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Absence of Peyer’s Patches and Abnormal Lymphoid Architecture in Chronic Proliferative Dermatitis (cpdm/cpdm) Mice

Harm HogenEsch,‡ Sarah Janke,* Dawnaly Boggess, † and John P. Sundberg ‡

The chronic proliferative dermatitis (cpdm) mutation causes inflammation in multiple organs, most prominently in the skin. Examination of the immune system revealed severe abnormalities in the architecture of lymphoid tissues. Peyer’s patches were absent. In contrast, the spleen, lymph nodes, and nasal-associated lymphoid tissues were present. The spleen had normal numbers of T and B cells, but the spleen, lymph nodes, and nasal-associated lymphoid tissues had poorly defined follicles and lacked germinal centers and follicular dendritic cells. The marginal zone in the spleen was absent. The total concentration of serum IgG, IgA, and IgE in cpdm/cpdm mice was significantly decreased, whereas serum IgM was normal. Fecal IgA was low to undetectable in mutant mice, and the concentration of fecal IgM was increased. The titer of DNP-specific Abs following immunization with DNP-keyhole limpet hemocyanin was significantly decreased for all IgG subclasses. In contrast, T cell function appeared normal as assessed by evaluation of the contact hypersensitivity response in cpdm/cpdm mice. The cpdm mutation causes a complex phenotype that is characterized by multiorgan inflammation and the defective development of lymphoid tissues. The cpdm/cpdm mouse may be a useful model to study the factors that control the development of lymphoid tissues, in particular the Peyer’s patches, and the mechanisms that control the humoral immune response. The Journal of Immunology, 1999, 162: 3890–3896.

S pontaneous mutations that cause immunologic abnormalities in inbred strains of mice continue to be extremely useful models to study the immune system and to dissect the mechanisms that contribute to immunodeficiency and autoimmune diseases.

Chronic proliferative dermatitis (gene symbol: cpdm) was discovered as a spontaneous mutation in a closed colony of C57BL/KaLawRij mice (1). Mutant mice develop a chronic persistent dermatitis characterized clinically by pruritus and scaling and light microscopically by epidermal hyperplasia and infiltration of the skin by eosinophils and macrophages (Mcph) (2). Similar lesions are present in the esophagus and forestomach. In addition, inflammation is present in the lung, the liver, and, in some mice, the perisynovial tissues of the knee and intervertebral joints (1). Affected skin from mutant mice transplanted onto normal littermates or nude mice remained abnormal, and skin transplanted from normal mice onto cpdm/cpdm mice remained free of lesions (3). This observation suggested that local factors play a critical role in the pathogenesis of the skin lesions. Although the presence of eosinophils in the inflammatory lesions is suggestive of an allergic reaction, the serum IgE concentration in three cpdm/cpdm mice was not increased and, in fact, was markedly lower than in normal littermates (4). The serum concentration of other Ig isotypes was not determined, and other immune functions were not evaluated.

We report here that, in addition to IgE, serum concentrations of IgG and IgA are also reduced in cpdm/cpdm mice compared with normal littermates. A further detailed investigation of the immune system of cpdm/cpdm mice revealed significant abnormalities in the humoral immune response and in the architecture of lymphoid tissues. Several spontaneous and induced mutations in mice with various defects in the humoral immune response and in the architecture of the lymphoid tissues have recently been reported (see Discussion). However, the particular combination of immune defects and inflammatory lesions in cpdm/cpdm mice appears to be unique, suggesting that this mutation affects a not yet identified pleiotropic gene that plays a role in both lymphoid and nonlymphoid tissues.

Materials and Methods

Animals

Colonies of cpdm/cpdm mice on a C57BL/KaLawRij background were maintained at The Jackson Laboratory and at Purdue University under conventional conditions. As reported previously (4), female cpdm/cpdm mice are infertile. Breeding was done by mating heterozygotes or by mating cpdm/cpdm males with heterozygote females (4). Normal littermate controls were either +/+ or +/cpdm. Because these control animals were phenotypically indistinguishable, they were designated +/+ . Mutant cpdm/cpdm mice and +/+ littermates of both sexes were used at 6–12 wk of age. No differences were observed between male and female mice.

