

Thank you. I'm delighted to ^{join you} have been asked to ^{to talk} talk today on immunodeficiency which is one of my favorite areas of immunology.

Objectives - selected ID

touch on principles & mgmt.

It is a topic which for better or worse has captured the imagination of the American public. In part this is due to T.V. specials like the Boy in the Plastic Bubble who appears on this slide.

The real ^{called} infant has the X-linked disorder severe combined immunodeficiency (SCID). ^{the 2nd child} At birth by C-section he was immediately transferred to protective isolation where he has remained ever since awaiting the treatment of choice, a BMT from and HLA-matched donor. ^{The parents had previously child who died of SCID in infancy.}

In part as a result of the increasing public awareness of ID through TV and magazines, we are seeing greater numbers of adults with frequent bacterial or viral infections referred for workup of ID.

Most, of course, turn out to be perfectly normal by our current standards of immune function, ~~many have been unlucky enough to have encountered a number of familiar viruses in a short period of time. We are also frequently asked to see patients who only have furunculosis. Most of these turn out to have chronic staph carrier states which is not an ID, or atopic dermatitis, with secondary skin infection.~~

However a surprising number of patients whom we see on referral do in fact prove to be immune deficient.

ID's can be divided into two categories, primary and secondary.

Primary ^{disorders} ID's are those in which the patient has no other obvious disease or condition known to increase susceptibility to infection. They are relatively rare, except in our clinics.

Secondary ID's are at least 300 times more frequent ^{than 10}

Among the 2nd order ~~causes of secondary include:~~

SLIDE The primary ID's are most easily thought of as falling into 4 categories although there is some overlap. Roughly 50% are

I would like to spend most of our time on the first two categories, the disorders of lymphocyte function since they are the most common. If there are specific questions about poly or complement disorders please feel free to raise them in the discussion period.

SLIDE

This slide should be familiar to you from Dr. Saxon's talk earlier this week. It shows the ontogeny of the immune system with pluripotent lymphoid stem cells migrating from the bone marrow to the thymus where they are educated to become T-cells important for helper function in the antibody response, and for killer cell responses against viruses and fungi. Some pluripotent cells migrate to the human equivalent of the Bursa of Fabricius (wherever it is) where they become B-cells, acquire surface Ig, and the capability of maturing to antibody secreting plasma cells.

In general the B-cell arm is most essential for defense against bacteria and to ^{alless} some extent against viruses. The Tcell arm is ~~the most critical~~ ^{critical} factor in host defenses against viruses, fungi including Candida, and protozoans such as Pneumocystis carinii.

SLIDE-LYMPH

This is of course a lymphocyte. They all look alike by light and electron microscopy. We can't tell if its a T or B-cell.

Human T-cells are identified by their capacity to form rosettes with sheep red blood cells, so-called e-rosettes. This SLIDE is an E.M. photo of a lymphocyte with 3 sheep erythrocytes bound to the membrane.

— B-cells are identified by an immunofluorescence ^{and react} which detects their sIg.
SLIDE-CLIN FEAT

Well then, what are the clinical features which should appropriately arouse your suspicion for ID?

In adults, chronic or recurrent sinopulmonary infection is highly suspicious for antibody deficiency.

Infections with unusual organisms. *P. carinii* or *Staph epi*.

In infants chronic diarrhea or failure to thrive may be underlying ID.

There are also specific features such as ataxia and ocular telangiectasias as in AT.

— In general the type of infections which occur provide important tipoff's to the type of ID. Recurrent otitis, sinusitis or pneumonia suggests ^{an} antibody deficiency problem. Viral fungal or parasitic infection suggests a T-cell ^{problem} deficiency. Infections with organisms of low virulence such as *Staph epi* in infants suggest chronic granulomatous disease, ^{a neutrophil killing defect}.

— Now on to some specific deficiency states.

SLIDE

The first is X-linked or Brutons agammaglobulinemia, the first ID disorder described by Bruton in 1952. Only males.

B-cell defect. Failure of development or function of the human equivalent of the bursa of Fabricius, the structure in birds that educates lymphoid stem cells to become B cells.

The infants are usually asymptomatic until age 6 mos at which time the passive transferred maternal IgG (the only Ig class to cross the placenta) reaches its lowest level. Rarely they are not symptoma-ic until early childhood.

The major problem is recurrent infection with *H. flu* and *Pneumococcus*. Diarrhea is frequently due to *Giardia*, a major problem for patients with antibody deficiency.

Laboratory findings are the absnece or marked deficiency of all Ig classes with total Qlg's less then 250 mg/dl. B cells are absent, but T-cells present in normal numbers. LN biopsy is rarely necessary to make the dx.

The Treatment ^{consists of} to ~~is~~ gammaglobulin injections for life, the liberal use of antibiotics and treatment of the giardiasis. Chronic lung disease may be a complication.

SLIDE

The major confusion in the dx of Brutons is something called "transient hypogamma of infancy." All infants go through a period of physiologic hypogamma at 5 or 6 months of age. Transient hypogamma results from a delay in the normal onset of Ig synthesis ^{after which synthesis by the infant begins} by the infants. They ^{can} be male or female, ^{they} have normal numbers of B-cells in the peripheral blood, ^{in contrast to Brutons where B-cells are absent}.

These kids may ^a require a 6 month course of gammaglobulin injections because of recurrent infections.

SLIDE

talk about at some length

The next syndrome is Common Variable ID or what I'd prefer to call Adult, Acquired Hypogammaglobulinemia. This is the second most common primary humoral ID of adults. The most common is, of course,.....

It is called variable because it appears to have a variable pathogenesis in some cases due to a B-cell defect and in others a T-cell problem.
30% have T_s, however not thought to be

The clinical manifestations are similar to Brutons with pyogenic sinopulmonary infections, skin infections, bacterial conjunctivitis but beginning usually in the age range of 13-35. ~~sporadic~~

Lymphadenopathy is common and pts are frequently subjected to unnecessary lymph node biopsies before the dx is made.

Most cases sporadic, rarely familial, Both sexes are affected.

In most instances QIg levels are very low, less than 400 mg/dl total. Usually not a subtle dx. However there are occasional pts with recurrent infection with borderline levels who may represent this disorder in evolution. We really have little idea of the kinetics of development of this disease. In these cases with borderline levels we frequently immunize once or several times with typhoid vaccine and measure fenrile agglutinins. If there is no antibody response, CVID is ptobably the correct dx.

Most pts have normal numbers of B cells. Thus it is not so much a quantitative B-cell deficiency as a failure of B-cek1 differentiation to plasma cells. Some suggestion that EBV may play a role in the pathogenesis.

Complications include an increased incidence of lymphoma, and pts may present with an associated thymooma which is best diagnosed by CT scan.

SLIDE

GI involvement is a common feature. Giardia mya be refractory to the usual Flagyl regimen..

General treatment is based on IM gamma in substantial doses given at 2-4 week intervals. Ig helps to use a little lidocaine.

We have recently tried larger doses given by portable syringe pumps such as are used for experimental insulin therapy. This approach has several potential advantages which include convenience of home adminis-tration and more frequent doses permitting achievement of higher serum levels.

~~IVIG~~ IVIG

We frequently need to add antibiotics often prophylactically to control sx of infection.

SLIDE OFF

We frequently are asked to see pts with recurrrent furunculosis sent in with the question of antibody or neutrophil deficiency. It is actially rather unusual to have either of these when the infections are totally confined to the skin.

The most frerquent cause is the chronic staphyloccoccal carruer state which is not a reflection of underlying ID. 30% of normal Caucasians carry Staphh aureus on the skin while only 1 in 10,000 have recurrent furunculosis as as result. The problem is due to colonization with specific strains of Staph which are particularly pathogenic. Colony count may be reduced with chronic antibiotics but infections frequently return. Scrubs may be helpful.

An interesting approach is to induce bacterial competition by introducing a non-path strain after several weeks of antibiotic treatment.

SLIDE

While on the subject of Staph I would like to briefly mention Hyper IgE Syndrome.

(4)

These pts are Children or young adults with recurrent skin and pulmonary staph infections.

Characteristically ^{these lesions} have "cold abscesses" lacking in redness and warmth.

Chronic dermatitis or eczema.

The laboratory finding of major interest is a sky-high IgE level usually 20-40,000. Other Ig's are normal. They also have a ~~poly~~ neutrophil chemotactic defect.

SLIDE-shows a young woman with this syndrome. Note the eczema and thick features said to be characteristic.

Most of these patients benefit from chronic antibiotics usually Bactrim or Septra.

I'd like to briefly mention the Wiskott-Aldridge Syndrome which consists of:

SLIDE

These patients have normal neutrophil ^{numbers and} function ~~and numbers~~. Infection is due to antibody deficiency.

A hypothesis linking eczema, antibody deficiency, and thrombocytopenia ^{has not been proposed} is not available.

I want to go on now and briefly mention 3 T-cell disorders: CMC, SCID, and a T-cell immunodeficiency syndrome of adults which we have just reported from UCLA.

CMC CHILD SLIDE

CMC is characterized by persistent Candida infection of mucous membranes, skin, hair and nails. The severity varies from single nail involvement to the entire skin. Candida esophagitis may occur, but they do not get deep systemic Candida infection. ~~septicemia~~.

Roughly 50% of cases have an associated endocrinopathy which usually appears several years after the start of Candida infection.

These include hypopara, hypothyroidism, Addisons disease, and diabetes

Family history is positive in only 20%.

A selective T-cell defect to Candida. ~~Only~~ Candida skin test is negative in 50%. The patients do make antibody to Candida.

response to
Candida
are intact.

SLIDE

Patients fall into 4 groups.

- I. early onset--most severe form-- hypothyroidism is the most frequent endocrine prob
- II. late onset --mildest--often a single nail or oral thrush
- III. Familial--mild to moderate--autosomal recessive--endocrinopathy rare
- IV juvenile/familial, the so-called Candida-endocrinopathy syndrome where hypopara and Addison's disease are frequent.

