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Macrophages, But Not T and B Lymphocytes, Are Critical for Subepidermal Blister Formation in Experimental Bullous Pemphigoid: Macrophage-Mediated Neutrophil Infiltration Depends on Mast Cell Activation

Ruoyan Chen,* Janet A. Fairley, † Ming-Lang Zhao,* George J. Giudice, † Detlef Zillikens, ‡ Luis A. Diaz,* and Zhi Liu*2

Bullous pemphigoid (BP) is a subepidermal blistering disease associated with autoantibodies against two hemidesmosomal proteins, BP180 and BP230. Numerous inflammatory cells infiltrate the upper dermis in BP. We have previously shown by passive transfer studies that Abs to the ectodomain of murine BP180 are capable of triggering blisters in mice that closely mimic human BP. Experimental BP depends on complement activation and neutrophil infiltration. In the present study, we investigated the relative contribution of neutrophils, mast cells (MCs), macrophages (Mφ), and lymphocytes and their functional relationship in the immunopathogenesis of this disease model by using mice deficient in these cells. Wild-type, T cell-deficient, and T and B cell-deficient mice injected intradermally with pathogenic anti-murine BP180 IgG exhibited extensive subepidermal blisters. In contrast, mice deficient in neutrophils, MCs, and Mφ were resistant to experimental BP. MCs play a major role in neutrophil recruitment into the dermis. Furthermore, Mφ-mediated neutrophil infiltration depends on MC activation/degranulation. The Journal of Immunology, 2002, 169: 3987–3992.

Bullous pemphigoid (BP) is an acquired autoimmune skin disease characterized by subepidermal blisters and autoantibodies against two hemidesmosomal Ags, BP230 (BPAG1) and BP180 (BPAG2) (1). These anti-hemidesmosomal autoantibodies are found in the circulation of patients, and can be detected, along with complement components, bound to the dermal-epidermal junction (DEJ) of perilesional skin. The skin blisters of these patients show detachment of basal keratinocytes from the underlying dermis and a dermal inflammatory infiltrate (2). A variety of cellular lineages have been identified in these inflammatory infiltrates, including eosinophils, neutrophils, lymphocytes, mast cells (MCs), and monocyte/macrophages (Mφ) (1, 3–8). MCs found in BP lesions exhibit morphological changes suggesting degranulation (7, 9). Lesional skin in BP patients exhibits several granular proteins derived from leukocytes, such as eosinophil cationic protein, eosinophil major basic protein, and neutrophil-derived myeloperoxidase (MPO) (10–12). Various inflammatory mediators that can activate MCs or leukocytes have been identified in lesional skin and/or blister fluids of BP patients, including C5a, eosinophilic/ neutrophilic chemotactic factors, histamine, leukotrienes, and various cytokines (e.g., IL-1, -2,-5,-6,-8, TNFs, and IFN-γ) (13–20). Several proteinases are also found in BP blister fluid, including plasmin, collagenase, elastase, and 92-kDa gelatinase (21–24).

An experimental model of BP that involves the passive transfer of anti-murine BP180 (mBP180) Abs into neonatal BALB/c mice reproduces the key immunopathological features of this human autoimmune disease, i.e., IgG and complement deposition at the DEJ, inflammatory infiltration of the upper dermis, and subepidermal blistering (25). We further showed that the pathogenicity of anti-mBP180 Abs is dependent on complement activation (26), MC degranulation (27), and neutrophil recruitment (28). In the present study, we investigated the role of Mφ, T and B lymphocytes, and compared the relative contribution of these inflammatory cells in subepidermal blistering in experimental BP.

Materials and Methods

Laboratory animals

Breeding pairs of C57BL/6J, MC-deficient WCB6F1-Mgf1/Mgf1−/−d (referred to as MC−/−) mice (29), T cell-deficient nude (referred to as T−/−) mice, T and B cell-deficient C57BL/6J Rag2tm1Mom (referred to as T−/−B−/−) mice (30) were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained at the Medical College of Wisconsin Animal Resource Center (Milwaukee, WI). Neonatal mice (24–36 h old with body weights between 1.4 and 1.6 g) were used for passive transfer experiments.

