

## Original Article

# The peripheral lymphoid compartment is disrupted in flaky skin mice

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**Summary** Flaky skin (*fsn*) is an autosomal recessive mutation on mouse chromosome 17 that causes severe anaemia, forestomach papillomatosis and a papulosquamous skin disease that resembles psoriasis in humans. In the present paper, it is reported that *fsn* causes peripheral lymphadenopathy, CD4/CD8 imbalance and hyper-responsiveness to T cell growth factors. Peripheral lymph nodes (PLN) of adult mutant (*fsn/fsn*) mice were found to contain almost 10-fold more leucocytes than PLN from phenotypically normal littermates (*+fsn* or *+/+*, hereafter referred to as *+/?*). Analysis of PLN cells using mAbs and flow cytometry revealed that this predominantly lymphoid hyperplasia was characterized by approximately equivalent increases in the numbers of CD3<sup>+</sup> T cells and CD19<sup>+</sup> B cells. However, expansion within the T cell compartment was non-random, because *fsn/fsn* PLN had a considerably reduced ratio of CD4<sup>+</sup> to CD8<sup>+</sup> T cells ( $1.08 \pm 0.37$ ) compared to *+/?* PLN ( $2.47 \pm 0.44$ ,  $P < 0.0001$ ). *In vitro* assays of cellular proliferation in response to T and B cell growth factors showed that *fsn/fsn* PLN cells were hyperresponsive to IL-2, IL-4 and IL-7 when compared with PLN cells from *+/?* mice. Studies using mesenteric lymph node and peripheral blood cells showed that hyperresponsive cells are widely distributed in *fsn/fsn* mice. Experiments in newborn mice showed that the lymphoid disturbances caused by *fsn* are established at least as early as 2 weeks of age, a time that precedes the onset of the earliest clinical skin lesions. These data implicate a role for the *fsn* gene product in regulating the size and content of the peripheral lymphoid compartment.

**Key words:** cytokines, development, lymphadenopathy, mutation, psoriasis, T cells.

## Introduction

Psoriasis is a chronic skin disease of unknown aetiology characterized by keratinocyte hyperproliferation, altered keratinocyte differentiation and inflammation of the dermis and epidermis. Although it is generally accepted that immunological factors contribute to the maintenance of the disease state, there is considerable debate as to whether human psoriasis is actually caused by immune system dysfunction<sup>1–3</sup> or by a genetic defect residing in epidermal keratinocytes.<sup>4</sup> Progress in identifying the primary aetiological lesion in human psoriasis has been hampered by a paucity of suitable *in vitro* and *in vivo* model systems.

Animal models have been used extensively to investigate mechanisms underlying human disease. Naturally occurring mouse mutations have been particularly informative in this regard. Flaky skin (*fsn*) is an autosomal recessive mutation on mouse distal chromosome 17 that arose spontaneously on an A/J inbred strain at the Jackson Laboratory in 1985.<sup>5,6</sup> The mutation was subsequently transferred to the BALB/cByJ and C57BL6/J strain backgrounds to overcome difficulties encountered in maintaining the mutation in the original A/J background. In all of these strains, *fsn* causes pleiotropic

defects of severe anaemia, forestomach papillomatosis and a progressive papulosquamous skin disorder that resembles psoriasis in humans.<sup>6</sup> The severity of epidermal dysregulation and the cellular composition of the inflammatory infiltrate are dependent on the genetic background.

The *fsn* mutation has been proposed as a suitable animal model for studying the aetiology of some forms of psoriasis in humans.<sup>6,7</sup> In preliminary studies with small numbers of *fsn/fsn* mice and their *+/?* littermates, we observed that all of the mutant mice had striking peripheral lymphadenopathy, an abnormality that had not previously been reported in studies on the immunological status of *fsn/fsn* mice.<sup>8</sup> Here we report the results of experiments we performed to characterize peripheral lymphadenopathy in *fsn/fsn* mice and to determine whether this disorder is associated with flaky skin disease.

## Materials and Methods

### Mice

Male and female BALB/cBy.A-*fsn*/J *fsn/fsn* mice and *+/?* littermate controls were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Mice were 4–6 weeks old when shipped and transit times to our facility were 3–4 days. The *fsn/fsn* and *+/?* mice were housed separately in polycarbonate cages on a bedding of autoclaved pine shavings and given free access to autoclaved rodent food (Camtech Nutrition, Auckland, New Zealand) and chlorinated water (10 p.p.m. residual chlorine). The 2- to 3-week-old mice used in some experiments were bred at our facility using tested *+fsn* × *+fsn* breeding pairs. Neonatal *fsn/fsn* mice were identified by their pallor due to anaemia.

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## Cells

Peripheral lymph nodes (PLN) harvested from each animal were the inguinal, axillary, and brachial pairs and two cervical pairs. In some experiments, PLN from two or more  $+/?$  mice were pooled to provide sufficient cells for assays. The PLN were gently minced through wire gauze to produce cell suspensions in PBS (Life Technologies, Auckland, New Zealand). The cells were washed in DMEM supplemented with 5% FCS (Life Technologies), L-glutamine, essential amino acids, penicillin (10 U/mL), streptomycin (10  $\mu\text{g}/\text{mL}$ ) and  $\beta$ -mercaptoethanol (50  $\mu\text{mol}/\text{L}$ ). Mesenteric lymph node (MLN) cells were processed using the same protocol as for PLN cells. Blood was obtained by cardiac puncture, and mononuclear cells isolated by centrifugation through Histopaque 1083 (Sigma, St Louis, MO, USA).