Histopathology and immunohistochemistry

Necropsy was performed on all animals, and tissues were fixed in 10% neutral buffered formalin and paraffin embedded. The entire small intestine was fixed and embedded as a “Swiss roll” (5) to permit a complete microscopic examination. Paraffin sections (5 μm) were stained with hematoxylin and eosin (H&E). Spleen fragments were embedded in optimal cutting temperature compound (Tissue-Tek, Miles, Elkhart, IN) and snap-frozen in liquid nitrogen. Sections (6–7 μm) were cut, collected on glass slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA), and air-dried for 1 h. Next, the sections
were fixed for 10 min in cold acetone and stored at −20°C for ≤4 wk. Staining was performed at room temperature. The slides were incubated for 15 min in PBS/1% BSA, incubated with mAbs against Thy-1 (30H-12, American Type Culture Collection (ATCC), Manassas, VA), B220 (RA3-6B2, ATCC), follicular dendritic cells (FDCs) (FDC-M2 (6), kindly provided by Dr. Koos-Vilbuis, Serono Pharmaceutical Research Institute, Plan-les-Ouates, Switzerland), marginal zone Mφ (ER-TR9, Bachem Bio- sciences, King of Prussia, PA), and marginal metallophilic Mφ (MOMA-1, Serotec, Oxford, U.K.) for 2 h, washed three times in PBS, and incubated with FITC-labeled donkey anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Other slides were incubated with FITC-labeled peanut agglutinin (PNA) (Sigma, St. Louis, MO). Slides were washed, covered slipped with Citifluor (Polysciences, Warrington, PA), and examined with a Nikon E400 microscope (Nikon, Tokyo, Japan) equipped with epifluorescence.

Paraffin sections of the intestine were stained for IgA-containing plasma cells as described previously (7). Briefly, paraffin sections were dewaxed, rehydrated, and treated with 1% H2O2 in methanol to block endogenous peroxidase. The sections were incubated with goat-anti-mouse IgA (Southern Biotechnology, Birmingham, AL), followed by donkey anti-goat IgG (Sigma) and goat peroxidase anti-peroxidase complex (Sigma). Peroxidase activity was visualized with the substrate 3,3-diaminobenzidine, and the sections were counterstained with 1% methylgreen.

Flow cytometry

Spleen cells were isolated by mechanical disruption followed by lysis of RBCs with Tris-buffered ammonium chloride. Cells (106) were washed in staining buffer (PBS with 1% BSA and 0.01% sodium azide) and stained with FITC-labeled anti-CD3, FITC-labeled anti-CD4, phycoerythrin-labeled anti-CD8, biotinylated anti-TCRαβ and anti-TCRγδ, phycoerythrin-labeled anti-B220 (all from Pharmingen, San Diego, CA), or FITC-labeled goat anti-mouse IgM (Kirkegaard and Perry, Gaithersburg, MD). Streptavidin-FITC or streptavidin Red670 (Life Technologies, Gaithersburg, MD) goat anti-mouse IgG1, IgG2a, IgG2b, or IgG3 (Southern Biotechnology) was added to the wells; 0.05% Tween, alkaline phosphatase-labeled goat anti-mouse IgM, IgG1, IgG2a, IgG2b, or IgG3 (Southern Biotechnology) was added to the wells; plates were incubated for 1 h with serial dilutions of serum or standards (Southern Biotechnology) overnight at 4°C. Plates were blocked for 15 min at room temperature with PBS supplemented with 1% BSA and incubated for 1 h with serial dilutions of serum or standards (Southern Biotechnology) in PBS/1% BSA at 37°C. After four washes with PBS/0.05% Tween, plates were incubated with alkaline phosphatase-labeled anti-mouse IgM, IgG1, IgA, or IgE (Southern Biotechnology) for 1 h at 37°C. The substrate nitrophenyl phosphate was used, and the OD was determined at 405 nm in a microplate reader (Molecular Devices, Menlo Park, CA).

Fecal pellets were collected at necropsy, mixed with cold PBS (15 µl/mg feces), and centrifuged. Supernatant was diluted 1/10 and 1/100 in PBS/1% BSA and used to measure IgM, IgG, and IgA by ELISA as described above.

