A Novel T Cell Receptor Transgenic Animal Model of Seborrheic Dermatitis-Like Skin Disease

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We have characterized a novel animal model of the common inflammatory skin disease seborrheic dermatitis (SD) that involves the expression of the self-specific 2C transgenic T cell receptor on the DBA/2 genetic background. Opportunistic fungal pathogens are present in the primary histological lesions and severe disease can be mitigated by the administration of fluconazole, demonstrating a role for infection in disease pathogenesis. Spontaneous disease convalescence occurs at 70–90 d of age and is preceded by an expansion of CD4^{+} T cells that partially restores the T cell lymphopenia that occurs in these animals. The adoptive transfer of syngeneic CD4^{+} T cells into pre-diseased DBA/2 2C mice completely abrogates the development of cutaneous disease. The pattern of disease inheritance in DBA/2 backcrosses suggests that one, or a closely linked group of genes, may control disease penetrance. Bone marrow reconstitution experiments demonstrated that the DBA/2 susceptibility factor(s) governing disease penetrance is likely non-hematopoietic since bone marrow from disease-resistant 2C mice can adoptively transfer the full disease phenotype to non-transgenic DBA/2 animals. This model implicates fungal organisms and CD4^{+} T cell lymphopenia in the development of a SD-like condition and, as such, may mimic the development of SD in acquired immunodeficiency syndrome.

Key words: AIDS-related opportunistic infections/animal/dermatitis/dermatomycoses/immunologic deficiency syndromes/mice/models/seborrheic/transgenic
J Invest Dermatol 124:151–159, 2005

Seborrheic dermatitis (SD) is a common medical condition afflicting approximately 3% of otherwise healthy individuals (Bergbrant, 1995; Faergemann, 1998; Ashbee and Evans, 2002; Gupta et al, 2004). The prevalence of this disease is dramatically higher among immunocompromised patients, particularly HIV-positive individuals, of whom 40%–80% have disease (Groisser et al, 1989). The high frequency among HIV-positive patients with CD4 cell counts between 400 and 500 cells per mm^3 has established SD as an early clinical marker of AIDS (Conant, 1994) and has spurred research into the pathophysiology of this enigmatic disease.

Unna first described SD as a clinical entity more than a century ago (Bergbrant, 1995). In 1874, Malassez demonstrated the presence of opportunistic fungi in lesional SD skin, which were later termed *Pityrosporum Malassezii* by Sabouraud (Bergbrant, 1995; Ashbee and Evans, 2002).

This led to the speculation that fungal pathogens played an active role in disease. Shortly thereafter it was found that these fungal organisms, the recently taxonomically revised *Malassezia* spp. (Gueho et al, 1996), represented normal inhabitants of the skin and a barrage of studies failed to conclusively demonstrate that an increased number of them existed in lesional tissue (Bergbrant, 1995; Ashbee and Evans, 2002; Gupta and Bluhm, 2004). The introduction of anti-fungal agents with potent activity against *Malassezia*, however, has reawakened interest in the fungal theory of SD pathophysiology (Gupta and Bluhm, 2004). Treatment studies have demonstrated that disease remission is associated with a reduction in the number of these organisms on the skin, and that recolonization with the fungus leads to a recurrence of disease (Bergbrant, 1995; Faergemann, 1998; Ashbee and Evans, 2002; Gupta and Bluhm, 2004). Although these studies seem to have reaffirmed Malassez's original hypothesis, several peculiar findings continue to perplex the understanding of SD pathophysiology. For example, the finding that asymptomatic individuals can harbor enormous numbers of *Malassezia* on the skin and yet never develop SD suggests that the number of yeast cells on the skin is only important for those with an inherent susceptibility to disease (Bergbrant, 1995). The high prevalence of SD among HIV-seropositive patients (Groisser et al, 1989) and the association of some SD cases with complement deficiency (Evans et al, 1977) suggests that various immune deficiencies can predispose to disease.

Here we describe a spontaneous murine model of SD-like disease that has a striking resemblance to human disease. The disease results from the expression of the 2C T cell receptor (TCR) transgenes (Sha *et al*, 1988b) in certain inbred mouse strains. The 2C TCR recognizes the naturally processed, ubiquitous p2Ca peptide (LSPFPFDL) presented by the class I molecule L^d (Udaka *et al*, 1993; Sykulev *et al*, 1994). This TCR is positively selected by H-2K^b in H-2^b mice and negatively selected by L^d in H-2^d mice (Sha *et al*, 1988a). On negatively selecting backgrounds, the 2C TCR recognizes the L^d-p2Ca ligand with high affinity ($\sim 2 \times 10^6$ per M) (Sykulev *et al*, 1994), which results in the comprehensive deletion of 2C TCR expressing double positive (DP) thymocytes (Sha *et al*, 1988a). This efficient elimination of T cell progenitor thymocytes precludes the rearrangement of endogenous TCR chain genes and the generation of a normal pool of T cells with diverse TCR specificities (Petrie *et al*, 1993). The effect of this extensive negative selection is a severe peripheral lymphopenia of $CD4^+$ and $CD8^+$ T cells. Although this is of little significance in some strains (Sha *et al*, 1988a), DBA/2 2C TCR transgenic mouse (D2C) mice develop an inflammatory skin disease with a striking resemblance to SD. Therefore the D2C mouse may be particularly well suited to study the pathophysiology of SD that occurs in the context of AIDS. Specifically, this model may illustrate how dysregulated systemic immunity interacts with cutaneous opportunistic pathogens to culminate in SD-like skin disease.

Results

D2C mice develop a spontaneous inflammatory skin disease D2C animals develop spontaneous cutaneous changes around the time of sexual maturity (32–38 d), which occurs within 1–2 wk of weaning (Whittingham and Wood, 1983), with males tending to develop disease earlier and more severely than female siblings. The course of disease is chronic with periodic flares. Disease in D2C mice occurs in a distinctive distribution involving primarily the ears, rostrum, and perineum. Although disease is often extremely inflammatory, only rarely does it extend beyond these "seborrheic areas" to cause generalized exfoliative dermatitis. Diseased mice are observed to scratch intensely at rostral skin, suggesting associated pruritus.

