Management of pemphigus
Tóth, Gábor Gellért

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Chapter I

General introduction

Pemphigus, derived from the term “pemphigoides pyertoi” used by Hippocrates (460-370 BC) to describe fever associated with blisters, refers to a group of autoimmune intraepidermal bullous diseases of the skin and mucous membranes. In Pemphigus vulgaris, the most common subtype, cutaneous lesions can be localized or generalized and usually present primarily as flaccid vesicles or bullae varying in size from less than 1 cm to several cm. The scalp, presternal, axillae and groin are predilection sites of involvement. The blisters may develop on normal skin and on erythematous macules or plaques and the fluid content is initially clear (serous). The blisters rupture easily and produce painful raw erosions. Pain of the blisters and erosions and the psychological loathing of a damaged skin, are important for patients suffering from pemphigus. Nikolsky’s sign is a typical clinical phenomenon in pemphigus: sheetlike removal of epidermis by pushing with a finger. The clinical diagnosis is funded by laboratory investigations. Histopathology is used to determine acantholysis in the epidermis in a skin biopsy. Furthermore, immunofluorescence techniques are used to detect in vivo depositions of IgG autoantibodies in the epidermis and anti-epithelial intercellular substance (ICS) IgG circulating in the blood.

Presentation

Pemphigus has a world-wide distribution with an incidence of approximately 0.1-0.42 per 100,000 per year (1-3). Pemphigus is a rare disease in Europe and North America. The prevalence and incidence of pemphigus in patients of Jewish origin is increased (1.6-3.2 per 100,000) and endemic regions are known in Tunisia, Iran and India (1;4).
Chapter I

The disease has a peak incidence of occurrence in patients between the fourth and sixth decade. About 0.8% of all dermatological patients suffer from pemphigus (4). For the Netherlands, the total year incidence of pemphigus is assessed to be about 46 casus (0.29 per 100,000), based on the counted number 177 patients who had a skin biopsy taken with the diagnosis of pemphigus examined by Dutch pathologists over the years 1995 and 1996 (unpublished data derived from the Dutch PALGA database).

Classification

The pemphigus spectrum is classified based on 1) clinico-pathological presentation, 2) type of autoantigens, and 3) subclass of autoantibodies. Table I and II show the pemphigus subtypes based on subclass of autoantibody (IgG or IgA) in combination with the targeted autoantigen.

The two main categories of pemphigus are pemphigus vulgaris (PV, vulgaris = ordinary) and pemphigus foliaceus (PF, folia = leaves). Pemphigus vulgaris (PV) is the most common type of pemphigus and comprises about 80% of patients with pemphigus (5). In about 50-70% (4;6) of the cases the disease begins with oral lesions, which may precede the cutaneous lesions by several months or be the major, if not only, manifestation in some patients (4). The mucous membranes are ultimately involved in most cases of PV. The histology of PV shows acantholysis in the lower epidermis with suprabasal blister formation.

Pemphigus foliaceus (PF) comprises about 20% of the patients with pemphigus. In PF only skin is affected, the mucous membranes are never involved (7). The allege that mucous membranes are always unaffected in PF is disputed by others (8). The histological level of blistering in PF is more superficial than in PV at the level above, in, or just beneath the granular layer (9). PF emerges with crusted squamous plaques in seborrhoic areas, and therefore may be mistaken for seborrhoic dermatitis, severe actinic keratosis, lupus erythematosus, psoriasis, or impetigo. However, PF may initially also erupt with generalized flaccid blisters. According to our experience the split level may then be observed in the mid-epidermis, 2-3 layers beneath the stratum granulosum. PF with flaccid blisters is differentiated from PV by the lack of mucous membrane involvement and the absence of anti-desmoglein 3 antibodies.
Pemphigus vegetans, a subtype of PV, has been divided classically in two subtypes, Neumann and Hallopeau (10). In the Neumann-type so-called vegetations (papillomatous granulations) sometimes hemmed with peripheral pustules develop on denuded areas surrounding orificiae. In the Hallopeau-type pustules are more common, they are rapidly followed by vegetations in often affected intertriginous areas. Also in PV vegetations on the face may develop, clinically and histopathologically characterized by papillomatosis. It is probably accurate to think of pemphigus vegetans as a clinical variant of PV.