## Antibodies

Monoclonal antibodies to murine CD4 (clone RM4-5, rat IgG2b), CD8 (53-6.7, rat IgG2a) and CD19 (1D3, rat IgG2a) and isotype controls were obtained as fluorescein or phycoerythrin conjugates from PharMingen (San Diego, CA, USA). Purified mAb to murine CD3 $\epsilon$  (500 A2, hamster IgG) was a gift of Dr M Widmer (Immunex Corporation, Seattle, WA, USA). Purified hamster IgG was purchased from Southern Biotechnology Associates Inc. (Birmingham, AL, USA). Binding of 500A2 mAb was detected using fluoresceinated goat F(ab')<sub>2</sub> antihamster IgG (G $\alpha$ H, mouse and rat serum adsorbed, Southern Biotechnology Associates Inc.). Each immunoglobulin reagent was used at a final concentration of 10  $\mu\text{g}/\text{mL}$ .

## Immunostaining and flow cytometry

Reactions were carried out with  $2\text{--}5 \times 10^5$  cells in cold PBS containing 1% FCS and 0.02% sodium azide (staining buffer). Cells were washed twice in staining buffer after each incubation step to remove unbound Ab. For examination of CD4, CD8 and CD19, cells were incubated with mAb(s) for 20 min. Coexpression of CD3 with CD4 or CD8 was determined using a two-step protocol in which cells were first incubated with 500A2 mAb and then incubated simultaneously with G $\alpha$ H and phycoerythrin-conjugated CD4 or CD8 mAbs. Cells were fixed in 3% paraformaldehyde in PBS after staining and analysed on a Coulter Elite ESP flow cytometer (Coulter Electronics, Hialeah, FL, USA) equipped with a 15 mW argon ion laser. All cell samples were gated on forward and side angle light scatter to exclude dead cells, debris and cell clumps. At least 5000 gated events were analysed for each sample. Mean values calculated

for  $fsn/fsn$  and  $+/?$  subsets were compared using Student's two-tailed *t*-test.

## Proliferation assays

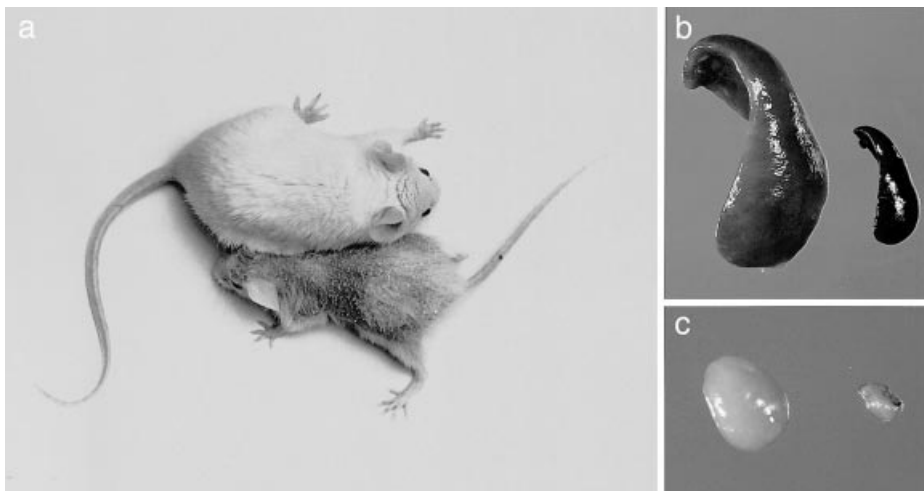
Recombinant human IL-2, murine IL-4 and human IL-7 were kind gifts of Dr M Widmer (Immunex). Growth factors were serially diluted in 50  $\mu\text{L}$  complete DMEM down the columns of Nunclon 96-well round-bottom microculture plates (Life Technologies) and then  $5 \times 10^4$   $fsn/fsn$  or  $+/?$  responder cells in 50  $\mu\text{L}$  complete DMEM were added to each well. Assay plates were incubated at 37°C in a mixture of 10% CO<sub>2</sub> in air for 54 h. Each assay well was then pulsed for 18 h with 10 kBq aqueous tritiated thymidine ([<sup>3</sup>H]-TdR, specific activity 37 MBq/mL; Amersham Life Sciences, Auckland, New Zealand). For determination of radioactivity, cell-associated [<sup>3</sup>H]-TdR was collected on glass fibre filtermats (Wallac Oy, Turku, Finland) using a Tomtec Harvester (Orange, CT, USA) and the filtermats assayed using a 1450 Microbeta Plus liquid scintillation counter (Wallac Oy). Uptake of [<sup>3</sup>H]-TdR, expressed as counts per minute (c.p.m.), was used as a measure of cellular proliferation.