The gross appearance of lesional skin is dependent upon the chronicity of the lesion. Acute disease invariably begins in the peri-ocular region with ill-defined erythema and peri-ocular weeping. No vesicles or pustules are obviously present; however, occasional inflammatory papules can be appreciated. This initial blepharitis is later accompanied by prominent peri-ocular edema that typically results in entropion. At this stage, serous exudate oozes from lesional tissue and yellow-brown crusts form over erythematous skin. Occasionally, purulent exudate is expressed from the conjunctiva. Frequently, the lid margins of diseased animals are sealed by exudate. Although acute skin disease initially has indistinct margins, the subsequent development of lesional alopecia sharply marginates disease (Fig 1*A*).

Ear disease is a prominent feature of acute pathology and can precede other grossly apparent signs of disease. Swelling of the pinna in D2C mice is a prominent early fea-
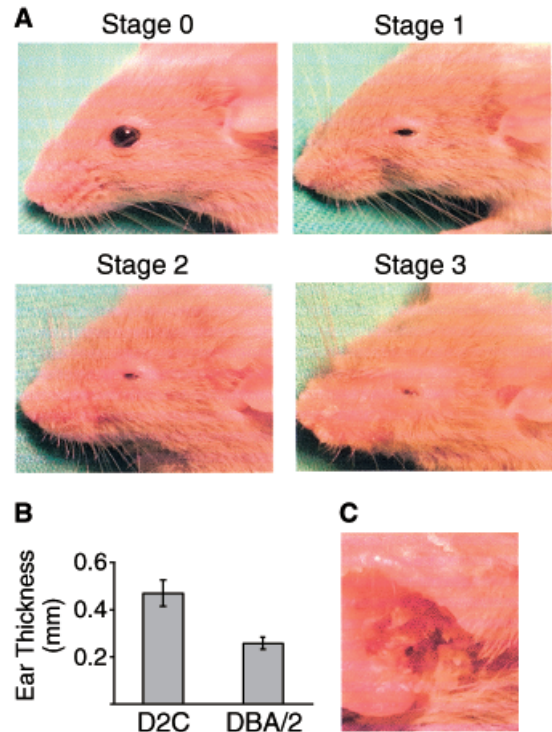

Figure 1
Gross pathology of disease. (*A*) Gross pathological changes were arbitrarily divided into four stages: stage 0, no disease; stage 1, minimal periocular disease; stage 2, major periocular swelling ± lid fusion with little contiguous spread to surrounding tissue; and stage 3, stage 2 features plus significant spread to contiguous tissue. (*B*) Pre-diseased 21-d-old DBA/2 2C T cell receptor transgenic mice (D2C) and age-matched DBA/2 controls were assayed for ear thickness. The difference was found to be statistically significant (p<0.05). (*C*) Ear disease in a stage 3 D2C mouse is shown. Note the concretion of hyperkeratotic debris occluding the external auditory meatus.

ture of disease (Fig 1*B*). Ear pathology can become quite severe, resulting in large concretions of hyperkeratotic crusted debris occluding the external ear canal (Fig 1*C*). Ear disease in these animals typically extends into the external auditory meatus, rather than out from it, and rarely involves the entire length of the canal.

In more chronic lesions, the prominent swelling and serous crusting of acute disease are replaced by thickening of the skin and the appearance of fine white scale. This lichenified appearance precedes convalescence, the onset of which is marked by a further reduction of swelling and the return of the normal dermatoglyphic pattern, and is followed by the regrowth of hair. Recovery from cutaneous disease begins at $\sim$ 70 d of age after which only subtle disease, if any, persists. After the establishment of remission, animals are resistant to recurrent disease.

To facilitate objective scoring of disease, we have arbitrarily divided disease into four stages: stage 0 (S0), no disease; stage 1 (S1), minimal peri-ocular erythema and edema; stage 2 (S2), major peri-ocular swelling ± lid fusion with little contiguous spread to surrounding tissue; and stage 3 (S3), S2 features plus significant spread to contiguous tissue (Fig 1*A*).

Microscopic pathology The microscopic features of disease are variable and dependent upon the clinical stage of
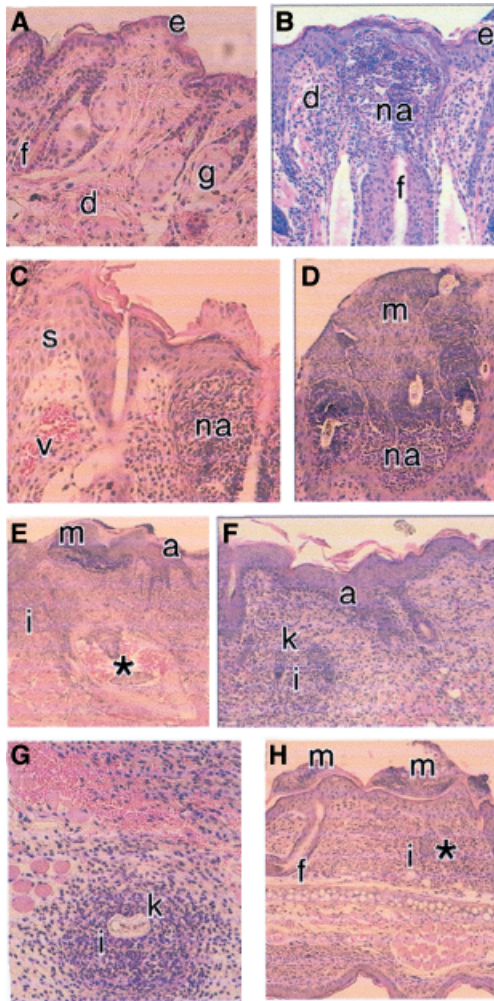
Figure 2
Microscopic pathology of disease. (*A*) Normally the epidermis (e) of non-lesional skin consists of one to two cell layers and overlays a non-inflamed dermis (d), containing plentiful sebaceous glands (g) and hair follicles (f). (*B*) The primary histological lesion in acutely diseased mice is a neutrophilic abscess (na) in the superficial follicle (f). (*C*) The neutrophilic abscesses are often situated adjacent to spongiotic (s) epidermis and edematous dermal papilla containing dilated blood vessels (v). (*D*) The neutrophilic abscesses coalesce into perifollicular mounds (m) of pyknotic neutrophilic debris. (*E*) Sub-acute lesions are characterized by primary and secondary histological changes. In these lesions, mounds of follicular debris (m) co-exist with acanthotic epidermis (a) and a multifocal coalescing inflammatory infiltrate (i) that is often concentrated around damaged follicles (*). (*F*) Chronically lesional skin is depleted of epidermal adnexa and has moderate acanthosis (a) co-existing with a dense dermal infiltrate (i). (*F, G*) Keratinacious debris (k) released from damaged adnexa is often present in the dermis of chronic lesions and is surrounded by a dense inflammatory infiltrate (i). (*H*) Ear pathology possesses similar histological features with mounds of debris (m) situated in a follicular distribution.