**Table I  Classification of pemphigus mediated by IgG [Jonkman, abstract EADV 2001]**

<table>
<thead>
<tr>
<th>Disease subtype</th>
<th>Antigen</th>
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<tbody>
<tr>
<td>Pemphigus vulgaris</td>
<td>desmoglein 3, (desmoglein 1), pemphaxin?</td>
</tr>
<tr>
<td>Pemphigus vegetans, Hallopeau type</td>
<td>desmoglein 3, (desmoglein 1)</td>
</tr>
<tr>
<td>Pemphigus vegetans, Neumann type</td>
<td>desmoglein 3, (desmoglein 1)</td>
</tr>
<tr>
<td>Neonatal pemphigus vulgaris</td>
<td>desmoglein 3, (desmoglein 1)</td>
</tr>
<tr>
<td>Paraneoplastic pemphigus / Paraneoplastic autoimmune multiorgan syndrome (PAMS)</td>
<td>desmoglein 3, (desmoglein 1) + plectin, desmoplakin I/II, 230-kDa bullous pemphigoid antigen, envoplakin, periplakin, 170-kDa antigen</td>
</tr>
<tr>
<td>Drug-induced pemphigus</td>
<td>desmoglein 1, (desmoglein 3)</td>
</tr>
<tr>
<td>Pemphigus foliaceus</td>
<td>desmoglein 1</td>
</tr>
<tr>
<td>Pemphigus herpetiformis</td>
<td>desmoglein 1, (desmoglein 3)</td>
</tr>
<tr>
<td>Pemphigus erythematodes</td>
<td>desmoglein 1</td>
</tr>
<tr>
<td>Neonatal pemphigus foliaceus</td>
<td>desmoglein 1</td>
</tr>
<tr>
<td>Fogo selvagem</td>
<td>desmoglein 1</td>
</tr>
</tbody>
</table>

Pemphigus herpetiformis is an unusual pruritic variant of pemphigus vulgaris. Clinically it resembles dermatitis herpetiformis, whereas histopathological and immunofluorescence examination yield findings diagnostic for pemphigus (11,12).

Another unusual manifestation of pemphigus is neonatal pemphigus. Transplacental passage of IgG during pregnancy in affected patients is thought to be the course of PV or PF (13,14).

The paraneoplastic subtype of pemphigus (PNP) is often associated with unusual lymphoreticular malignancies (15). Diagnostic for PNP is the presence of circulating autoantibodies against plakins (plectin, desmoplakin I/II, 230-kDa bullous pemphigoid antigen, envoplakin, periplakin, 170-kDa antigen).
antigen, envoplakin, periplakin, and an uncharacterized 170-kDa antigen) which are detectable by immunoprecipitation or immunoblot (15). It is suggested that paraneoplastic pemphigus (PNP) is a heterogeneous autoimmune syndrome involving several internal organs: paraneoplastic autoimmune multiorgan syndrome (PAMS) (16).

In the skin a spectrum of at least 5 different clinical and immunopathological mucocutaneous variants are noted (i.e. pemphigus-like, pemphigoid-like, erythema multiforme-like, graft-vs-host disease-like, and lichen planus-like). The pathophysiological mechanisms of PAMS involve both humoral and cellular autoimmunity responses. Epithelial cell membrane antigens other than Dsg1 or Dsg3 are targeted by effectors of PAMS autoimmunity. Apoptosis of damaged basal cells mediates epithelial clefting, and respiratory failure results possibly from obstruction of small airways with sloughed epithelial cells.

Drug-induced pemphigus was first described in 1969 by Degos in patients using penicillamin (17). It is demonstrated that autoantibodies from drug-induced pemphigus patients have the same antigenic specificity, on a molecular level, as autoantibodies from other pemphigus patients (18,19). The chance of acquiring pemphigus after penicillamin intake of least 6 months is 7% (20). Since then, more medications were reported to evoke pemphigus, such as penicillin, ampicillin, rifampicin, pyrazolon derivates, a combination of aspirin and indomethacin, and a combination of propanolol and mepbromate (21). Drugs ‘at risk’ for pemphigus are sulphhydryl (SH)-group containing drugs, known as thiol-drugs (i.e. captopril) (22). Drug-induced and drug-triggered pemphigus are considered to be separate entities (22). In case of drug-induced, exogenous, non-autoimmune factors play a major role, and the disease regresses when the offended drug is discontinued (23). In drug-triggered pemphigus, the drug only stimulates a predisposition (endogenous and genetic factors) to develop active autoimmune disease. It seems that penicillamin and SH-containing drugs actually induce pemphigus, whereas other drugs only trigger a disimmune mechanism previously programmed and ready to be set off (22). Drug-triggered pemphigus is known to be refractory to therapy if the offending drug is not stopped immediately. Dietary factors, containing chemical compound resembling the above mentioned drugs, such as thiols
(garlic, onion, celery), isothiocyanates (mustard, horseradish), phenols (mango, cashew), and tannins (cassava, red chillies, tea, red wine) are also mentioned as exogenous factors to trigger pemphigus in genetically predisposed persons (24).