## Results

### Peripheral lymphadenopathy

All of the adult  $fsn/fsn$  mice used in this study were runty in comparison with their  $+/?$  littermates and displayed clinical skin disease (Fig. 1a), anaemia and splenomegaly (Fig. 1b) and forestomach epithelial hyperplasia, as described previously.<sup>6</sup> The haematological and epithelial lesions became increasingly severe as the mice aged.

Because the skin is drained by lymphatics that enter PLN, our interest in the immunological status of  $fsn/fsn$  mice with clinical skin disease prompted us to examine the draining PLN for cellular abnormalities. This led to the observation that  $fsn/fsn$  mice have massively enlarged PLN (Fig. 1c). We compared the number of PLN cells obtained from  $fsn/fsn$  and  $+/?$  mice. The  $fsn/fsn$  mice were found to contain  $120 \times 10^6$  PLN cells per animal. This was almost 10-fold more than the number of PLN cells obtained from  $+/?$  littermates. As shown in Fig. 2, the cellularity of  $+/?$  PLN was relatively constant from 6 to 18 weeks of age. In contrast, the cellularity of  $fsn/fsn$  PLN increased until about 14 weeks of age and then declined, even as skin disease became more severe thereafter.

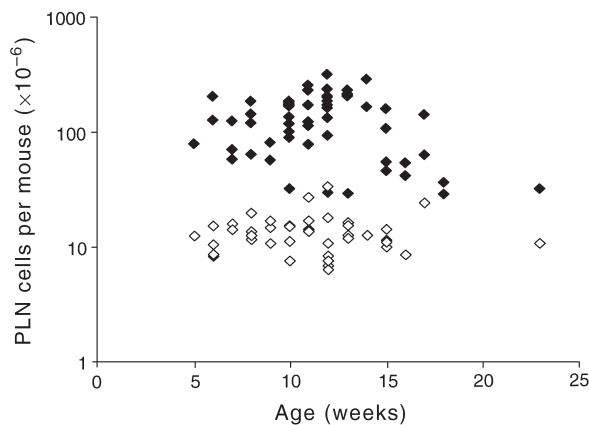


**Figure 1** Flaky skin (*fsn*) causes psoriasis, splenomegaly and peripheral lymphadenopathy. (a) Comparison of a 12-week-old BALB/cBy.A-*fsn*/J *fsn/fsn* mouse with typical flaky skin disease (bottom) and a phenotypically normal  $+/?$  littermate (top). (b) Splens and (c) axillary lymph nodes (LN) from *fsn/fsn* (left) and  $+/?$  (right) mice.

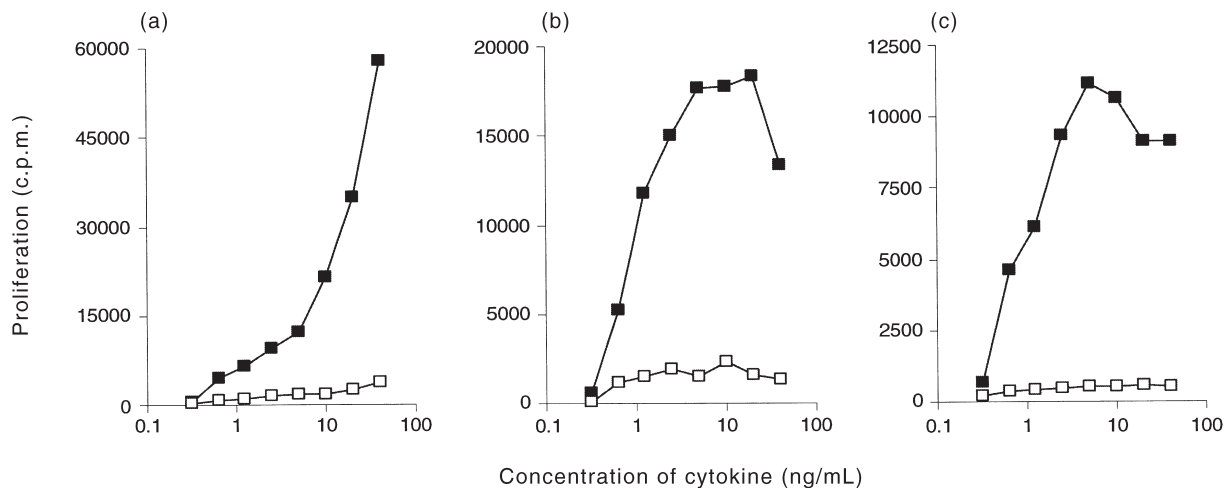
Data from a single experiment using 23-week-old *fsn/fsn* and *+/?* mice (Fig. 2) indicated that peripheral lymphadenopathy in *fsn/fsn* mice persisted throughout life. When we examined the MLN of *fsn/fsn* mice, we found that they were slightly atrophic in comparison with MLN from *+/?* mice. Thus, lymphadenopathy in *fsn/fsn* mice appeared to be confined to PLN.