the animal. Lesional tissue obtained from acutely affected animals possesses: neutrophilic abscesses within the follicles and surrounding epidermis (Fig 2*B, C*); dilated vessels within edematous dermal papilla; and scattered areas of spongiosis involving the follicular infundibulum and epidermis adjacent to foci of follicular inflammation (Fig 2*C*). The neutrophilic abscesses coalesce into mounds of pyknotic neutrophilic debris and, together with prominent globules of eosinophilic serum and compact parakeratotic squames, form the mound-like scale-crusts that are situated near the

ostia of hair follicles (Fig 2*D*). Lesions, which clinically have a lichenified appearance, demonstrate epidermal thickening from the typical one to two cell layer thickness in normal murine skin (Fig 2*A*) to greater than 10 cell layers, and is sometimes accompanied by finger-like projections of acanthotic epidermis extending into the dermis (Fig 2*E*). Dense infiltrates of mixed inflammatory cells that surround glands and hair follicles are a feature of chronic pathology (Fig 2*E–H*). The extent of this pyogranulomatous inflammation is largely dependent upon the integrity of the adnexal structures since keratinacious debris released from damaged follicles is often at the center of inflammatory foci (Fig 2*F–H*).

Pattern of inheritance and genetics of disease susceptibility To investigate the genetic susceptibility factors that predispose to disease, we first backcrossed the 2C TCR transgenes onto various H-2^d expressing inbred strains. H-2^d congenic C57BL/6 inbred mouse strain (B6) 2C animals were found to be resistant to disease, whereas BALB/c 2C mice developed a mitigated disease phenotype ($<$50% of BALB/c 2C mice have clinical disease, n $=$ 10) (Fig 3*A*). Non-H-2^d expressing backgrounds, such as H-2^b expressing B6 and H-2^s expressing SJL mice, are resistant to disease (Sha *et al*, 1988a).

To further investigate the contribution of the DBA/2 genetic background to disease development, we determined the frequency and severity of disease development in successive backcrosses from the B6 to the DBA/2 background. Although (B6 × DBA/2) $N_1$2C ($N_1$2C) mice are resistant to disease (n $>$ 100), approximately 50% of $N_2$2C (n $=$ 50) animals, and 100% of $N_3$2C (n $=$ 25) and further DBA/2 2C backcrosses, develop variable degrees of spontaneous cutaneous pathology (Fig 3*B*). There was no difference in the incidence or severity of disease in H-$2^{b/d}$ and H-$2^{d/d}$ N_2 DBA/2 2C backcrosses, indicating that an increased dose of the cognate 2C transgenic TCR antigen (Ag) is not a factor in disease pathogenesis (data not shown). The extent of disease in successive backcrosses to the DBA/2 genetic background became progressively worse, up to approximately the fourth backcross generation at which point the typical D2C pattern of disease penetrance was established (Fig 3*B*). This pattern of inheritance is consistent with as few as one susceptibility factor, or a group of closely linked genes, controlling disease penetrance. To determine whether these susceptibility factor(s) were of hematopoietic origin, we attempted to adoptively transfer disease to H-2^d congenic B6 mice with T cell depleted D2C bone marrow. Interestingly, none of these recipient mice developed gross pathological changes or histological stigmata of disease (Fig 3*C*). To ensure that the recipient's hematopoietic systems were in fact donor derived (B6 × DBA/2) N_1 mice (BDN$_1$) were also used as recipients. BDN$_1$ mice normally express both the "b" and "d" H-2 haplotypes (H-$2^{b/d}$); however, after being reconstituted with D2C bone marrow, lymphocytes from these recipients failed to stain with the anti-H-2^b monoclonal antibody (mAb) HB51, indicating that their hematopoietic systems were donor derived (data not shown). These results indicated that the DBA/2 defect(s) could be non-hematopoietic and that disease might be transferred to non-transgenic DBA/2 recipients with marrow from H-2^d congenic B6 2C mice. Strikingly, 100% of the
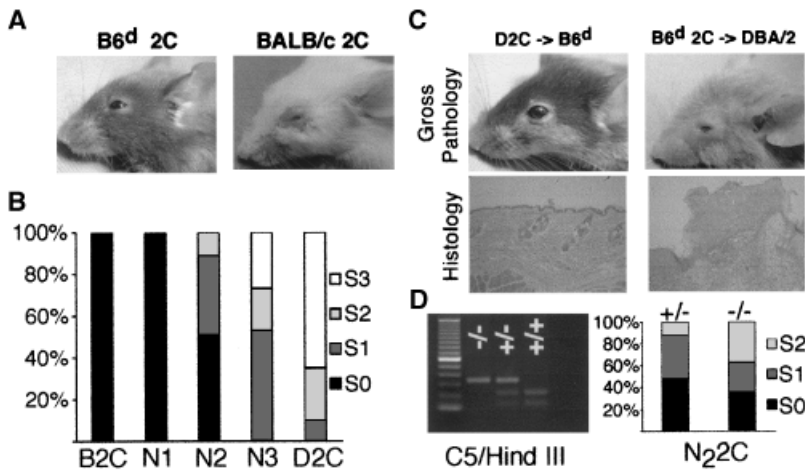
Figure 3
Strain susceptibility and disease modulating factors.
(*A*) H-2^d B6 2C mice are resistant to disease whereas BALB/c 2C mice develop a mitigated form of disease with reduced penetrance. (*B*) Disease incidence and severity increases with successive backcrosses of the 2C T cell receptor transgenes from the H-2^d congenic B6 background (B2C) to the DBA/2 background (DBA/2 2C T cell receptor transgenic mice (D2C)). (*C*) Bone marrow from D2C and B6 H-2^d 2C animals was adoptively transferred to lethally irradiated non-transgenic B6 H-2^d and DBA/2 recipients, respectively. Photographs of the chimeric mice are shown along with representative histology from the rostral skin. (*D*) DNA from C5-sufficient, C5-deficient, and an animal heterozygous for these C5 alleles was amplified and digested with *Hind*III demonstrating how the C5 genotype can be determined using this technique. This assay was used to determine the C5 genotypes of N$_2$2C backcrosses to the DBA/2 background, which were compared with the extent of clinical disease.

