Immunologic Studies in Patients with Darier's Disease. JOHN M. HUMENNIK, WENDY HUWITZ, AND BRIAN V. JEGASOTHY, Dept. of Medicine, Division of Dermatology, Duke University Medical Center and the V. A. Medical Center, Durham, North Carolina.

Darier's disease (Keratosis follicularis) is an uncommon inherited cutaneous disease. Many patients with this disorder develop severe and progressive viral illnesses which may even result in death.

We investigated patients with Darier's disease for immunologic compromise to explain their unusual susceptibility to viral infections. Skin tests performed on 3 patients with severe Darier's disease revealed a total anergy. White blood and differential counts were entirely normal. T and B cell counts in peripheral blood were normal. However, the lymphocyte proliferative response in vitro to antigens and to the T cell mitogen Concanaavalin A, was greatly decreased but, the response to pokeweed mitogen (a B cell mitogen) was increased above normal.

Further, stimulated lymphocytes, from one individual who was studied, were unable to produce migration inhibitory factor (MIF). In vitro lymphocyte studies in a patient whose disease was improved by treatment with 13 cis-Retinoic Acid, remained abnormal. An unaffected family member and two control volunteers had normal lymphocyte function.

Our studies suggest that patients with Darier's disease have a functional defect of T cells which might explain their poor ability to handle viral infections.

Staphylococcal Adherence to Human Corneocytes. GARY W. COLE. Dept. of Dermatology, Univ. of California, Irvine and the V. A. Medical Center, Long Beach, California.

Staphylococcus aureus can be isolated in increased frequency and numbers from the normal or involved skin of patients with atopic dermatitis. The development of staphylococcal micro-pustules may be important in the pathogenesis of atopic dermatitis. The adherence was evaluated between staphylococci and corneocytes of patients with various skin diseases including atopic dermatitis.

Human corneocytes, obtained from skin scrapings, were shaken in 0.4 m EDTA-PBS buffer with glass beads, centrifuged 3 times to separate cells and to remove contaminating bacteria and adjusted to an O.D. of 15 in PBS at 530 nm using a spectrophotometer. Staphylococcus aureus was grown to stationary phase in Trypsinase soy broth, washed 3 times in PBS and adjusted to an OD of 0.5 at 530 nm. 0.5 ml of each suspension was mixed and incubated at 37°C for 45 min after which nonadherent bacteria were separated from corneocytes by 8 slow speed centrifugations. Corneocyte suspensions were then placed on slides, stained by the Gram's method and examined microscopically.

The adherence index (AI) was defined as the percent of corneocytes coated with 20 or more cocci.

The mean AI for a group of atopic dermatitis patients was 48.4 ± 12.9% (n = 5) as compared to a AI of 10.4 ± 5.9% (n = 6) for similar group of patients with other scaling diseases and normal controls (p < 0.005).

Perhaps these preliminary experiments indicate that one reason for high staphylococcal colonization of atopic skin is due to the increased adherence of these bacteria to atopic stratum corneum.

Cold Urticaria. PROFESSOR M.W. GREEVES, DR. A. KOBZA-BLACK, AND DR. R.A.J. EADY.

The clinical and laboratory date of 36 patients with idiopathic cold urticaria is reviewed. Cold desensitization has been unsuccessful as a maintenance therapy. Histamine and other mediator release has been studied before and after systemic drug administration. Some medications can reduce the histamine liberated but have little effect on the urtications produced. Doxantrazole, Prednisone and the protease inhibitor transhemaxic acid were all inferior to cyproheptadine. The results of combinations of H1 and H2 antihistamines as well as aminophylline and salbutamol are discussed.

Heat, Not Caffeine, Induces Flushing in Erythematotelangiectatic Rosacea. JONATHAN K. WILKIN, M.D. Department of Dermatology, University of Texas Medical School at Houston, Houston, Texas.