#### *The PLN cells from fsn/fsn mice are hyperresponsive to T and B cell growth regulators*

The increased cellularity of *fsn/fsn* PLN was suggestive of either developmental or functional abnormalities, or both. To gain some insight into molecular mechanisms, we compared the proliferative responses of *fsn/fsn* and *+/?* PLN cells to known T and B cell growth factors. As illustrated by the representative result shown in Fig. 3, *fsn/fsn* PLN cells consistently displayed greater proliferative responses to IL-2,



**Figure 2** Comparison of the total number of peripheral lymph node (PLN) cells obtained in 6- to 18-week-old *fsn/fsn* (◆) and *+/?* (◇) mice.



**Figure 3** Flaky skin (*fsn*) causes hyperresponsiveness to growth factors. *fsn/fsn* and *+/?* peripheral lymph node (PLN) cells were cultured in the presence of either IL-2 (a), IL-4 (b), or IL-7 (c) for 72 h, the last 18 h in the presence of [<sup>3</sup>H]-thymidine. As shown in this representative experiment, *fsn/fsn* (■) PLN cells displayed greater proliferative responses to the cytokines examined than did *+/?* (□) PLN cells.

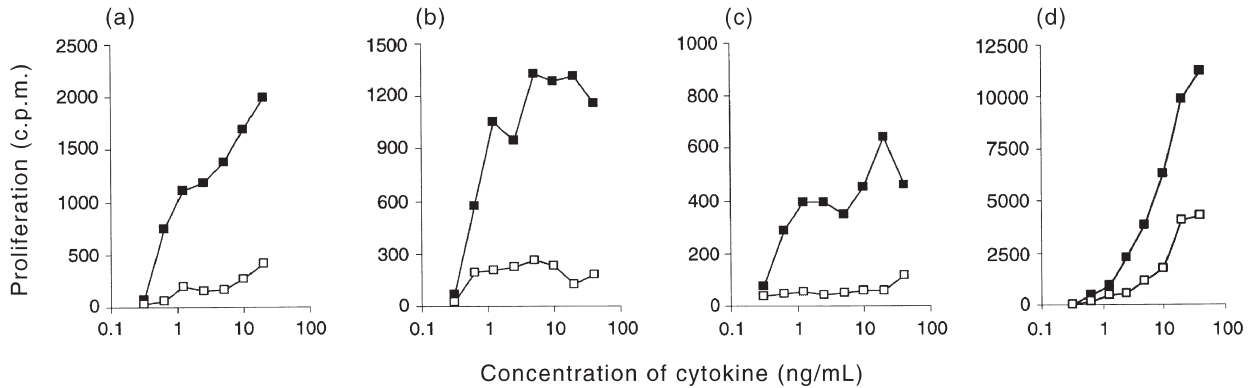
IL-4 and IL-7 than did *+/?* PLN cells. Surprisingly, *fsn/fsn* PLN cells were not hyperresponsive to IL-15 (data not shown), a growth factor that shares a number of biological properties with IL-2.<sup>9</sup> The degree of hyperresponsiveness of *fsn/fsn* PLN cells was variable from one animal to the next, but this variability did not appear to correlate with either the ages of the mice examined, the magnitude of the lymphadenopathy or the severity of skin disease. In 12 out of 15 experiments, IL-2 stimulated greater proliferative responses by *fsn/fsn* PLN cells than did IL-4 or IL-7, but in three other experiments the most potent stimulator was IL-4. In two experiments using CD4<sup>+</sup> and CD8<sup>+</sup> cells purified to > 99.5% homogeneity by cell sorting, we found that hyperresponsiveness to IL-2 was a feature of both CD4<sup>+</sup> and CD8<sup>+</sup> cells.

#### *Hyperresponsive cells are widely distributed in fsn/fsn mice*

To determine whether hyperresponsive cells were confined to PLN or were distributed throughout the lymphoid apparatus of *fsn/fsn* mice, we examined the proliferative responses of MLN cells (Fig. 4a) and peripheral blood mononuclear cells (Fig. 4b) in *fsn/fsn* and *+/?* mice. The proliferative responses of MLN cells to cytokines were much lower than those seen using PLN cells (compare Fig. 4a with Fig. 3). However, *fsn/fsn* MLN cells displayed greater proliferative responses to IL-2, IL-4 and IL-7 than did *+/?* MLN cells. Further, mononuclear cells isolated from *fsn/fsn* blood displayed greater proliferative responses to IL-2 than did *+/?* blood cells (Fig. 4b). These data indicated that hyperresponsive cells were not confined to the PLN of *fsn/fsn* mice, but were widely distributed.

#### *Expansion of T and B cells*

Single colour flow cytometry using mAbs to CD3, CD19 and CD11b revealed that peripheral lymphadenopathy in *fsn/fsn* mice was mainly due to expansion of lymphoid rather than myeloid cells. We found that the proportions of CD3<sup>+</sup> T cells



**Figure 4** Hyperresponsive cells are widely distributed in *fsn/fsn* mice. (a, b, c) mesenteric lymph node (MLN) cells from *fsn/fsn* and *+/?* mice were cultured with cytokines and their proliferative responses assayed as described for Fig. 3. The results of this representative experiment show that *fsn/fsn* (■) MLN cells displayed greater proliferative responses to IL-2 (a), IL-4 (b) and IL-7 (c) than did *+/?* (□) MLN cells. (d) Mononuclear cells isolated from the blood of *fsn/fsn* and *+/?* mice were cultured with IL-2 and their proliferative responses assayed as described above. The results show that the proliferative response of *fsn/fsn* (■) cells was of greater magnitude than that of *+/?* (□) cells.

