What's New in Clinical Research

A Symposium to be held December 9, 1980, in New York at the Annual Meeting of the American Academy of Dermatology in Association with The Society for Investigative Dermatology, Inc.


Access to the content of the seaceous follicle is important to the study of diverse follicular disorders: such as acne, keratosis pilaris, large pores, perioral dermatitis, trichostasis spinulosa. We have developed a procedure for performing follicular biopsies noninvasively. The follicular biopsy is an extension of the surface biopsy technique originated by Marks & Dawber.

The follicular biopsy consists of applying a quick-setting cyanoacrylate adhesive to the skin and allowing it to polymerize on a glass slide. Lifting off the slide removes a thin sheet of the stratum corneum and the keratinous lamella.

The keratinous casts can be analyzed by the horny follicular casts. The keratinous casts can be analyzed by the following approaches: (1) morphologic studies with the stereomicroscope, light, scanning, and transmission electron microscopes, (2) bacteriologic and mycologic determinations, and (3) chemical composition by thin-layer chromatography.

The follicular biopsy provides a tool for sampling the follicular contents of normal and abnormal skin in a convenient, simple, noninvasive manner.

Penicillamine-Induced Pemphigus. Daniel J. Santa Cruz, Philip G. Pholerau, and Jouni Utto, Divisions of Surgical Pathology and Dermatology, Washington University School of Medicine, St. Louis, Missouri.

Penicillamine has been used for treatment of a variety of conditions, and pemphigus is a known, albeit rare, complication of penicillamine therapy. Reports of its occurrence have been limited to the British and European literature. Recently, we have examined 2 patients with penicillamine-induced pemphigus bringing the total number of reported cases to 29. Analysis of these cases demonstrated an age range from 31 to 75 yr, and an almost equal male-female ratio. Twenty-two received the drug for rheumatoid arthritis, 3 for scleroderma, 2 for psoriatic arthritis, 1 for generalized morphea and 1 for Wilson’s disease. The length of treatment varied from 1 mo to 2 yr, and the dosage ranged from 250 to 1000 mg per day. Twenty-one of the 29 developed follicleus, 4 vulgaris, 1 erythematosus, and in 3 cases the authors did not specify the type of pemphigus. Direct and indirect immunofluorescence studies demonstrated intercellular autoantibodies in the majority of the cases. We have been able to obtain followup information on 26 patients: 19 with follicleus, 3 with vulgaris, 1 with erythematosus and the 3 with unspecified type of pemphigus have resolved; 4 with follicleus did not resolve, and 2 patients have died, 1 as a direct result of immunosuppressive therapy and the other of Goodpasture’s disease. We conclude that pemphigus is a significant complication of penicillamine therapy, and that when it occurs, withdrawal of the drug usually leads to resolution of the disease.


The role of the autonomic nervous system is felt to be important in the pathogenesis of idiopathic Raynaud’s Disease, a cold induced vasospastic phenomenon in distal extremities. We quantitated the therapeutic effects of autogenic training and/or reserpine in order to learn about the mechanism of this disease.

Four groups of eight matched patients with Raynaud’s received the following therapy: I. Reserpine. II. Reserpine plus autogenic training. III. Saline. IV. Saline plus training. Response was assessed by cold stress test. The patient sat in a chamber where temperature was dropped from 80°F to 58°F in 30 min while changes in blood flow, extremity temperature, heart rate, and cardiac output were monitored. Blood samples were assayed for catecholamines, cortisol, prolactin, dopamine beta hydroxylase and thyroid stimulating hormone. Autogenic training consisted of relaxation exercises and the use of a digital band thermometer.

Initial results suggest that Raynaud’s patients have a marked elevation of baseline thyroid stimulating hormone and a biphasic elevation during cold exposure. The patients’ TSH levels were approximately 10 times that of controls. Raynaud’s patients have decreased digital blood flow on cold exposure and fail to recover 25 min following cold stress. They can be trained to increase their digital blood flow by the use of biofeedback techniques. The role of thyroid stimulating hormone in the pathogenesis of Raynaud’s has never been described and has important therapeutic implications.

Immunogenetic Principles Apply to Clinical Contact Sensitivity: Autologous Factors Are Required for In Vitro Lymphocyte Activation to Nickel. William R. Lewis, Alan M. Dattner, and Steven K. Shama, Dermatology Branch, NCI, NIH, Bethesda, Maryland.