Immune response to DNP

Mice were immunized i.p. with 100 µg of DNP-keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, CA) mixed with aluminum hydroxide according to the manufacturer’s instructions (Pierce). After 3 wk, the mice received an i.p. injection of 100 µl of DNP-KLH in PBS. Serum was collected at 1 wk after the booster and analyzed for DNP-specific Abs by ELISA. Microtiter plates (Costar) were coated overnight with 10 µg/ml of DNP-BSA (Calbiochem) at 4°C. The plates were blocked with PBS/1% BSA for 15 min at room temperature and incubated with 10-fold dilutions of serum in PBS/1% BSA for 1 h at 37°C. After four washes with PBS/0.05% Tween, alkaline phosphatase-labeled goat anti-mouse IgM, IgG1, IgG2a, IgG2b, or IgG3 (Southern Biotechnology) was added to the wells; plates were incubated for 1 h at 37°C. Nitrophenyl phosphate was used as substrate, and the OD was determined at 405 nm in a microplate reader (Molecular Devices). The DNP-specific titer was calculated as the inverse of the serum dilution that gave an OD equal to the mean plus 2 SD of preimmunization sera.

Contact hypersensitivity (CHS) response

The abdominal skin of anesthetized mice was shaved and depilated. A 20-µl solution of 0.5% 2,4-dinitrofluorobenzene (DNFB) (Sigma) in acetone/olive oil (4:1) was applied to the skin. After 5 days, the ear thickness of the left ear was measured with a spring-loaded micrometer (Mitutoyo, Tokyo, Japan) and 10 µl of 0.2% DNFB in acetone/olive oil was applied to the dorsal surface of the ear. Ear thickness was measured after 2, 24, and 48 h. An increase in ear thickness is reported following subtraction of the thickness of the ear before challenge.

Intradermal (i.d.) injection of TNF

The dorsal side of the left ear of the mice was injected with 20 µl of recombinant mouse TNF (Peprotech, Rocky Hill, NJ) diluted in to 5 µg/ml in PBS/0.1% BSA. The right ear was injected with the 20 µl of PBS/0.1% BSA. This process was repeated the following day, and ear thickness was measured after 24 h. An increase in the thickness of the left ear is reported following subtraction of the thickness of the right ear. The ears were subsequently fixed in formalin and embedded in paraffin; sections were stained with H&E.

Results

Total Ig in serum and feces

Sera from cpdm/cpdm mice and +/? littermates were assayed for total IgM, IgG, IgA, and IgE. The concentration of IgM was not significantly different between the two groups of mice, but the concentrations of IgG, IgA, and IgE were significantly lower in the cpdm/cpdm mice. The concentration of IgA in fecal samples was greatly reduced in cpdm/cpdm mice compared with +/?
mice (Fig. 2). In contrast, there was a significant increase of IgM in the feces of cpdm/cpdm mice. The concentration of IgG in the fecal samples of normal mice varied widely. Although the average concentration of IgG in normal mice was fivefold higher than in the cpdm/cpdm mice, this difference was not statistically significant ($p = 0.12$). Immunohistochemical staining of the intestines of cpdm/cpdm mice revealed an absence of IgA-containing plasma cells. In contrast, numerous IgA plasma cells were present in the intestines of +/? mice (data not shown).

**Humoral immune response to DNP-KLH**

To evaluate the Ag-specific immune response, mice were immunized i.p. with DNP-KLH absorbed on alum and 3 wk later with DNP-KLH in PBS. Serum samples were collected at 1 wk after the second immunization and analyzed for DNP-specific Abs. The DNP-specific serum Ab titers were lower in cpdm/cpdm mice for all isotypes (Fig. 3). There was a modest reduction of DNP-specific IgM (2-fold) and IgG3 (4-fold) and a greater reduction of DNP-specific IgG1 (12-fold), IgG2a (37-fold), and IgG2b (7-fold).

**Flow cytometric analysis of spleen cells**

The percentage of B and T lymphocytes was slightly or moderately reduced in the spleens of cpdm/cpdm mice, reflecting the increased proportion of myeloid cells (Fig. 4). In absolute numbers, there were no statistically significant differences in the number of lymphocytes in the spleen. The number of CD3$^+$ cells was $9.2 \pm 1.1 \times 10^6$ (mean $\pm$ SEM) in +/? mice and $11.1 \pm 3.5 \times 10^6$ in cpdm/cpdm mice. The number of B220$^+$ cells was $33.3 \pm 8.1 \times 10^6$ in +/? mice and $52.4 \pm 8.0 \times 10^6$ cells in cpdm/cpdm mice. The percentage of CD4$^+$ T cells was significantly reduced ($16.8 \pm 0.6$ in +/? mice vs $8.5 \pm 0.8$ in cpdm/cpdm mice; $p < 0.01$), but there was no significant change in the absolute number of CD4$^+$ T cells ($9.2 \pm 0.5 \times 10^6$ vs $8.2 \pm 1.7 \times 10^6$). There was no significant difference in either the percentage ($9.6 \pm 0.7\%$ vs $5.9 \pm 1.6\%$) or absolute number ($5.3 \pm 0.7 \times 10^6$ vs $6.1 \pm 2.3 \times 10^6$) of CD8$^+$ T cells.