**Table 1** Expression of CD3 and CD19 in BALB/cByA-*fsn/J fsn/fsn* and *+/?* PLN

Mice	CD3 <sup>+</sup>		CD19 <sup>+</sup>	
	(%)	( $\times 10^{-6}$ )	(%)	( $\times 10^{-6}$ )
<i>+/?</i>	78.2 $\pm$ 4.4 (13)*	9.4 $\pm$ 3.1 (13)	18.2 $\pm$ 4.1 (10)	2.5 $\pm$ 0.8 (8)
<i>fsn/fsn</i>	74.3 $\pm$ 5.6 (16)	71.1 $\pm$ 46.7 (14)	21.5 $\pm$ 8.4 (13)	20.5 $\pm$ 15.6 (10)
<i>P</i>	0.0516	< 0.0001	0.2660	0.0052

Peripheral lymph node (PLN) cells from 6- to 18-week-old flaky skin (*fsn/fsn*) and *+/?* mice were stained with mAbs to CD3 or CD19 or with isotype-matched control mAb and analysed by flow cytometry as described in Materials and Methods. Data are the mean  $\pm$  SD. Percentage values for CD3 and CD19 were corrected after subtracting background staining with control mAbs. Total CD3<sup>+</sup> or CD19<sup>+</sup> cells were calculated by multiplying the percentage values by the total number of cells obtained. \*Numbers of experiments are indicated in parentheses. In some experiments, PLN from individual mice were pooled. *P* values given are comparing *fsn/fsn* and *+/?* mice using a two-tailed *t*-test.

in *fsn/fsn* and *+/?* PLN were not significantly different (Table 1), but that *fsn/fsn* PLN contained about 8-fold more CD3<sup>+</sup> cells than *+/?* PLN. The proportions of CD19<sup>+</sup> B cells in *fsn/fsn* and *+/?* PLN were not significantly different, but *fsn/fsn* PLN contained approximately 10-fold more CD19<sup>+</sup> cells than *+/?* PLN. Thus, peripheral lymphoid hyperplasia in *fsn/fsn* mice was characterized by equivalent increases in the numbers of both T and B cells, but the ratio of T cells to B cells was not affected by the *fsn* mutation.

#### CD4<sup>+</sup> and CD8<sup>+</sup> T cells

Flow cytometric analysis of the T cell compartment in *fsn/fsn* PLN revealed a major disruption in the balance of CD4<sup>+</sup> and CD8<sup>+</sup> cells (Table 2). The proportion of CD8<sup>+</sup> cells was significantly higher in *fsn/fsn* than in *+/?* PLN, while the proportion of CD4<sup>+</sup> cells was significantly lower in *fsn/fsn* than in *+/?* PLN. Consequently, the CD4<sup>+</sup>/CD8<sup>+</sup> ratio was markedly reduced in *fsn/fsn* PLN (1.08  $\pm$  0.37 mean  $\pm$  SEM) compared with *+/?* PLN (2.47  $\pm$  0.44, *P* < 0.0001). Two-colour analysis demonstrated that CD3<sup>+</sup>CD4<sup>+</sup>, CD3<sup>+</sup>CD8<sup>+</sup> and CD4<sup>+</sup>CD8<sup>+</sup> cells were equally rare in *fsn/fsn* and *+/?* PLN (data not shown). However, CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>-</sup> cells, which were essentially undetectable in *+/?* PLN, accounted for

approximately 10% of CD3<sup>+</sup> cells in *fsn/fsn* PLN. This is illustrated by comparing data in Tables 1 and 2. Analysis of PLN from adult mice ranging from 6 to 17 weeks of age (Fig. 5) showed that the CD4<sup>+</sup>/CD8<sup>+</sup> imbalance in *fsn/fsn* mice was established by 6 weeks of age and remained remarkably constant as *fsn/fsn* PLN cellularity increased and subsequently decreased as mice aged (as shown in Fig. 1). Despite the fact that *fsn/fsn* PLN contained a considerably reduced proportion of CD4<sup>+</sup> cells compared with *+/?* PLN, the massively increased cellularity of *fsn/fsn* PLN meant that these PLN contained four-fold more CD4<sup>+</sup> cells and 10-fold more CD8<sup>+</sup> cells than *+/?* PLN (Table 2).

