and TSK/TCR-$\alpha^{-/-}$ mice exhibit marked subcutaneous hypertrophy (panels A, B, respectively) compared to B6 and non-TSK TCR-$\alpha^{-/-}$ mice (panels C, D, respectively). High power images of the subcutis depict varying morphological features of this layer characteristic of non-TSK animals. In some areas this layer consists of unorganized fibrous bundles intermixed with haphazardly oriented cells in a typical loose connective tissue pattern (not shown), while other areas have a more ordered arrangement of fine fascicles aligned parallel to the skin surface (panels G, H). This latter organization was chosen to make a fair comparison to the large and highly ordered, faintly staining fibrous bundles that dominate the subcutis in TSK animals (panels E, F). Panels E and F show the similarity in fiber structure between TSK and TSK/TCR-$\alpha^{-/-}$ mice. Note the location of the hypodermal tear in panel D, with the majority of the subcutaneous tissue adherent to the underlying musculature, illustrating the importance of deep biopsy.

was to determine the most reliable dermatohistological features of the condition, which has been controversial. Second, we wanted to reevaluate the dogma that unmitigated skin tightness could develop in the absence of typical histological changes seen in TSK mice[8].

In 1976 Green, *et al* published observations on a new autosomal dominant mutation affecting the "tightness" of mouse skin[1]. They characterized the cutaneous pathology as a massive increase in the subcutaneous tissue with no obvious changes present in the dermis or epidermis. Electron microscopy revealed that the hypertrophied subcutis consisted largely of tangled microfibrillar structures, which is consistent with the assembly of TSK *Fibn-1* into microfibrils that undergo an abnormal degree of aggregation[3]. This suggested that the excessive matrix deposition might be responsible for binding the skin to the underlying musculature. Menton, *et al* agreed with this premise and proclaimed that the extraordinarily well developed lamellar layer of connective tissue, which corresponds to the thin

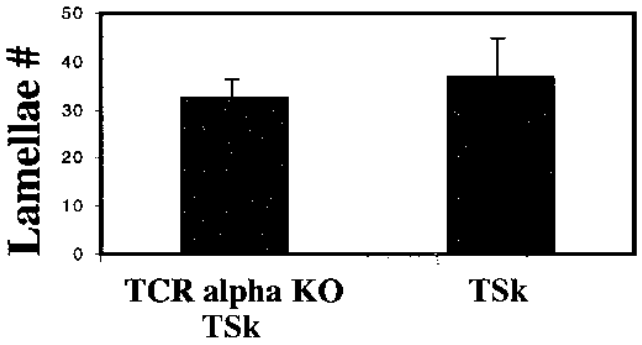

Figure 4. Subcutaneous fibrous lamellae were counted as a surrogate marker for hypodermal thickness in age and sex matched TSK mice with or without the TCR-$\alpha^{-/-}$ mutation (n = 4 per group). The TCR-$\alpha^{-/-}$ mutation was associated with a 12% reduction in subcutaneous thickness as measured by this assay; however, this difference was not statistically significant ($p > 0.05$). The fascial layers in non-TSK mice were too fine and branching to make such measurements (see Figures 3G, 3H).

layer of hypodermal fascia in normal skin, appeared to be continuous with the epimysium of the body wall musculature, forming a very firm attachment. They speculated that the difficulty experienced in grasping a skinfold on TSK mice was probably attributable to this[13]. However, Menton, *et al* disagreed with Green's assessment of the dermis, which they felt was both thicker and denser than control skin and lacked the normal fibrous architecture. This, they felt, explained the abnormal tensile properties of excised TSK skin[13]. Although the issue of TSK dermal changes is still unresolved, the fact that dramatic subcutaneous hypertrophy, but not skin tightness, is abrogated by the absence of CD4+ cells[8] argues strongly in favor of such changes, and against the idea that subcutaneous matrix deposition is mediating the skin tightness. However, the authors of this $CD4^{-/-}$ study[8] argued against any TSK mediated dermal alterations, which reopened the issue of tight skin etiology.

We had doubts about the dissociation of subcutaneous changes from skin tightness and reevaluated the effect of severe immunodeficiency on the development of diagnostic changes in the subcutis. Our results indicate that the elimination of all lymphocytes bearing αß TCR, which includes all CD4+ lymphocytes, did not prevent the development of skin tightness or subcutaneous hypertrophy. We speculate that biopsies of inadequate depth may have adversely affected previous conclusions[8]. If full thickness sections extending down to the body wall musculature had been obtained, it is likely that characteristic histological changes would have been seen in the $CD4^{-/-}$ TSK animals. It is uncertain why only the $CD4^{-/-}$ TSK and not regular TSK animals were affected by this artifactual tearing when the skin was peeled off the underlying musculature, but we feel that a CD4+ dependent immune response and resultant sclerosis might fortify the abnormal matrix, making it less likely to be torn. Similar errors in sampling may have contributed to the disregard of diagnostic subcutaneous changes in the study of TSK mice with the $IL4^{-/-}$ mutation[9] and TSK mice that expressed a highly restricted TCR repertoire[9], all of which had unperturbed development of subcutaneous hypertrophy and typical skin tightness (Oble DA, unpublished data).

These results, together with the report of Siracusa, *et al*[12], strongly suggest that the early features of the tight skin syndrome can arise independently of an adaptive immune response. There is abundant evidence to support an autoimmune component of this disease[4-10,14], and it remains to be seen whether this component is an inconsequential epiphenomenon or a significant factor capable of altering the natural progression of disease. We did not investigate the histological changes seen in TSK mice older than 4 months, which are reported to have serological evidence of autoimmunity[14] and progressive changes in the subcutis and dermis[14,15]. These late changes might represent an immune mediated enhancement of TSK pathology, a hypothesis that is supported by studies that found the titer of anti-topoisomerase 1 autoantibodies correlated with the degree of cutaneous sclerosis[15]. If, and how much, the core features of disease are modified by autoimmunity needs clarification, and experiments using older, profoundly immunodeficient TSK mice, such as IL-2 receptor $\gamma_c^{-/-}$/TSK, will facilitate this inquiry.

ACKNOWLEDGMENT

We would like to acknowledge Julie Chow at the UBC Hospital for tissue sectioning, and Soo-Jeet Teh for her invaluable assistance in the lab. Special thanks to Edward Kim for typing of experimental mice.

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