Pemphigus erythematosus (Senear-Usher), a subset of PF, usually presents with concomitant deposition of immunoglobulins and complement along the epidermal basement membrane zone in lesional skin in addition to pemphigus staining pattern in the epidermis (25).

Endemic pemphigus foliaceus, fogo selvagem, wildfire, or Brazilian PF, occurs predominantly in central and southern Brazil. In contrast to PF, fogo selvagem occurs in endemic foci, and often affects children and young adults (26). The etiology of fogo selvagem is still unknown. The frequent association with insect bites has lead to the concept of fogo selvagem being a transmissible disease with acquired immunity in adulthood. However, the infectious agent and possible vectors have not yet been identified (27).

**Table II Classification of pemphigus mediated by IgA only**

<table>
<thead>
<tr>
<th>Disease subtype</th>
<th>Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcorneal pustular dermatosis</td>
<td>Desmocollin 1</td>
</tr>
<tr>
<td>Intraepidermal neutrophilic IgA dermatosis</td>
<td>Desmoglein 3?</td>
</tr>
<tr>
<td>IgA-pemphigus vulgaris</td>
<td>Desmoglein 3 (Desmoglein 1)</td>
</tr>
<tr>
<td>IgA-pemphigus foliaceus</td>
<td>Desmoglein 1</td>
</tr>
</tbody>
</table>

Recently IgA-pemphigus was identified, characterized by immunoglobulin A (IgA)-type autoantibodies directed against keratinocyte cell surfaces (28). There is now evidence that IgA pemphigus encompasses at least two subgroups: a subcorneal pustular dermatosis (SPD)-type, characterized by subcorneal pustules and autoantibodies to desmocollin 1; and the intra-epidermal neutrophilic dermatosis (IEN)-type cases which show intraepidermal pustules and in whom the autoantigen is variable but may be desmoglein 3, the pemphigus vulgaris antigen (29). The treatment of IgA-mediated pemphigus is different from that of IgG-mediated pemphigus.
Transformation between the PV and PF categories is possible, although rare. Nevertheless if such transformation does occur, then a shift from PV to PF is more common (30-36) than, vice versa, a shift from PF to PV (33;36;37). The disease period before transition may vary between 1-20 years (30). In chapter 7 a patient is reported transforming from PV to PF.

Pathogenesis

The molecular pathomechanism disregulating keratinocyte adhesion in pemphigus are to a far extend elucidated. The unraveled pathogenesis makes pemphigus a model of organ-specific humoral autoimmune diseases. Figure I depicts the desmosomal complex, which plays the major role in the cell-cell adhesion of keratinocytes (38). Despite the solid evidence that anti-desmoglein (Dsg) antibodies play a central role in the pathophysiology of PV and PF (39;40), the significance of non-desmoglein antibodies in the pathogenesis is raised in recent reports (41;42).

Stanley and Udey formulated the desmoglein compensation hypothesis, which explains the differences in skin and mucous membranes involvement between PV and PF (40). Patients with PF have antibodies exclusively reactive with Dsg1. Patients with PV have antibodies reactive to Dsg3, but may also have antibodies to Dsg1. It has been demonstrated that early in the course of PV, when lesions are limited to mucous membranes, patients tend to have antibodies only against Dsg3. Furthermore, it is not inevitable that all patients with Dsg 3 antibodies will ultimately develop Dsg 1 antibodies. Anti-Dsg1 antibodies originate later in the disease progress of PV, coinciding with skin involvement (40). In figure II the triangles represent the distribution of Dsg1 and Dsg3 in adult skin and mucous membranes. In PF the patients’ serum contains only anti-Dsg1 antibodies and cause blisters by interfering with the function of Dsg1 in the upper epidermis, where there is no Dsg3 to compensate. In early pemphigus vulgaris, when patients produce only anti-Dsg3 antibodies, suprabasal blisters develop only in mucous membranes, where Dsg 1 cannot compensate for the loss of Dsg3 mediated adhesion. Later in the course of PV, when the patients’ sera contains anti-Dsg1 and anti-Dsg3 antibodies, the function of both Dsgs is compromised and blisters occur also on the skin. The desmoglein compensation hypothesis does not cover the observation that
patients with Dsg 3 antibodies alone can get mild blistering of the skin (patient 4, chapter V). The validity of the compensation theory has been confirmed in experiments with PF and PV antibodies in normal and Dsg3 null mice (44). This study suggest that pemphigus autoantibodies inhibit the adhesive function of desmoglein proteins, and demonstrates that either Dsg1 or Dsg3 alone is sufficient to maintain keratinocyte adhesion.