DBA/2 recipients of H-2^d B6 2C bone marrow developed the disease phenotype (0% S0, 45% S1, 30% S2, 25% S3, n = 20), with gross and microscopic features indistinguishable from those of D2C mice (Fig 3*C*).

The striking resemblance between D2C and human SD pathology (Rook *et al*, 1992) and the realization that non-hematopoietic factor(s) may control disease penetrance suggested that the natural DBA/2 deficiency in the fifth component of complement (C5) might play a role in disease pathogenesis (Wetsel *et al*, 1990). A null mutation of C5 was previously implicated in an inflammatory form of SD (Evans *et al*, 1977) and the mitigated phenotype of C5 sufficient BALB/c 2C mice provided further support for this hypothesis. To further address this possibility, we used a PCR-RFLP-based assay on N$_2$2C DBA/2 backcrosses to determine whether the segregation pattern of the deficient DBA/2 C5 allele was similar to the pattern of disease inheritance (Fig 3*D*). C5-deficient N$_2$2C animals had a slightly worse phenotype relative to C5-sufficient animals, suggesting that C5 deficiency may modulate disease expression. However, several C5 sufficient N$_4$2C mice developed severe pathology (data not shown), and many N$_2$2C mice homozygous for the defective DBA/2 copy of the C5 gene were completely asymptomatic (Fig 3*D*), demonstrating that the defect is neither necessary nor sufficient for disease.

Identification of fungi in the primary histological lesion Since the overgrowth of opportunistic basidiomycetes fungi is a feature of SD pathophysiology (Ashbee and Evans, 2002), anti-fungal staining of lesional skin was performed to determine whether this was also true for D2C mice. Grocott's methenamine silver (GMS) and periodic acid schiff (PAS) staining consistently revealed numerous small ovoid structures in the superficial layers of keratin and within the neutrophilic abscesses in lesional tissue. These were often organized in clusters and possessed pale centers. No deep invasion or mycelial shift was appreciated in any of the sections (Fig 4*B–F*). These positively stained structures were not readily apparent in non-lesional skin from diseased animals or from DBA/2 control skin (Fig 4*A*).

Reversion of cutaneous pathology with anti-fungal treatment The presence of fungal material in the primary

histological lesion suggested that opportunistic fungal pathogens might be playing an active role in disease pathogenesis. Imidazole anti-fungal agents have excellent activity in the skin (Faergemann and Laufen, 1993) and are used to treat SD (Gupta and Bluhm, 2004). Using an established dosing strategy for azole-responsive murine fungal infections (Louie *et al*, 1998), we found that a considerable degree of clinical disease in S3 animals was reversed after a 9-d course of fluconazole whereas the condition of phosphate-buffered saline (PBS)-treated animals remained unchanged or deteriorated (Fig 5*A*, *B*). Importantly, the clinical resolution of disease was associated with the mitigation of the typical histological changes and a reduction of PAS staining in tissue sections taken from previously lesional skin (Fig 5*C*).

D2C mice are severely immunocompromised Given that immunocompromised patients are known to suffer from a high incidence of SD (Groisser *et al*, 1989), we investigated the effect of the strongly self-reactive transgenic 2C TCR on immune competence. Based upon data from N$_1$2C mice (Sha *et al*, 1988a), we expected and found that the expression of the 2C TCR in the H-2^d expressing DBA/2 background induces massive central deletion and a peripheral lymphopenia (Fig 6*A*). D2C mice were found to have a 10-fold reduction in the total number of thymocytes (3.8 × 10^6 vs 3.8 × 10^7, p < 0.05) and have 500-fold fewer DP thymocytes (6.1 × 10^4 vs 3.2 × 10^7, p < 0.05) compared with DBA/2 controls. The D2C thymus was also characterized by a marked reduction of CD8$^+$ and CD4$^+$ single positive cells relative to non-transgenic controls (Fig 6*A*). The peripheral immunophenotype of D2C mice reflects the negatively selecting thymic environment (Fig 6*A*) with a 4-fold reduction in total lymphoid CD4$^+$ T cells (7.5 × 10^6 vs 2.9 × 10^7, p < 0.05) accompanying a 13-fold reduction in total lymphoid CD8$^+$ T cells (1.4 × 10^6 vs 1.8 × 10^7, p < 0.05). We speculated that this T cell lymphopenia reflected a reduced thymic output of mature T cells. Supporting this hypothesis, there were virtually no CD4$^+$ T cells present in the spleens of 10-d-old D2C mice, whereas spleens from age-matched DBA/2 animals contained significant numbers of these cells (3.6 × 10^4 vs 4.6 × 10^5 cells, respectively, p < 0.05). To ascertain whether this level of T cell lymphopenia was
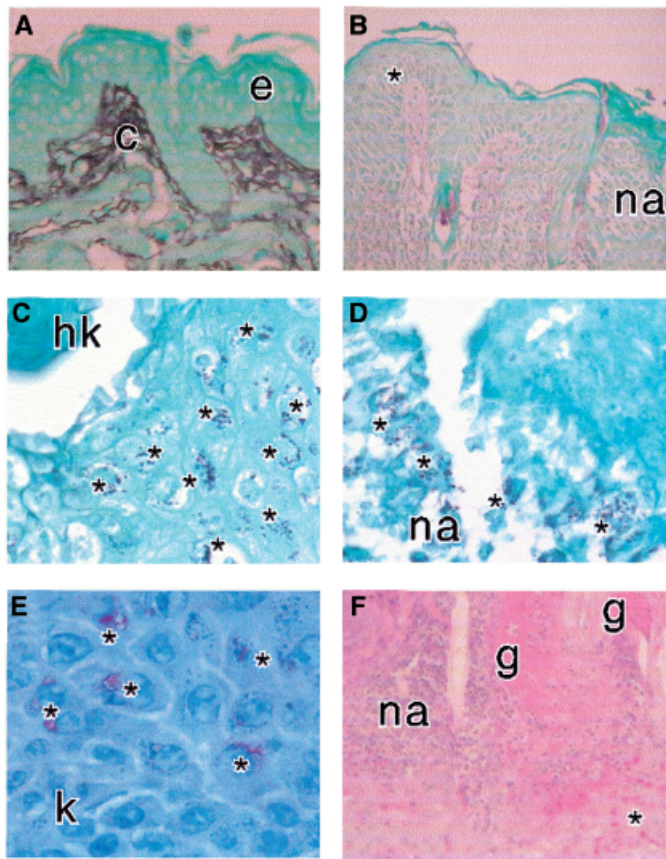
Figure 4
Anti-fungal staining of lesional skin. (*A*) Non-lesional epidermis (e) does not stain with Grocott's methenamine silver (GMS); however, GMS stains dermal connective tissue (c). (*B*) Finely speckled staining with GMS is frequently observed in lesional epidermis and often surrounding follicular abscesses (na). (*C, D*) High-powered views of lesional skin demonstrate small round GMS-stained structures (*) in the epidermis below hyperkeratotic (hk) mounds and within the neutrophilic abscesses (na). (*E, F*) Small structures (*) clustered together around keratinocytes (k) from lesional skin stain with periodic acid schiff (PAS) as well. Note the PAS-positive globules of serum (g) within the neutrophilic abscess (na) and nascent scale-crusts.