**Immunohistology of the spleen**

Immunohistochemical staining of the spleens of normal littermates with B220- and Thy-1-specific mAbs revealed a normal distribution of B cells in the follicles and marginal zone as well as T cells in the periarteriolar lymphocyte sheath (Fig. 6). Staining with PNA revealed any histologic evidence of rudimentary PPs (Fig. 5). Cross-sections through the noses of +/? mice revealed bilateral lymphoid follicles (nasal-associated lymphoid tissue (NALT)) associated with the ventral meatus of the nasal cavity (Fig. 5). In contrast to the complete absence of PPs from the intestine, variably sized collections of lymphocytes, without follicular structure, were present in the noses of mutant mice at the same sites as the follicles in the +/? mice (Fig. 5).

The spleens of cpdm/cpdm mice contained Thy-1$^+$ lymphocytes that were present around arterioles as a periarteriolar lymphocyte sheath similar to normal mice. Small clusters of B220$^+$ lymphocytes were localized adjacent to the periarteriolar lymphocyte sheath, but there were no well-defined follicles (Fig. 6). GCs were absent, as demonstrated by the lack of clusters of PNA$^+$ cells, and there were no cells that stained with the FDC-specific mAb FDC-M2 (Fig. 6). A few isolated cells reacted with the mAbs...
MOMA-1 and ERTR-9, but there was no evidence of a marginal zone (Fig. 6).

CHS response

To assess the cell-mediated immune response in the mutant mice, the CHS response to DNFB was determined. After elicitation of CHS in sensitized mice, the ear swelling response is biphasic (8). The early response at 2 h after elicitation is mediated by a sub-population of cells that is different from that seen for the later response at 24 and 48 h (8). There was no difference between the cpdm/cpdm mice and +/? littermates at 2, 24, and 48 h after the challenge of sensitized mice with DNFB (Fig. 7).

Response to i.d. injection of TNF

An i.d. injection of TNF induces inflammation through signaling via TNF receptor 1 (TNFR1) (9). To determine whether TNFR1 is functional in cpdm/cpdm mice, 100 ng of TNF was injected into the ears of cpdm/cpdm and +/? mice. There was no significant difference in the magnitude of ear swelling at 24 h after two injections of TNF. The increase in ear thickness was 17.8 ± 2.1 × 10⁻² mm (mean ± SEM) for the mutant mice and 21 ± 0.9 ×
10^{-2} mm for the +/- mice. Light microscopic examination revealed marked edema and infiltration by neutrophils and fewer lymphocytes and Mφ in the left ears of cpdmc/cpdm and +/- mice. However, there was marked apoptosis of keratinocytes in cpdmc/cpdm mice, but not in the epidermis of +/- mice.

**Discussion**

The phenotype of cpdmc/cpdm mice is complex and characterized by mixed cellular inflammation of multiple tissues (most prominently of the skin), abnormal architecture of lymphoid tissues, and defects in the humoral immune response. Although the overall numbers of lymphocytes of the various subpopulations in the spleen appeared to be normal as determined by flow cytometry, immunohistochemical analysis indicates that the organization of the lymphoid tissues is defective. Because immune responses depend upon cognitive interactions between B cells, T cells, and APCs, it is likely that the lack of cellular organization causes the failure of the humoral immune response in cpdmc/cpdm mice.

PPs were completely absent from cpdmc/cpdm mice, whereas peripheral LNs, mesenteric LNs, and NALTs were present. This suggests that the organogenesis of PPs is entirely independent of the development of other mucosal and systemic lymphoid organs. Recent studies have revealed a role for both lymphotoxin (LT) and TNF in the development of secondary lymphoid organs in the mouse. LTα- and LTβ-deficient mice completely lack PPs. LNs

**FIGURE 6.** Immunofluorescent staining of spleens of +/- (A, C, and E) and cpdmc/cpdm (B, D, and F) mice. Light areas represent the labeled cells. Staining for B220 (A and B) reveals well-developed follicles in the spleens of +/- mice and small clusters of B cells in the cpdmc/cpdm mice. Staining with the mAb FDC-M2 (C and D) demonstrates FDCs in +/- mice and not in cpdmc/cpdm mice. The mAb ER-TR9 (E and F) against marginal zone Mφ outlines the marginal zone in +/- mice and stains a few scattered cells (arrowhead) in cpdmc/cpdm mice.