Flushing reactions are characteristic of rosacea and are widely iniminated in the genesis and exacerbations of rosaceous stigmata. The elimination diet is currently regarded as an important component of the regimen for rosacea. The diets usually proscribe "coffee" or "caffeine" and suggest substitution by a "decaffeinated coffee." We examined the flushing responses in 24 patients with erythematotelangiectatic rosacea to a variety of putatively provocative agents from the clinical literature and anamneses. Ambient, axillary and malar temperatures were measured by thermistors with telethermometers and continuously recorded on a multichannel polygraph after low-level DC amplification. The malar thermal circulation index, which is directly proportional to malar blood flow, was calculated prior to challenge (Rb) and again at maximum change (Rb) during a 45-min observation period. Comparisons of the changes in calculated malar thermal circulation indices (P/Rb) and the times between the onset of stimulus and the point of one-half the maximal temperature change (LT50) were made among the different agents. The results showed that drinking hot coffee at 60°C induces flushing, i.e., an increase in P/Rb (p < 0.01), but that caffeine, 200 mg, or coffee in water at 22°C do not. Further, ingesting water at 60°C leads to the same flushing as coffee at 60°C, viz., an identical increase in P/Rb (p < 0.01) and an identical LT50 (p < 0.05). Deplification is not a requisite. Water at 60°C held in the mouth produces the same increase in P/Rb (p < 0.05) and an identical LT50 (p < 0.05).

It is concluded that in rosacea (1) increased heat in the oral cavity may cause flushing, (2) the probable mechanism includes a counter current heat exchange at the level of the internal jugular vein and common carotid artery, increased heat in the blood bathing the anterior hypothalamus, and heat dissipating responses, including flushing, (3) heat is the agent in hot coffee that induces flushing, (4) caffeine need not be proscribed, and (5) hot, decaffeinated coffee is rejected as an allowable substitute.

Excessive Helper T-Cell Function in Progressive Systemic Sclerosis. DANIEL SAUDEI, M.D., R. L. BAILEN, M.D., RANDALL S. KRAKAUER, M.D. Departments of Dermatology & Immunology, Cleveland Clinic Foundation, Cleveland, Ohio.
Derived from Various Skin Layers, R. Fleischmajer, T. Krieg, R. Timp, J. S. Perlsh, Division of Dermatology, Hahnemann Medical College, Philadelphia, PA and Max Planck Institute für Biochemie, Munich, West Germany.

The purpose of this study was to estimate collagen and fibronectin synthesis of scleroderma and normal fibroblasts derived from the papillary, reticular and adipose layers of the skin. Fibronectin and procollagen were measured by a radioimmuno assay. Collagen types were estimated by pulse with H3-proline. The synthesis of pepsin-resistant protein was studied in an attempt to preserve phenotypes. Following a 24 hr pulse with H3-proline we estimated the synthesis of pepsin-resistant protein in fibroblasts derived from all layers studied.

The most significant increase in collagen synthesis was noted in fibroblasts derived from the papillary layer. Type III procollagen and fibronectin were measured by indirect immunofluorescence (IIF) with Type I and Type III procollagen antibodies.

**Results**

<table>
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<th></th>
<th>OH-proline</th>
<th>Pepsin-resistant protein %</th>
<th>Type I Type III procollagen III ×10−6</th>
<th>Fibronectin ng DNA</th>
<th>Type I Type III procollagen III ×10−6</th>
<th>Fibronectin ng DNA</th>
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**Selective Deficiency of the C4 Component of Complement Associated with Dermatologic Disease. Jonathan Gell,1, Mauray L. Tylor,2, and Vincent Agnello.**

Recent studies have shown that selective genetic deficiencies of the Clr, Cbs, or C4 complement components may be associated with certain clinical syndromes including discoid lupus (DLE), systemic lupus erythematosus (SLE), anaphylactoid purpura and systemic lupus-like illnesses. In the course of screening complement component levels in 1,730 sera from patients with a wide range of diseases, 32 patients (excluding patients with SLE) were found with a selective marked depression of C4 levels. Ten of these patients had dermatologic disease: 5 had rashes typical of DLE, 2 had urticaria, 2 had urticaria and angioedema and 1 had dependent purpura. None of the patients fulfilled the American Rheumatism Association's preliminary criteria for SLE and none had antibodies to native DNA. Studies of C4 levels in family members did not provide evidence supporting an hereditary basis for the C4 deficiencies in the index cases. Immunofluorescence studies of skin biopsies of the lesions in 6 of 7 patients thus far studied were negative for basement membrane deposits of immunoglobulin, Clq, C4 and C3. One had only IgM deposits. Thus far no substances have been detected in the sera of these patients which react with C4. These findings suggest that there is a high incidence of C4 deficiencies among patients with selective C4 deficiencies. Whether the C4 deficiency is acquired or on a hereditary basis is not apparent from family studies of C4 levels alone is under further study.