associated with functional immunocompromise, the T cell-dependent Ag hen egg lysozyme (HEL) was used to immunize D2C mice and syngeneic DBA/2 littermates. Ten days after challenge, DBA/2 mice had mounted a strong humoral response but D2C mice had not (Fig 6*B*). Interestingly, despite a failure in Ag-specific antibody generation, D2C mice possess massively increased levels of serum IgG (Fig 6*C*) and enlarged lymphoid organs (Fig 6*D*) that contain normal to increased numbers of B lymphocytes (Delaney, 1999).

Correction of CD4$^+$ T cell lymphopenia correlates with disease remission and occurs as a result of massive Ag-driven peripheral expansion To first investigate the possible link between T cell lymphopenia and disease, we examined the peripheral blood CD4 counts immediately before the time of pubescence (Whittingham and Wood, 1983), when animals become susceptible to disease. The CD4 counts in these mice were nearly 30-fold lower than control counts (Fig 6*E*). Since CD4$^+$ T cells increase over time in D2C mice, we speculated that disease convalescence might occur as a result of the acquisition of a protective number of
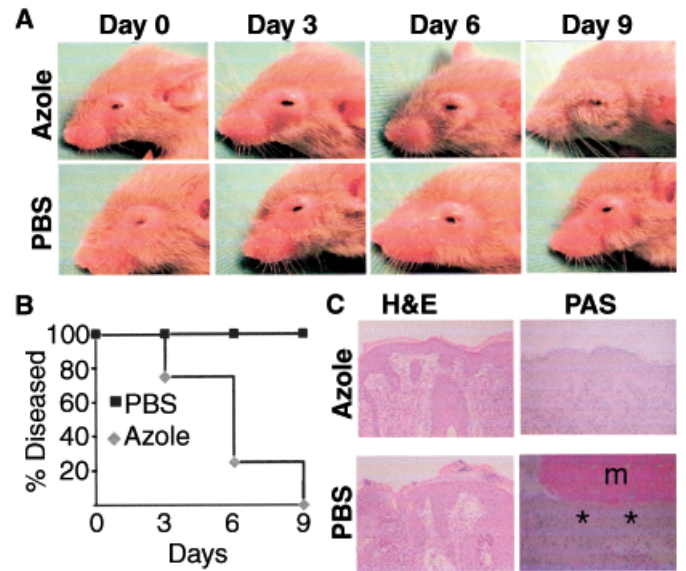

Figure 5
Treatment of diseased DBA/2 2C T cell receptor transgenic mice (D2C) with anti-fungal medication. (*A*) Acutely ill D2C mice with severe pathological changes were treated for 9 d with phosphate-buffered saline (PBS) or fluconazole. (*B*) Recovery of mice was monitored over the 9-d treatment period. Mice were considered to be disease free when hair regrowth began over lesional rostral skin. (*C*) Representative histological sections stained with hematoxylin & eosin (H&E) and periodic acid schiff (PAS) are shown. Note the punctate epidermal PAS staining (*) in the PBS control skin, which underlies a large mound of debris (m) that also stains prominently with PAS because of the inclusion of serum.

CD4$^+$ T cells. Examination of convalescent D2C mice revealed that the CD4$^+$ T cells in these animals had expanded over 400% since pubescence whereas the CD4$^+$ T cells from DBA/2 control animals had expanded a meager 28% over this same window of disease susceptibility (Fig 6*E*). Complete disease remission occurs when CD4$^+$ T cells have accumulated to between 400 and 1000 cells per mm^3, which takes place several weeks after the onset of convalescence.

To better demonstrate the extent of CD4$^+$ T cell peripheral expansion in D2C mice, we administered a 10-d course of bromodeoxyuridine (BrdU) to 50-d-old D2C mice and age-matched DBA/2 controls. Consistent with previous studies (Tough *et al*, 1999), we found that this short administration of BrdU labels negligible numbers of peripheral CD4$^+$ T cells from non-transgenic mice whereas 30% of the CD4$^+$ T cells from D2C mice incorporated the marker, indicating that massive peripheral T cell expansion was occurring (Fig 6*F*). To determine whether this represented homeostatic expansion to fill a lymphopenic environment or whether these cells were dividing after exposure to cognate Ag, we stained these cells with the memory markers CD44 and CD62L as well as the acute activation marker CD69. The vast majority of CD4$^+$ T cells from DBA/2 mice expressed an immunophenotype typical of naive cells (CD44^{lo}, CD69$^-$, and CD62L^{hi}), whereas the majority of CD4$^+$ T cells from D2C mice were CD44^{hi}, CD69$^+$, and CD62L^{lo} (Fig 6*F*). This pattern suggests that the expansion of CD4$^+$ T cells in D2C mice is Ag driven (Goldrath *et al*, 2000).