**FIGURE 7.** CHS response in normal C57BL/Ka and mutant cpdmc/cpdm mice. Data indicate the mean ± SEM of five mice.
are undetectable in LTα-deficient mice, except for mesenteric LNs in a few animals (10, 11). In contrast, LTβ-deficient mice have mesenteric and cervical LNs, but other LNs are absent (12, 13). The LTα1β2 heterotrimer binds to the LTβ receptor (LTβR). LTβR-deficient mice lack PPs and all LNs (14). Interestingly, mice that are heterozygous for both }ltα and }ltβ have normal LN development, but completely lack PPs (15). However, these mice are different from }cpdm/cpdm mice, because the microarchitecture of the spleen and LNs in }ltα−/−/ltβ−/− mice is relatively normal in contrast to the severe defects in the }cpdm/cpdm mice. TNF-α and TNFR-1-deficient mice have a reduced number of PPs, and the TNFs that are present are small in size (16, 17). LN organogenesis is normal in these mice. However, mice that are deficient in both TNFR1 and LTβ lack all LNs, including the mesenteric and cervical LNs, suggesting redundancy in the signals for LN development (15). Further evidence for a role of LT and TNF comes from elegant experiments using in utero treatment of pregnant mice with blocking receptor fusion proteins and receptor-agonist mAbs. Treating }ltα-deficient mice with an agonist anti-LTβR mAb induced all LNs, indicating a crucial role of the LTβR in LN organogenesis (18). However, mice treated in utero from gestational day 9 onward with an LTβ receptor (LTβR)-Ig fusion protein developed mesenteric, cervical, sacral, and lumbar LNs, but not other LNs nor PPs (19). Treating mice with a combination of LTβR-Ig fusion protein and TNFR1-Ig fusion proteins completely inhibited LN development, again indicating a role for both LT and TNF in LN organogenesis (18). Given the importance of TNFR1 in the development of PPs and the similarities in lymphoid organ development between TNFR1-deficient mice and }cpdm/cpdm mice, the function of TNFR1 in }cpdm/cpdm mice was determined by an i.d. injection of TNF. The inflammation caused by TNF is dependent upon a functional TNFR1 (9). There was no difference in the degree of ear swelling between }cpdm/cpdm and +/+ mice, indicating that TNFR1 is functional. Previous studies showed that }cpdm/cpdm mice express TNF (4), suggesting that the defective lymphoid organ development cannot be attributed to a defect in TNF or TNF signaling. An i.d. injection of TNF caused apoptosis of keratinocytes in the epidermis of }cpdm/cpdm mice but not in the epidermis of +/+ mice. Apoptosis of keratinocytes is a feature of the dermatitis that is part of the phenotype of }cpdm/cpdm mice, but the ears normally do not develop this dermatitis (1, 2). The TNF-induced apoptosis in the ears suggests a general increased susceptibility of }cpdm/cpdm keratinocytes to apoptosis.

Mice deficient for the B cell-specific chemokine receptor BLR1 have a reduced number or a complete absence of PPs. LNs develop normally in these mice, with the exception of the inguinal LNs (20). Mice deficient for β1 integrin also have rudimentary PPs (21), reflecting the important role of β1 integrins in the homing of lymphocytes in gut-associated lymphoid tissues. NALT consists of paired lymphoid tissue in the ventral meatus of the nasal cavity of rodents (22). It is considered part of the mucosa-associated lymphoid tissues, and the primary inductive site for immune responses in the upper respiratory tract. As such, NALT is often considered to be the functional equivalent of PPs. Interestingly, NALT is present in }cpdm/cpdm mice, suggesting that the organogenesis of PPs is controlled by mechanisms other than those that control the NALT. NALT has not been investigated in the aforementioned gene-deleted mouse mutants with defects in LN and PP development.

Another spontaneous mouse mutant, the alymphoplasia (aly/aly) mouse, with defective development of lymphoid tissues, has been described recently (23). The immunodeficiency in these mice is much more severe than that of }cpdm/cpdm mice. The aly/aly mice completely lack LNs as well as PPs and have severe defects in both the humoral and cellular immune response (23).