HFA-B8 and DRW3 in Subacute Cutaneous LE (SCLE). James N. Gilliam, M.D. and Peter Stastny, M.D. University of Texas Health Science Center at Dallas, Dallas, Texas.

Patients with lupus erythematosus (LE) can be grouped into cutaneous LE subsets. We have recently studied a subset of LE characterized by a distinct form of skin disease which we have called subacute cutaneous LE (SCLE). Patients with SCLE usually have mild multisystem disease involving principally the joints and skin. Skin biopsies show perivascular and peripapillary lymphoid infiltrates and hydropic degeneration of the basal layer of the epidermis characteristic of LE. HLA typing was performed in 15 patients with SCLE, 50 patients with discoid LE (DLE), 20 patients with systemic LE (SLE) and 68 normal controls, all white and residing in the same area. The SCLE group had a marked increase in the antigens HLA-B8 and DRW3. The frequency of B8 was 60% in SCLE patients compared to 29% in controls (p < 0.02); and DRW3 was 77% in SCLE compared to 31% in normal individuals (p < 0.005). The HLA-A1, B8, DRW3 haplotype was found in only 24% (p < 0.02). The strongest association in these patients was with the HLA-D region, with DRW3 giving a relative risk of 7.5 (p < 0.005). The HLA-A1, B8, DRW3 haplotype was not increased in SLE and DLE patients compared to the normal controls. The differences between SCLE and SLE as well as SCLE and DLE for these antigens were statistically highly significant. These findings confirm the clinical impression that SCLE constitutes a distinct entity and suggest a strong immunogenetic factor.

The HLA-B8, DRW3 antigens known to be associated with other forms of autoimmunity, signal the presence of an immunologic reaction involving the skin. SCLE is a subset of LE with distinct clinical and laboratory features.

Subpopulations of Lymphocytes in Vesiculobullous Diseases. A. Razzague Ahmed, Division of Dermatology, UCLA School of Medicine, Los Angeles, California.

The contribution of several authors has established the autoimmune nature of pemphigus and bullous pemphigoid. In both diseases there are in vivo bound immunoglobulins and/or complement at the site of pathology. Using indirect immunofluorescence techniques, circulating antibodies against intercellular substance and the basement membrane zone have been demonstrated. There is evidence in the literature, to suggest that the production of antibodies in normal humans and several diseases states is controlled by the interaction of subpopulations of lymphocytes and possibly macrophages. Such studies have not been reported in vesiculo-bullous diseases.

The aim of this study was to examine the peripheral blood lymphocytes of patients with pemphigus and bullous pemphigoid. Studies included characterization of surface markers and certain in vitro function. 18 untreated patients with pemphigus and 19 patients with bullous pemphigoid were studied along with 42 age and sex matched controls. The study involved measurements of T cells, B cells, Fc receptor cells, PHA stimulation and antibody dependent cell mediated toxicity (ADCC). T cells were measured using sheep red blood cell (SRBC) rosette forming assay. B cells were measured by using fluoresce conjugates polyvalent antihuman immunoglobulin antisera and measuring cells with membrane fluorescence. Fc receptor bearing cells were measured using one cell culture system; 1/2500 normal levels of circulating T cells and ADCC (P value less than 0.05). Patients with bullous pemphigoid have normal levels of circulating T cells, B cells, Fc receptor cells, and ADCC (P value greater than 0.05) but considerably reduced response to PHA (P value less than 0.05).

These studies indicate that patients with vesiculo-bullous diseases have abnormalities of lymphocyte functions. This study illustrates the need for further investigations to detect molecular and cellular abnormalities of lymphocytes to better understand the triggering mechanisms and the regulatory mechanism in the production of autoantibodies against the skin.

Reactions to Jellyfish Stings—Toxic or Allergic? K.R. Hartman, G.J. Calton, and J.W. Burnett. Division of Dermatology, University of Maryland School of Medicine, Baltimore, Maryland.