The adoptive transfer of syngeneic CD4$^+$ T cells abrogates the development of disease To see whether disease
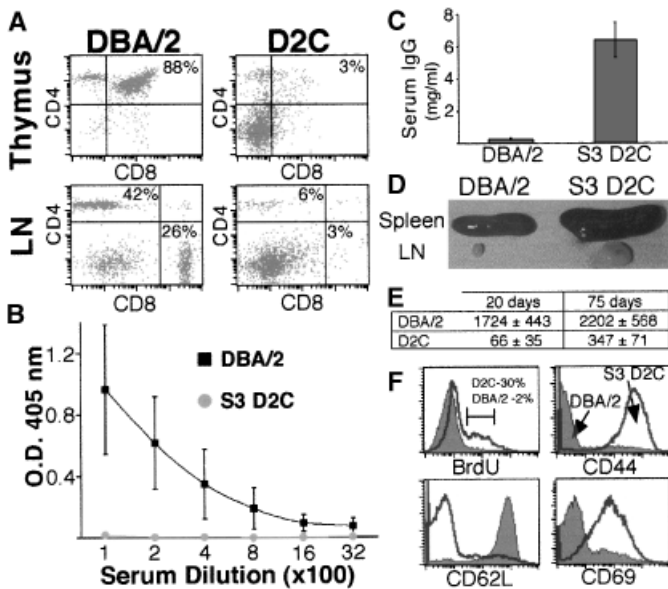
Figure 6
Immunological defects of DBA/2 2C T cell receptor transgenic mice (D2C). (*A*) Thymocytes and lymph node (LN) cells from DBA/2 and D2C mice were stained with monoclonal antibodies directed against CD4 and CD8. Dot plots are gated on live cells. (*B*) Forty days old DBA/2 and D2C mice were immunized with hen egg lysozyme (HEL) and assayed for HEL-specific IgG by ELISA. (*C*) Total serum IgG was determined in DBA/2 and diseased D2C mice by ELISA. (*D*) Representative photographs are shown of lymphoid organs from DBA/2 and diseased D2C mice. (*E*) Peripheral blood CD4 counts were determined for 20-d-old pre-diseased D2C mice, 75-d-old convalescent D2C mice, and age-matched DBA/2 controls. (*F*) LN cells from 50-d-old D2C and DBA/2 mice were stained with the indicated markers. Histograms are gated on live CD4+ T cells. For bromodeoxyuridine (BrdU) staining, animals were first fed BrdU for 10 d.

remission in D2C mice is induced by the acquisition of sufficient numbers of CD4+ T cells, we sought to abrogate the development of pathology in pre-diseased D2C mice by the adoptive transfer of syngeneic DBA/2 CD4+ T cells. These recipient mice were completely resistant to the development of disease (100% S0, n = 12) whereas D2C recipients of PBS developed typical pathological changes (Fig 7A, B). Sections taken from the skin of these CD4+ cells recipients were devoid of any microscopic stigmata of disease and were indistinguishable from DBA/2 skin sections (Fig 7C). This transfer reconstituted D2C recipients with a functional humoral immune system (Fig 7D), and also resulted in the amelioration of other phenotypic abnormalities such as the development of lymphadenopathy and splenomegaly (Fig 7E). The hypergammaglobulinemia typical of D2C mice, however, was only partially corrected by the adoptive transfer of CD4+ T cells (Fig 7F). Although this reduction was found to be statistically significant (p < 0.05), the concentration of serum IgG in recipients of CD4+ T cells was still nearly 10-fold higher than in age-matched DBA/2 controls.

Discussion

Herein we have described a novel spontaneous animal model of SD-like disease, which arises in 2C TCR expressing DBA/2 mice (D2C mice) housed under specific pathogen-free conditions. Histological sections of diseased skin
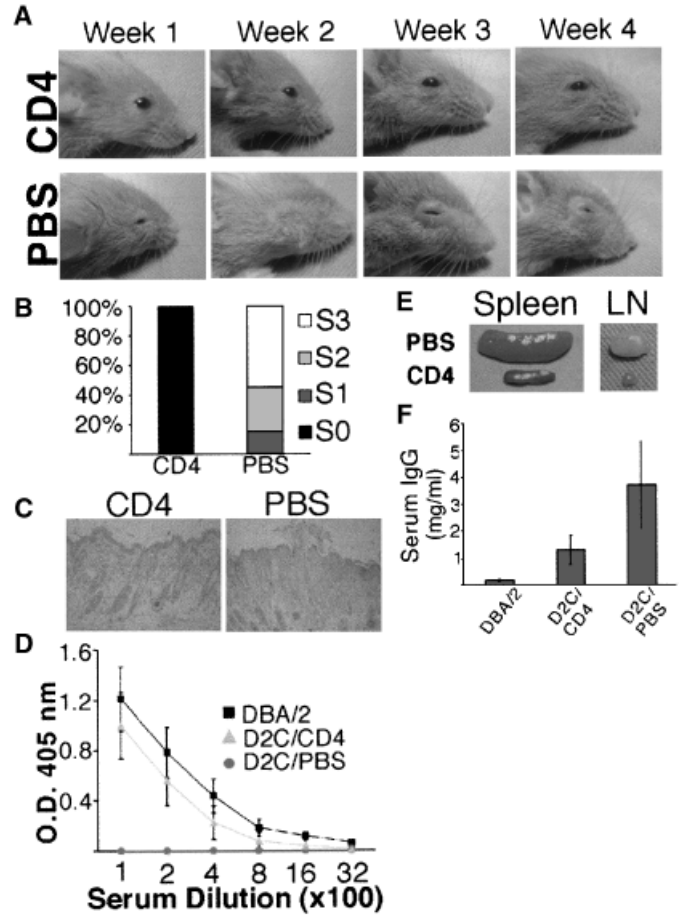

Figure 7
CD4+ T cell adoptive transfer. (*A*) Twenty-one day old pre-diseased DBA/2 2C T cell receptor transgenic mice (D2C) received phosphate-buffered saline (PBS) or 2×10^7 syngeneic DBA/2 CD4+ T cells. Representative photographs are shown. (*B*) The effectiveness of the cell transfer was quantified by classifying resulting disease according to stage. (*C*) Representative hematoxylin & eosin-stained histological sections from the CD4+ T cell and PBS groups are shown. (*D*) Four weeks after transfer, the recipients and DBA/2 control animals were immunized with hen egg lysozyme (HEL) and assayed for HEL-specific IgG (the difference between the anti-HEL response of DBA/2 mice and D2C recipients of CD4+ T cells was not statistically significant). (*E*) Representative lymphoid organs from D2C recipients of PBS and syngeneic CD4+ T cells are shown. (*F*) Total serum IgG is shown for DBA/2 mice, as well as for D2C recipients of either syngeneic CD4+ T cells or PBS (the serum IgG concentration in D2C recipients of CD4+ T cells was found to differ significantly from this same measure in both DBA/2 mice and D2C recipients of PBS (p < 0.05)).