Preparation of pathogenic rabbit anti-murine IgG

The preparation of recombinant mBP180 and the immunization of rabbits were performed as previously described (25). The titers of rabbit anti-mBP180 Abs in the rabbit sera and in the purified IgG fractions were assayed by indirect immunofluorescence (IF) using mouse skin cryosections as substrate (25). The pathogenicity of these IgG preparations was tested by passive transfer experiments as described below. One pathogenic anti-mBP180 IgG (referred to as R530) and one control IgG (referred to as R50) were used (26).
A 50-µl dose of sterile IgG in PBS was administered to neonatal mice by intradermal (i.d.: 2.64 mg IgG/g of body weight). The injection techniques have been described elsewhere (25, 28). The skin of neonatal mice from the test and control groups was examined 12 h after the IgG injection. The extent of cutaneous disease was scored as follows: ---, no detectable skin damage; +, minimal evidence of epidermal detachment* sign (this sign was elicited by gentle friction of the mouse skin which, when positive, produced, fine, persistent wrinkling of the epidermis); 2+, intense erythema and epidermal detachment sign involving 20–50% of the epidermis in localized areas; and 3+, intense erythema with frank epidermal detachment sign involving >50% of the epidermis. The animals were then sacrificed and the following specimens were obtained. Skin sections were taken for light microscopy (H&E staining) and for direct IF analysis to detect rabbit IgG and mouse C3 deposition at the basement membrane zone (BMZ). Sera of injected animals were obtained for indirect IF assay to determine the circulating titers of anti-mBP180 IgG. Direct and indirect IF analyses were performed as previously described (25). Monospecific FITC-conjugated goat anti-rabbit IgG was obtained commercially (Kirkeggard & Perry Laboratories, Gaithersburg, MD). Monospecific goat anti-mouse C3 was purchased from Cappel Laboratories (Durham, NC).

Analysis of the inflammatory cells in the dermis by flow cytometry

Isolation, identification, and quantification of the dermal inflammatory cells were done by following published protocols (31, 32) with some minor modifications. Briefly, skin sections (8 × 8 mm) at the IgG-injecting sites were obtained and rinsed in 70% ethanol. The skin sections were cut into 4 × 4-mm sheets and submerged in culture medium into a 6-well plate. Each well contained 4 ml of RPMI 1640 with 25 mM HEPES, 10% heat-inactivated FBS and penicillin/streptomycin. The plate was incubated at 37°C for 6 h in a 5% CO2 incubator. The cells spontaneously emigrating out of the skin sections were pooled and incubated for additional 20 min at 37°C in PBS with 2 mg/ml glucose and without calcium and magnesium. The pooled cells were washed twice in HBSS without phenol red. The dermal inflammatory cells were identified by characteristic size (forward scatter) and granulosity (side scatter) combined with two-color flow cytometric analysis (31, 32). The neutrophils were identified as small cells, Ly-6G bright (RB6-8C5; BD PharMingen, San Diego, CA), and negative for F4/80 (A3-1; Caltag Laboratories, Burlingame, CA) or MHC class II (25-9-17; BD PharMingen). The Mϕ were identified as F4/80 positive or Mac-3 positive (M58/4; BD PharMingen) and MHC class II low or negative. The T lymphocytes were identified by their characteristic size and by CD3 (145-2C11; BD PharMingen) expression; B lymphocytes were identified by CD19 (1D3; BD PharMingen); the eosinophils were identified by their granulosity associated with F4/80 lightly positive and MHC class II negative. Rat IgG2b (A95-1; BD PharMingen) and IgG2a (R35-95; BD PharMingen) were used as the isotype controls. One million cells were washed once with PBS/10% FBS/0.4% sodium azide and then incubated with Fc Blocker (CD16/CD32; BD PharMingen) for 15 min at 4°C. The suspension was then centrifuged and the pellet was resuspended in 30 µl of PBS/3% BSA, followed by adding 1.5 µl of labeled Ab. After incubation for 30 min at 4°C, the cells were washed three times with PBS/1% FBS/0.4% sodium azide, and the cells were analyzed by flow cytometry. For each sample, 104 cells were analyzed. Each cell population was expressed as number of cells per skin section.