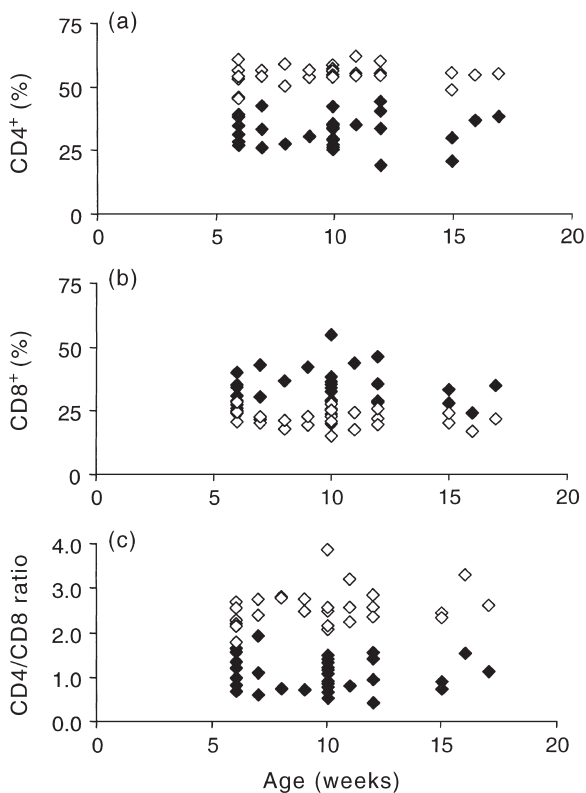
#### Peripheral lymphadenopathy and CD4<sup>+</sup>/CD8<sup>+</sup> imbalance are established prior to the onset of clinical skin disease

Although we did not attempt to score clinical skin disease in the *fsn/fsn* mice used in the present study, it was evident to us that the severity of the lymphoid abnormalities did not correlate with the severity of skin disease. This was particularly true in 6- to 10-week-old mice that displayed severe lymphoid lesions but only mild skin disease. This suggested that development of lymphoid lesions in *fsn/fsn* mice was not necessarily a direct effect of skin disease. We tested this

**Table 2** Expression of CD4 and CD8 in BALB/cBy.A-*fsn/fsn* and +/? PLN

Mice	CD4 <sup>+</sup>		CD8 <sup>+</sup>		CD4 <sup>+</sup> /CD8 <sup>+</sup> ratio
	(%)	( $\times 10^{-6}$ )	(%)	( $\times 10^{-6}$ )	
+/?	55.3 $\pm$ 3.4 (30)*	6.9 $\pm$ 3.0 (26)	23.1 $\pm$ 3.8 (30)	2.8 $\pm$ 1.2 (26)	2.47 $\pm$ 0.44 (30)
<i>fsn/fsn</i>	33.8 $\pm$ 6.4 (39)	34.2 $\pm$ 21.1 (35)	33.1 $\pm$ 7.8 (39)	31.6 $\pm$ 19.6 (35)	1.08 $\pm$ 0.37 (39)
<i>P</i>	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Peripheral lymph node (PLN) cells from 6- to 18-week-old flaky skin (*fsn/fsn*) and +/? mice were stained with mAbs to CD4 or CD8 or with isotype-matched control mAbs and analysed by flow cytometry as described in Materials and Methods. Data are the mean $\pm$ SD. Percentage values for CD4 and CD8 were corrected after subtracting background staining with isotype controls. Total CD4<sup>+</sup> or CD8<sup>+</sup> cells were calculated by multiplying the percentage values by the total number of cells obtained. CD4<sup>+</sup>/CD8<sup>+</sup> ratios were calculated by dividing percentage CD4<sup>+</sup> cells by percentage CD8<sup>+</sup> cells for each experiment. \*Number of experiments are indicated in parentheses. In some experiments, PLN cells from individual mice were pooled. *P* values given are comparing *fsn/fsn* and +/? mice using a two-tailed *t*-test.



**Figure 5** CD4<sup>+</sup>/CD8<sup>+</sup> imbalance caused by flaky skin (*fsn*) is established early and persists throughout life. Comparison of the proportions of (a) CD4<sup>+</sup> and (b) CD8<sup>+</sup> cells and (c) the CD4<sup>+</sup>/CD8<sup>+</sup> ratios in *fsn/fsn* (◆) and +/? (◇) peripheral lymph node cells.

hypothesis by examining PLN from 2- to 3-week-old (newborn) *fsn/fsn* and +/? mice bred in our own animal facility. The *fsn/fsn* mice were distinguished from their +/? littermates on the basis of their pallor at birth and by their slower growth rates thereafter. We examined a total of seven *fsn/fsn* and 10 +/? newborn mice in four separate experiments. None of the newborn *fsn/fsn* mice displayed clinical skin disease, but all had marked splenomegaly and peripheral lymphadenopathy. As shown in Table 3, PLN from newborn *fsn/fsn* mice contained approximately  $30 \times 10^6$  cells per

animal, being almost 4-fold more than the number of PLN cells obtained from their +/? littermates and more than twice the number of PLN cells routinely obtained from adult +/? mice. Like PLN cells from adult mice, PLN cells from newborn *fsn/fsn* mice were found to be hyperresponsive to IL-2 (data not shown). Analysis of these cells by flow cytometry showed that the proportion of CD4<sup>+</sup> cells was considerably lower in *fsn/fsn* than in +/? PLN and that the proportion of CD8<sup>+</sup> cells was higher in *fsn/fsn* than in +/? PLN. Consequently, the average CD4<sup>+</sup>/CD8<sup>+</sup> ratio observed in the newborn *fsn/fsn* mice studied in the present paper ( $0.87 \pm 0.22$ ) was less than half the value seen in their +/? littermates ( $1.98 \pm 0.26$ ,  $P < 0.0006$ ). Extrapolation of data in Table 3 indicated that PLN of newborn *fsn/fsn* mice contained a population of CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>-</sup> T cells analogous to that observed in adult *fsn/fsn* mice. Collectively, these data indicated that most, if not all, of the lymphoid lesions observed in adult *fsn/fsn* mice were established at least as early as 2 weeks of age, prior to the appearance of clinical skin lesions.

