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A Randomized Trial and the Treatment of Pemphigus Vulgaris

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Pemphigus vulgaris is a rare, life-threatening autoimmune disease. Mycophenolate mofetil is a potent immunosuppressant medication approved by the Food and Drug Administration to be used after solid organ transplantation and to treat pemphigus vulgaris. Mycophenolate mofetil has not become the "wonder drug" that had been anticipated based on initial clinical reports. Studies like that reported by Beissert *et al.* in this issue are essential to improve dermatologic care.

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Pemphigus vulgaris is a severe, life-threatening autoimmune disease associated with antibodies to desmoglein 1 and 3 (Amagi, 2008; Stanley, 2008). These antibodies cause blisters that originate just above the basal cell layer of the epidermis. The science behind this association is extensive, elaborate, and elegant. The relationship between desmoglein antibodies and the clinical phenotype is so profound and investigators' ability to manipulate and study this relationship in culture and nonhuman species is so absolute that pemphigus vulgaris is often described as a "model" autoimmune illness.

Pemphigus vulgaris is rare, however—its incidence is often estimated to be 0.5 to 4 per million person-years, and it has a point prevalence of 1 to 4 per 100,000 people (Amagi, 2008; Groves, 2009; Marazza *et al.*, 2009; Stanley, 2008). The disease was thought to be fatal until Lever's introduction of systemic corticosteroid therapy, which has become a mainstay of treatment. However, most dermatology textbooks now recommend that, in order to minimize side effects, maximize early control of the disease, and decrease mortality, systemic corticosteroid therapy should be concurrently augmented with an additional immunosuppressive agent such as mycophenolate mofetil, azathioprine, or cyclophosphamide (Amagi, 2008; Groves, 2009; Stanley, 2008).

About 10 years ago, I heard a presentation about a patient with pemphigus vulgaris. The resident presenter was adamant about using a wonderful new treatment, mycophenolate mofetil, noting that it was superior to sole corticosteroid therapy or the combination of corticosteroid therapy with any other agent. I asked about the evidence supporting this therapy and was told that current scientific evidence made it obvious that this was the correct approach. Within two years, I was told by peers that in treating pemphigus vulgaris it was never proper to use a systemic corticosteroid alone, that it should always be used with mycophenolate mofetil. Furthermore, I was told that mycophenolate mofetil was essential for sparing patients from side effects of systemic corticosteroid therapy and that mycophenolate mofetil was an important part of any adequate therapeutic regimen. A few years later, I spoke to a colleague who was trying to develop a randomized clinical trial to evaluate pemphigus vulgaris treatment regimens. The proposal included the randomization of patients with pemphigus vulgaris to standard of care (corticosteroids) or to standard of care plus another agent that was not mycophenolate mofetil. The review panel was supportive of the study, but indirect feedback related to the decision not to fund indicated that there was already a cure for pemphigus vulgaris—mycophenolate mofetil—making the study unnecessary.

Mycophenolate mofetil is a potent immunosuppressant medication approved by the Food and Drug Administration (FDA) for the prevention of solid-organ rejection after transplantation (renal, cardiac, and hepatic). It is an inosine monophosphate dehydrogenase inhibitor (de Jonge *et al.*, 2009; *Physicians' Desk Reference*, 2009). Therefore, it inhibits guanosine nucleotide synthesis, which is important for T- and B-cell proliferation and function. Mycophenolate mofetil has often been used as a substitute for azathioprine, and it has been endorsed by several authors as an effective as well as steroid-sparing agent to treat many autoimmune illnesses (de Jonge *et al.*, 2009; Villarroel *et al.*, 2009). Commonly reported side effects of this drug include dizziness, headache, nausea, diarrhea, leukopenia, tremors, and vomiting (de Jonge *et al.*, 2009; *Physicians' Desk Reference*, 2009). The FDA issued a "black box" warning describing concerns about lymphoma, malignancy, and infection after use of the drug and, more recently, a warning about increased risk of opportunistic infections such as progressive multifocal leukoencephalopathy. Its use is also associated with an increased risk of pregnancy loss and congenital malformations.

