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Immune Modulation in Pemphigus Vulgaris: Role of CD28 and IL-10

Paola Toto, Claudio Feliciani, Paolo Amerio, Hirotake Suzuki, Binghe Wang, Gulnar M. Shivji, David Woodley, and Daniel N. Sauder

Pemphigus vulgaris (PV) is an autoimmune bullous skin disease characterized by Abs to the desmosomal cadherin desmoglein-3. Although the autoantibodies have been shown to be pathogenic, the role of the cellular immune system in the pathophysiology of pemphigus-induced acantholysis is unclear. To further delineate the potential role of T cell-signaling pathways in the pathogenesis of PV, we performed passive transfer experiments with PV IgG in gene-targeted mutant mice. Our results demonstrated that CD28-deficient mice (lacking a costimulatory signal for T cell activation) are 5-fold more sensitive to the development of PV than wild-type mice. To evaluate whether the higher incidence of disease was due to an impairment in intercellular adhesion of keratinocytes, we performed in vitro acantholysis, using CD28−/− mice keratinocytes. No alteration in in vitro adhesion was detected in CD28−/−-type keratinocytes. Because the CD28 molecule plays a pivotal role in the induction of Th2 cytokines, we examined the levels of a prototypic Th2 cytokine (IL-10) in CD28−/− mice. Lower levels of IL-10 mRNA were found in lesions from CD28−/− mice. To determine whether pemphigus susceptibility in CD28−/− was related to IL-10 deficiency, we performed passive transfer experiments in IL-10−/− mice that demonstrated increased blisters compared with controls. To confirm that IL-10 is involved in the pathogenesis, rIL-10 was given with PV IgG. IL-10 significantly suppressed the disease activity. These data suggest a potential role of IL-10 in PV. The Journal of Immunology, 2000, 164: 522–529.

Pemphigus vulgaris (PV) represents a potentially life-threatening autoimmune skin disease, characterized histologically by intraepidermal blisters and immunopathologically by in vivo bound and circulating IgG directed against the cell surface of keratinocytes (KC) (1). Significant advances have been made in understanding the pathogenesis of PV (2, 3). It is one of the few autoimmune diseases where the Ag is known, the Ab has been identified and passive transfer studies have demonstrated that the Ab mediates the disease process (4). IgG autoantibodies on the cell surface of keratinocytes are demonstrated in virtually all patients with active disease (5). In addition, the majority of patients have circulating IgG autoantibodies directed against keratinocyte surface molecules (6, 7). There is a direct correlation between the titer of the circulating Abs and disease activity. Pemphigus IgG, when added to skin organ cultures, have been shown to induce lesions similar to that seen in vivo (8, 9). However, more direct evidence demonstrating that pemphigus is an autoantibody-mediated disease comes from passive transfer studies. In their landmark studies, Anhalt et al. (10) demonstrated that pemphigus IgG could induce blister formation in normal mice that clinically and histologically was virtually identical to pemphigus.

Pemphigus autoantibodies are targeted against an adhesion molecule expressed on the surface of KC named desmoglein-3 (11, 12). Dsg3 is a 130-kDa transmembrane glycoprotein of the calcium-dependent cell adhesion molecule family known as cadherins (13). As a consequence of the Ab binding to the target Ag, KC lose their reciprocal adhesion properties. This process is termed acantholysis and results in the development of blisters.

Before the availability of corticosteroids, PV had a mortality rate of ~75% (14). Corticosteroids significantly improved the prognosis of PV patients, lowering the mortality rate to 21.4% (15). More recently, the introduction of other immunosuppressive drugs such as azathioprine, cyclophosphamide, and cyclosporin A, used as adjuvant therapies, exerting a steroid-sparing effect, has further decreased the mortality rate of PV. Nevertheless, even with aggressive immunosuppressive therapy, there is a mortality of ~10% (16, 17). Moreover, long-term immunosuppression and corticosteroid therapy are associated with significant morbidity (18, 19). The lack of knowledge concerning the precise immunopathogenesis of PV hampers the ability to target specific mediators and effectors involved in the disease.