In the last year there has been a major development in the treatment of CMC. KETOCONAZOLE SLIDE

Kilpatrick 12/80 Annals

conf

Manija
Pattay

Severe combined immunodeficiency, the disease of the boy in the plastic bubble, essentially a failure of development of both humoral and cellular immunity. Many variants exist. Survive to adulthood only with extraordinary measures or successful BMT. Major difficulty with Pneumocystis and Candida infection.

At UCLA we have recently seen an acquired cellular ID of adult homosexual men which resembles SCID.

SKIN DISEASE IN IMMUNE DEFICIENCY DISORDERS

1) 1 ID's most easily considered in terms of 4 major arms of the Isystem. Some overlap.
1 ID's are those in which...

2) 2 ID's are 300x as common.

3) Infantile X-linked agamma first of the ID's reported by Bru in 1952.

As you are all well aware in the chicken there is a gut-assoc lymphoid organ the B of F

May have pyogenic skin infections. Also a dermat-M-like syndrome rptd. in several pts. Since T-cell immunity intact smallpox vac went smoothly without dissemination as was seen in infants with combined B and T defects. Even so we recommend against live-virus vaccines.

4) Common variable ID, the most common symptomatic ID in adults.

Bacterial skin infection is uncommon unless it represents superinfection, e.g. herpes zoster with ~~associated~~^{superimposed} cellulitis or another breakdown of the skin barriers.

There is an increased incid. of RA-like arthritis, dermat-M, scleroderma an SLE-like illnesses in this population, and also in the families of patients.

I have seen rare patients with generalized erythroderma and eczema.

Some patients have an associated T cell defect which can be severe. Many may have seen our pt. D.K. who has chronic warts and molluscum contagiosum as well as laryngeal candida.

This dx is unlikely in young individuals with chronic furunculosis who are otherwise well, ie lack sinopulmonary disease or history of S-P disease. Most patients with chronic furunculosis alone are chronic staph carriers, a condition which does not represent a documented ID.

Just why particular strains of Staph. aureus are pathogenic for certain individuals is unknown and this area needs further research. Routine measurement of QIg's in patients who present this way is probably not warranted.

5) Selective deficiency of IgA

increased incidence of SLE; 1 case candida granuloma; 1 case disseminated varicella
as well as RA and Sjogrens

less than 5 mg/DL, no other Ig class deficiency

many in fact most are healthy, other S-P infection
assoc with gluten enteropathy and malabsorption

nl # of IgA B cells

antibodies to IgA spontaneously arise, anaphylactoid rx to blood tx

IgG₂ subclass defic in sick IgA deficient but not healthy ones
may explain

7) Wiskott-Aldrich - *x-linked* recessive of boys

- triad of eczema, thrombocytopenic purpura, recirrent infection
melena then excema
- high IgE
- infection may not begin until age 6 mos
- eczema in the usual locations frequently secondarily infected
a pruritic eczematoid dermatitis in the usual locations with purpura at the sites of scratching.
- also have herpetic skin infections. hypercatab of G, A and M, anergy
12% malignancies poor response to imm c polysacc
BMT has corrected

8) Ataxia telangiectasia

1:40,000 births heterozygous state 1:100
kids are normal at birth and sometime after walkinh begins clumsiness is noted
usually come to the attn of a neurologist by age 4 who makes the dx.
loss of Purkinje cells and granular cell layer in the cerebellum
IgA low in 70% , as ij pts with other ID's they get lymphoid cancers
and IgE elevated alpha fetoprotein

telangiectasias occur 1st on the bulbar conjunc. With time they involve lateral
nose, antecubital and popliteal fossae and dorsa of the hands and feet
may have S-P infection but neurologic usually dominates
may have a progressive cutaneous atrophy resemblin scleroderma

also areas of hypo and hyperpigmentation

Samuel Gotoff a radiotherapist made a sharp observation when he published
that there were untoward responses to radiation in AT skin. AT may in fact be
a DNA repair defect analogous in some way to xeroderma pigmentosum altho AT
pts do not get skin cancers. Some pts have a 14q tandem transloc and AT fibro-
blasts do not repair radiation injury well.

9) Chronic mucocutaaneous candidiasis

6) Hyper IgE syndrome; its not clear where the major immunologic defect resides. I mention it here because of the prominent antibody abnormality, an enormous hypersecretion of IgE.

in children and young adults the abscesses cluster about the scalp, face and neck
Said to be "cold" and lacking in the normal PMN response.

Eczema but not typical atopic dermatitis. Pruritic, lichenified but not the usual AD dis

--cutaneous and pulmonary infections with Staph -lifelong
--cold abscesses furunculosis
--"coarse" facies both sexes
--chronic dermatitis or eczema
--Lab: sky-high IgE. Others normal. blood&tissue eosinophilia
defective poly chemotaxis, but normal bacterial killing and NBT test
--Rx: continuous antibiotics have been used.

defect in oxidative metabolism of neutrophil
Chronic Granulomatous Disease
--X-linked (altho a rare female variant exists) onset in infancy
--enzyme deficiency (NADH or NADPH reductase) leads to abnormal neutrophil
metabolism and impaired intracellular killing
--draining lymphadenitis with unusual pathogens in child under 2 is almost
diagnostic
--Lab: decreased bacterial killing leukocytosis, anemia, hyperglob
absent NBT (nitrobluetetrazolium dye) reduction test 80-90% nl neut reduce to F
carrier state can be diagnosed 10% CGD neut reduce
--may survive to adulthood with aggressive therapy

abscesses and granulomas in liv, spleen, lung and bones

nl chemotaxis, phago but can't kill worth a damn

except non-catalase producing bacteria (hem strep and pneumococci)

SYSTEMIC DISEASES WITH IMM FEATURES

rheumatic diseases, vasculititides, autoimmune hemolysis and thrombo, MGravis

association with MHC, ie HLA

A B C and D loci with D locus viewed as analog of Ir gene region in msc

B-27 with AS strongest

but most interesting associations are with D locus

SLE c DR2 and DR3 alleles

RA c DR4

suggest genetic predisp + unknown env fact

autoantibodies are characteristic

SLE anti DS-DNA, SS-DNA, nucleoproteins, saline extractable nuclear ag's RnP (MCTD) and Sm (SLE)

ANA + in 90% SLE

LE cell test obsolete

RA RF+ 70% lymphocyte reactivity to collagen in DR4+ normals

Sjog lymphocytic infilt of exocrine glands sicca syndrome
Primary-alone Secondary-with RA, SLE or others

anti-sal duct ab in 70% of 2°, 25% 1°

+ CIC's of ? signif.

Sclero 40% ANA +
anti-centromere ab in CRST

PA anti parietal, anti-intrinsic factor or both

Primary biliary cirrhosis 90% AMAntibody, also antiSM, ANA

Graves anti-TSH receptor on thyroid follicular cell

Hashi - lymph infiltration + increased antithyroglob ab

MG anti-ACH receptor in 80%, 75% have thymic abns usually hyperplasi
but also thymomas

Immunosuppressive rx

Steroids are not that potent suppressors of immunity.

Ig decreases only modest

redistrib of cells

most important is inhibition of migration of inflamm cells

(less prominent with god)

Leuko or lymphopheresis.

ALG used; monoclonals coming in.

Plasmapheresis widely used but minimally effective in RA, SLE

useful in diseases with high circ anti-tissue ab's (Goodpastures
with anti-GBM or MG).

Cytotoxics fall into 2 groups:

antimetabolites aza, methotrexate which act on dividing cells
(aza+ allo a no-no)

alkylating agents cytox, chlor act on resting and dividing cells

in general alk in high doses are most potent, but also most toxic

Steroid adequate for AHD except cold, and ITP, some SLE

Cytosan drug of choice for vasculis (Weg, PAN) except for small vessel
which usually respond to steroids.

Immunopotential really a thing of the future.

BCG, Levam disappointing.

Oncofetal Ag's

CEA non-specific cigs, IBD

maybe OK for monitoring ca rx S/P lung, colon surgery

Alphafetoprotein-nl adult levels by 2 yrs

hepatoma, germ cell tumors of testis & ovary

follow with B-HCG in non-seminoma testicular tumors

IMMUNODEFICIENCY States

A. Introduction

Immunodeficiency diseases result from disturbances in the body's host defense mechanisms. They can be divided into primary and secondary categories. Secondary are at least 300 times as frequent (malnutrition, splenectomy, burns, steroid and cytotoxic drug treatment, radiotherapy, etc.)

For primary, 4 basic categories (although there is considerable overlap)

- | | |
|---------------------------------------|-----|
| 1. humoral | 50% |
| 2. cellular (T-cell) | 40% |
| 3. phagocytic ^{dysfunctions} | 6% |
| 4. complement deficiency | 4% |

1. B. Humoral immunodeficiency

(A) 1. X-linked agammaglobulinemia

- the first described ID disorder (Bruton, 1952)
- B-cell defect (possible failure development of bursa-equivalent in man)
- asymptomatic until age 6 months when maternal IgG reaches ~~near~~ lowest point
- X-linked recessive (^{males} boys only)
- recurrent H. flu, pneumococcus infections of ears, sinus, lungs.
- Lab: marked deficiency of all Ig classes. Total Ig less than 250 mg/dl.
- absent isohemagglutinins
- absent B-cells, normal to increased T-cells
- Delayed DTH skin tests ^{become +}
- Lymph node biopsy ^{rarely necessary} _{infrequently} for diagnosis.

--Rx: gammaglobulin injections or plasma, antibiotics, giardia treatment

(B) 2. Transient hypogammaglobulinemia of infancy

- major differential dx for X-linked ^{globulinemia}
- ^{normal} infants have a period of transient hypogammaglobulinemia at age 5-6 months
- ^{these patients have a longer} there may be a lag in the onset of normal Ig synthesis ^{and develop bacterial} with subsequent infections
- may require ⁶ months of gamma globulin rx.
- both sexes
- normal B-cell numbers in blood.