Severe constitutional reactions to marine venoms may be induced by allergic as well as toxic mechanisms. To determine the role of allergy, sera from 2 patients with exaggerated reactions to stings of a jellyfish (sea nettle, Chrysoura quinquecirrha) were studied by a radiol allergosorbent test (RAST) adapted for use to identify the immune response to sea nettle venom. Allergen-specific IgE concentrations in sera of exposed persons who reacted to stinging by developing a mild eruption were similar to those determined in nonexposed individuals. The 2 patients with severe envenomations revealed allergen-specific IgE concentrations over 5 times the average normal level. These extremely high levels persisted for at least 4 yr after a major sting. These patients also tested positively in a RAST similarly developed for the Portuguese man-ow’-war (Physalia physalis) toxin, indicating some level of cross-reactivity for the antibodies. Liquid antigen was successful.
in diminishing the apparent antigen-specific IgE levels, confirming the specificity of the antigen-bound Sepharose beads. These results indicate the capability of these jellyfish proteins to induce an IgE response in humans and the utility of the RAST as a screening device to detect susceptible persons, or to possibly identify the offending species in case of stinging due to unknown causes.

**Increased PMN Activation by Serum from Patients With Psoriasis.** Julie B. Sedgwick, Paul R. Bergstresser, M.D., and Eric R. Hurd, M.D. University of Texas Health Science Center at Dallas, Dallas, Texas.

Granulocytes (PMNs) are a prominent feature of the epidermal and papillary dermal infiltrate in psoriasis. Our observations of increased *in vitro* PMN adherence and altered morphology of circulating PMNs suggest that psoriasis is not confined to cutaneous lesions but has systemic manifestations as well. To determine the capacity of psoriatic serum with zymosan activation to stimulate normal PMNs, we measured superoxide (O₂) generation by ferricytochrome C reduction. PMNs from normal donors, incubated with serum from untreated psoriasis patients, induced a significant (p < .001) increase in cytochrome C reduction by 10 minutes when compared to normal sera. This difference increased in sequential measurements. The nmoles of cytochrome C reduced are tabulated.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>n</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
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<tr>
<td>Normal controls</td>
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<td>Psoriasis patients</td>
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<tr>
<td>Untreated</td>
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<td>4.0</td>
<td>16.0</td>
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</tr>
</tbody>
</table>

Following systemic treatment of psoriasis, cytochrome C reduction approached normal values. Complement inactivation of test sera before incubation or omission of zymosan from the reaction resulted in minimal cytochrome C reduction (<10 nmoles at 25 min). These data suggest that patients with psoriasis have a complement-dependent serum factor which leads to increased PMN activation after zymosan stimulation. O₂ generation has been reported to cause auto-oxidation, and depolymerization of hyaluronic acid and bovine synovial fluid. Therefore, increased PMN stimulation may play a role in the pathogenesis of the skin lesions and joint destruction in psoriasis patients.

**Serum Factor with T-Cell Inducing Activity in Mycosis Fungoides and Sezary Syndrome.** B. Safai, J.T. Twomey, V. Lewis, G. Goldstein, and R.A. Good*. *Memorial Sloan-Kettering Cancer Center, New York City, 10021. †V.A. Hospital Baylor College of Medicine, Houston, Texas.

Mycosis Fungoides (MF) is a lymphoproliferative disorder with distinct clinical and histological features. Sezary Syndrome (SS) is considered a leukemic variant of MF. Central to this disease is a proliferation of lymphocytes with characteristic of thymus-derived T-cell markers. These neoplastic lymphocytes manifest aneuploidy, impaired proliferation, failure to produce lymphokines and reduced cytotoxic reactivity. The abnormal T-cell proliferation in MF and SS may result from excessive inductive stimulation, an intrinsic derangement of T-cell precursors or combination of both. In the present study, serum from patients with these syndromes was tested for activity that promotes lymphocyte differentiation. Sera from 13 patients with MF and 2 with SS were tested for activity that induces lymphocyte differentiation. Thy 1.2 antigen and surface immunoglobulin were used respectively to measure T and B cell differentiation. The indicator cells were null lymphocytes from the spleens of congenitally athymic nude mice. The T-cell inducing activity is ascribed to the thymic hormone and declines with advancing age.

Normal serum induced some T-cell but no B-cell differentiation. T-cell inducing activity was significantly more active than in normal serum (P < .001). This high T-cell inducing activity was observed even in elderly individuals with MF whose serum thymic hormone activity normally should have been absent or very low. This serum factor present in MF and SS which is a potent inducer of T lymphocyte differentiation might be associated with the neoplastic proliferation in cutaneous T-cell lymphomas. The site of production and possible role of it in MF is not as yet known. The possibility of hyperserection by thymic epithelim or its production by epidermal cells must be considered and investigated.