All lacked plasma cells and cells exhibiting *cytoplasmic* fluorescence with fluorescine-labeled antibodies to IgM, IgG, and IgA in biopsies of ileum and stimulated lymph node. In contrast to the patient with XLA, patients with SA had prominent germinal centers in stimulated nodes; one had the syndrome of nodular lymphoid hyperplasia (NLH). Using criteria established for identification of bursa-derived lymphocytes in chickens, B-lymphocytes were identified by electron microscopy in the lymph node of one patient with SA; only T-lymphocytes were found in the patient with XLA. *Membrane-bound* immunoglobulins (IgM, IgG, and IgA) were detected by immunofluorescent studies of living peripheral blood lymphocytes of both patients with SA, but were not found on cells from the patient with XLA. The results indicate that in these patients with SA, lymphocytes of the plasma cell line are able to synthesize surface immunoglobulins and to respond to antigenic stimulation by germinal center formation but fail to differentiate to the stage of antibody secretion; lack of antibody-mediated "feedback" inhibition of antigen-induced proliferation may account for the NLH often observed in SA. Our observations also provide preliminary evidence that B and T lymphocytes can be distinguished at an ultrastructural level in man.

Phytohemagglutinin (PHA) skin test: A measure of intact cell-mediated immunity. RICHARD J. BONFORTE, R. MICHAEL BLAESE, MARCEL TOPILSKY, LOUIS E. SILTZBACH, and PHILIP R. GLADE. *Mt. Sinai Sch. Med., City Univ. N. Y., N. Y.*

PHA is a non-specific stimulant of normal lymphocytes resulting in vitro in their blast-like transformation with increased RNA and DNA synthesis, mitosis, and the release of putative mediators of cell-mediated immunity. Histologically the skin reaction to intradermal PHA shows perivascular infiltration of mononuclear cells, the hallmark of delayed-type hypersensitivity. Since prior exposure is not required—only the capacity to respond—PHA appears to be the ideal agent to assess cell-mediated immunity in vivo. In a continuing series of more than 30 individuals 2 μg of PHA intradermally produces erythema and induration in 24–48 hrs., despite the presence or absence of cutaneous reactivity to other skin-test antigens (PPD-S, histoplasmin, mumps, SK-SD, candida, and trichophyton). In a selected group of patients in vivo skin reaction to PHA correlates with in vitro response of the individual's peripheral blood lymphocytes to PHA. PHA in vivo thus appears to be an effective measure of intact cell-mediated immunity and is especially useful in infants and children who have had limited prior exposure to those antigens normally used as skin-test reagents.

Pernicious anemia, concordant twins, and immune dysfunction. ERWIN W. GELFAND, A. IZZET BERKEL, HERMAN A. GODWIN, ROSS E. ROCKLIN, JOHN R. DAVID, and FRED S. ROSEN. *Children's Hosp. Med. Ctr., Boston City Hosp., and Robert B. Brigham Hosp., Boston, Mass.*

The pathogenesis of pernicious anemia (PA) may be mediated by auto-immune mechanisms. However, the presence or absence of antibodies to intrinsic factor has not been clearly correlated with the clinical course of the disease. Three members of one family, a mother and identical twin daughters, were found to have classical PA and achlorhydria. Schillings tests revealed less than 1% excretion of the $Co^{57}B_{12}$ isotope. Repeat tests with orally administered intrinsic factor increased the isotope excretion to 10% or more. The twins, unlike their mother, had low levels of serum γG, absent γA, and normal γM globulins, and impaired antibody responses. The serum of the mother and only one of the twins contained antibodies to intrinsic factor and gastric parietal cells. Lymphocytes from the mother and the twins, cultured in the presence of intrinsic factor, underwent transformation with increased uptake of H^3-thymidine into DNA (stimulation indices—9.8, 2.5, 1.4). Production of migration inhibition factor in similar cultures in these three patients resulted in 17, 37, and 9% inhibition of macrophage migration. These studies suggest that cell-mediated immunity, rather than humoral immunity, may play a critical role in the development of PA.