3. Selective IgA deficiency

- 1:600-1:800 normal blood donors
- IgA ^{usually} less than 5 mg/dl. ^{of IgA are} Less severe ^{depressions} of unclear significance.
- most asymptomatic
- some ^{have} recurrent sinopulmonary infection, severe nasal allergy
- autoimmune disease (RA, SLE)
- gamma globulin ^{therapy is} contraindicated (pts have antibody to IgA-30%)

4. Common variable immunodeficiency (adult acquired hypogammaglobulinemia)
 - common
 - variable pathogenesis
 - present usually ages 13-35
 - pyogenic infection (D. pneumo, H. flu, staph)
 - malabsorption, giardia, autoimmune disease, malignancy in some cases
 - normal B cell numbers. Some have T cells which suppress antibody secretion.
 - may have associated T-cell defects which worsen with age
 - Rx: gamma globulin injections, antibiotics

C. Cellular (T-cell) Immunodeficiency

1. Severe combined immunodeficiency disease (SCID)
 - lymphoid stem-cell defect leads to complete absence of T and B-cell immunity.
 - susceptible to any microbe.
 - oral candida, otitis, pneumocystis carinii, CMV, graft vs. host disease
 - 2 forms of inheritance
 - can be corrected completely with bone marrow transplantation
 - partial correction with fetal liver or thymus transplant
2. SCID variants
 - adenosine deaminase or nucleoside phosphorylase deficiency
 - cellular immunodeficiency with abnormal immunoglobulins (Nezelof's)
 - immunodeficiency with short-limbed dwarfism
 - Type I-combined
 - Type II-cellular immunodeficiency
 - Type III- humoral immunodeficiency
3. Wiskott-Aldridge Syndrome^{ca}
 - triad of eczema, thrombocytopenia, recurrent infection
 - high IgE
 - infection may not commence until age 6 mos.
 - eczema in usual locations, frequently secondarily infected
 - Rx: plasma, antibiotics, platelets. Marrow transplantation-1 case.
4. Chronic Mucocutaneous Candidiasis (CMC)
 - persistent Candida of mucus membranes, skin, hair, nails
 - 50% have associated endocrinopathy
 - 20% have positive family history.
 - narrow defect in T-cell mediated immunity (to Candida, sometimes T. rubrum and Epidermophyton floccosum)
 - varying severity, ie 1 fingernail vs. diffuse cutaneous
 - systemic Candidiasis does not occur because of otherwise intact host defenses
 - onset usually in infancy, but anytime in first 20 years
 - Lab: most tests WNL. Negative Candida skin test in 50%. Pts have antibody to Candida.

3. G6PD deficiency
 - similar clinically to CGD
4. Myeloperoxidase deficiency
5. Lazy-leukocyte syndrome
 - defective chemotaxis and neutropenia
 - abnormal random migration
 - susceptible to severe bacterial infection

E. Complement Deficiency

Complement is necessary for normal opsonization, bacterial killing, and neutrophil chemotaxis. However some component deficiencies are not associated with infection.

Evaluate complement system in patients with recurrent infection in whom you have already looked at everything else. CH50 is a good screening test.

C2 deficiency is the most common component deficiency.

Codominant inheritance.

Many pts. with homozygous C2 deficiency have SLE-like illness with prominent discoid lesions.

11) Complement defects manifest in the skin in several ways.

Patients with homozygous C6, C7, or C8 deficiency are unusually susceptible to meningococcemia or gonococcal sepsis. Lytic effect of these terminal components apparently essential for killing of Neisseria species. Patients who have these infections should be screened with a CH 50 or 100 which should be 0. If this is the case further studies to pinpoint the missing component are recommended, and studies of asymptomatic family members as well.

Also patients with homozygous C2 deficiency have a lupus like illness.

12) Last but not least the immunodeficient homosexual patients with AID.

Some of these patients have Kaposi's sarcoma as seen on the next slides. We have also seen unusual manifestations of infection in the skin as seen on the next slide

In patients with lesser severity of ID fungal skin infection has been common. Also have observed a high incidence of rashes to Bactrim which is administered for rx of Pcp, and of facial seborrhea.

CMC

° T cell arm has

- a deficiency of CMI to a specific organism - ~~blind~~, blind
- persistent, chronic candida infection (mucous membranes, scalp, skin, nails)
- majority of cases are sporadic, familial autosomal recessive forms exist
- does not represent complete tolerance to Candida
- pts make vigorous Ab response - precipitins
- etio and patho are unknown
- ~~association with endocrine disorders~~ paradox of autoimmunity / immunodef.

1st 1929 Thorne & Hankley - 54% ~~candidiasis~~ + hypopara

1978 Stadium classification

I. Early onset - most severe, C. granulomas may occur

50% endocrinops - usually hypothyroid

II Late onset - mildest

IV endocrine disorders predominate and may precede candida

~~mother & daughter - ↓ pituitary chromatin and ↑↑↑ ISE~~

Chin features

~~mucous membranes (mouth, esoph, vagina) nails skin less often~~

~~abnormal immune (↓ cortisol), hypopara, diabetes, hypothyroid, pernicious anemia
(autoab to parietal cells)~~

~~{ swallowing difficulties 2° esophagitis
hoarseness 2° larynx involvement~~

~~chronic vaginitis / candida described~~

~~- not sepsis~~

Lab

candida easily cultured, bx → superficial layers of epid or epith
lymphs, PC's, polyp

- autoab to endocrine organs - may precede detection of hormone defic.
diffuse lymphoid infiltration
- Ig's usually ↑, rare pt IgA deficient
- Nl. ab response to immuniz ~~̄~~ tet or typhoid vaccine

- candida skin tests + prolifer, ↓↓ MIF to candida even if not transformed.
- basophils - poor DNCB sensitization
- T cell #'s normal.
- PHA, MLR normal.

Rt

symptomatic { continuous antifungal drugs
amphotericin B
itraconazole
nails particularly affected

transfer factor - from Can + or Can - pts

Thymosin

levamisole - ↑ MIF in vitro, but no clinical

early onset rarely survive beyond 3rd decade (cardio, liver failure)

Pathogenesis

- 1) plasma factors which inhibit lymphocyte function
 - a) ↓ prolifer of all lymphocytes to Candida ag (Pataconi, 1971)
inhib development of Amphotericin B, also recovery of ST + candida prolifer
 - b) ↓ MLR and mitogen responses (Twomey 1975)
- 2) suppressor T cells - Ag specific induction in fungal diseases
also leprosy
- 3) endocrine pathologies not 2° to Candida infection - tendency toward autoimmunity
- 4) minimal congenital thymic defect - hypoplasia
rel to DiGeorge

Objectives for "Immunodeficiency States" Section
Immunology Minicourse, Daniel Freeman Memorial Hospital
June 4, 1981 - Michael S. Gottlieb, M.D.

List of Objectives:

1. To review the clinical manifestations of the most frequently encountered adult and pediatric immunodeficiency syndromes.
2. To describe current concepts of the pathophysiology of immunodeficiency states.
3. To present guidelines for the cost-effective laboratory assessment of the patient with suspected immunodeficiency.
4. To discuss the principles of management of patients with documented humoral and cellular immunodeficiency.

Test Questions for "Immunodeficiency States"
Immunology Minicourse, Daniel Freeman Memorial Hospital

Test Questions:

1. The most useful test in the evaluation of a patient with suspected antibody deficiency is:
 - 1) serum protein electrophoresis
 - 2) immunoelectrophoresis
 - 3) quantitative immunoglobulins by radial immunodiffusion
 - 4) challenge with typhoid immunization
2. Features of the Wiskott-Aldrich syndrome include:
 - 1) eczema
 - 2) neutropenia
 - 3) thrombocytopenia
 - 4) elevated IgE level
3. The most useful drug in the management of severe chronic mucocutaneous candidiasis is:
 - 1) amphotericin B
 - 2) mycostatin suspension
 - 3) ketoconazole
 - 4) transfer factor
4. Frequent causes of diarrhea in adult acquired hypogammaglobulinemia include:
 - 1) giardia
 - 2) bacterial overgrowth
 - 3) mucosal atrophy
 - 4) non-granulomatous jejunoileitis

Neurologie abn - Not mentally retarded
immunologie

DNA repair 1974 chromosome 14 q tandem
endocrine - diabetes, autoantibodies both long arms translocate to each other
at least 14 cases since then with no 14q

deletions of 5, 9 + transy
DNA repair defect

Sam Gotoff - burns in irradiated skin

"untoward responses to irradiation" in AT paper 8
irradiated AT fibroblasts don't repair well.

Painters + young PNAS 12/60
shortened mitotic delay
N=12

	%	
AdK ↑ acid phosph.	87	T-segg ↓ 25
En ↓	83	
IsG ₂ ↓	78	
AFP ↑ α ₂ Beto protein	75	
LA Dehyd ↑ LDH	75	
PMN chem	73	
IsA ↓	67	
CAMP/CGMP ↑	60	
capping ↑	58	
Smig ↑	58	
DNA repair ↓	45	
T cells	36	

Cancer is lymphoid - one T cell leukemia
not many others have been studied

T cells are $\downarrow$ but not in all pts - E rosettes
~~less~~ $\downarrow$ Con A, PHA $\downarrow$

T cell function does not clearly $\downarrow$ $\bar{c}$ ag
skin tests generally pretty good.

α $\left\{ \begin{array}{c} \delta 4 \\ \delta 3 \delta 1 \delta 2 E \delta 1 \alpha 2 \end{array} \right.$

Levinson raises E rosettes, correct/pla responses in
2 children, suggestion slowed development

AT 1 in 40,000

heterozygote 1:100 by calculation

↑ freq of ca

≡ Swift suggests ↑ ca among heterozygotes (parents) if true may be a common ca suspect trait

dx at ~ 5-6 yo, usually die by puberty

interesting model 1) 1° ID disease

2) ataxia - progressive neuro deterioration

Born ~ 1, sometime after walking begins ~ 3 yo → clumsiness in walking at 4-5 they have seen a neurologist - progressive ataxia in childhood is usually AT so neurologist makes dx

discovered 1926

rediscovered 1941 Madame Louis Bar

disease discovered by 2 pediatric neurologists in LA school septen
Sedgewick and Boda (LA) 1958

rediscovered old reports

noted - 150 autopsies

Purkinje layer in cerebellum very depleted - almost absent
+ dysplasia, small vestigial thymuses. Nuclei various
tissues (liver, myocardium) varied in size



seen in
elderly, MTX in liver
pts

suggestion that they age prematurely
= grey hairs =

chronic Staphylococcal carrier state

~30% of Caucasian pops are chronic carriers of staph a.

adult pt strains only ~ 1:10,000 develop chronic furunculosis
due to SA particularly pathogenic to that individual and sometimes to others
= chronic furum alone and no other symptoms unlikely
to have ID, but rather a chronic staph carrier
perhaps unorthodox to ^{readily avail} QIG's but not ^{the} more
sophisticated tests of neutrophil function
hidradenitis suppurativa, atopic dermatitis frequently
superinfected

tx difficult - occurs a long course of ab's
Maiden in ^{UC} EF good results by rx antib and ~~moderate~~
seeding skin at various sites = a non-path
SA str 502A. Colonized and 502A deep down
growth of prev. strain.