The multiple hit hypothesis is another hypothesis to explain acantholysis in pemphigus and fosters development of non-steroidal treatments. Both Dsg and non-Dsg autoantibodies are required to induce blisters. As recently reviewed (45), a list of known autoantigens in pemphigus includes both adhesion molecules (Dsg1, Dsg2, and Dsg3, desmocollins, plakoglobin, collagen XVII/BP180) and receptor molecules (α3 AchR, α9 AchR, pemphaxin and other annexins) (46).

Data supporting a role for non-Dsg autoantigen role in the pathogenesis of pemphigus include the following (47):

1. PV sera devoid of anti-Dsg1 activity produce new blisters in Dsg3 (-/-) mice. Dsg3 (-/-) mice develop spontaneous few blisters, but after injection of PV sera develop massive new blistering, similar to that in Dsg3 (+/+ ) mice. This confirms the importance of inactivating Dsg3 to produce PV-like blisters, but also indicates that anti-Dsg antibodies are not the sole pathogenic antibodies in PV sera.

2. PV sera contain multiple non-Dsg antigens from both normal, and Dsg3 (-/-) keratinocytes, including an antigen (not Dsg3) that migrates at 130 kDa.

3. Absorption of PV sera with Dsg3-Ig fusion protein removes pathogenicity; however, antibodies eluted from the Dsg3-column react with multiple antigens from both Dsg3 (-/-) and normal keratinocytes (47).

4. From 34 to 71% of first relatives of PV patients have anti-Dsg1 or anti-Dsg3 antibodies without any clinical signs of pemphigus (47;48).

The above data suggest that PV is also mediated by non-Dsg autoantibodies acting in concert with those against Dsg. One of the non-Dsg antigens is the keratinocyte α9 acetylcholine receptor (47) and a 130 kDa antigen pemphaxin (45). This notion may open a novel approach for the treatment of pemphigus using cholinergic agonists (pyridostigmine or Mestinon®) (49,50).
The desmoglein compensation theory is incompatible with the “older” protease (plasmin) theory of blister formation. This theory suggests that after binding of autoantibodies production of urokinase type plasminogen activator is stimulated (51). Plasminogen is converted to plasmin, which may be responsible for loss in strength of intra- or extracellular components of the desmosomal complex, resulting in acantholysis (52). Mahoney et al. demonstrated however that plasminogen activator is not necessary for pemphigus immunoglobulin to induce acantholysis in the neonatal mouse model of pemphigus (53). Caldelari et al (54) recently demonstrated in an in vitro model that the intracellular component, plakoglobin, plays a major role in the pathogenesis of pemphigus. Binding of PV IgG to plakoglobin knockout keratinocytes did not induce acantholysis. When full-length plakoglobin was reintroduced into the plakoglobin knockout cells, responsiveness to PV IgG was restored. This study excludes the steric hindrance-only-hypothesis of IgG binding to the extracellular portion of the desmosome.

Therapy

‘How to treat pemphigus?’ has always been a difficult question, since the disease may break through milder treatment modalities, whereas aggressive immunosuppressive drugs require careful monitoring of the patient. Choice of treatment, i.e. first and second line immunomodulators, depends on disease phase. For instance, in mild orally affected patients, topical or intralesional corticosteroids or tetracycline mouthwashes may be started as first line therapy. Whereas the severe affected patient needs to be treated with systemic high dose immunosuppressive therapy.