We have studied the in vitro second culture response of lymphocytes from 2 nickel-sensitive individuals in the presence of autologous and allogeneic irradiated leukocytes. Lymphocytes were incubated in appropriate leukocyte culture for 13 days with 10 µg/ml NiSO₄. Lymphocytes, primed in this manner, were harvested and recultured in the presence of varying concentrations of NiSO₄ plus irradiated autologous or allogeneic cells. The primed lymphocytes responded best to NiSO₄ in the presence of irradiated leukocytes from the same donor (autologous).

These findings indicate that self-specific or histocompatibility products are required for in vitro lymphocyte activation to nickel. From these results and by analogy to other studies with the experimental sensitizer dinitrochlorobenzene, it is proposed that major histocompatibility products are involved in immune recognition of many and possibly all contact sensitizers.

Combination PUVA-Methotrexate Therapy of Psoriasis. Warrick L. Morison, Khosrow Montaz, John A. Parrish, and Thomas R. Fitzpatrick, Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, MA.

A combination of 2 very effective treatments for psoriasis has been studied in 30 patients with the aim of clearing the disease, and more importantly maintaining control of the disease, using a minimal number of PUVA treatments and dose of radiation. Patients were given a preliminary course of low doses of methotrexate and then PUVA plus methotrexate until their disease was controlled. Methotrexate was then withdrawn while the frequency of PUVA treatments was gradually reduced to weekly exposures. This schedule of therapy was very effective in rapidly clearing disease even in patients who had not shown a satisfactory response to PUVA therapy. The total clearance doses, the
Experimental Infections in Rabbits and Humans with *Pityrosporum orbiculare* and *P. ovale*. Jan Færgemann, and Torsten Fredriksson, Department of Dermatology, Central Hospital Vasteras, Vasteras, Sweden.

The purpose of this investigation was to produce experimental tinea versicolor in rabbits and humans with *Pityrosporum orbiculare* and *P. ovale*.

*P. orbiculare* and *P. ovale* were inoculated, with and without occlusion, on the glabrous follicle-rich inside of the ear in 10 male rabbits and on the lateral aspect of the upper arm in 5 patients with a history of tinea versicolor, and in 3 healthy volunteers.

After 1 week tinea versicolor-like lesions were produced with both *P. orbiculare* and *P. ovale* in 8 of 10 rabbits. Likewise experimental infections, similar to those found clinically in tinea versicolor, were seen in all of the 5 patients with a history of tinea versicolor and in the 3 healthy volunteers. Experimental infections were only produced under occlusion. Microscopically, short hypoe and transformation between round and oval forms were seen in both *P. orbiculare* and *P. ovale*.

This investigation adds to the identity of *P. orbiculare* and *P. ovale* the fungus seen in tinea versicolor. Spontaneous healing and the fact that experimental infections were produced only under occlusion illustrates the importance of predisposing factors in tinea versicolor.

Immune-Mediated Activation of Leukocytes at the Dermal Epidermal Junction In Vitro. W. Ray Gammon, W. Mitchell Sams, Jr., and Clayton E. Wheeler, Jr., The University of North Carolina School of Medicine, Department of Dermatology, Chapel Hill, North Carolina.

It has been proposed that immune complex-mediated, leukocyte activation at the dermal-epidermal junction may be an important event in the pathogenesis of skin injury and blister formation in bullous pemphigoid. To determine the validity of this hypothesis, cryostat sections of fresh frozen normal human skin were incubated with pemphigoid sera containing complement-fixing antibodies to the cutaneous basement membrane zone and subsequently overlaid with human peripheral blood leukocytes suspended in fresh or heat-inactivated (56°C × 30 min) normal human serum containing 0.2% nitro blue tetrazolium (NBT). Following a 30-min incubation, leukocytes suspended in fresh but not heat-inactivated serum were attached in a specific linear pattern to the dermal-epidermal junction and contained heavy intracellular deposits of reduced NBT. To determine if the reaction was specific for cells attached to the junction compared to cells randomly distributed on tissue, development of the NBT reaction was continuously observed. Cells at the junction showed a faster rate of NBT reduction compared to randomly distributed cells. These results provide evidence for a selective activation of leukocytes attached immunologically at the dermal-epidermal junction and further support a role for immune-mediated cellular inflammation in the pathogenesis of pemphigoid skin lesions.

Human Skin Fibroblast in Culture: The Effects of Proline Analogues on Growth Kinetics and Collagen Production. Jouini Uitto and Elaine M. L. Tan, Division of Dermatology, Department of Medicine, Harbor UCLA Medical Center, Torrance, California.