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ID is a topic which for better or worse

Bubble

As a result of increased public awareness

Tonite discuss clinical features, pathophysiology, rx of more common ID encountered in practice of adult medicine with emphasis on primary ID's.

Increased suscept to infection is

What should increase suspicion that you are dealing with an ID patient?

Chronic or recurrent infection, failure to respond to usual rx, unusual organisms.

Primary ID's Def.

Most are congenital, but with improved mgmt. and new antibiotics... like cystics

Relatively rare, however many systemic diseases are associated 300x

Evaluation of humoral ID. B cell or humoral immunity

Qlg's are single best and cost effective test for

not D

IEP and SPEP less adequate.

Pt. with borderline G.

Response to immuniz.

chronic sinusitis debb dx

allergic
VM thin
Aspirin
Polyps
CPES
Ciliary defect.

Adult acquired. (CVID) autoimmunity in families

Unpaired (but also lymphoma)

THERAPY

overgrowth

human immune globulin replacement therapy - COHN Fx II Ig traces of A.M.

IM-0.6cc/kg/3-4 weeks because of T 1/2 70Kg = 42ml/mo 10cc/wk 20 g 2wks

IVIG, subcu infusion pump.

antibiotics - spec on pts c bronchiectasis, sinus dist.
postural drg.

IgA

<5

ataxia distinguish

Hyper Ig E

ml upper limit 250

① Primary ID - DEF

T cell in wall

② Causes of Secondary

GRID 1

③ CLASSIFIC OF 1°

2

INFECTIONS IN
IMMUNE ABN'S IN

④ Evaluation of Humoral

CORTICOSTEROIDS

⑤ COMMON VARIABLE

NEUTROPHIL DISORDERS

⑥ GI INVOLVEMENT

⑥a Protein LOSING - causes

CGD

⑦ ISA DEFIC

COMPLEMENT eval

⑧ Hyper IgE

⑨ PHOTO 1

PHOTO 2

cell mediated - eval

CME FACE

CME KID

CME CLASSIFICATION

LAB EVAL OF CME

KETO

HANDS before

HANDS after

Nails before & after

COMBINED IMMUNODEFICIENCY DISEASES (Severe Combined Immunodeficiency (SCID),
Swiss-type Agammaglobulinemia, Cellular Immunodeficiency with
Abnormal Ig Synthesis (Nezelof's Syndrome)

Historical - 1950 1st description
Sibs with diarrhea, candidiasis, lymphopenia, decreased
lymphoid tissue.
1958 Definition of inheritance patterns autosomal recession.
1963 x-linked.
1968 Reconstitution with bone marrow transplant (Gatti).
1972 ADA deficiency - heterogeneity.
1973 Thymus transplant.
1978 Thymic epithelium

Pathogenesis - Varied

Constant feature of thymic hypo/dysplasia suggested thymic defect, but fetal thymus
transplant alone did not reconstitute.

Complete correction by BMT suggested absent lymphoid stem cell

but demonstration of thymic helper defect or intrathymic defect, and detection of
cells early in T-lineage in some patients is inconsistent with absent stem cell
idea.

Possible role of intrauterine infection interrupting normal stem cell differentiation

Clinical

Candida, Pneumocystis, CMV or measles pneumonia - bacterial pneumonias occur later
skin problems - fungal infections - resistant to therapy; intrauterine GVHD (erythro-
derma) hepatitis - CMV or other

Chronic diarrhea → malabsorption

Hematologic - neutropenia, red cell aplasia

Neurologic - chronic viral encephalopathy

Laboratory

Lymphopenia usually - though not necessary.

May have nl percentage E-rosettes, detectable PHA & MLC.

Some degree of normality does not necessarily indicate milder disease.

Absent proliferative response to antigens is a constant feature.

IgG <200; IgA & IgM non-detectable. Occasional hyper IgM.

Occ. monoclonal paraproteins - IEP may be useful

Lo ADCC (Sanal & Buckley, 1978)

Lo Clq levels.

Treatment

1. BMT - treatment of choice - HLA-A, B, C sibling and D match - 10/16 successful
with 6 mo. followup.
Only 1 of 19 D mismatched survived.
2 patients have received unrelated D matched - 1 survived.
2. Other approaches. Fetal thymus, fetal liver, liver and thymus, thymosin,
cultured thymic epithelium transplants, transfer factor.

THYMIC HYPOPLASIA (DiGEORGE SYNDROME)

Congenital immunodeficiency -- hypocalcemia seizures usually in 1st 24 hrs. of life
congenital heart disease
unusual facial configuration
infections-chronic nasal drainage, Pneumocystis
pneumonia, oral candida, diarrhea, "failure to thrive."
thymus and parathyroid absence or hypoplasia

Historical - 1829 Harrington - anatomic abnormalities and seizures
1965 DiGeorge - association with immunodeficiency
1968 Cleveland, August - fetal thymus transplants
1975 Lischer and Huff - partial syndromes

Pathogenesis - abnormal embryologic development of pharyngeal pouches during weeks 6-10 of embryogenesis.

Thymus develops from 3rd and 4th pouches during weeks 6-8.
Parathyroid from 3rd, 1st and external ear from 1st and 2nd,
pulmonary artery from 6th.

Spectrum of thymic deficiency (attached Table). Why? Degree of hypoplasia varies, and hypertrophy or further trophy can occur.

Probable deficiency of a "humoral" inducer substance.
T-cell reconstitution can be accomplished with fetal thymus transplant in a Millipore chamber-occurs as soon as 6 hrs. after implantation and is permanent. Suggests that the substance is not required continually-Steel, 1972.

Wara, 1975 and Wara and Ammann found that Thymosin Fraction 5 (bovine) increases the number of T cells in vitro. Suggests DiGeorge patients have an inducible substrate, but unclear if it is pre- or post-thymic.

Laboratory

Low serum calcium and elevated phosphorus (PTH deficiency).

Absent thymus shadow on xray.

Usually decreased rosette percentage (can be WNL "in partial").

PHA severely depressed to normal.

Depressed antibody response to immunizations, however nl infants often have poor responses at <3 mos. Hypogamma in several cases (Gatti, 1972).

Treatment

10-14 week old fetal thymus implanted in muscle or intraperitoneally, T cell reconstitution usually within 4 days. Permanent in 1 of 1st 2 patients with 6 yr. followup.

Millipore approach if thymus is >14 weeks to avoid GVHD.

Bovine thymosin may be useful.

cultured thymic epithelium led to partial reconstitution (Thong, 1978, Hong 1976)

Prognosis

Reasonable for correction of the ID. Congenital heart disease, hypopara, and frequently mental retardation are major problems.

TABLE 16-2. Summary of Selected Cases of Chronic Mucocutaneous Candidiasis without Endocrinopathy

Immunologic Studies											References
Age	Sex	Age of Onset	Antibody to Endocrine Organs	PHA*	MIF	D.H.† Skin Tests	Candida Skin Tests	Lymphocyte Response to Candida	Serum Candida Antibody	Beneficial Treatment	
17	F	1 yr.		+	Neg.	Neg.	Neg.	+	+	Lymphocytes	Chilgren et al., 1967, 1969
10	F	1 yr.				Neg.	Neg.		+	Amphotericin Marrow	Buckley et al., 1968
21	F	12 yr.		+		Neg.	Neg.				Marmor and Barnett, 1968
25	M	2 yr.		+		Neg.	Neg.	Neg.	+	Amphotericin	Hermans et al., 1969
25	F	?		+	Neg.	Neg.	Neg.	+	Neg.		Valdimarsson et al., 1970
9	F	1 yr.		+	+	Neg.	Neg.	Neg.	+		Goldberg et al., 1971
10	M	1 mo.	None	+	+	+	+	+	+		Kirkpatrick et al., 1971a
20	M	6 mo.	None		↓	Neg.	Neg.	↓	+		Kirkpatrick et al., 1971a
6	M	8 mo.	+	↓		+	Neg.	↓	+	Transfer Factor	Kirkpatrick et al., 1971a
26	F	3 yr.	None	+	Neg.	+	Neg.	↓	+	Lymphocytes	Kirkpatrick et al., 1971a
9	M	6 yr.	None	+	↓	+	+	+	+		Kirkpatrick et al., 1971a
23	F	8 yr.	+	+		+	+	↓	+	Amphotericin	Kirkpatrick et al., 1971a
16	F	10 yr.	None	+	↓	+	Neg.	+	+	Amphotericin	Kirkpatrick et al., 1971a
9	F	1 mo.	None	+	+	?	Neg.	+	+	Transfer Factor	Schulkind et al., 1972

PHA: phytohemagglutinin; MIF: migration inhibitory factor; D. H.: delayed hypersensitivity.

*Considered decreased if response was less than that of skin test positive controls.

†Response to antigens other than Candida (such as PPD, mumps).