Although the immunopathology of pemphigus clearly involves autoantibodies, the cellular arm of the immune system is also involved in the pathogenesis of pemphigus (20, 21). To clarify the role of cell-mediated immunity, we utilized a passive transfer of PV Abs utilizing gene-targeted mutant mice lacking T cell-signaling pathways and cytokines. Our results demonstrate that IL-10 plays a suppressive role in acantholysis and thus provide a potential novel therapeutic approach to treatment.
Materials and Methods
Preparation of pemphigus IgG
Plasma was obtained from the plasmapheresis of one patient with the clinical, histologic, and immunologic features of pemphigus during the acute phase of the disease. Total IgG concentration was measured by nephelometry using monospecific goat anti-human IgG (Beckman Instruments, Mississauga, Ontario, Canada). Pemphigus Ab titers were measured by indirect immunofluorescence using monkey esophagus epithelium as the tissue substrate (22). As a negative control, IgG fractions were isolated and removed from PV plasma using protein A (PA). Isolation of IgG fractions from PV plasma was achieved by standardized technique using staphylococcal protein-A coupled to Sepharose 4B (23) (Pharmacia Biotech, Uppsala, Sweden). PA was washed four times in cold PBS and finally incubated with PV plasma overnight at 4°C. The supernatant was then collected and used as negative control. Absence of IgG fractions in the control plasma was assessed by staining monkey esophagus epithelium substrate and confirmed by nephelometry. Plasma and control plasma were filter sterilized with Millex (pore size, 0.22 mm; Millipore, Bedford, MA) and stored at −20°C.

Mice
The following strains were used: BALB/c, CD28−/−, C57BL/6 (CD28−/− control), IL-10−/−, and C57BL10/IL-10−/− control). BALB/c were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were housed and bred under specific pathogen-free conditions in the animal facility of the Sunnybrook Health Science Centre. Neonates (<24 h of age) were used. An average of 15 mice within each experimental group was used, and each experiment was repeated at least three times. All animal procedures were approved by the Sunnybrook Health Science Centre animal care committee.

CD28−/− mice. The generation of mice homozygous for CD28 gene mutations (CD28−/−) has been described previously (24), and this mutation was interbred six times into the C57BL/6 background before generating CD-28-deficient (CD28−/−) mice.

Mice homozygous for the defect were used as the knockout (KO) mice, with the wild-type animals serving as the nondeficient controls.

IL-10−/− mice. Results were purchased from The Jackson Laboratory. IL-10−/− mice, by disruption of the IL-10 gene (25), were back-crossed onto C57BL/10 background for six generations and maintained under pathogen-free conditions. Mice homozygous for the defect were used as the KO mice, with the wild-type animals serving as the nondeficient controls.

Passive transfer model
To induce PV in mice, we utilized the model of Anhalt et al. (10) with minor modifications. Briefly, plasma was injected intradermally in the dorsal area, into neonatal mice through a 30-gauge needle. The total dose administered ranged from 30 to 70 μl/g (177–413 μg/ml IgG) of body weight per day. We chose a dose of 30 μl/g because this was the minimum dose inducing the disease in WT mice. When the volume of fluid to be injected exceeded 65 μl, half of the total amount was given twice in the day. Early strain of gene-targeted mutant mice, and WT mice were injected with the same dose of PV plasma. As a negative control, gene-targeted mutant mice and WT mice were injected with plasma depleted of IgG by treatment with protein A-beaded agarose.

Mice were examined 24 h after the injections. Cutaneous lesions consisting of intact blisters or erosions were enumerated. The lesions were arbitrarily scored as follows: 1+, detachment of a small area; 2+, definite blister; and 3+, multiple blisters.

