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

Darryl A. Oble,*1 Elisabeth Collett,* Mindy Hsieh,* Malene Ambjørn,* Jennie Law,* Jan Dutz,† and Hung-Sia Teh*
Departments of *Microbiology and Immunology and †Medicine, University of British Columbia, Vancouver, Canada

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
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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 mm³ 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^d in H-2b mice and negatively selected by L^d in H-2d 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 “seborrhoeic 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 periocular 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 1A).

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 feature of disease (Fig 1B). Ear pathology can become quite severe, resulting in large concretions of hyperkeratotic crusted debris occluding the external ear canal (Fig 1C). 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 sebaceous crusting of acute disease are replaced by thickening of the skin and the appearance of fine white scale. This thickened 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 periocular disease; stage 2, major periocular swelling \( \pm \) 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.

**Microscopic pathology**  The microscopic features of disease are variable and dependent upon the clinical stage of disease. The microscopic changes can be arbitrarily divided into four stages: stage 0 (S0), no disease; stage 1 (S1), minimal periocular erythema and edema; stage 2 (S2), major periocular swelling \( \pm \) lid fusion with little contiguous spread to surrounding tissue; and stage 3 (S3), S2 features plus significant spread to contiguous tissue. (A) Stage 0 and Stage 1 disease are seen. (B) Stage 2 and Stage 3 disease are illustrated. (C) Stage 3 disease is shown.
ostia of hair follicles (Fig 2D). Lesions, which clinically have a lichenified appearance, demonstrate epidermal thickening from the typical one to two cell layer thickness in normal murine skin (Fig 2A) to greater than 10 cell layers, and is sometimes accompanied by finger-like projections of acanthotic epidermis extending into the dermis (Fig 2E). Dense infiltrates of mixed inflammatory cells that surround glands and hair follicles are a feature of chronic pathology (Fig 2E–H). The extent of this pyogranulomatous inflammation is largely dependent upon the integrity of the adnexal structures since keratinocytic debris released from damaged follicles is often at the center of inflammatory foci (Fig 2F–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-2d expressing inbred strains. H-2d 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 3A). Non-H-2d expressing backgrounds, such as H-2b expressing B6 and H-2a 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) N12C (N12C) mice were resistant to disease (n > 100), approximately 50% of N22C (n = 50) animals, and 100% of N32C (n = 25) and further DBA/2 2C backcrosses, develop variable degrees of spontaneous cutaneous pathology (Fig 3B). There was no difference in the incidence or severity of disease in H-2d/d and H-2b/d free 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 3B). 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-2d 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 3C). To ensure that the recipient’s hematopoietic systems were in fact donor derived (B6 × DBA/2) N1 mice (BDN1) were also used as recipients. BDN1 mice normally express both the “b” and “d” H-2 haplotypes (H-2b/d); however, after being reconstituted with D2C bone marrow, lymphocytes from these recipients failed to stain with the anti-H-2b 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-2d congenic B6 2C mice. Strikingly, 100% of the

the animal. Lesional tissue obtained from acutely affected animals possesses: neutrophilic abscesses within the follicles and surrounding epidermis (Fig 2B, 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 2C). 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

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). (G) Keratinocytic 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.
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 3C).

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 PCRRFLP-based assay on N2 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 3D). C5-deficient N2 2C animals had a slightly worse phenotype relative to C5-sufficient animals, suggesting that C5 deficiency may modulate disease expression. However, several C5 sufficient N2 2C mice developed severe pathology (data not shown), and many N2 2C mice homozygous for the defective DBA/2 copy of the C5 gene were completely asymptomatic (Fig 3D), demonstrating that the defect is neither necessary nor sufficient for disease.

Identification of fungi in the primary histological lesion Since the overgrowth of opportunistic basidiozymycetes 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 4B–F). These positively stained structures were not readily apparent in non-lesional skin from diseased animals or from DBA/2 control skin (Fig 4A).

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 5A, 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 5C).

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 N2 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 6A). D2C mice were found to have a 10-fold reduction in the total number of thymocytes (3.8 x 10\(^6\) vs 3.8 x 10\(^7\), p < 0.05) and have 500-fold fewer DP thymocytes (6.1 x 10\(^4\) vs 3.2 x 10\(^5\), 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 6A). The peripheral immunophenotype of D2C mice reflects the negatively selecting thymic environment (Fig 6A) with a 4-fold reduction in total lymphoid CD4\(^+\) T cells (7.5 x 10\(^6\) vs 2.9 x 10\(^7\), p < 0.05) accompanying a 13-fold reduction in total lymphoid CD8\(^+\) T cells (1.4 x 10\(^6\) vs 1.8 x 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 x 10\(^5\) vs 4.6 x 10\(^5\) cells, respectively, p < 0.05). To ascertain whether this level of T cell lymphopenia was...
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 6B). Interestingly, despite a failure in Ag-specific antibody generation, D2C mice possess massively increased levels of serum IgG (Fig 6C) and enlarged lymphoid organs (Fig 6D) 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 6E). 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 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 6E). Complete disease remission occurs when CD4⁺ T cells have accumulated to between 400 and 1000 cells per mm³, 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 6F). 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 (CD44lo, CD69⁻, and CD62Llo), whereas the majority of CD4⁺ T cells from D2C mice were CD44hi, CD69⁺, and CD62Lhi (Fig 6F). 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
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 hyperggammaglobulinemia 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 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 spilloage 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 congenic 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 congenic B6 2C and N2C 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 vigorously 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 mm3 are nearly 10-fold lower than the 400–500 cells per mm3 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 gammapathy 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).
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 Dennis Loh (then at Howard Hughes Medical Institute, Washington University, St Louis, Missouri). 2C TCR transgenic mice were bred from an H-2b expressing C57BL/6 (B6) background onto the H-2d expressing BALB/c, DBA/2, and B6 (B6.C-H-2d7bBy) backgrounds. N2C, N2cC, N2cC, 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-dis eased 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 x 10⁷ cells were injected by tail vein into irradiated (1150 cGy) recipients (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 TAT CCA GGC-3', and 5'-ATA ATG GGA TGC CTG TGT T-3' (Nucleic Acid-Protein Service Unit, University of British Columbia). This mutation, occurring in C5-deficient strains, disrupts a HindIII restriction site and, as such, digestion of the resulting amplicons with HindIII (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 = 6 animals per group). Animals were considered to be convalescing at the first observation of new hair growth on rostral skin.

Histology

Ear thickness determination

Histology

Adoptive transfer of bone marrow

Genotyping alleles of the C5

Fluconazole administration

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Address correspondence to: Hung-Sia Teh, Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada V6T 1Z3. Email: teh@interchange.ubc.ca

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