TABLE 16-3. Summary of Selected Cases of Chronic Mucocutaneous Candidiasis with Endocrinopathy

TABLE 16-3. Summary of Selected Cases of Chronic Mucocutaneous Candidiasis														
Immunologic Studies														
Age	Sex	Age of Onset	First Abnormality	Endocrine Organs Involved or Assoc. Disease	Antibody To Endocrine Organs	PHA*	MIF	D.H. Skin Tests†	Candida Skin Tests	Lymphocyte Response To Candida	Serum Candida Antibody	Beneficial Treatment	Reference	
25	F	9 yr.	Endocrine	Parathyroid parietal cell				+	+		+		Chilgren et al., 1967	
25	M	6 yr.	Candida	None		+			Neg.	+	+	Lymphocytes	Chilgren et al., 1967	
13	F	8 yr.	Endocrine	Parathyroid				+	+		+		Chilgren et al., 1967	
17	F	6 yr.	Candida	Diabetes		+	Neg.	Neg.	Neg.	+	+	Amphotericin	Chilgren et al., 1969	
23	M	13 yr.	Endocrine	Adrenal		+	Neg.	Neg.	Neg.	+	+		Chilgren et al., 1969	
19	F	2 yr.	Candida	Chronic hepatitis, adrenal, thyroid	+				+			Amphotericin	Wuepper and Fudenberg, 1967	
4	F			Pituitary		+		Neg.	Neg.	+	+		Castells et al., 1971	
23	F	1 mo.	Candida	Thyroid	None	↓	↓	Neg.	Neg.	↓	+	Transfer factor	Kirkpatrick et al., 1971a	
18	M	1 yr.	Candida	Parathyroid	+	+	↓	Neg.	Neg.	+	+	Transfer factor	Kirkpatrick et al., 1971a	
24	M	2 yr.	Candida	Adrenal, thyroid, diabetes	+	+		Neg.	+	+	+		Kirkpatrick et al., 1971a	
16	M	2 yr.	Candida	Adrenal	None	↓		+	Neg.	↓	+		Kirkpatrick et al., 1971a	
18	M	3 yr.	Candida	Adrenal	+	↓		+	Neg.	↓	+		Kirkpatrick et al., 1971	
14	M	1 yr.	Endocrine	Pernicious anemia, adrenal	+	↓		Neg.	Neg.	↓	+	Thymus transplant	Levy et al., 1971	

PHA: phytohemagglutinin; MIF: migration inhibitory factor; D. H.: delayed hypersensitivity.

*Considered decreased if response was less than that of skin test positive controls.

†Response to antigens other than Candida (such as PPD, mumps).

TABLE 16-4. Clinical and Genetic Classification of Chronic Mucocutaneous Candidiasis*

Group Type	Severity of Candidiasis	Endocrinopathy	Inheritance
I. Early-onset chronic mucocutaneous candidiasis	Moderate-severe	Common	Sporadic
a. <i>Candida</i> granuloma	Severe	Common	Sporadic
II. Late-onset chronic mucocutaneous candidiasis	Mild	Rare	Sporadic
III. Familial chronic mucocutaneous candidiasis	Mild-moderate	Rare	Autosomal recessive
IV. Juvenile familial polyendocrinopathy with candidiasis (<i>Candida</i> -endocrinopathy syndrome)	Mild-moderate	Always	Autosomal recessive

*From Stiehm, E. R., et al.: *Ann. Intern. Med.*, 89:91-106, 1978.

trick et al., 1971a; Valdimarsson et al., 1970). In general, the laboratory findings suggest an immunologic defect more severe than the clinical symptoms would indicate.

Antibody defects are unusual in CMC. These include isolated IgA deficiency (Claman et al., 1966; Aralanantham et al., 1979), deficiency of serum candidacidal activity (Louria, 1967; Chilgren et al., 1967; Wuepper and Fudenberg, 1967; Lonsdale and Gavan, 1970), and deficient IgG (but normal IgM) response to diphtheria immunization (Cahill et al., 1974).

Some CMC patients have plasma factors that inhibit lymphocyte function. Paterson et al. (1971) reported a plasma factor in a patient with CMC which inhibited proliferation of normal lymphocytes to *Candida* antigen. The inhibitor disappeared after amphotericin B therapy, and its loss was associated with the development of a positive skin test and a normal *in vitro* proliferative response to *Candida* antigen. Twomey et al. (1975) studied a similar inhibitor and showed that it also inhibited lymphocyte responses to mitogens or allogeneic cells.

Defective regulatory mechanisms may be present in CMC. Stobo et al. (1976) reported the presence of antigen-specific suppressor T cells in patients with other chronic fungal infections. By contrast, Aralanantham et al. (1979) found deficient non-specific suppressor function in two children with the *Candida*-endocrinopathy syndrome.

One anergic CMC patient had abnormal mononuclear leukocyte chemotaxis to C5a and abnormal production of lymphocyte-derived chemotactic factor (Snyderman et al., 1973). Another CMC patient has also been reported with macrophage dysfunction and a plasma inhibitor (Twomey et al., 1975). Rosenblatt et al. (1979) noted defective monocyte-mediated antibody-dependent cellular cytotoxicity (ADCC), using human erythrocytes as targets, in several severe CMC patients. A mother and daughter with CMC have defective neutrophil chemotaxis and marked elevation of IgE (Van Scoy et al., 1975).

The variability of the laboratory abnormalities in CMC and their frequent return to normal after antimicrobial treatment suggests that many are epiphenomena which do not represent the basic immunologic defect.

The endocrinopathies are not a result of *Candida* infection of endocrine organs. Repeated attempts to culture *Candida* or to demonstrate *Candida* organisms histologically in involved organs are unsuccessful. The endocrinopathies are more likely secondary to the tendency toward autoimmunity. A triad of thymoma, candidiasis, and myositis has been reported (Montes et al., 1972). Autoantibody formation is particularly common in the *Candida*-endocrinopathy syndrome (Blizzard and Gibbs, 1968) and may precede overt endocrine deficiency.

An attractive unifying hypothesis suggests an autoimmune disorder directed at the lymphoid apparatus, leading to a cell-mediated defect that in turn results in chronic candidiasis and progressive endocrinopathy. Another hypothesis suggests that the entire syndrome is a manifestation of a minimal congenital thymic defect.

Clinical Manifestations. Stiehm (1978) has classified CMC into four clinical variants (see Table 16-4). The most severe and typical form is early onset CMC; *Candida* granuloma may occur in this form, and about 50 percent of the patients have an endocrinopathy, usually hypothyroidism. Late-onset candidiasis is the mildest form and may be limited to paronychia or buccal mucosal involvement. A third form is familial candidiasis, an autosomal recessive disorder in which endocrinopathies are uncommon. The fourth form is the autosomal recessive juvenile familial polyendocrinopathy with candidiasis, the *Candida*-endocrinopathy syndrome. In this syndrome, the endocrine disorder (usually hypoparathyroidism) predominates and may even precede the onset of the *Candida* infections.

The *Candida* infection is most commonly present on the oral mucous membranes. Fingernails and toenails are frequently involved and, less commonly, the skin of the face, hands, or feet (Figs. 16-25,

Polymyxin B Reactions, IgE Antibody, and T-Cell Deficiency

Immunochemical Studies in a Patient after Bone Marrow Transplantation

JAMES D. LAKIN, Ph.D., M.D., DOUGLAS M. STRONG, Ph.D., and KENNETH W. SELL, M.D., Ph.D.,
Bethesda, Maryland

A patient with aplastic anemia was immunosuppressed with cyclophosphamide and transplanted with allogenic bone marrow. While lacking demonstrable T-cell activity posttransplantation, he developed a generalized macular erythematous eruption and fever, clinically attributed to intranasal polymyxin B. A specific IgE antibody, demonstrated by direct skin testing, Prausnitz-Küstner passive transfer, and indirect passive hemagglutination was temporally related to the reaction. Discontinuation of the drug led to prompt defervescence and resolution of the drug eruption.

HYPERSENSITIVITY to the polypeptide antibiotic polymyxin B sulfonate is a rare clinical occurrence (1). To our knowledge, documented immediate allergy to polymyxin had not been recorded in the literature before our recent investigations of an IgE-mediated type-1 hypersensitivity reaction. The reaction occurred in a functionally T-cell suppressed patient after bone marrow transplant who received repeated intranasal aerosolization of the material (2). Subsequently, a second and alternate form of adverse reaction to polymyxin B has been observed after bone marrow transplant in a patient who also received the antibiotic by intranasal insufflation.

Case History

A 15-year-old white boy had developed acute infectious hepatitis with impending hepatic coma in November 1973. He was treated with neomycin and prednisolone. His course was complicated by *Staphylococcus aureus* sepsis, treated with a 5-week course of methicillin. Progressive, persistent depression of all formed elements of the blood was noted and a bone marrow biopsy showed severe hypoplasia. The patient was given oxy-methalone, prednisone, folic acid, and multivitamins. His ane-

mia did not improve, and he was transferred to the National Naval Medical Center on 9 March 1974 for bone marrow transplantation. His sister, the potential donor, was HL-A identical to the patient on histocompatibility testing. History of the patient and his immediate family was negative for atopy. On admission, the leukocyte count was 300/mm³; platelets, 15 000/mm³; hemoglobin, 8.5 g/dl; hematocrit, 25 volume %. Results of immunologic studies were as follows: IgG, 1525 mg/dl; IgA, 170 mg/dl; IgM, 125 mg/dl. On delayed skin testing the patient showed 8 mm of duration at 48 hours to PPD-S, 5 tuberculin units. Mixed lymphocyte culture and cell-mediated lymphocytotoxicity assays showed T-cell activity against a standard lymphoblast culture equal to normal positive control levels. The patient's prednisone was discontinued the next day. He was maintained with platelet, leukocyte, and erythrocyte infusions.

The patient was placed in a "germ-free" laminar flow unit on 26 April 1974 and subjected to a regimen of oral vancomycin, gentamicin, and nystatin; skin cleansing with Betadine®, vancomycin, polymyxin B, and neomycin solutions; oropharyngeal sprays of solutions of vancomycin, polymyxin B, neomycin, and nystatin to eradicate the endogenous flora. A 4-day immunosuppressive course of cyclophosphamide, 1.9 g/m² body surface area per day, was administered, followed by infusion of 2.1×10^6 of his sister's bone marrow cells. On the next day and again 1 and 6 weeks later, methotrexate, 20 mg, was given intravenously. Sixteen days after marrow infusion (18 May 1974), a generalized macular erythema, blushing easily with pressure, was observed (Figure 1A) with fever to 38.8 °C. No T cells could be shown in the peripheral blood by rosette formation. Repeat delayed skin test battery was completely negative. Bone marrow biopsy showed continuing aplasia without evidence of engraftment. Total serum IgG, IgA, and IgM were normal. Direct scratch testing was done on the patient with solutions of polymyxin B, vancomycin, neomycin, kanamycin, gentamicin, and Betadine at 1 mg/ml each. A strongly positive wheal and flare reaction to polymyxin B was noted at 20 minutes. Patch testing to these solutions was negative at 48 and 72 hours. Polymyxin B was omitted from the regimen after 20 May 1974, at which time the eruption had progressed to discrete erythematous maculopapular lesions (Figure 1B). Over the next 72 hours, the patient defervesced and the rash resolved. A repeat bone marrow biopsy on 30 May 1974 was aplastic, the initial marrow transplant having failed to engraft. Three weeks later a second, subsequently successful marrow transplant was done, using the same donor. The patient is currently doing well 8 months posttransplantation.