Consistent with the importance of PPs in the production of IgA-secreting plasma cells, serum IgA was significantly reduced and fecal IgA was nearly undetectable in }cpdm/cpdm mutant mice. The concentration of IgM in feces was increased in }cpdm/cpdm mice. Both IgA and IgM are transported across epithelial cells via the polymeric Ig receptor to the mucosal surface (24). Deficient production of IgA in }cpdm/cpdm mice may result in increased availability of the polymeric Ig receptor for IgM and increased transport of IgM into mucosal secretions.

A large number of induced mouse mutations with defective GC formation have been described in recent years (6, 25). GCs are the main sites for the somatic mutation and affinity maturation of B lymphocytes, for isotype switching, and for the generation of memory B cells (25, 26). Indeed, affinity maturation is impaired in CD19-deficient mice (27) and bcl-6-deficient mice (28). However, similar to the }cpdm/cpdm mice, many null mutations produce some, albeit often diminished, concentrations of Ig isoforms other than IgM, indicating that some isotype switching occurred in the absence of GCs. Somatic mutation and affinity maturation were observed following immunization in LTα- and Lyn-deficient mice (29, 30). These studies indicate that GCs are not absolutely necessary for these B cell functions and suggest that the defective Ig switching in }cpdm/cpdm mice is caused by defects other than the absence of GCs.

Staining spleen sections with the FDC-specific mAb FDC-M2 indicated an absence of GCs in the spleen. FDCs are also absent in LTα- (11), LTβ- (12, 13), TNF- (31), and TNFR1- (32) deficient mice as well as NF-κB-deficient mice (33, 34). The origin of FDCs is controversial. They may be derived from stromal cells or from the bone marrow. Regardless of their origin, the precursors require signals from B and T lymphocytes for differentiation into FDCs. The FDC-deficient mice are instrumental in defining these signals. Recent studies suggest that expression of LTα by B cells is a crucial signal for FDC differentiation (35, 36). Another signal is provided by TNFR1 expression on nonhemopoietic (stromal) cells (6).

The marginal zone is the part of the spleen immediately adjacent to the periarteriolar lymphocyte sheath and B cell follicles, in which many branches of the arterial blood supply to the spleen open. The marginal zone is strategically located for phagocytosis and the processing of Ags in the blood (37). The marginal zone of the mouse is composed of an inner layer of metallophilic Mδ, the marginal sinus, and an outer reticular network with marginal zone Mφ and B cells (37). The metallophilic Mφ react with the mAb MOMA-1, and the marginal zone Mδ react with the mAb ER-TR9 (37, 38). Staining with both MOMA-1 and ER-TR9 did not identify a marginal zone in }cpdm/cpdm mice. MOMA-1 is expressed in the spleens of TNFR1- and TNFR2-deficient mice (39), but not in the spleens of LTα- and LTβ-deficient mice (12, 13, 39) or in the spleens of mice treated with an LTβR-Ig fusion protein that blocks the LTβR (40). The data are consistent with a critical role of the LTαβ heterotrimer and the LTβR in the development of the marginal zone.

The CHS response in }cpdm/cpdm mice was similar to that of +/+ littermates. This response depends upon the interaction of various cell types, and the normal CHS response in }cpdm/cpdm mice indicates that the lymphoid tissue organization is adequate for this response to occur. The CHS response has a sensitization phase, in which DCs from the skin migrate to the LN and present the hapten, in this study DNFB, to CD4+ and CD8+ T cells. The elicitation phase is mediated by a poorly characterized, early acting cell type that causes mast cell and platelet degranulation and is responsible
for the ear swelling at 2 h after application of DNFb to the ear (8, 41). This is followed by inflammation caused by the sensitized CD4+ and CD8+ T cells resulting in ear swelling at 24 and 48 h (42). Both the early and late elicitation phases were normal in the cpdm/cpdm mice. This finding suggests that the defective organization of lymphoid tissues in cpdm/cpdm mice primarily affects the humoral immune response and leaves the cell-mediated response intact.

In summary, these studies of the immune system in cpdm/cpdm mice have demonstrated a selective absence of PPs and abnormal intact. The mice have demonstrated a selective absence of PPs and abnormal intact. The humoral immune response and leaves the cell-mediated response (42). Both the early and late elicitation phases were normal in the cpdm/cpdm mice. The manuscript.

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