Mϕ depletion

Carraigeenan (Sigma-Aldrich, St. Louis, MO), a sulfated polysaccharide that destroys Mϕs, was used to selectively deplete Mϕs (33). Carraigeenan was dissolved in PBS and injected i.p. (0.2 mg/g body weight) into C57BL/6J mice according to previously described protocols (34, 35). Carraigeenan treatment resulted in a severe depletion of Mϕs (8- to 9-fold reduction). A total of 2 h later, the Mϕ-depleted mice (referred to as Mϕ−/−) were injected i.d. with pathogenic anti-mBP180 IgG. The animals were examined 12 h post IgG injection.

Neutrophil depletion

Polymorphonuclear leukocyte (PMN) depletion and injection procedures were performed as described (28). Briefly, a polyclonal rabbit anti-murine PMN Ab, AI-A31140, which selectively depletes mouse neutrophils in vivo, was purchased from Accurate Chemical & Scientific Corporation (Westbury, NY). Mice were depleted of PMN by two i.p. injections of AI-A31140 (10 mg IgG in 50 µl of PBS per injection) given 12 h apart. The control groups received 2 × 50 µl of normal rabbit IgG (10 mg IgG in 50 µl of PBS). PMN-depleted animals received one 50-µl i.d. (2.64 mg/g body weight/day) injection of pathogenic anti-mBP180 IgG, 12 h after anti-PMN treatment. The animals were then sacrificed and the skin and sera of mice from the test and control groups were examined 12 h later as described above.

Quantitation of MCs and MC degranulation

Lesional and nonlesional skin sections of IgG-injected mice were fixed in 10% Formalin. Paraffin sections (5 µm thick) were prepared and stained with toluidine blue and H&E. Total numbers of MCs were counted in five fields and classified as degranulated (>10% of the granules exhibiting fusion or discharge) or normal as described previously (27, 36). The results were expressed as percentage of MC degranulation.

In vivo inhibition of MC degranulation

Neonatal +/- mice were pretreated with cromyolin sodium (10 µg/g body weight), a MC degranulation inhibitor (37, 38), and 2 h later injected i.d. with pathogenic IgG (2.64 mg/g body weight). Twelve hours after IgG injection, the animals were examined for clinical blisters and the skin sections were analyzed by IF, H&E, and toluidine blue staining. Skin neutrophil infiltration was quantified by MPO assay.

Quantitation of skin site PMN accumulation

Tissue MPO activity in skin sites of the injected animals was assayed as described (39, 40). A standard reference curve was first established using known concentrations of purified MPO. The skin samples were extracted by homogenization in an extraction buffer containing 0.1 M Tris–Cl (pH 7.6), 0.15 M NaCl, and 0.5% hexadecyl trimethylammoniumbromide. MPO activity in the supernatant was measured by the change in OD460 resulting from decomposition of H2O2 in the presence of o-dianisidine. MPO content was expressed as relative MPO activity (OD460 reading per milligram of protein). Protein concentrations were determined by the Bio-Rad dye binding assay (Bio-Rad, Hercules, CA) using BSA as a standard.

Neutrophil isolation

Mouse neutrophils were isolated from heparinized blood by dextran sedimentation followed by separation on a density gradient as described (41). RBCs were removed from the cell preparation by hypotonic lysis in 0.2% NaCl. Neutrophils were washed and resuspended in cold PBS/0.1 M glucose, counted in a hemocytometer, and adjusted to a concentration of 1 × 107 cells/ml. Neutrophil purity was determined by cell-cytospin and LeukoStat staining (Fisher Diagnostics, Orangeburg, NY). The viability of the neutrophils was >96% as determined by trypan blue exclusion.

i.d. injection of neutrophils

Mϕ-deficient mice were injected i.d. with pathogenic anti-mBP180 IgG (2.64 mg/g body weight/50 µl of PBS). Two hours later, these mice received 5 × 105 neutrophils i.d. (in 50 µl of PBS/10 mM glucose) at the same site (42). The animals were analyzed 12 h after the IgG injections as described above.