► From Research Work Unit No. MR041.06.01.0025AYGI, the Clinical Immunology Division and the Tissue Bank Division, Department of Clinical and Experimental Immunology, Naval Medical Research Institute, Bureau of Medicine and Surgery, Navy Department, Bethesda, Maryland.

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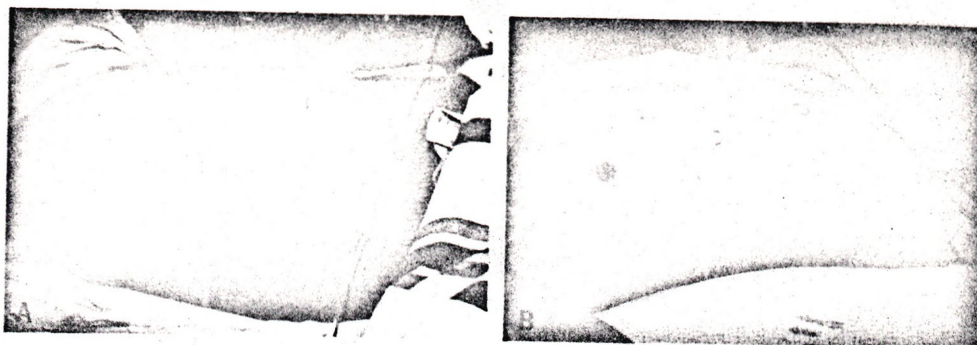
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Figure 1A. Initial dermal eruption noted in association with polymyxin B administration. B. Dermal eruption 4 days after onset.



Materials and Methods

POLYMYXIN-BOVINE SERUM ALBUMIN AND POLYMYXIN-KEYHOLE LIMPET HEMOCYANIN PREPARATION

Polymyxin was conjugated to bovine serum albumin and to keyhole limpet hemocyanin as previously described (2).

PRAUSNITZ-KÜSTNER SKIN TESTING

Passive transfer skin testing was done in normal adult male chimpanzees using the method as adapted for the rhesus monkey (3).

MEASUREMENT OF HUMAN IGE ANTIPOLYMYXIN B ANTIBODY BY INDIRECT HEMAGGLUTINATION

Polymyxin-coated sheep erythrocytes were prepared and reacted with patients' serum as previously recorded. IgE antibody specific for polymyxin B was measured by a micro-hemagglutination method developed by our laboratory for this purpose (2, 4). Briefly, fresh sheep erythrocytes were treated with chromic chloride, coated with polymyxin, and reacted with serial twofold dilutions of patients' serum. The cells were then washed three times and reacted with an equine antiserum that, after adsorption, has been shown to be highly specific for epsilon-chain determinants and devoid of detectable cross-reacting anti-light chain activity (2).

Results

PRAUSNITZ-KÜSTNER PASSIVE TRANSFER OF REAGINIC ACTIVITY

To confirm that the positive immediate skin test to polymyxin B was indeed mediated by specific reagin rather than by nonimmunologic mast-cell degranulation and histamine release, Prausnitz-Küstner passive transfer to normal chimpanzees was done. When challenged at 48 hours with free polymyxin B, 50 µg intradermally, the sites showed immediate wheal and flare reactivity to a titer of 1:50. Serum samples obtained 4 weeks before the development of the generalized maculopapular eruption and 8 weeks after the episode were found to contain no skin-sensitizing activity. Control sites previously having been injected with normal human serum were negative when challenged with this concentration of antigen (Table 1).

MEASUREMENT OF IGE ANTIPOLYMYXIN ANTIBODY

Using indirect passive hemagglutination, IgE antibody specific for polymyxin B was demonstrable during the period in which the patient experienced his generalized eruption. Samples of serum obtained 4 weeks before and 8 weeks after this period were devoid of IgE antibody directed against the antibiotic, correlating with those results obtained with Prausnitz-Küstner testing.

A serum sample obtained from the bone marrow donor at the time of marrow removal was also negative for IgE antipolymyxin B antibody. Likewise, blood component donors' serums did not contain detectable IgE antipolymyxin activity (Table 1).

Discussion

Immunologically mediated adverse reactions to polymyxin B have rarely been reported. Intrabronchial aerosolization of the antibiotic has been associated with bronchoconstriction and severe hypoxia (5). The mechanism of this reaction was unclear, however, as polymyxin B is a well-documented nonspecific mast cell-degranulating and histamine-releasing agent (6). To our knowledge, the only previously documented type-1 hypersensitivity reaction to polymyxin B associated with specific IgE antibody was presented in our recent study (2). That patient, an acute myelocytic leukemic in relapse, was transplanted with related-donor bone marrow after whole body irradiation and rabbit antithymocyte globulin administration. This produced a state of functional T-cell suppression during which she developed increasingly severe bouts of rhinorrhea, perinasal and periorbital pruritis, erythema, and edema immediately after intranasal insufflation of low-concentration polymyxin B. The reaction was temporally associated with specific IgE antibody (2).

The classic dermal reactions produced by combination of IgE antibody and specific antigen are those of urticaria

Table 1. IgE Antipolymyxin B Antibody Levels Measured by Prausnitz-Küstner Skin Testing and Indirect Hemagglutination

Source	Date	Titer	
		Prausnitz-Küstner Skin Testing	Indirect Hemagglutination
Patient	26 April 74	0	0
	22 May 74	1:50	1:64
	29 July 74	0	0
Marrow donor	26 April 74	0	0
Normal human serum		0	
Phosphate buffered saline		0	
Blood component donors			
1			0
2			0
3			0
4			0
5			0
6			0

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COMPLEMENT DEFICIENCY STATES

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In recent years the study of complement deficiency states has revealed that deficiency of specific complement components may predispose to certain diseases, much like the predispositions that occur with selective deficiencies of the immune system. In addition to the hereditary deficiencies of complement, there are acquired deficiencies of complement which persist over long periods and which also appear to predispose to certain diseases. It is the purpose of this review to discuss both the hereditary and acquired complement deficiency states of man.

The recent advances which have occurred in this field can be directly related to the rapid expansion of our understanding of the complement system. There have been major advances in the isolation and study of the biochemistry of individual complement components, delineation of the various pathways of activation, and elucidation of the biological activities associated with complement activation (10, 83, 84, 100, 128). To facilitate the discussion of complement deficiency states, a brief summary of the complement system with emphasis on its biologic activities will be given.

Activation of the Complement System

The complement system consists of a series of discrete plasma proteins which are present as inactive precursors until they are activated by highly specific biochemical reactions, some of which involve limited proteolytic cleavage. Thus far 19 components have been isolated and characterized (Table 1). There are two known pathways of activation, the classical and the alternate or properdin pathway. The classical pathway is more thoroughly characterized (Fig. 1). It is activated by antigen-antibody complexes. The C1 component consists of three subunits, C1q, C1r and C1s, which are held together in the presence of calcium ions. C1q contains the combining site for a specific receptor on the Fc portion of IgG1, IgG2, IgG3 and IgM. These immunoglobulin molecules, when complexed to an-

tigen, will fix and activate C1q. The activation does not result in cleavage. However, its fixation does activate an enzymatic activity in C1r that cleaves C1s and thereby generates the first major enzyme in the complement system, C1 or C1 esterase as it is commonly called. A serum inhibitor for this enzyme exists, the C1 esterase inhibitor. C1 then cleaves C4 and C2 whose major cleavage products form a bimolecular complex, C4b2a which has a new enzyme activity. This is the classical C3 convertase, which is capable of splitting C3 into a small fragment C3a and a larger fragment C3b. The attachment of C3b to the C4b2a complex leads to the formation of the last enzyme in the classical pathway, C4b2a3b, which is capable of binding and cleaving, C5 (73, 85). Following the binding of C5b, the remainder of the terminal components of the complement sequence are assembled without enzymatic activation.

The alternate pathway of complement activation was first described in 1954 by Pillemer and his colleagues as a mechanism whereby complement could be activated without the presence of antigen-antibody complexes. Zymosan, a yeast cell wall polysaccharide was found to activate C3 and the terminal components of complement without utilization of C1, C4, or C2 (96). Pillemer thought that this pathway may represent a natural defense system against foreign organisms in the unimmunized host. In the last few years intensive renewed study of this pathway has confirmed many of his original observations and impressions. The alternate pathway does appear to be a mechanism for activation of the complement system by yeast and bacterial wall components as well as possibly other substances without the presence of specific antibody (47, 51, 69, 84). A number of components of this pathway have been isolated and identified (Table 1), including properdin. The pathway has not as yet been completely delineated and for the purpose of this discussion a simplified scheme (Fig. 1) is presented. The activating substance, which may be zymosan, a variety of polysaccharides including bacterial lipopolysaccharides, aggregated IgA or aggregated IgE, interact with a serum protein called the initiating factor (IF), which appears to be identical with C3NeF or nephritic factor (77, 125). This protein was originally identified in the serum of patients with mesangiocapillary glomerulonephritis and lowered serum levels of C3 (123). Following the interaction of this complex with Factor B, Factor D, C3 and C3b, a C3 convertase is formed, C3bB. This enzyme, like the classical C3 convertase, can split

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C3 into C3a and C3b. A most unusual aspect of this pathway is that the product of the substrate cleavage is incorporated into the cleavage enzyme establishing a potential positive feedback system (86). A serum inhibitor, the C3 inactivator (C3INA), can cleave C3b into C3c and C3d. These fragments cannot participate in the formation of a C3 conver-

tase. The C3bB enzyme combines with properdin to form a new enzyme activity, C3bBP, which can cleave C5. As in the classical pathway, this in turn activates the terminal components of the complement sequence.