In the Cochrane Controlled Trials Register and PubMed database are no systematic reviews (meta-analysis), or large randomized clinical trials (RCT) available for treatment of pemphigus. There are only 4 RCT’s with a Sackett level of evidence of at least II (55).
Introduction

Sackett levels of evidence and clinical recommendation

<table>
<thead>
<tr>
<th>Grade of recommendation</th>
<th>Level of evidence</th>
<th>Evidence Type</th>
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<tbody>
<tr>
<td>A</td>
<td>Ia</td>
<td>meta-analysis of RCT’s</td>
</tr>
<tr>
<td>A</td>
<td>Ib</td>
<td>large RCT with clear cut results and low risk of error</td>
</tr>
<tr>
<td>B</td>
<td>II</td>
<td>small RCT with uncertain results and moderate to high risk of error</td>
</tr>
<tr>
<td>B</td>
<td>III</td>
<td>non-randomized contemporaneous controls</td>
</tr>
<tr>
<td>C</td>
<td>IV</td>
<td>historical controls</td>
</tr>
<tr>
<td>C</td>
<td>V</td>
<td>no controls, case series</td>
</tr>
<tr>
<td>D</td>
<td>VI</td>
<td>expert opinion, and case report</td>
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</table>

Since their introduction in the 1950s of the previous century, the cornerstone of therapy remains corticosteroid treatment at minimal effective dosage.

To minimise iatrogenic effects of cumulative corticosteroids, there has been a continuous search for alternative therapies regarding treatment during the last 40 years (56-58). These include azathioprine, cyclophosphamide, methotrexate, mycophenolate mofetil, pulse glucocorticoid therapy, gold, tetracyclines, dapsone, intravenous immunoglobines, plasma exchange, immunoapheresis, and intralesional steroids. Because these modalities are normally used in conjunction with oral daily systemic steroids, rather than as monotherapy, these modalities can be called adjuvant therapies.

Adjuvant therapy is started immediately with the glucocorticoid treatment, or after an initial period of treatment with oral glucocorticoids only. Reasons for adding adjuvant in a later stage are:

- unsatisfactory initial clinical effect of oral steroids,
- sustained need for high dose maintenance steroid therapy,
- presence of steroid related adverse events,
- intention to reduce steroid related adverse events.

Chapter II summarizes current therapy in pemphigus.

Prognosis

Without therapy, pemphigus is fatal within the first year of onset in 75% of the patients due to sepsis and loss of body fluids (59;60). Since the use of steroids in 1950,
pemphigus has transformed from an almost invariably fatal disease into one whose mortality is now about 5% (61;62). Mortality at present is determined by the complications of the therapy. Evaluation of three recent studies show that sepsis and lung embolism are the main causes of death, caused by the steroid treatment (59;62;63).

Morbidity of pemphigus did not further decrease since the introduction of steroids, attributed to the iatrogenic effects of the therapy (57;59;63-65). The iatrogenic morbidity is responsible for the largest part of costs that these patients generate. Therapy can be discontinued in approximately 75% of all patients after 10 years (61). About 25% of the patients remain dependant of both steroids and adjuvant treatment during life.

The most relevant adverse events are steroid-induced diabetes mellitus, hypertension, infection (cave masking of symptoms), ulcer pepticum (bleeding, perforation, cave masking), increased clotting tendency, osteoporosis, and delayed wound healing. Furthermore, adverse events are provoked by the scala of adjuvant therapies (66).

The induction of complete remission was studied in a long-term longitudinal study in 40 patients with pemphigus vulgaris treated conventionally and followed up for an average of 7.7 years by the same investigator (61). The course of the disease follows different patterns, with respectively 25%, 50%, and 75% of the patients reaching complete remission 2, 5, and 10 years after the diagnosis pemphigus. Induction of complete remission is related to initial severity, extend of disease, and early response to treatment. The rate of remissions in pemphigus is unclear because these are usually reported at a single point in the evolution of the disease. Thus it is uncertain whether treatment simply suppresses the manifestations of the disease and consequently must be continuously administered, or induces complete and long-lasting remissions that permit therapy to be discontinued.

**Pulse therapy**

Pulse therapy, the ‘big shot’ (67), refers to discontinuous intravenous infusion of very high doses glucocorticoids over a short time. Doses of each pulse are not standardised, but are usually 500-1000 mg methylprednisolone or 100-200 mg dexamethasone. The aim of pulse therapy is getting quicker and stronger efficacy and decreasing the need for
long-term use of steroids. The contradiction is that pulsed administration of high dose steroids is used to achieve the steroid-sparing effect.