## Discussion

In contrast to psoriasis in humans, flaky skin disease in mice homozygous for the *fsn* mutation is accompanied by anaemia, forestomach papillomatosis and other organ system disturbances. Nevertheless, several lines of evidence support the view that the flaky skin mutant mouse may be a useful animal model for understanding the etiopathogenesis of psoriasis in humans. First, *fsn/fsn* mice develop a chronic skin disorder that is characterized by keratinocyte hyperproliferation, abnormal keratinocyte differentiation and inflammation of the dermis and epidermis.<sup>6,10</sup> These characteristics are hallmarks of psoriasis in humans. Second, the histopathological features of skin disease in *fsn/fsn* mice are influenced by background modifying genes,<sup>6</sup> as appears to be the case in human disease.<sup>11</sup> Third, full-thickness skin grafts from *fsn/fsn* mice maintain the psoriasiform phenotype when transplanted onto immunodeficient mice,<sup>7</sup> a feature shared with human psoriatic skin grafts.<sup>12,13</sup> Fourth, *fsn/fsn* mice display an epidermal growth factor receptor profile<sup>14</sup> that is identical to that of lesional and non-lesional human psoriatic epidermis.<sup>15</sup> Finally, cyclosporin A and ultraviolet B radiation, two treatments that are known to produce clinical improvement in human psoriasis, induce remission of skin disease in *fsn/fsn* mice.<sup>14</sup>

**Table 3** Peripheral lymph node data for 2- to 3-week-old BALB/cBy.A-*fsn/J* *fsn/fsn* and *+/?* mice

Mice	Total cells ( $\times 10^{-6}$ )	CD4 <sup>+</sup> (%)	CD8 <sup>+</sup> (%)	CD4 <sup>+</sup> /CD8 <sup>+</sup>
<i>+/?</i>				
1	4.7	54.5	24.0	2.27
2	ND	63.3	29.6	2.14
3	12.2	57.0	31.9	1.79
4	6.7	57.0	32.9	1.73
Mean $\pm$ SD	7.9 $\pm$ 3.8	58.0 $\pm$ 3.8	29.6 $\pm$ 4.0	1.98 $\pm$ 0.26
<i>fsn/fsn</i>				
1	24.3	40.8	36.4	1.12
2	ND	38.3	52.4	0.73
3	41.1	35.4	36.6	0.97
4	24.4	31.4	48.1	0.65
Mean $\pm$ SD	29.9 $\pm$ 9.7	36.5 $\pm$ 4.0	43.4 $\pm$ 8.2	0.87 $\pm$ 0.22
<i>P</i>	0.0214	0.0002	0.0227	0.0006

Peripheral lymph node (PLN) cells from 2- to 3-week-old flaky skin (*fsn/fsn*) and *+/?* mice were processed with mAbs to CD4 or CD8 or isotype control mAbs and analysed by flow cytometry as described in Materials and Methods. Percentage values for CD4 and CD8 were corrected after subtracting background staining with isotype controls. Total CD4<sup>+</sup> or CD8<sup>+</sup> cells were calculated by multiplying the percentage values by the total number of cells obtained. ND, not done. *P* values given are comparing *fsn/fsn* and *+/?* mice using a two-tailed *t*-test.

In view of the association of immune system activation with psoriasis in humans, we sought to obtain further information about the immunological status of *fsn/fsn* mice. To do this, we examined over 100 *fsn/fsn* mice and their phenotypically normal *+/?* littermates. These mice were obtained in 12 shipments from The Jackson Laboratory over a 15 month period. Tissues from approximately half of these mice were used in the studies described in the present paper. Peripheral lymphadenopathy was a consistent and striking abnormality in *fsn/fsn* mice. When we assayed the growth properties of *fsn/fsn* PLN cells *in vitro*, we found that they were hyper-responsive to several cytokines known to regulate the growth of T and B cells. Flow cytometric analyses revealed that the increase in *fsn/fsn* PLN cellularity was predominantly due to 8- and 10-fold increases in the numbers of T and B cells, respectively. Further analysis of the T cell compartment showed that *fsn/fsn* PLN had a considerably reduced CD4<sup>+</sup>/CD8<sup>+</sup> ratio. This was reflected by a 10-fold increase in CD8<sup>+</sup> cells but only a 4-fold increase in CD4<sup>+</sup> cells. Although all of these abnormalities occurred in association with flaky skin disease of variable severity in adult mice, studies in newborn mice showed that peripheral lymphadenopathy, CD4<sup>+</sup>/CD8<sup>+</sup> imbalance and hyperresponsiveness to IL-2 were established prior to the appearance of clinical skin disease.