Biologic Activities of the Complement System

The major biologic activities associated with various complement components are shown in Table 2. These activities are generated as a result of activation of the complement system. All nine components of the classical pathway are required for cytolysis to occur. A less efficient cytolytic process can be generated by activation of C5-C9 via the alternate pathway. The actual lysis of the membrane is a function of the C8 and C9 molecules, C8 resulting in slow lysis while the addition of C9 markedly enhances the process (118). While cytolysis appears to be the main function of the complement system in that a complete sequence of activation of the components is required, it is by no means the most important biologic activity that is mediated by the complement system.

It is apparent from the serum concentrations of complement components (Table 1) and the scheme shown in Figure 1 that C3 is the key complement component in both pathways of activation. The split product of C3, C3b, mediates a major biologic function of the complement system, opsonization. This results in enhancement of phagocytosis. Since opsonic activity can be generated in the absence of specific antibody by cell wall components of microorganisms, it is a potential defense system in the unimmunized host.

Other defense mechanisms may be mediated by the early components of the complement system. *In vitro* studies demonstrated that C1 and C4b are sufficient for neutralization of certain viral orga-

TABLE 1
Molecular Weights and Serum Concentrations of Complement Components (83)

Component	Molecular weight	Serum concentration $\mu\text{g/ml}$
Classical		
C1q	400,000	180
C1r	180,000	?
C1s	86,000	110
C2	117,000	25
C3	180,000	1,600
C4	206,000	640
C5	180,000	80
C6	95,000	75
C7	110,000	55
C8	163,000	80
C9	79,000	230
Alternate		
Properdin	184,000	25
Properdin Factor B	93,000	200
Properdin Factor D	24,000	?
Initiating Factor (IF)	150,000	?
Inhibitors		
C1	90,000	180
C3b Inactivator	100,000	25
C6	?	?
C7	?	?

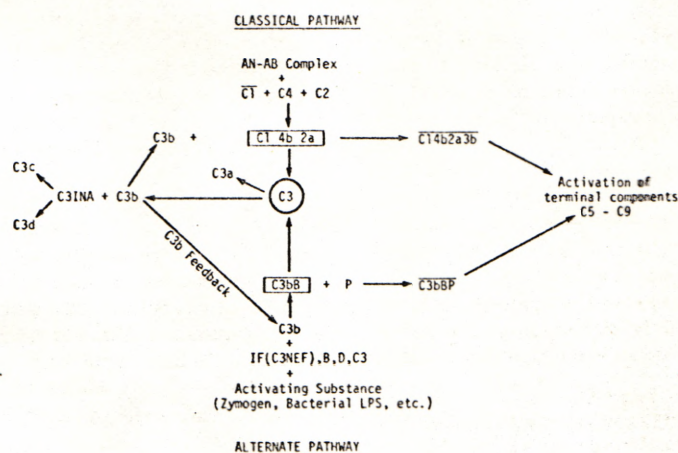


FIG. 1. Simplified scheme of the classical and alternate pathways of complement adopted from (77). The pathways merge on C3. The complexes shown in boxes are analogous C3 convertases.

- 1) Enhancer
- 2) Viral neu
- 3) Viral lysis
- 4) Enhancer
- 5) Mediator
- a) Chem
- b) Anaph
- c) Enhanc
- d) Marro
- 6) Cell lysis
- 7) Modulator

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TABLE 2
Major Biologic Activities of Complement Components

	Component
1) Enhancement of phagocytosis (opsonization)	C3b
2) Viral neutralization	C1 C4b
3) Viral lysis initiation	C1q
4) Enhancement of yeast phagocytosis	C5b
5) Mediators of Inflammation	
a) Chemotactic factor	C3a, C5a, C5b67
b) Anaphylotoxin	C3a, C5a
c) Enhancer of vascular permeability	? C2 fragment (C2 kinin)
d) Marrow leukocyte release	C3b
6) Cell lysis	C8, C9
7) Modulators of immune response	?C4b, ?C3b, ?C3d

nisms coated with non-neutralizing antibodies (23). Potentially, an important mechanism for the unimmunized host was suggested by studies in which oncornaviruses were lysed by human complement without antibody (21). C1q appears to react directly with these viruses. Other studies have demonstrated that C5b enhances phagocytosis of yeast (80).

Other biological activities are mediated by cleavage products of the early components. Patients with hereditary angioneurotic edema produce a peptide, perhaps by cleavage of C2, that enhances vascular permeability (34). A small fragment from C3 cleavage, C3a has both chemotactic activity and anaphylotoxin activity (31, 127). C5a also has relatively weak anaphylotoxin activity, but potent chemotactic activity (57, 72). Since there is no enzymatic cleavage of 6, 7, 8 and 9, there are no split products produced. However, the complex C5b67 is thought to have chemotactic activity in certain pathological situations (133).

Hereditary Deficiencies of the Complement System

Thus far, hereditary deficiencies have been described for 9 of 11 of the classical components and 2 inhibitors (Table 3). None so far has been described for components unique to the alternate pathway.

C1q Deficiency

Genetic deficiency of C1q has not been clearly established. C1q has been reported to be absent or depressed in severe combined immunodeficiency (Swiss type of lymphopenic agammaglobulinemia) (87). Recent studies of this disease showed that C1q as well as gamma globulin levels were restored following bone marrow transplantation (12). However, turnover studies were not done and the possibility

TABLE 3
Clinical Findings in Human Complement Deficiency States

Component	Clinical findings
C1q?	Combined Immunodeficiency
C1r	SLE, Glomerulonephritis
C1s	SLE
C4	SLE
C2	None, Discoid LE, SLE, Anaphylatoxin Purpura, Hodgkins, Dermatomyositis, Glomerulonephritis
C3	Infections
C5	SLE
C5 Dysfunction?	Infection and Eczema (Leiner's syndrome)
C6	None, Infections
C7	None, Raynaud's
C8	Disseminated Gonococcemia, SLE
C1 INH	Hereditary angio-edema, SLE
C3b INA	Infections

exists that the C1q levels vary directly with the gamma globulin levels, as indicated by Kohler's studies (61). Kohler showed an increased catabolism of C1q, probably secondary to low IgG levels, in adult patients with common variable immunodeficiency. There has not been any report of patients with selective C1q deficiency.

C1r Deficiency

There are three known cases of selective C1r absence in two kindreds. There appears to be an autosomal pattern of inheritance of the C1r deficiency (C1rD) gene. However, on pedigree analysis expected heterozygotes do not uniformly have C1r levels in the 50% of normal range (10). No linkage between the HLA system and the C1rD gene was found, in contrast to the linkage with HLA and other complement components (30).

In one kindred, two siblings were homozygous for C1rD (82). Other complement components were normal except for C1s which in all three homozygous C1r deficient patients has been found to be approximately 50% normal. The proband in this family, a 16-year-old Puerto Rican boy, had an illness suggestive of systemic lupus erythematosus (SLE) with fever, skin lesions, vasculitis and arthralgias. Nephritis was not present clinically; however, renal biopsy showed mild mesangial deposits typical of immune complexes by electron microscopy, and immunofluorescent studies demonstrated immunoglobulins and C3 along the basement membrane. The severe skin lesions which clinically were typical for discoid lupus erythematosus (DLE) did not, however, have the deposits of immunoglobulins and complement usually found in immunofluorescent studies of DLE lesions.

The second sibling in this family with complete absence of C1r, a sister to the proband, had a history of arthralgias and skin rash. A skin biopsy in this case also was negative for immunoglobulin and complement deposits. Both siblings had histories of frequent chronic infections consisting of otitis media and upper respiratory infections. An additional sibling died at age 12 with "SLE."

A third case of complete C1r deficiency which has been reported was an 11-year-old female with chronic glomerulonephritis (95). Biopsy studies done at the time of end stage disease showed deposits of IgG, C1q, C2 and C3. Following transplantation, the selective C1r deficiency persisted.

C1s Deficiency

There are five known cases of selective C1s absence in two kindreds which have been reported, thus far only in abstract form. The first case is a 6-year-old child with SLE (97). An hereditary basis for the C1s deficiency was not established. At the time of these studies quantitative C1s studies were not available; however, C1q and trace C1r were noted. The deficiency was most likely a selective deficiency of C1s since C1s restored hemolytic activity while C1q or C1r did not. Clinically, there were no features atypical for SLE noted. The ANA was positive and immunoglobulins were detected in the basal membrane of the skin.

In the second kindred there are four siblings with selective absence of detectable C1s (17).

Two are normal and the others, a male and female, have lupus-like disease consisting of polyarthritis, alopecia, discoid lupus, Raynaud's phenomenon and positive antinuclear antibodies. LE cell tests were negative. An additional sibling with normal complement levels has a similar syndrome. Two other siblings died in childhood of infectious diseases (infantile enterocolitis and acute meningitis).

C1 Inhibitor Deficiency

The genetic deficiency of C1 inhibitor is associated with the syndrome of hereditary angioneurotic edema (HANE) which consists of recurrent bouts of noninflammatory swelling which may involve the subcutaneous tissues, intestinal walls, airways, and lungs. Laryngeal edema may often be fatal and intestinal involvement may result in painful abdominal crises often with obstruction (35).

The deficiency has been thought to be the most common genetic abnormality in the complement system. However, this may be due to the prominent associated clinical manifestations. The actual gene frequency is not known. The lack of detections of individuals homozygous for this defect suggests that gene frequency may actually be lower than other complement deficiency states which are not as clinically apparent.

The C1 inhibitor inhibits the enzymatic activity of C1 component including the subunits C1r and C1s. It also inhibits a number of other enzymatic activities in other plasma protein systems (58, 99). There include kallikrein, Hageman factor and its active fragments, plasma thromboplastin antecedent and permeability factor of dilution (PF-dil). The inhibition of C1 results by formation of an irreversible complex with C1s. No split products result from this interaction. The inhibitor does not react with denatured C1s, suggesting a major role for conformational factors in the complex formation (54).