Tissue specimens staining
Lesional and perilesional skin were obtained for light microscopy and direct immunofluorescence 24 h postinjection with PV IgG. At the time of biopsies, serum was also obtained and assayed for indirect immunofluorescence on a monkey esophagus epithelium to detect the IgG titer.

Direct immunofluorescence
Perilesional skin was biopsied and specimens were snap frozen in liquid nitrogen until use. Cryostat sections (5 μm) were used, and direct immunofluorescence studies were performed according to standard techniques (26). Briefly, specimens were washed in PBS for 10 min, incubated for 30 min with FITC-conjugated F(ab')2 fragment of rabbit anti-human IgG, specific for γ-chains (1:25; Dako, Glostrup, Denmark), and washed in PBS for 15 min. Slides were covered with buffered glycerol, and results were read in a Nikon Optiphot immunofluorescence microscope (Nikon, Melville, NY).

Indirect immunofluorescence
Sera were collected 24 h after PV IgG treatment, PA treatment (IgG depleted), or sham treatment. Indirect immunofluorescence studies were performed according to standard techniques (22, 26). Cryostat sections (5 μm) of monkey esophagus were employed as substrate, washed for 10 min in PBS, incubated for 30 min with different concentrations of sera (1:1–1:600), washed in PBS for 15 min, labeled with FITC-conjugated F(ab')2 fragment of rabbit anti-human IgG (Dako) for 30 min, and then washed again in PBS for 15 min. Slides were covered with buffered glycerol, and results were examined using a Nikon Optiphot immunofluorescence microscope.

Histologic technique
Skin biopsies from mice were fixed in 10% formalin and stained with hematoxylin and eosin.

Cell culture and acantholysis measurement
In vitro acantholysis was assessed using a modification of the previously described methodologies (8, 9, 27–29). Primary KC cultures were prepared from newborn C57BL/6 and CD28−/− mice by treating skin samples with 1% dispase II (Boehringer Mannheim, Laval, Quebec, Canada) solution overnight at 4°C. Epidermal sheets were separated from the dermis and stirred in a trypsin-EDTA solution (0.05% trypsin and 0.53 mM EDTA) for 20 min at room temperature. Cell suspensions were filtered through nylon mesh and centrifuged at 300 × g for 10 min. Cell pellets were resuspended in Eagle’s MEM with 10% FBS, incubated at 37°C in a humidified atmosphere containing 5% CO2. The medium was removed and replaced with fresh medium every 2 days. Third passage cells were then plated onto 35-mm tissue culture dishes (6 × 103 cell/dish) in MEM supplemented with 10% FBS. At confluence, the cultures were washed with two changes of PBS. Cells were then incubated in 2 ml MEM containing 1/50 dilutions of PV plasma, PA-treated plasma, or FCS. After a 9-h incubation at 37°C in a humid atmosphere of 5% CO2 in air, the cells were gently washed with their overlying medium 15 times with a Pasteur pipette. The medium was removed and replaced with fresh MEM containing 10% FCS. The plates were then stained in a Leica Q500 MC image processing and analyzed by Quantimet 500 software system (Leica, Cambridge, U.K.). Briefly, the system was calibrated to measure the number of cells attached to the plate and the area free of cells. Measurements were applied in 10 random areas for each plate, and an average of measures was calculated in both PV sera-impregnated cells and control cells (30, 31).

Statistical analysis
Data regarding the incidence of the disease in KO and control mice were analyzed using the χ2 test; a p value <0.05 was considered to be significant.