from D2C mice consistently demonstrated the presence of small round fungal structures in diseased epidermis. These structures were not apparent in non-lesional skin from D2C mice or control DBA/2 skin. The direct visualization of fungal overgrowth in conventionally processed histological sections, without usage of the specialized techniques normally utilized to demonstrate fungal overgrowth in SD (Bergbrant, 1995), suggested that this was a significant aspect of the model. Moreover, treatment with the anti-fungal drug fluconazole reduced the extent of PAS staining in previously lesional skin and hastened clinical recovery. Fungal overgrowth and skin lesions in D2C mice occur in areas known to have high concentrations of sebaceous glands (Gude *et al*, 1982). Moreover, D2C disease has a slight male

predominance and does not occur before puberty, suggesting that an influence of androgens on the pilosebaceous unit may play a significant role in pathogenesis. These findings are consistent with the distribution, sex predilection, and timing of disease in human SD (Rook et al, 1992; Faergemann, 1998) and the premise that disease in D2C mice is initiated by lipophilic fungi. The identification of the pathogenic organisms in this condition, however, has been complicated by the fact that culturing lipid-dependent fungi is notoriously difficult. Previous studies have indicated that Malassezia spp. cannot be cultured from rodent integument (Guillot et al, 1994); however, further studies will determine whether an unusual susceptibility to these organisms allows the skin of D2C mice to be colonized.

It is likely that trauma may also affect the pattern as well as the ultimate phenotype of disease since we have observed that diseased D2C mice scratch rostral skin more frequently than non-transgenic controls (data not shown). The frequent occurrence of ruptured follicles and spillage of keratinaceous debris in histological sections suggest that trauma participates in lesion induction. The intense scratching of diseased tissue by D2C mice may account for the lichenification noted in chronic lesions as well.

The results of this study suggest that an impairment of adaptive immunity, namely a deficiency of CD4$^+$ T cells in D2C mice, is necessary for disease. This CD4$^+$ T cell lymphopenia is induced by the comprehensive negative selection of 2C TCR$^+$ DP thymocytes. In non-H-2^d expressing backgrounds where this extensive central deletion does not occur, 2C mice are resistant to disease (Sha et al, 1988a), attesting to the requirement of a self-reactive TCR in disease pathophysiology. CD4$^+$ T cell lymphopenia is, however, clearly not sufficient for disease since H-2^d congenic B6 2C and N$_1$2C mice possess a similar level of CD4$^+$ T cell lymphopenia yet remain resistant to disease. Familial clustering of human SD is known to occur, suggesting that genetic alterations may predispose to disease; however, the pattern of inheritance of this condition has not yet been determined (Braun-Falco et al, 1991). It is clear from the results of this study that DBA/2 genetic susceptibility factor(s) are important for D2C disease and our backcross and bone marrow transfer experiments suggest that as few as one non-hematopoietic DBA/2 defect may control disease penetrance.

Given the role of opportunistic fungi, we reasoned that the susceptibility factor(s) controlling disease penetrance most likely compromise resistance to infection. A mutation in the DBA/2 C5 gene had previously been found to predispose to fungal infection and has been the basis for using these animals as a model organism for in vivo fungal studies (Hector et al, 1990). Although the DBA/2 C5 defect was not found to be critical for disease pathogenesis in D2C mice, it did modulate disease severity. The possibility that multiple genetic defects and environmental insults contribute to disease pathophysiology suggests that a more complex mode of inheritance may be at work in the model.

Although the aforementioned data support a role for immunodeficiency and opportunistic pathogens in the model, immune dysregulation may also be of paramount importance. The finding that sentinel BALB/c nude mice (T, B, and NK lymphocyte deficient) remained disease resistant (data not shown) when co-housed with affected D2C mice suggests that immunopathology participates in generating the cutaneous phenotype. BALB/c nude mice share the genetic background that predisposes BALB/c 2C mice to disease, but lack the population of vigourously expanding CD4$^+$ T cells as well as the potential to generate autoreactive 2C TCR$^+$ T cells. 2C mice on negatively selecting genetic backgrounds possess peripheral T lymphocytes expressing high levels of the clonotypic 2C TCR (Sha et al, 1988a). Although such cells appear to be anergized (Sha et al, 1988a; Caveno et al, 1999), it is possible that these cells may exacerbate immunopathology, and thereby contribute to the ultimate phenotype of disease.

The association between disease and CD4$^+$ T cell lymphopenia in D2C mice closely parallels that which occurs in AIDS-related SD. When D2C mice become susceptible to disease at approximately 28 d of age, their CD4 counts of 66 ± 35 cells per mm^3 are nearly 10-fold lower than the 400–500 cells per mm^3 threshold at which HIV-positive patients begin to develop SD (Conant, 1994). The finding that disease convalescence in these animals occurs around the time that this critical threshold is acquired and that the restoration of the CD4$^+$ T cell compartment in D2C mice suppresses cutaneous pathology provides further support for this parallel. Furthermore, D2C mice possess a number of additional phenotypes that are hallmarks of AIDS, such as lymphoid organomegaly and intermittent diarrhea (Delaney, 1999), as well as the coexistence of hypergammaglobulinemia and humoral immune dysfunction (Pahwa, 1989; Johanson, 1996; Mindel and Tenant-Flowers, 2001). The finding that these mice could not mount a specific response against HEL suggested that their gammopathy resulted from the dysregulation of B cells rather than from a greater exposure to environmental Ag because of associated skin inflammation and barrier disruption. Interestingly, the hypergammaglobulinemia that occurs in D2C mice was mitigated, but not completely abrogated, by the adoptive transfer of CD4$^+$ T cells. The complete abrogation of this B lymphocyte defect, however, may have necessitated an earlier transfer of CD4 T cells because this hematological alteration likely begins at an early age. The cutaneous disease in these animals may be particularly well suited to study the pathophysiology of SD that occurs during the course of HIV infection. However, D2C cutaneous disease overlaps somewhat with a number of additional conditions such as Malassezia folliculitis, atopic dermatitis, and the veterinary conditions Malassezia otitis and dermatitis; thus, the model could have implications for a number of additional clinical entities. The elevation of IgE seen in D2C mice is particularly intriguing (Delaney, 1999), indicating a possible overlap with atopic dermatitis of the head and neck region, a condition previously associated with Malassezia fungi (Ashbee and Evans, 2002).