Lymphopenia, dysgammaglobulinemia and decreased cellular immunity: A genetic lymphocyte defect. ARMOND S. GOLDMAN, C. WAYNE SMITH, and ELTON DUPREE. *Univ. of Tex. Med. Branch and Shriner's Burns Inst., Galveston, Tex.*

A previously unrecognized genetic immunologic disorder is reported. The patient a 13-year-old female, presented with recurrent viral and bacterial infections, eczema and acute allergic reactions. Humoral and cellular immunity were reduced, the serum IgG and IgM were low; IgD was normal; IgA and IgE were increased. She was lymphopenic, but her thymus appeared normal microscopically. In comparison to normal lymphocytes, her blood lymphocytes in culture did not suppress the life-span of PMNs. In those leukocyte cultures, the PMNs inhibited blastogenesis due to phytohemagglutinin, concanavalin A, pokeweed mitogen and antigens. However, in PMN-depleted cultures the relative frequency of blastogenesis became normal but lymphocyte survival and quantitative blastogenesis due to antigens and mitogens were reduced.

The parents' lymphocytes were unable to limit PMN survival *in vitro* and the relative frequency of transformation of their lymphocytes was reduced. However, the functions of their lymphocytes in PMN-depleted cultures seemed normal. These findings suggest that the principal defect in this disease is in the lymphocytes and that the propositus is homozygous for the defective gene, whereas, the asymptomatic carriers are heterozygous. Although this disorder resembles the Wiskott-Aldrich syndrome, the genetic aspect of the disease, the lack of thrombocytopenia, the low IgG and other features indicate that this is a previously undescribed primary immunologic disorder.

Macrophage function in severe combined immunodeficiency. RICHARD A. GATTI and ROBERT A. GOOD. *Univ. of Minn., Minneapolis, Minn.*

In patients with severe combined immunodeficiency (i.e., lymphopenic agammaglobulinemia), both humoral and cell-mediated forms of immunity are lacking as a result of absence of immunoblasts or lymphoid precursor cells capable of differentiating into plasma cells and small lymphocytes. The small lymphocytes of these infants do not respond to stimulation in vitro with phytohemagglutinin, allogeneic cells or antigens. This situation can be corrected by transplanting bone marrow from an HL-A compatible healthy donor. This finding establishes that the differentiative sites are intact. Macrophage function has not been evaluated in these patients. Nor is there clear evidence to indicate whether macrophages derive from the same defective precursor stem cells as lymphocytes. In these experiments, a glass-adherent cell population was obtained from two patients with severe combined immunodeficiency. After three days incubation, these adherent cells (i.e., macrophages) were added to highly purified lymphocytes from two PPD-sensitive donors. Such highly purified lymphocytes do not respond to antigens in the absence of at

least one macrophage per 100 lymphocytes. The macrophages of our patients restored this response to PPD. In addition, a soluble factor (CMRF) collected from macrophage cultures of both patients, as described by Bach et al., also reconstituted the response of purified lymphocytes to PPD. These studies indicate that macrophages in severe combined immunodeficiency are capable of 1) restoring the response of purified lymphocytes to PPD and 2) synthesizing CMRF (conditioned medium reconstituting factor). They also confirm Bach et al.'s observation that CMRF can replace the macrophage in its interaction with purified lymphocytes.

Incompatible bone marrow transplantation in lymphopenic immunologic deficiency. REBECCA H. BUCKLEY, D. BERNARD AMOS, WILLIAM P. KREMER, and DELFORD L. STICKEL. *Duke Univ. Sch. of Med., Durham, N. C.*

Fatal graft-versus-host (G-V-H) disease has been an invariable accompaniment of incompatible bone marrow transplantation in infants with lymphopenic defects. This report describes an approach which permitted circumvention of this complication. Histocompatibility studies demonstrated no HL-A compatible donors for a female infant with lymphopenic agammaglobulinemia (very low IgG, absent IgA, IgM, and IgE). Serum from her mother (para 16) contained antibodies (1:64 titer) to paternal HL-A antigens inherited by the patient. Maternal lymphocytes were not stimulated by paternal or infant's leukocytes in mixed leukocyte culture in media containing maternal plasma. Immunologic enhancement was attempted at age 5 months by pretreating the infant with cell-free maternal plasma I.V. 24 hours before the I.V. infusion of 5×10^6 immature albumin-gradient-separated maternal marrow cells. Appetite and growth increased dramatically during the first 3 months, thrush and pneumonia cleared, and no overt signs of G-V-H were noted. Delayed cutaneous responsiveness to *C. albicans* was seen by the 13th day and persists. Lymph node biopsy at 90 days contained plasma cells which stained with fluoroscein-conjugated anti-IgG, IgA, IgM or IgE. A second maternal marrow transplant was given at age 11 months, again without overt G-V-H. This 19-month-old infant has gained 4 kg and grown 20 cm since the first transplant 14 months ago. A normal serum IgA concentration and somewhat low but constant levels of IgG and IgM are present now 5 months after the last plasma infusion. These findings suggest possible usefulness of immunologic enhancement in future attempts when compatible donors are unavailable.