Determination of IL-10 mRNA
Since CD28 signaling has been demonstrated to influence IL-10 expression, we investigated the mRNA level for this cytokine in CD28−/− mice. Total RNA was extracted from CD28−/− neonatal mouse skin by the acid guanidinium thiocyanate-phenol-chloroform method. RT-PCR were performed as described previously (32). Primers sets and positive cDNA template controls for mouse IL-10 and β-actin were obtained from Ciontech Laboratories (Palo Alto, CA). The sequences for each primer were as follows: IL-10 primers: upstream, 5′-AAG CTG AGA ACC AAG ACC CAG ACA TGA CGG CG-3′; downstream, 5′-GAG TAT CCC AGA GCC CCA GAT CCG ATT TGT G-3′; and β-actin primers: upstream, 5′-GTT GGC CGC CCT TGG ACC CAA A-3′; downstream, 5′-CTC TTT GAT TTC GTC ACC GAT TTC-3′. Specific cDNA obtained from RT was amplified in a total volume of 10 μl containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl2, 200 μM each of all four dNTPs, 10−6 M tetramethyl ammonium chloride, 10 pmol of each primer, and 0.5 U of Taq DNA polymerase (Pharmacia Biotech, Baie d’Urfe’, Quebec, Canada). The mixture was overlaid with 15 ml of mineral oil and PCR cycles were performed in a Perkin-Elmer/Cetus thermal cycler 480 (Perkin-Elmer/Cetus, Norwalk, CT) with denaturation for 1 min at 94°C, annealing for 1 min at 60°C, and extension for 1 min at 72°C. PCR signals for β-actin and IL-10 were obtained after 30 cycles. An aliquot (5 μl) of the PCR product was electrophoresed on a 1.5% agarose gel. After photographing the gel, relative amounts of PCR products were determined by scanning the negative films using a Utrascan XL LKB 2222-020 laser densitometer (Pharmacia Biotech). For relative quantitation, the densitometric value of each sample was normalized to β-actin.

Administration of recombinant murine IL-10 protein
Murine rIL-10 was obtained from Genzyme (Cambridge, MA). Lyophilized powder was reconstituted with sterile distilled water to a final concentration of 100 μg/ml and diluted with PBS including carrier protein (0.1% BSA) to a final concentration of 3 μg/ml. A total volume of 15 μl...
(50 ng) was injected intradermally in neonatal C57BL/10 in the dorsal area. The administration of rIL-10 was followed, within 15–60 min, by intradermal injection of PV plasma (50 μl/g body weight per day). Negative controls consisted of injections of PV plasma (50 μl/g body weight) and rIL-10 vehicle. Mice were examined 24 h later to detect the presence of cutaneous lesions. Samples were collected for immunofluorescence staining and routine histology.

**Results**

**Pemphigus vulgaris IgG**

Pemphigus plasma was obtained as described in *Materials and Methods*. Indirect immunofluorescence using monkey esophagus as substrate demonstrated a titer of 1:2460 and was shown to have an IgG concentration of 5.9 mg/ml using nephelometric analysis. PA-treated plasma showed absence of intercellular staining on monkey esophagus, and IgG levels were below the level of detection using the nephelometric analysis.

**Passive transfer of PV**

A dose-response study utilizing passive transfer of PV IgG demonstrated a direct correlation between the amount of PV IgG injected and the incidence of the disease. Characteristic clinical (Fig. 1a), histological (Fig. 1b), and immunological (Fig. 1c) findings in neonatal mice injected with PV IgG are shown in Fig. 1. Acantholytic changes accompanied by an inflammatory infiltrate were evident in mice given PV plasma (Fig. 1b) and absent in mice injected with control plasma (Fig. 1d) or PA-treated plasma (Fig. 1e). The epidermis of all mice injected with PV IgG showed human IgG bound to the intercellular cell surface by direct immunofluorescence (Fig. 1c).
No staining was found in mice injected with PA-treated plasma. No difference was observed in the intensity of fluorescence in different strains of mice treated with an equal dose of PV IgG. The IgG titer in all mice injected with PV plasma, as detected by indirect immunofluorescence, ranged between 1:100 in mice injected with 30 μl/g body weight/day PV plasma and 1:200 in mice injected with 50 μl/g body weight/day. No circulating PV IgG were detected in mice injected with PA-treated plasma. In all of the WT strains, IgG deposits were observed with a minimal dose of 30 μl/ml/day PV plasma (177 μg/g PV IgG). At that dose, ~10% of mice displayed clinical evidence of disease, with a mean disease severity scored 1.5. When a dose of 50 μl/g/day (295 μg/g PV IgG) body weight of PV plasma was administered, >75% of WT mice developed blisters, with a mean disease severity of 2.5 (Fig. 2).