The study by Beissert *et al.* (2010, this issue) is a fine example of a randomized clinical trial. Randomized clinical trials are often considered the best study design for proving the efficacy of a therapy. The random treatment selection is helpful in controlling treatment selection bias, and other design features, such as precise definition of

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the disease to be studied, longitudinal follow-up, a proper understanding of drug use/exposure, the proper acquisition of end points (often by blinding the evaluator), adherence to a well-written protocol, and statistical analysis plan, help to ensure that this type of study will provide the highest level of evidence. In fact, the use of this design has recently been advocated for basic science studies as well (Bart van der Worp *et al.*, 2010).

The goal of Beissert *et al.* (2010) was to evaluate the efficacy and safety of mycophenolate mofetil when administered as 2 or 3 g/day in addition to about 1 mg/kg of corticosteroid per day to individuals with pemphigus vulgaris. The authors randomized 96 patients, and 75 completed the study. For a disease as rare as pemphigus vulgaris, this was a Herculean effort. To summarize, the investigators noted that after 48 to 52 weeks of follow-up, roughly an equal number of subjects (any mycophenolate mofetil use, 69%, versus corticosteroid use only, 64%, $P = 0.66$) had responded to therapy. In contrast, on the basis of historical data, the study had been designed assuming that 30% of patients receiving only corticosteroids would respond versus a 70% response rate in the mycophenolate mofetil–plus-corticosteroid arm. However, patients who received mycophenolate mofetil did respond more quickly (by about 7 weeks), had more durable responses (lasting about 3 months longer), and over the course of the study used about half a gram less corticosteroid (about 10 mg/week less) and were maintained on low-dose corticosteroid (prednisone <10 mg/day) almost 2 months longer. With respect to safety, there were few differences in adverse events, serious adverse events, or dropout in subjects who were randomized to receive mycophenolate mofetil with corticosteroid versus those who received corticosteroid alone.

These fascinating results show that the use of mycophenolate mofetil may help a patient achieve an earlier, more durable response when administered with corticosteroid therapy as compared with corticosteroid therapy alone. It is important to note that these analyses were not part of the primary end point; some would therefore suggest that the findings are more akin to the results presented in a cohort study than the primary results of a randomized clinical trial. In addition, the total amount of corticosteroid required to achieve this response may be clinically similar and the overall rate of adverse and serious adverse events may be similar to those for patients receiving corticosteroid therapy alone. The study did not appear to demonstrate the dramatic differences that would have been expected based on "expert opinion."

> Mycophenolate mofetil helped patients with pemphigus achieve earlier and more durable responses when administered with corticosteroids.

Randomized clinical trials also have limitations. As in bench science, all studies must be replicated. One study never answers all questions, and sometimes the results of a single study are not reproducible. Although randomized clinical trials maximize the internal validity of a study (e.g., by reducing bias due to treatment selection or information errors), the external validity of a randomized clinical trial may be compromised as compared with other study designs. External validity is related to how well information gained from the study generalizes outside of the study. For example, this study had inclusion and exclusion criteria that might have rendered the study population distinct from a general patient population with pemphigus vulgaris, and it used a precise plan for tapering corticosteroid. The reader needs to decide whether subject selection and treatment are importantly different from their general practice. In addition, were the reported outcomes clinically meaningful and not just statistically meaningful?

Judging by the results presented by Beissert *et al.* (2010), mycophenolate mofetil is certainly not the wonder drug described to me several years ago, nor did it work as well as had been anticipated in the investigators' power analysis, but, to be fair, very few drugs work as well as initially discussed by clinicians. The results of this study demonstrate that it is important for physicians contemplating the use of mycophenolate mofetil to consider carefully the benefits and risks of the drug as well as its risks when used in combination with corticosteroids. It appears that, after a year of care, clinical responses are similar whether or not mycophenolate mofetil is added to corticosteroid treatment. What must be considered is whether the potential for a quicker and more durable response is advantageous to patients and whether the steroid-sparing effect is more important than the added risks of using mycophenolate mofetil, which include malignancy and fatal opportunistic infections. Finally, it is essential to consider whether mycophenolate mofetil offers an advantage over other steroid-sparing agents that have not been objectively compared with it in a similar randomized clinical trial setting. In fact (again, based on limited evidence), another promising therapy based on rituximab is now being championed for the treatment of pemphigus vulgaris (Cianchini *et al.*, 2007).