After the experiments described in the present report had been completed, Pelsue *et al.* published a report describing peripheral lymphadenopathy and other immunological abnormalities in 8-week-old *fsn/fsn* mice.<sup>16</sup> The magnitude of peripheral lymphadenopathy described by these authors was similar to that found in the 8-week-old mice used in our study (Fig. 2). While Pelsue *et al.* reported that *fsn/fsn* PLN had a reduced CD4<sup>+</sup>/CD8<sup>+</sup> ratio, their data showed that this was due to a decrease in the proportion of CD4<sup>+</sup> cells which, in contrast to our findings, was not accompanied by an increase in the proportion of CD8<sup>+</sup> cells. Pelsue *et al.* also found that

*fsn/fsn* PLN contained a decreased proportion of T cells and an increased proportion of B cells in comparison with *+/?* PLN; however, both the *fsn/fsn* and *+/?* PLN examined by these investigators appeared to contain an unusually low proportion of lymphocytes and, particularly in the case of *fsn/fsn* PLN, an unusually high proportion of granulocytes. The reason for the discrepancies between our data and the findings reported by Pelsue *et al.* are not clear, but may relate to differences in the number of mice examined, colony variation, possibly including the influence of background A/J strain genes,<sup>6</sup> housing conditions and other factors.

Peripheral T cell pool sizes and CD4<sup>+</sup>/CD8<sup>+</sup> ratios are regulated by homeostatic mechanisms that remain poorly understood.<sup>17</sup> Although peripheral lymphadenopathy has been associated with numerous spontaneous and induced mouse mutations, abnormal CD4<sup>+</sup>/CD8<sup>+</sup> ratios have not been reported in murine models of psoriasiform skin disease<sup>18-20</sup> or in gene-targeted mice that develop peripheral lymphadenopathy.<sup>21-23</sup> To the best of our knowledge, *fsn* is the only mouse mutation known to cause both peripheral lymphadenopathy and CD4<sup>+</sup>/CD8<sup>+</sup> imbalance.

The mechanisms leading to lymphadenopathy and CD4<sup>+</sup>/CD8<sup>+</sup> imbalance in *fsn/fsn* mice are impossible to determine on the basis of the available data. We do not know if the disruption in the balance between CD4<sup>+</sup> and CD8<sup>+</sup> cells in *fsn/fsn* PLN occurs during thymic ontogeny or after thymic emigrants have seeded the peripheral tissues. Preliminary studies using thymic tissue from newborn and adult *fsn/fsn* mice failed to identify abnormalities in CD4<sup>+</sup>/CD8<sup>+</sup> ratios. Further studies in thymectomized mice may resolve this issue. One possible explanation for the lymphadenopathy is suggested by our finding that *fsn/fsn* PLN cells were hyper-responsive to IL-2, IL-4 and IL-7. One interpretation of this result is that cytokine receptor expression is up-regulated in *fsn/fsn* PLN, possibly due to the presence of an abnormally

high proportion of activated and/or cycling cells. Studies designed to address this issue more directly and to identify the hyperresponsive cell type(s) in *fsn/fsn* PLN are in progress.

The etiopathogenesis of psoriasis in humans is highly controversial. Two fundamentally different hypotheses have been proposed to explain the available human data. The first is that keratinocyte hyperproliferation and altered keratinocyte differentiation are caused by a primary genetic defect residing in epidermal keratinocytes and that inflammation and T cell activation are secondary, albeit highly destructive, phenomena. Familial association,<sup>24,25</sup> epidemiological findings<sup>11</sup> and data obtained from studies in transgenic mice expressing  $\beta 1$  integrin in the suprabasal layer of the epidermis<sup>19</sup> provide support for this theory. The second hypothesis is that psoriasis is primarily a disorder of the immune system and that pathological alterations in keratinocyte biology result from inappropriate stimulation by growth and other regulatory factors produced by activated T cells, dendritic cells, macrophages and mast cells.<sup>26,27</sup> This hypothesis is supported by clinical investigations showing improvement in psoriasis patients treated with immune-modulating agents<sup>1,28</sup> and by T cell adoptive transfer studies in mice.<sup>29</sup> The data presented in the present report do not support one of these hypotheses over the other. Although we have shown that peripheral lymphadenopathy and CD4<sup>+</sup>/CD8<sup>+</sup> imbalance predate the appearance of clinical skin lesions, Sundberg<sup>6</sup> has previously reported that histopathological changes in the skin of *fsn/fsn* mice are evident in the second week of life. Thus, the relationship between lymphoid abnormalities and flaky skin disease in *fsn/fsn* mice remains to be established.

The *fsn* mutation causes pleiotropic defects of anaemia, psoriasiform skin disease and forestomach papillomatosis. The data presented in the present study indicate that *fsn* is also required for regulating the size and content of the peripheral lymphoid compartment. The *fsn* mutation is therefore likely to reside in a gene for which expression influences the development of both epithelial and haematopoietic tissues. Further analysis of lymphoid abnormalities in *fsn/fsn* mice may provide novel opportunities to identify the defective gene and to search for homologous genes in humans.

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