CD28−/− mice are more sensitive to the development of pemphigus

When a dose-response study was performed on CD28−/− mice, a higher incidence of disease was observed. In particular, with a dose of 30 μl/g body weight/day PV plasma, 53% of the CD28−/− mice injected developed PV lesions (mean disease severity, 2) as compared with 9.5% of their WT (C57BL/6) (mean disease severity, 1.5; Fig. 3). With an administered dose of 50 μl/g body weight/day, 100% of the CD28−/− mice showed evidence of disease (mean disease severity, 3) compared with 75% of the controls (mean disease severity, 2.5).

CD28−/− mice KC display the same level of acantholysis as control mice KC

KC express the counterligand for CD28, namely, B7. Although we hypothesized the CD28−/− mice were more susceptible to PV due to alteration in immune function, the absence of the CD28 receptor could affect the adhesion between KC (which have been shown to express the B7-3 counterligand). To determine whether the increased susceptibility of CD28−/− mice to the development of PV was due to an immune-mediated mechanism or to a direct influence on KC adhesion, we conducted in vitro acantholysis studies using KC cultures from CD28−/− mice.

As shown in Figs. 4 and 5, after a 9-h incubation with PV plasma, CD28−/− KC displayed the same degree of acantholysis as controls (Fig. 5, b and d). KC incubated with PA-treated plasma did not exhibit acantholysis (Fig. 5, a and c). The results of this study suggest that alteration in KC adhesion cannot account for the higher sensitivity to PV development in CD28−/− mice.
Lower levels of IL-10 mRNA are found in CD28−/− mice skin with PV IgG-induced lesions

Because the CD28 molecule plays a pivotal role in the induction of IL-10 by Th2 lymphocytes (33, 34), we hypothesized that an impairment of IL-10 could explain the higher incidence of pemphigus in CD28−/− mice. To test this hypothesis, we performed a RT-PCR on CD28−/− mice. Skin from CD28−/− mice before and after PV IgG treatment demonstrated a lower level of IL-10 mRNA compared with that of controls (Fig. 6).

IL-10−/− mice display a higher incidence of pemphigus compared with controls

To further evaluate whether a deficiency in IL-10 production is linked to a higher sensitivity to the development of disease, we performed passive transfer studies. When a dose of 30 μl/g body weight/day of PV plasma was administered to IL-10−/− mice, pemphigus lesions were detected in 42% (mean disease severity, 2.5) compared with 10% of the controls (C57BL/10) (mean disease severity, 1.5; Fig. 7).

Intradermal administration of rIL-10 suppresses the development of pemphigus

To further support the hypothesis that IL-10 plays an inhibitory role on PV, we injected rIL-10 protein in WT mice (C57BL/10) and then performed passive transfer studies as above. When 50 ng/mouse of rIL-10 was administered to mice with 50 μl/g of PV plasma, blisters developed in 14.2% of the mice compared with 100% of controls (Fig. 8). A lower incidence of blisters was seen also in IL-10−/− mice (17 vs 100%) and CD28−/− mice (18.5 vs 100%) after administration of rIL-10. IL-10 administration lowered disease severity in all mice strains compared with controls (1.5 vs 3).