Pulse therapy was initially proposed for the emergency treatment of acute rejection of kidney transplants 30 years ago (67), and it is still first choice for the treatment of acute rejection. Now, it is also widely used in the treatment of many inflammatory or autoimmune diseases.

The largest experience with glucocorticoid pulse therapy in dermatology is obtained in patients with pemphigus vulgaris. Pasricha et al. described both a steroid-sparing effect, 84% complete remission rate, and long-term remission of up to 9 years, using pulse therapy (68). We retrospectively studied the sequelae of 14 patients with pemphigus who were treated by pulse therapy (chapter 4).

Pulse therapy is mostly given intravenously rather than orally, without evidence to support the necessity of the intravenous route. Oral pulse therapy is preferable, since it avoids vena puncture, reduces costs, and is more convenient for the patient. An oral formulation for dexamethasone pulse therapy was also necessary for blinding the clinical trial by placebo, in which the therapeutic effect of corticosteroid pulses will be evaluated. Intravenous placebo pulses may be considered unethical. Dexamethasone was chosen for use in oral pulses. To develop a suitable dosage for oral pulses, bioavailability of high-dose dexamethasone had to be determined. Chapter 3 explains the pharmacokinetics of high-dose oral dexamethasone pulse therapy.

**Glucocorticosteroids**

Glucocorticosteroids are important anti-inflammatory and immunosuppressive drugs with three distinct mechanisms of action: 1) genomic, 2) specific non-genomic, and 3) unspecific non-genomic. The (classical) genomic action mechanism is glucocorticoid receptor mediated. Glucocorticoids bind to the cytosolic expressed glucocorticoid receptor. After binding the activated steroid-receptor complex translocates to the nucleus, where synthesis of regulating proteins, e.g. lipocortin-1, an inhibitor of phospholipase A2, is initiated. The steroid receptor complex also interacts with transcription factors, modulating transcription of messenger RNA and subsequent decreased synthesis of certain proinflammatory cytokines and increased synthesis of anti-inflammatory
cytokines. These genomic actions are observed at any corticosteroid dose, and occur later than 30 minutes after binding of the glucocorticoid at the receptor. Additional non-genomic effects have been shown (in vitro) to occur only at high-doses, above 250 mg prednisolone equivalent per day (69-71).

Specific non-genomic effects occur rapidly (seconds or minutes) and result mainly from direct interaction on biologic cell membranes and are supposed to interfere with activation and maintenance of immune cells. In therapeutically relevant concentrations, methylprednisolone instantaneously inhibits Ca\(^{2+}\) and Na\(^+\) ions cycling across the membranes and decreases intracellular free Ca\(^{2+}\) concentration, but has little effect on protein synthesis (71). Further non-genomic effects are a decreased phospholipid turnover in the cell membranes and a decreased production of free radicals (72).

Pulse therapy of high-dose glucocorticoids thus may have additional unspecific non-genomic effects, and therefore may lead to rapid and intense immune responses. This was demonstrated in a recent study in children with autoimmune diseases where additional non-genomic effects were observed due to pulse therapy despite a significant down-regulation of glucocorticoid receptors in these children (69).

How does pulse therapy work in pemphigus is uncertain. Corticosteroid pulses reduce skin blistering within days, when autoantibody titres are not yet lowered. Recently, Stanley hypothesized that high dose corticosteroids pulses might induce transcription of Dsg isoforms, providing protection from anti-Dsg autoantibodies (76). Alternatively, we speculate that corticosteroids may strengthen desmosomes by modulating intracellular calcium oscillations, which for instance may revert plakoglobin-dependant acantholysis.

Clinical and laboratory monitoring

At present, no consensus is available for clinical scoring disease activity, and therefore monitoring pemphigus. There is a need for a uniform simple scoring system with a small interobserver bias, since pemphigus is a rare disease.