The pages of this journal are a testament to the high level of investigation common to cutaneous science, yet dermatologists are often eager to accept as effective therapies that have not been tested in a well-designed clinical study. Reading the *JID* makes me certain that we have a profound understanding of keratinocyte biology and the biology of many human skin diseases, as represented by mouse models and cell culture. Unfortunately, studies like that of Beissert *et al.* (2010) are uncommon because of our discipline's habit of underfunding, and seemingly undervaluing, clinical science. The investigators are to be congratulated for their effort, the quality of their work, and the contribution they have made to our understanding of clinical dermatologic care.

CONFLICT OF INTEREST
The author states no conflict of interest.

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Grabbing Amphiregulin by the Tail to Better Understand Keratinocyte Growth

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Amphiregulin (AREG) is an important regulator of cellular growth in keratinocytes, carcinomas, and hyperproliferative epidermal disorders, including psoriasis. Stoll and colleagues present data suggesting that the cytoplasmic carboxy-terminal domain of amphiregulin plays an important role in regulating autocrine keratinocyte growth through the epidermal growth factor receptor. These observations raise novel and interesting biological questions regarding the function of the cytoplasmic C-terminal region of AREG.

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Amphiregulin (AREG), a member of the EGFR ligand family, is important for regulating keratinocyte growth (Stoll *et al.*, 2010, this issue). *In vivo* studies of transgenic mice overexpressing AREG in the epidermis demonstrate a complex yet psoriasiform phenotype including epidermal hyperplasia, cutaneous inflammation, and arthritis (Cook *et al.*, 1997). Ligand-dependent signaling through EGFR in keratinocytes is complex, because these cells can produce at least five members of the EGFR ligand family, including AREG, epiregulin, heparin-binding EGF-like growth factor, betacellulin, and transforming growth factor-α (Coffey *et al.*, 1987; Cook *et al.*, 1991; Hashimoto *et al.*, 1994; Shirakata *et al.*, 2000; Strachan *et al.*, 2001). The expression of multiple EGFR ligands in keratinocytes raises a question regarding the specific biological role of AREG in regulating keratinocyte growth. If AREG plays a specific role in regulating keratinocyte growth, then what structural motifs of AREG are responsible for this important biological effect? The data presented by Stoll *et al.* (2010) provide insights into this complex scenario.

The AREG (also known as schwannoma-derived growth factor) gene is located on human chromosomal band 4q13.3 and yields a 1.4-kb transcript composed of six exons that can produce a 252–amino acid (aa) transmembrane glycoprotein. This polypeptide is also known as the pro-form of AREG (Pro-AREG). Pro-AREG is composed of multiple domains, including a signal sequence (aa 1–19), an N-terminal domain (aa 20–101), an EGF-like domain (aa 102–184), a membrane stalk (aa 185–198), a transmembrane domain (aa 199–221), and a cytoplasmic domain (aa 222–252).

Transmembrane EGFR ligands such as AREG are released from their membrane anchors by metalloproteinases and ADAM family proteases; this processing adds another layer of regulation (Lu *et al.*, 2009; Sternlicht *et al.*, 2005). In fact, ADAM17-null mice, to some extent, phenocopy EGFR-null mice (Sternlicht *et al.*, 2005). As expected, proteolysis of AREG results in four membrane-bound and four soluble isoforms (Brown *et al.*, 1998). Hypothetically, each of these eight isoforms could have a unique signaling potential; such molecular diversity raises the possibility of complex signaling paradigms.

To better understand the role of AREG in regulating keratinocyte growth, Stoll and colleagues (2010) utilized a knockdown approach with lentiviruses that express a tetracycline-inducible small hairpin RNA (shRNA) targeting AREG. Keratinocytes transduced with this lentivirus downregulated AREG mRNA and cell-associated AREG protein levels by 83 and 71%, respectively, and shed AREG by more than 95%, all in a doxycycline-dependent manner. Previous work by this group has shown that AREG antibodies can block the autocrine-stimulated ERK phosphorylation and keratinocyte proliferation induced by shed AREG (Kansra *et al.*, 2004, 2005). The AREG-dependent growth of these keratinocytes was also inhibited by metalloproteinase inhibitors and ErbB tyrosine kinase inhibitors,

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