Discussion

Most studies on the immunopathogenesis of PV have focused on the role of the humoral immune system in the disease pathogenesis (35). Some studies have suggested that a cell-mediated response is also involved (20, 21, 36). These studies have implicated alterations in T cell-signaling pathways in PV. Recently, Lin et al. (36) demonstrated that Dsg3-specific lymphocytes proliferate in response to antigenic stimulation. Characterization of the Dsg3-responding lymphocytes showed that they were Th2-like cells secreting Th2 cytokines like IL-4 and IL-10. These cells expressing a CD4 memory phenotype have been suggested to be relevant in the autoimmune response in PV patients by modulating the B cell isotype switch (34). The same authors demonstrate that T lymphocytes from a subset of patients respond to both Dsg3 and Dsg1, and hypothesized that the T cell-immune response, initially directed to an epitope on Dsg3, may subsequently involve other Dsg3 epitopes or other desmosomal Ags such as desmocollins and Dsg1 (37).
Therefore, as already proposed for other autoimmune disorders (38, 39), a “determinant spreading” phenomenon of the autoimmune response might also be invoked in PV. Although the induction of the autoimmune response in PV is clearly a T cell-dependent phenomenon, the role of T cells in the development of acantholysis is still controversial. Several reports demonstrated elevated serum levels of soluble IL-2R in pemphigus patients which correlated with the extent and severity of the disease (21). Higher levels of sIL-2R are found in pemphigus blister fluid than in corresponding serum samples, suggesting the presence of activated T cells in pemphigus lesions. Other studies have shown that IL-2 is found in lesional and perilesional skin of pemphigus patients and is not found in clinically uninvolved skin (21). Immunohistology studies have demonstrated that 60% of the mononuclear cells in the dermis of pemphigus lesions express the pan T cell marker CD3 and the ratio CD4:CD8 is 2:1 (21, 40). Recently, a role for a mononuclear cell infiltrate has been suggested in a murine model of paraneoplastic pemphigus, in fact a cytotoxic lichenoid infiltrate producing inducible nitric oxide synthase has been shown within paraneoplastic pemphigus lesions in mice (41). Further evidence of cell-mediated immune mechanisms in the pathogenesis of pemphigus is the demonstration that lymphocytes from pemphigus patients are needed to induce pemphigus-like lesions in SCID mice engrafted with human skin (42). An alternative model for the study of this disease consists in the injection of PV IgG in athymic nude mice engrafted with human oral mucosa. In this model, Buschard et al. (43) demonstrated intercellular binding of pemphigus Abs to human mucosal grafts in all cases; however, suprabasilar blisters were found in <20% of the mice injected with pemphigus IgG. Thus, pemphigus IgG is not sufficient to induce acantholysis in the absence of T cells.

One of the major mechanisms involved in T cell communication is through release of cytokines. Cytokine dysregulation has been reported in pemphigus. Increased levels of IL-6 and TNF-α have been found in the sera of patients with PV, and these findings correlate with disease severity (44). Cytokines have also been shown to play a role in another autoimmune blistering skin disease, bullous pemphigoid. Systemic treatment with neutralizing Abs to murine IL-1 or IL-6 completely blocked the development of blisters in BALB/c mice injected with rabbit IgG directed against the 180-kDa bullous pemphigoid Ag. Similar results were achieved by administration of IL-10, a cytokine known to down-regulate the expression of IL-1 and IL-6 (45–47).

T cell activation requires a signal to be transduced through the Ag-specific T cell receptor as well as a costimulatory molecule (48). Costimulatory molecules may influence quantitatively and qualitatively T cell activation and induce the maturation of a preferential subset of T lymphocytes. An important costimulatory molecule for T cells is the transmembrane receptor CD28, whose ligands B7-1 (CD80) and B7-2 (CD86) are expressed on APC (49). KC also express the B7-3 (BB-1) receptor (50). For this reason, to investigate the role of T cells in the development of acantholysis, we chose to target the CD28-B7 pathway by performing a passive transfer of PV IgG in CD28−/− mice. The sentinel work of Anhalt et al. (10) has been the gold standard for an animal model of the disease. The passive transfer model established the pathogenic role of pemphigus Abs. Nevertheless, there are several inherent limitations with the passive transfer model. Among these is the limitation that one cannot directly study the cellular immune aspect of the disease since human IgG are passively transferred to a murine host. For that reason, an active model of pemphigus has long been sought. Recently, Amagai et al. (51) have demonstrated an active immune model for PV by transferring splenocytes from Dsg3−/− mice, immunized with Dsg3, into Dsg3+/+ mice, RAG2−/− to prevent rejection. However, this model has yet to be fully characterized. Thus, for these studies, we relied on the passive model. Our results indicate that CD28−/− mice are significantly more sensitive to the development of PV than controls.