In summary, the D2C mouse develops skin disease that has morphological and pathological similarities to SD. It is likely that a combination of factors confers to these mice a unique susceptibility to develop spontaneous psoriasiform skin disease when housed under specific pathogen-free conditions. We propose that the D2C mouse may serve as a veritable model for SD-like disease, particularly disease that occurs in AIDS, and its timely development will elucidate

the interplay between infection and immunity in this poorly understood disease.

Materials and Methods

Mice Breeders for 2C TCR transgenic mice (Sha *et al*, 1988b) were kindly provided by Dr Denis Loh (then at Howard Hughes Medical Institute, Washington University, St Louis, Missouri). 2C TCR transgenic mice were bred from an H-2[b] expressing C57BL/6 (B6) background onto the H-2[d] expressing BALB/c, DBA/2, and B6 (B6.C-H-2[d]/bBy) backgrounds. $N_2$2C, $N_3$2C, $N_4$2C, and subsequent backcrosses to the DBA/2 background were analyzed for the development of disease (at least 15 animals were scored for each backcross generation). 2C mice were genotyped by PCR on ear punch DNA using primers specific for the Vβ 8.2 (5′-AGA TAT CCC TGA TGG ATA CAA GGC-3′), and Jβ 2.5 (5′-CTA ACA CGA GGA GCC GAG TGC CTG-3′) TCR chains (Nucleic Acid-Protein Service Unit, University of British Columbia). A 250 bp band is amplified from 2C TCR-positive animals whereas amplification of non-transgenic control DNA results in a faint smear. UBC's Committee on Animal Care approved the animal studies described.

Ear thickness determination The pinna of 21-d-old, pre-diseased D2C and age-matched DBA/2 mice (n = 6 animals per group) were measured using a Mitutoyo pocket thickness gage (Long Island Indicator Service, New York, NY, USA).

Histology Tissue was fixed as previously described (Oble and Teh, 2001) and sections were stained with hematoxylin & eosin (H&E), GMS, or PAS stains.

Adoptive transfer of bone marrow Bone marrow cells were depleted of mature T cells using the anti-Thy-1.2 mAb (J1j.10, American-type culture collection (ATCC), Manassas, Virginia) and Low-Tox-M rabbit complement (Cedarlane, Ontario, Canada) according to company specifications. 1×10^7 cells were injected by tail vein into irradiated (1150 cGy) recipients (n = 20 animals per recipient group).

Genotyping alleles of the C5 A 328–330 bp fragment of the C5 gene, containing the 2 bp deletion known to induce C5 deficiency (Wetsel *et al*, 1990), was amplified using the following primers: 5′-CCA TCT GTC TCC AGA TGA ATA TGT-3′ and 5′-ATA ATG GGA GTC ATC TGC GTT T-3′ (Nucleic Acid-Protein Service Unit, University of British Columbia). This mutation, occurring in C5-deficient strains, disrupts a *Hind*III restriction site and, as such, digestion of the resulting amplicons with *Hind*III (Life Technologies, Burlington, Canada) was used to genotype the animals. C5 sufficient strains have a 211 and 119 bp band whereas deficient animals have a single 328 bp band. Animals heterozygous for the C5 alleles possess all three bands.

Fluconazole administration Fifty days old severely diseased D2C mice were treated once daily for 9 d with i.p. injections of fluconazole (12 mg per kg, Pfizer, Quebec, Canada) or PBS (n = 4 animals per group). Animals were considered to be convalescing at the first observation of new hair growth on rostral skin.

Flow cytometry mAb used for FACS are as follows: HB51 (H-2 K[b]D[b], ATCC); 53.67 (CD8α, BD PharMingen, San Diego, California); GK1.5 (CD4, BD PharMingen); H1.2F3 (CD69, Cedarlane); IM7.8.1 (CD44, Cedarlane); MEL-14 (CD62L, BD PharMingen); 145.2C11 (CD3, BD PharMingen); and B44 (BrdU, BD PharMingen).

HEL immunization and HEL ELISA HEL (Sigma, St Louis, Missouri) was diluted in PBS and emulsified with an equal volume of TiterMax (Sigma). 0.1 mL of the emulsified Ag (50 μg HEL) was administered i.p. After 10 d, serum was collected. Immulon plates (Dynatech Laboratories, Indianapolis, Indiana) were coated with 100 μL of HEL at a concentration of 0.5 μg per mL in 50 mM

carbonate buffer (pH 9.6). Plates were then blocked with 2% BSA in PBS after which serum was diluted in blocking solution and incubated. Alkaline phosphatase-conjugated goat anti-mouse IgG anti-sera was used for detection (1030–04, Southern Biotechnology, Birmingham, Alabama). Plates were read at 405 nm on a Spectra Max 250 plate reader (Molecular Devices, Sunnyvale, California).

Serum IgG ELISA Immulon plates were coated with 100 μL of goat F(Ab′)$_2$ anti-mouse Ig (1012–01, Southern Biotechnology) at a concentration of 4 μg per mL in carbonate buffer. Blocking, sample incubations, and detection of bound IgG were performed as described for the HEL ELISA. The Thy1.1-specific murine IgG2a mAb HIS51 (eBioscience, San Diego, California) was used to develop a standard curve.

CD4 counts An aliquot of heparinized tail vein blood was diluted in 0.83% NH$_4$Cl RBC lysis buffer after which the total WBC counts were determined using a hemocytometer. The remaining blood was centrifuged over histopaque (Sigma) for the collection of interface cells. Staining with GK1.5, and 145.2C11 was used to identify the percentage of these that were CD4^{+} T cells.

BrdU incorporation *in vivo* Mice were fed BrdU (Sigma) in their drinking water (0.8 mg per mL) for 10 d before being sacrificed and assayed for BrdU incorporation as previously described (Tough *et al*, 1999).

Adoptive transfer of purified CD4^{+} T cells Purified CD4^{+} T cells were obtained by incubating lymph node (LN) cells with biotinylated GK1.5 (BD PharMingen) and subsequently with streptavidin-conjugated magnetic microbeads (Miltenyi Biotec, Auburn, California) before being applied to a Macs Separation Column (Miltenyi Biotec). This procedure yields >95% purity of CD4^{+} T cells. 2×10^7 purified CD4^{+} T cells in 0.5 mL PBS were administered by tail vein to 20-d-old pre-diseased D2C recipients. Control animals received an i.v. injection of PBS (n = 12 animals per group).

Statistics Student's two-tailed *t* tests were used for the statistical analysis of data.

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