IL-8 pretreatment of Mϕ-deficient mice

Recombinant human IL-8, purchased from R&D Systems (Minneapolis, MN), was stored at 1 mg/ml in sterile PBS. Single i.d. injection of IL-8 (50 ng in 50 µl of PBS) or an equivalent amount of BSA, was given into neonatal Mϕ-deficient mice 60 min before i.d. injection of rabbit anti-mBP180 IgG (2.64 mg/g body weight in 50 µl) (28). Control animals received an equivalent amount of normal rabbit IgG in place of the anti-mBP180 IgG. Twelve hours after the IgG injections, the animals were analyzed as described above.

Statistical analysis

The data were expressed as mean ± SEM and were analyzed using the Student’s paired t test. A value of p < 0.05 was considered significant.

Results

Analysis of dermal inflammatory cells in mice injected with pathogenic anti-BP180 IgG

Pathogenic anti-BP180 IgG induces intensive cutaneous inflammation and recruits numerous inflammatory cells into the dermis. To quantify these cells within the dermis, mice were injected i.d. with anti-BP180 or control Abs. Twenty-four hours later, the cells...
To compare relative contributions of different inflammatory cells in experimental BP, neonatal control, and mice deficient in neutrophils, MΦ, T lymphocytes, and B lymphocytes were significantly increased in mice injected with pathogenic IgG than mice injected with control Ab. The influx of eosinophils remains the same between the pathogenic IgG-treated and control groups. These results were similar to findings obtained by routine histology staining of the mouse skin sections.

**Neutrophils, MCs, MΦ, but not B and T lymphocytes, are required for subepidermal blistering in experimental BP**

To determine whether MΦ play a role in MC activation/degranulation, MΦ-sufficient and MΦ-deficient mice were injected i.d. with pathogenic IgG. The total number of MCs and the percentage of MC degranulation in the IgG-injected skin were quantified at different time periods after IgG injection. As expected, wild-type mice (n = 5) injected with pathogenic IgG developed subepidermal blisters 12 h after IgG injection (Table I), while MΦ-deficient mice (n = 5) injected with pathogenic IgG did not show any sign of skin blistering (Table I). Toluidine blue staining showed a slightly (but not significantly) higher degree of MC degranulation in the skin of MΦ-sufficient as compared with MΦ-deficient mice injected with pathogenic IgG at 2 and 12 h post-IgG injection (Fig. 4Aa, a and c). As expected, MC degranulation in the skin of MΦ-sufficient and MΦ-deficient mice coinjected with pathogenic IgG and cromolyn was minimal (Fig. 4Ab, b and d). We also failed to see any significant difference in total number of MCs and percentage of MC degranulation in paired groups of mice (MΦ-sufficient vs MΦ-deficient group and cromolyn-treated MΦ-sufficient vs cromolyn-treated MΦ-deficient mice) across all time points (Fig. 4B). These results suggest that depletion of MΦ does not affect MC degranulation.

*MΦ are not involved in MC activation, but play an accessory role in recruiting neutrophils in experimental BP*

FIGURE 1. Quantification of inflammatory cell recruitment in the dermis of mice injected with pathogenic anti-BP180 IgG. Neonatal mice were injected i.d. with pathogenic or control IgG (2.64 mg/g body weight). The skin sections at the IgG-injection site were obtained 24 h postinjection and processed to recover infiltrating cells. The cells obtained from four mice were pooled and the different populations of inflammatory cells were identified by staining and flow cytometry as described in Materials and Methods. The data shown are the mean ± SE, n = 4 for each group. *, p < 0.05, and **, p < 0.01, Student t test for paired samples.