Transfer factor in the treatment of chronic mucocutaneous candidiasis. MARTIN L. SCHULKIND, WILLIAM H. ADLER, III, WILLIAM A. ALTEMEIER, III, and ELIA M. AYOUB. *Univ. Fla. Coll. Med., Gainesville, Fla.*

The use of "transfer factor" to correct a partial defect in cellular immunity to *Candida albicans* was studied in an 8-year-old girl with chronic granulomatous mucocutaneous candidiasis.

The patient first presented at age 5 years with extensive deforming encrusted lesions on her face, head, trunk and extremities. No evidence of endocrinopathy or antibodies to endocrine tissue was found. Her general humoral immunity was intact. She had a normal complement of granulocytes and lymphocytes. Candida aggregation activity was present in her serum. Her skin test response to *C. albicans* extract was consistently negative. Her lymphocytes underwent blastogenesis to PHA, diphtheria toxoid and *C. albicans* extract. However, in the presence of exogenous transfer factor, blastogenesis to candida increased. Transfer factor extracted from her cells did not transfer immunity to non-sensitized cells.

Amphotericin B therapy cleared her skin lesions temporarily, but neither fresh frozen plasma injections nor 5-fluorocytosine was effective. Following 2 injections of transfer factor, she developed a positive skin test response to *C. albicans* extract, and her lymphocytes produced leukocyte inhibition factor (LIF) to candida. After a third injection there was appreciable clearing of the skin lesions.

These findings indicate that exogenous transfer factor can restore cellular immunity to candida in a patient with chronic mucocutaneous candidiasis and may be an effective treatment for this disease.

A primitive immunologic marker of intrauterine virus infection. JOSEPH W. ST. GEME, JR., CATHERINE W. C. DAVIS, and LLOYD F. VAN PELT. *UCLA Sch. of Med., Harbor Gen. Hosp., Torrance, Calif.*

Retrospective and prospective studies of the human have suggested that intrauterine mumps virus infection evokes an incomplete immunologic response of delayed hypersensitivity, without humoral antibody. Experimental infection of the subhuman primate during the first third of pregnancy also leads to the development of only cellular immunity in the infant offspring. The significance of delayed hypersensitivity as an immunologic marker of fetal infection is strengthened by the observation of an anamnestic neutralizing antibody response in 2 of 4 infant monkeys following a second skin test. Repeated skin testing of 5 seronegative adult monkeys failed to induce a primary antibody response.

Intrauterine mumps virus infection illustrates the phylogenic and ontogenic concept that cellular immunity is the most primitive immunologic response. Experimental infection of 9 monkeys between the 25th–40th day of gestation, with subsequent cesarean section after 1, 2 and 3 weeks, has demonstrated that virus multiplies in the young fetus for only 1 week. Fetal interferon response does not occur, so the termination of viral replication seems to result from transplacental distribution of abundant 7S maternal neutralizing antibody. This conclusion is supported by the restrictive effect of antibody on mumps virus replication in vitro.

Thus, the immature fetus confronts minimal antigenic mass and accrues immunopoietic instruction for only the more primitive response of delayed hypersensitivity.

Demonstration and replacement of a functional defect of the fifth component of complement in newborn serum. A major tool in the therapy of neonatal septicemia. MICHAEL E. MILLER. *Univ. of Pennsylvania, Philadelphia, Pa.*

Previous studies from this and other laboratories have shown a relative deficiency in the opsonic activity of neonatal serum. The nature of this deficiency has been incompletely understood. The studies now reported show that the impairment of opsonic activity in neonatal serum involves a *functional* deficiency of the fifth component of serum complement (C5). (1) Opsonic activity of neonatal sera towards baker's yeast phagocytosis (Miller, 1969) was restored to normal by the addition of sera from mice with normal amounts of C5 (B10D2 new line) but not by addition of sera from a co-isogenic strain lacking C5 (B10D2 old line); (2) Utilizing highly purified human C3 & C5, re-constitution of