Both for diagnosis and follow-up immunological techniques are used to detect specific in vivo depositions of IgG in the epidermis and circulating IgG in blood. In 1964, Beutner and Jordon (73) first demonstrated antibodies to the cell surface of epidermal cells in the sera of patients suffering from PV. Since then indirect IF is used widely for
monitoring disease activity, however pemphigus antibody titres do not always correlate with actual disease activity (74). Classically, immunoprecipitation is used to identify pemphigus antigens in a research setting. The technique is available in few centers worldwide. PV is characterized by antibodies against the 130 kDa PV antigen (desmoglein 3), and PF by antibodies against the 160 kDa PF antigen (desmoglein 1). Alternatively, immunoblot may be used, but this technique is often false-negative for anti-desmoglein antibody detection. The usefulness of immunoblotting in pemphigus diagnostics is mainly restricted to identifying paraneoplastic pemphigus, where an highly specific plakin pattern can be found, and to IgA-pemphigus were IgA against desmocollins and desmogleins can be detected (15). The breakthrough in pemphigus diagnostics came in 1997, with the development of an ELISA (enzyme-linked immunosorbant assay (MBL, Nagoya, Japan) to detect specific autoantibodies against the ectodomains of desmoglein 1 and 3 (43). The technique is highly sensitive and specific, and also quantitative. Monitoring pemphigus by quantifying circulating anti-desmoglein antibodies with ELISA therefore seems to be an attractive option.

Chapter 5 proposes a model for robust staging of disease activity for pemphigus vulgaris. Uniform monitoring pemphigus vulgaris, with a small interobserver bias, due to a simple clinical definition seems now possible. This clinical definition is currently used in our PEMPULS trial, an international, prospective, multi-centre, double-blind, placebo-controlled, parallel-group, randomized clinical trial, in which the efficacy of oral high-dose dexamethasone pulse therapy is studied. Participating countries are: the Netherlands, Belgium, France, Germany, Hungary, Italy, Poland, Spain, and the United Kingdom. All information for participants and others who are interested is public available on our website http://www.pempuls.nl.

**Safety of High-dose Azathioprine**

Standard therapy in the Netherlands for pemphigus comprises maintenance dosage prednisolone in combination with azathioprine. Myelotoxicity and drug efficacy are now known to be related to the activity of the key enzyme in azathioprine metabolism, thiopurine methyltransferase (TPMT). The drug has well-documented toxic effects on hematopoietic cells that may be acute or chronic, including: macrocytosis, anemia,
trombocytopenia, leukopenia, pancytopenia, and acute bone marrow failure. About 1 of 300 patients are homozygous for the inactive TPMT allele (75). Azathioprine should not be prescribed to such patients that will otherwise develop acute myelosuppression. TPMT as measured in erythrocytes also discloses patients with an high TPMT activity. On standard dosage of 100-150 mg azathioprine per day, patients with a high azathioprine metabolism would be suboptimal treated, and should receive more azathioprine.

We reviewed the sequelae of 14 dermatological patients on high-dose azathioprine, and we assessed the clinical value and suitability of the TPMT assay in clinical practice in this small group of patients who need sufficient immunosuppression (chapter 6).

**Aims of this study**

The groundwork for this thesis started in May, 1997 with the design of the PEMPULS trial that was initially applied to the Dutch ‘Ontwikkelingsgeneeskunde’ program. Since treatment of pemphigus is not an economic issue in public health care, the proposal was rejected. Pemphigus is an orphan disease. Dexamethasone is an orphan drug lacking patent protection so that no pharmaceutical industry was interested either. With limited financial support of the Faculty of Medical Sciences of the University of Groningen and the University Hospital Groningen we embarked for an international, prospective, multi-centre, double-blind, placebo-controlled, parallel-group, randomized clinical trial, in which the efficacy of oral high-dose dexamethason pulse therapy is studied. The academy driven trial is carried out by the network of the European Society of Autoimmune Bullous Diseases on a non-profit basis. The scientific question whether pulse therapy really works as adjuvans in pemphigus apparently raised the enthusiasm of the participants. First, the results of dexamethasone pulse therapy in our department were studied retrospectively in a pilot-study (Chapter 4). A literature search was performed to evaluate all therapies in pemphigus (Chapter 2). An attempt was made to design definitions for monitoring disease activity (Chapter 5). To design the oral substitute (necessary for placebo control) for intravenous dexamethasone pulse therapy, we started pharmacokinetic studies on our ward (Chapter 3). Since high-dose azathioprine is used as first choice adjuvant in the Netherlands, we started measuring TPMT-enzyme activity to avoid serious adverse events (Chapter 6).
Fig I. Transmembrane desmosomal adhesion proteins of the desmosomal complex.
Fig II. The desmoglein compensation theory. The triangles represent the distribution of Dsg1 and Dsg3 in adult skin and mucous membranes.

Desmoglein Compensation Hypothesis

Pemphigus foliaceus

Skin

Mucous membrane

Pemphigus vulgaris

Skin

Mucous membrane

Early

Only Anti-Deg3

Late

Anti-Deg3 + Anti-Deg1

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