**FIGURE 2.** Clinical and histological examination of neonatal mice deficient in different inflammatory cells injected with pathogenic anti-mBP180 IgG. Neonatal mice (24–36 h old) were injected i.d. with anti-mBP180 IgG R530 (2.64 mg/g body weight). Twelve hours later, wild-type C57BL/6J (A and B), T cell-deficient (T−) (see Table I), T and B cell-deficient (T&B−) (G and H) mice developed subepidermal blisters. In contrast, MC-deficient (MC−) (C and D), MΦ-deficient (MΦ−) (E and F), and neutrophil-deficient (PMN−) (I and J) mice injected i.d. with pathogenic IgG showed no evidence of skin disease. d, dermis; e, epidermis; v, vesicle; filled arrowhead, blister (×200). Insets. Higher magnification of H&E staining sections (×800) demonstrate the absence (D, F, and J) and presence (B and H) of infiltrating PMN in the dermis (arrowhead).
We found that M\(\phi\)-deficient mice reconstituted with neutrophils become susceptible to experimental BP either by i.d. injection of pathogenic IgG plus 5 \(\times\) 10\(^5\) mouse neutrophils or i.d. injection of pathogenic IgG plus IL-8. Twelve hours after IgG injection, the injected M\(\phi\)-deficient mice \((n = 5)\) developed subepidermal blisters (Table I). As a control, M\(\phi\)-deficient mice injected with IL-8 alone exhibited neutrophil infiltration in the skin but no skin lesions (Table I). Taken together, these results suggest that M\(\phi\) mainly participate in PMN recruitment in experimental BP.

M\(\phi\)-mediated neutrophil recruitment is MC dependent

To determine whether M\(\phi\)-mediated neutrophil infiltration depends on MCs, wild-type, MC-deicient, and M\(\phi\)-deficient mice with or without pretreatment of carrageen or cromolyn were injected i.d. with pathogenic IgG. Twelve hours later, neutrophil infiltration in the skin of the injected animals was quantified by the MPO assay. We found that M\(\phi\)-deficient mice \((n = 5)\) with and without M\(\phi\) depletion showed similar MPO activities \((0.34 \pm 0.04\) for untreated vs 0.39 \(\pm\) 0.05 for carrageen-treated mice; Fig. 5A). In contrast, M\(\phi\)-deficient mice pretreated with cromolyn exhibited a significant reduction in tissue MPO activity as compared with M\(\phi\)-deficient mice without cromolyn treatment \((0.68 \pm 0.11\) for untreated vs 0.52 \(\pm\) 0.04 for M\(\phi\)-deficient plus cromolyn).

![Figure 3](image)

**FIGURE 3.** Relative contribution of inflammatory cells in experimental BP. Neonatal wild-type (WT; \(bar 1\)), neutrophil (PMN)-deficient \((bar 2)\), MC-deficient \((bar 3)\), M\(\phi\)-deficient \((bar 4)\), T cell (T)-deficient \((bar 5)\), and T and B cell (T&B)-deficient mice \((bar 6)\) received 2.64 mg/g body weight pathogenic anti-mBP180 IgG. Tissue MPO activities (mean \(\pm\) SEM) in the injection sites were determined 12 h after the IgG injection. \(n = 8\) for each group. \(*, p < 0.05;**, p < 0.01\). Student t test for paired samples \((bar 1 vs 2, 3, or 4)\). The MPO values shown were corrected for control IgG controls. Each group of mice injected with control IgG yielded an average MPO activity of \(-0.1\) OD\(_{500}\)/mg protein.

![Figure 4](image)

**FIGURE 4.** M\(\phi\) deficiency has no effects on MC degranulation. M\(\phi\)-sufficient (M\(\phi^+\)) and M\(\phi\)-deficient (M\(\phi^-\)) mice were pretreated with the MC degranulation blocker cromolyn sodium (CS) for 2 h and injected i.d. with pathogenic IgG R530. The injected animals were examined at different time points after IgG injection. A, Toluidine blue staining. At 12 h post IgG injection, M\(\phi^+\), but not M\(\phi^-\), CS-treated M\(\phi^+\), and CS-treated M\(\phi^-\) mice developed blisters (Table I). At 2 h post IgG injection (peak of MC degranulation), M\(\phi^+\) \((a)\) and M\(\phi^-\) \((c)\) mice showed extensive MC degranulation in Toluidine blue-stained skin sections. In contrast, CS-treated M\(\phi^+\) \((b)\) and CS-treated M\(\phi^-\) \((d)\) mice exhibited background levels of MC degranulation. B, Time course (0–12 h postinjection) of MC degranulation. The MCs in the dermis were counted and classified as degranulated (>10% of the granules exhibiting fusion or discharge) or normal (see Materials and Methods). Toluidine blue-stained sections showed a similar pattern of progression of MC degranulation between comparable groups of mice: M\(\phi^+\) vs M\(\phi^-\) and CS-treated M\(\phi^+\) vs CS-treated M\(\phi^-\).
Each group of mice injected with control IgG yielded an average MPO vs PBS (and mice pretreated with the M