Adult human skin fibroblasts were incubated in a medium containing fetal calf serum, ascorbic acid and β-aminopropionitrile, and the effects of 2 proline analogues, L-azetidine-2-carboxylic acid (ACA) and cis-4-hydroxy-L-proline (cHyp) on the cell growth and collagen synthesis were examined. Addition of 1-100 µg/ml ACA into the medium of early log-phase cultures decreased the subsequent cell proliferation in a time- and dose-dependent manner. The decrease in cell number appeared to be, at least in part, due to an inhibition of attachment of the cells on the plastic substratum, since fewer cells were attached in the presence of 1, 25 or 100 µg/ml ACA than in the controls incubated without the analogue. This effect was also dose-dependent. Similar effects were obtained with cHyp, but relatively higher concentrations were required to achieve the same degree of inhibition.

To study the effects of the analogues on collagen production, the cells were incubated with H proline or H glycine, and procollagen...
formation was assessed by synthesis of H hydroxyproline or bacterial collagenase-degradable \(^{14}\)H-polypeptides. In the presence of 25 \(\mu\)g/ml ACA, procollagen synthesis was inhibited, and the secretion of procollagen into the medium was also decreased with increasing concentration of ACA. Similar effects by the analogues were also observed in skin fibroblast cultures derived from patients with progressive systemic sclerosis.

In summary, the results indicate the proline analogues, such as ACA, inhibit the fibroblast growth in culture. At the same time, procollagen production by these cells is reduced. The results suggest, therefore, that proline analogues may prove useful in limiting the excessive accumulation of collagen in fibrotic processes, as such as progressive systemic sclerosis.

Erythema Nodosum Leprosum—An Immune Complex-Mediated Necrotizing Vasculitis. N. P. Sanchez, M. C. Mihm, Jr., N. A. Soter, P. Davila, and J. L. Sanchez. Harvard Medical School, Boston MA and University of Puerto Rico, San Juan, PR.

Erythema nodosum leprosum (ENL) is a form of cutaneous vasculitis which occurs in patients with lepromatous leprosy. The skin lesions are generally erythematous or indurated nodules, but may become necrotic, pustular or hemorrhagic. Four patients with ENL were studied histologically with the 1 micrometer section technique, direct immunofluorescence, and with assays for complement factors and circulating immune complexes. In each of 4 patients necrotizing vasculitis was identified by the presence of endothelial cell swelling and necrosis, matted fibrin in the vessel walls in the deep dermis and a neutrophilic infiltrate in vessels associated with nuclear debris. In the superficial dermis perivascular lymphocytic and mononuclear infiltrates predominated associated with endothelial cell hypertrophy. Basophils and rare eosinophils were noted. Throughout the dermis epithelial cells exhibiting vacuoles containing fragmented organisms—lepra bacilli—were observed. Direct immunofluorescence studies showed granular deposits of IgG and C3 in the vessels. Circulating immune complexes as assessed by \(^{125}\)I-Clq binding assay were detected in 3 patients. Levels of Clq, C4, C5, C6 and factor B were elevated or normal. The unequivocal presence of vasculitis with granular deposits of immunoglobulin and complement as shown by the fluorescence microscopy suggest that ENL is an immune complex-mediated necrotizing vasculitis.


We have previously demonstrated that 405 nm irradiation of human serum, to which uroporphyrin had been added, resulted in the generation of C, derived chemotactic activity for polymorphonuclear leukocytes. To investigate the significance of this observation in the pathogenesis of cutaneous lesions in patients with porphyrias, sera from 13 patients with erythropoietic protoporphyria (EPP) were exposed to 405 nm irradiation in vitro. The light source was GEF40BL, the irradiation dose was 2.5J/cm\(^2\), measured at 400–410 nm and 15 cm. There was marked diminution of total hemolytic activity (CH\(_{50}\)) and hemolytic titers of C, in the irradiated sera. The diminution of hemolytic activities was proportional to the concentration of protoporphyrin in the serum. In addition, chemotactic activity for human polymorphonuclear leukocytes was generated in all irradiated sera; no chemotactic activity was detected in patients' sera that were not irradiated, nor in irradiated normal human serum. Using the crossed immunoelectrophoresis technique, cleavage products of C, were detected in irradiated patients' sera. Similar results were obtained when sera from 4 patients with porphyria cutanea tarda (PCT) were irradiated. It is suggested that in patients with EPP and PCT, exposure to 405 nm irradiation results in the activation of the complement system with generation of chemotactic activity, which eventuates in the development of the cutaneous lesions.