The CD28 receptor is known to play a determining role in the adhesion of T cells to fibronectin and VCAM-1 by mediating the up-regulation of β1 integrins on the surface of T lymphocytes (52). β1 integrins are expressed on the plasma membrane of KC and play a central role in controlling adhesion and terminal differentiation within the epidermis. The β1 integrin family (αvβ3 and αvβ5) has been localized to the lateral aspects of basal keratinocyte plasma membranes where they maintain cell-cell aggregation into an intact epithelium. In vitro experiments using Abs to β1 integrins demonstrated an impairment in lateral aggregation of cultured KC (53, 54). Therefore, we needed to rule out the possibility that an alteration in KC adhesion properties could be responsible for the increased level of acantholysis detected in CD28−/− mice. The results of the in vitro acantholysis performed on CD28−/− mice KC suggest that this is not the case. In particular, the absence of CD28 did not alter KC cell-cell adhesion.

The CD28 receptor is involved in the control of the Th1/Th2 switch during the development and progression of autoimmune diseases (55, 56). It has been shown that blockade of the CD28-B7 pathway affects these pathological conditions by influencing the pathway of T cell-derived cytokines (57, 58). The disruption of the CD28-B7 signal early in nonobese diabetic mice promoted the development of spontaneous autoimmune diabetes associated with an enhancement in the production of Th1 type cytokines and an inhibition of the Th2 subset (59). It has recently been shown that CD28 ligation, achieved by administration of anti-CD28 Abs, prevents bacterial toxin-induced septic shock in mice by inducing IL-10 expression (60). Thus, the CD28 costimulation appears to be crucial for the development of a Th2 response. Furthermore, in vivo models suggest that T cells from newborns generate predominantly Th2-immune responses (61–63). Therefore, the inhibition of the CD28 costimulatory pathway in a neonatal model definitely produces a Th2-deficient environment. Our model of PV in CD28−/− is characterized by a stronger susceptibility to the development of PV, thus suggesting the lack of a Th2-inhibitory cytokine, like IL-10.

Using a semiquantitative RT-PCR technique, we demonstrated significantly lower IL-10 mRNA expression in PV-induced CD28−/− mice compared with controls. This prompted us to examine the susceptibility of IL-10−/− mice to pemphigus. Consistent with the above finding, IL-10−/− mice display a stronger susceptibility to the development of pemphigus. IL-10 displays a significant immunoregulatory role by promoting the development of a Th2 cytokine pattern and inhibiting proinflammatory cytokines such as TNF-α and IL-1α. This is further supported by the fact that IL-10 KO have increased IL-1 and TNF-α (64), and IL-1 and TNF play a role in acantholysis seen in pemphigus (65). Thus, we propose a model in which T cells display a mainly inhibitory role in acantholysis by producing the anti-inflammatory cytokine IL-10. To further confirm that IL-10 induction may have control in the development of acantholysis, we directly administered IL-10 to mice passively transferred with PV IgG. The IL-10 treatment significantly reduced the development of PV in these mice. Since KC-derived cytokines have also been shown to be involved in PV, IL-10-immunoregulatory influences could be directed at both T cell and KC-derived cytokines. Our data demonstrate that IL-10 has a “therapeutic” effect on murine experimental PV. It is conceivable that administration of IL-10 may be a useful treatment for pemphigus in humans.
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