was 2.64 mg/g body weight. Tissue MPO activity (mean ±SEM) in skin at the injection site was determined 12 h after IgG administration. \( n = 8 \) for each group, \( * p < 0.05 \). Student’s \( t \) test for paired samples (bar 1 vs 2; bar 3 vs 4). The MPO values shown were corrected for control IgG controls. Each group of mice injected with control IgG yielded an average MPO activity of \(-0.1 \text{ OD}_{460} / \text{mg protein}\).

FIGURE 5. \( \Phi \) acts downstream of MCs in experimental BP. A. MC-deficient (MC-) (bars 1 and 2) and MC-sufficient (MC+) (bars 3 and 4) mice pretreated with the M\( \Phi \) depleting reagent carrageen (bars 1 and 3) or PBS (bars 2 and 4) were injected i.d. with pathogenic R530. B. M\( \Phi \)-deficient (M\( \Phi \)-) (bars 1 and 2) and M\( \Phi \)-sufficient (M\( \Phi \)+) mice (bars 3 and 4) pretreated with the MC degranulation blocker CS (bars 1 and 3) or PBS (bars 2 and 4) were injected i.d. with pathogenic R530 IgG. The IgG dose was 2.64 mg/g body weight. Tissue MPO activity (mean ± SEM) in skin at the injection site was determined 12 h after IgG administration. \( n = 8 \) for each group, \( * p < 0.05 \). Student’s \( t \) test for paired samples (bar 1 vs 2; bar 3 vs 4). The MPO values shown were corrected for control IgG controls.

Discussion
Our study demonstrates that neutrophils, MCs, and M\( \Phi \), but not T and B lymphocytes, are required for full expression of the disease phenotype in experimental BP. We also established the relationship between MCs and M\( \Phi \) in the development of the cutaneous disease. MCs play a major role in recruiting neutrophils, while M\( \Phi \) amplifies the neutrophil inflammation in a MC-dependent fashion.

Based on immunohistological evidence, it has long been hypothesized that anti-BMZ autoantibody-triggered subepidermal blister formation in BP is mediated by inflammatory cells (43). Our previous and current findings provide in vivo evidence that DEJ separation is initiated by anti-BP180 IgG (25, 44) and dependent on neutrophils (28), MCs (27), and M\( \Phi \) (Fig. 2). We have previously established a causal relationship between neutrophil infiltration into the skin and subepidermal blistering in experimental BP (28). Depletion of circulating neutrophils completely abolishes the skin disease. Neutrophil elastase and gelatinase B mediate BMZ tissue damage and DEJ separation (42, 45). We further showed that neutrophil recruitment triggered by anti-BP180 Abs depends mainly on MCs (27). MC-deficient mice are resistant to experimental BP and exhibit \(-70\% \) reduction of neutrophil infiltration into the skin.

The fact that MC deficiency does not totally impair neutrophil recruitment in experimental BP suggests that there are at least two neutrophil recruitment pathways, MC-dependent and MC-independent pathways. Our present data show that M\( \Phi \) are also involved in anti-BP180 IgG-triggered neutrophil infiltration in mice.

To determine the functional relationship between MCs and M\( \Phi \) in mediating neutrophil infiltration, we first tested whether M\( \Phi \) deficiency could impair MC activation/degranulation. We found that 1) M\( \Phi \) did not affect MC degranulation (Fig. 4); 2) MC-deficient mice and mice deficient in both MCs and M\( \Phi \) exhibit the same disease scores and similar levels of neutrophils (Fig. 5A); and 3) inhibition of MC degranulation further reduced neutrophil infiltration in M\( \Phi \)-depleted mice (Fig. 5B). Thus, MCs act upstream of M\( \Phi \) in the inflammatory cascade in experimental BP.

MCs can produce a variety of inflammatory mediators such as leukotrienes, platelet-activating factor, and cytokines that contribute directly or indirectly to neutrophil recruitment (46, 47). In fact, levels of histamine, leukotriene B4, IL-1, -2, -5, -6, and TNF-\( \alpha \) are present in BP blister fluids (13–20). It is likely that some of these mediators are released from MCs and are involved in the recruitment of neutrophils directly by themselves and indirectly by activating M\( \Phi \). Both activation and degranulation of MCs are associated with matrix degradation (47). MC-specific serine protease MCP-4 (chymase) also activates gelatinase B (48, 49).

Therefore, MCs could also contribute to tissue damage in experimental BP directly by cleaving structural proteins in the DEJ or indirectly by activating gelatinase B. Similarly, M\( \Phi \) are also capable of releasing a variety of proinflammatory mediators, proteolytic enzymes, and reactive oxygen species (50), and could participate in either recruiting neutrophils or directly damaging BMZ. How MCs regulate M\( \Phi \) pathological functions in experimental BP is currently under investigation.

Lymphocytes are found in the lesional/perilesional skin of human BP (1) and the skin of experimental BP (25). However, our data show that mice deficient in T cells and T and B cells are susceptible to experimental BP, suggesting that these cells do not participate, at least during the early stages of the disease induced by passive transfer of pathogenic anti-BP180 IgG. Interestingly, T cell-deficient mice show more infiltrating neutrophils in the dermis and more severe disease activity relative to wild-type mice (Fig. 2). T cell-deficient mice show more infiltrating neutrophils in the dermis and more severe disease activity relative to wild-type mice (Fig. 2). T cells contain \( \alpha \)-proteinase inhibitor, the physiological inhibitor of neutrophil elastase (51). We have shown that \( \alpha \)-proteinase inhibitor is critical to the down-regulation of subepidermal blistering (52). Therefore, infiltrating T lymphocytes may secrete this inhibitor at the skin site to block neutrophil elastase activity, which subsequently inhibits neutrophil recruitment (44). It is also worth noticing that eosinophils are absent in the lesional skin of mice during the first 24 h of blister development, although they are the predominant cells in the inflammatory infiltrate in human BP. The difference in predominant cell infiltration between human BP and mouse BP could be due to the difference in their disease stages: skin biopsies of mouse BP are obtained at 12 or 24 h post-IgG injection, while most BP patient biopsies are obtained more than days or weeks after the initial onset of clinical disease activity. In fact, eosinophils are identified in the lesional skin of mouse BP when the mouse model is maintained long term (>96 h; our unpublished observations). It is also possible that eosinophils are not directly involved or play only a secondary role in the initiation of human BP. Because a pathogenic role of eosinophils in human BP remains to be proved, we cannot rule out the possibility that there are critical differences in the immunopathology between human and mouse BP. Whether eosinophils play a role in the late stages of the disease process, e.g., wound healing after tissue damage, needs further investigation.

In summary, this study provides direct evidence that M\( \Phi \), in collaboration with MCs and neutrophils, are required for subepidermal blistering in experimental BP and establishes a functional relationship between these proinflammatory cells. These findings provide new insights into the immunopathogenic mechanisms in
BP and have significant implications for therapeutic intervention for this disease.

References

9. Kawana, S., A. Ueno, and S. Nishiyama. 1990. Increased levels of immunoreactive BP and have significant implications for therapeutic intervention for this disease.

References

9. Kawana, S., A. Ueno, and S. Nishiyama. 1990. Increased levels of immunoreactive BP and have significant implications for therapeutic intervention for this disease.

References

9. Kawana, S., A. Ueno, and S. Nishiyama. 1990. Increased levels of immunoreactive BP and have significant implications for therapeutic intervention for this disease.