Two major forms of the genetic deficiency exist (102, 103). In approximately 85% there is low serum concentration of the inhibitor by both functional and antigenic assay. In the variant form, which comprises approximately 15% of cases, there is normal or elevated concentration of a functionally inactive inhibitor. The abnormal inhibitor shares similar antigens with the functional protein; however, it has a molecular weight of 109,000 compared to

105,000 for the normal complex is the same.

The genetic deficiency of C1 inhibitor is understood to be a hereditary autosomal recessive disorder. There is no simple clinical test for the deficiency. The high levels in the blood have been reported.

The pathogenesis is not entirely understood. It is known that the deficiency is caused by a variety of factors. Trauma, which causes tissue damage, of Hageman factor, kininogen, the gene is activated by the (EACA) inhibitors, attacks of 111).

During development cannot be identified. There are chronic solute C1 deficiency severity drops in activation. C4 is not C1 inhibitor ruled out. The recent release is molecularly increased. It is present in evidence peptide.

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105,000 for the normal inhibitor and does not complex with C1s (54). The clinical syndrome is the same in both forms.

The genetics of this disorder is not completely understood. Pedigree analysis indicates an autosomal dominant pattern of inheritance with all affected members being heterozygous (39). There is evidence for diminished synthesis of the inhibitor protein in the common form but a simple deficiency of a structural gene controlling C1 inhibitor synthesis does not account for the highly variable levels of C1 inhibitor that occur in these patients including undetectable levels in some. A disorder of a regulatory gene has been postulated (112).

The pathophysiology of the disease is also not entirely delineated. Attacks are accompanied by activation of C1; however, it is not known how bouts of disease activity are initiated. Since multiple plasma enzymes are inhibited by the C1 inhibitor, in addition to C1s, a variety of potential activating pathways exist. Trauma, particularly dental work, is known to precipitate attacks. One pathway which has been postulated is that trauma causes tissue damage that results in activation of Hageman factor which triggers clotting, kinin generation and fibrinolysis resulting in the generation of plasmin which is known to activate C1 (93). This hypothesis is supported by the fact that epsilonaminocaproic acid (EACA) and tranexamic acid, two plasmin inhibitors, provide effective drug therapy in attacks of hereditary angioneurotic edema (41, 111).

During attacks, patients with HANE usually develop detectable levels of free C1s which cannot be found in the circulation of normal individuals. C4 and C2, the substrates of C1s, are chronically depressed in most patients. Absolute C4 levels do not correlate well with the severity of disease activity; however, relative drops in C4 levels can be demonstrated with activation of the disease. It can be noted that C4 is never normal during attacks and if both C1 inhibitor and C4 are normal, HANE can be ruled out (39). How activation of early component relates to the pathophysiology of the disease is not yet clear. It appears that a low molecular weight peptide which can cause increased vascular permeability and angioedema is present in the serum during attacks. There is evidence, although not conclusive, that this peptide is derived from C2 (33). Because of this

and the kinin-like activity of the peptide, the fragment has been termed C2 kinin.

C4 Deficiency

C4 deficiency has been extensively studied in the guinea pig (38, 40); however, only one fully reported case is known in man and another recently has been reported in part. The proband of the family reported in detail was an 18-year-old female with typical clinical findings of SLE including extensive rash (55). There was complete absence of C4, with other complement components being normal. Addition of isolated C4 to the serum restored the hemolytic activity. The mother, who is healthy, had half normal C4 levels by antigenic analysis suggesting an autosomal codominant type of inheritance for the C4 deficiency (C4D) gene. Serologically the SLE present in the proband was atypical in that no LE cells were present. The ANA was negative and no immunoglobulins were deposited in the skin. HLA studies on this family have shown linkage of the C4D and HLA genes (101).

The second case, apparently a homozygous C4 deficiency, is a five-year-old boy with a "lupus-like syndrome" (19). Antinuclear antibodies were present. Clinically he did not have increased susceptibility to infection, although certain *in vitro* chemotactic and opsonic functions were abnormal.

C2 Deficiency

C2 deficiency appears at present to be the most commonly occurring complement component deficiency. Although the evidence is as yet incomplete, the gene determining this deficiency, C2D, appears to be inherited as a codominant with the gene(s) determining C2 synthesis. The gene frequency is estimated at 1% based on the finding of five heterozygous C2D individuals among 250 normal blood donors (44) and one homozygote among 10,000 normal blood donors (5).

It now appears that C2 deficiency is associated with certain disease states. This was not, however, apparent from the first four kindreds reported (Table 4). In 21 of the next 22 kindreds studied the proband had a disease state (Table 4). The studies of 26 families with homozygous C2 deficient probands are listed in Table 4. The clinical findings among the individuals homozygous for C2D are shown in Table 5. Twenty-three of 38 have diseases mainly of the

TABLE 4
HLA Types and Clinical Findings in Kindreds of Hereditary C_2 Deficiency

	Homozygous individuals		HLA			Clinical findings	Number heterozygous individuals		Clinical findings
	Age	Sex	A	B	D		F	M	
1) M. R. Klemper et al 1966 (60)	45	M				Normal	10	5	Normal
	43	F		N.R.		Normal			
	40	M				Normal	1	2	Normal
2) M. R. Klemper et al 1967 (59)	40	M		N.R.		Normal	1	2	Normal
3) N. R. Cooper et al 1968 (20)	7	F		N.R.		Normal	4	5	Normal
4) S. Ruddy et al 1970 (107)	19	M				Normal			
J. Kumate 1976 (63)	12	M		N.R.		Normal			
	10	F				Normal	3	2	Normal
5) V. Agnello et al (3)	55	F	10	18	W2	SLE with Discoid	15	16	Normal
6) N. K. Day et al 1973 (26)	18	M	10	18	W2	SLE with Discoid			
			2	4A2	W2				
7) M. Sussman et al 1973 (119)	24	F*		N.R.		Anaphylactoid Purpura	—	—	—
8) J. Wild et al 1974 (131)	31	F		N.R.		Normal	2	2	Normal
	37	M		N.R.		Discoid LE	—	—	—
9) E. Gelfand et al 1974 (46)	11	M*		N.R.		Anaphylactoid Purpura	—	—	—
10) N. K. Day et al 1974 (27)	18	M		N.R.		Normal	3	2	Normal
11) J. Leddy 1974 (5)	25	F		N.R.		Idiopathic Membranous GN	5	6	Normal
12) K. Osterland et al 1975 (90)	22	F	10	18	W2	SLE with Discoid			
J. Atkinson et al 1975 (11)	65	F		N.R.		Discoid LE	3	2	Normal
13) J. Leddy et al 1975 (66)	50	M		N.R.		Normal			
	60	M				Dermatomyositis	1	2	Normal
14) L. Einstein et al 1975 (37)	10	M	10	18	N.R.	Normal			
	13	F	1	17	N.R.	Anaphylactoid Purpura	—	—	—
15) P. Friend et al 1975 (42)	44	F*	10	18	W2	Chronic Vasculitis	2	6	Normal
16) K. Wolski et al 1975 (132)	17	F	3	5		SLE			
17) N. Day et al 1976 (28, 29)	2	M	W24	18	W2	Normal	1	2	Normal
			2	18					
	24	M	W24	18	N.R.	Hodgkins Disease			
			2	18					
18) D. Gibson et al 1976 (48)	23	F	10	18	N.R.	SLE	1	0	Normal
			1	8					
19) R. Stern et al 1976 (117)	37	F	10	18	W2	Discoid LE	4	0	Normal
			9	5	W2				

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TABLE 5

Clinical Findings among Individuals with
Homozygous C₂ Deficiency

None	15
Discoid LE	5
SLE with DLE	3
SLE	5
Probable SLE	1
Anaphylactoid Purpura	3
Dermatomyositis	1
Chronic Vasculitis	1
Idiopathic Membranous GN	1
Hodgkins	1
Recurrent Infections	2
Total	38

autoimmune type. Fourteen had SLE or DLE. The sex distribution among affected and normal individuals is shown in Table 6. The female-male ratio for normals was 1:2, while for affected individuals the ratio was approximately 5:1. The ratios for those individuals with SLE and DLE were 8:1 and 4:1, respectively, which is consistent with predominance of females generally seen in these diseases. The ratio for the total group, however, was approximately 2:1 which may reflect a sampling bias resulting from screening rheumatic disease patients.

Only 2 of 38 cases listed in Table 5 had recurrent infections as the primary clinical problem (family 21, Table 4). However, frequent infections have been noted in several other cases. Unusual susceptibility to infection transiently in childhood has been noted for both male homozygotes in family 4 and the propositus in family 6. Minor recurrent infections of the sinopulmonary and urinary tracts have been noted in the probands in families 5, 19 and 20.

Clinically the patients with SLE fulfill the currently accepted criteria for this disease; however, many are atypical in some respects as shown in Table 7. Five of eight patients with SLE tested had weak or absent antibodies to native DNA. Only two of nine had renal disease. In addition, five of seven tested had no immunoglobulins or complement deposited in the skin and the two positives had only IgG.

Those with DLE also appear typical clinically (Fig. 2). However, three of four patients with DLE tested did not have any immunoglobulins

15) P. Friend et al 1975 (42)	44	F	5	W2	Normal	1	2	Normal
16) K. Wolski et al 1975 (132)	17	F	3	W24	Normal			
17) N. Day et al 1976 (28, 29)	2	M	2		Hodgkins Disease			
	24	M	2	W24	N.R.	1	0	Normal
			2		SLE			
18) D. Gibson et al 1976 (48)	23	F	10	W2	Discoid LE	4	0	Normal
19) R. Stern et al 1976 (117)	37	F	10	W2				
			9					

20) V. Agnello 1976	62	F	10	W2	Discoid LE	0	1	Normal
			10	?				
21) M. Glosky et al 1976 (50)	1 1/2	F	11	N.R.	Frequent Infections	2	1	Normal
	7	F	W24	N.R.	Frequent Infections	1	1	Normal
22) R. McLean et al 1976 (75)	48	F	10(W25)	N.R.	SLE			
			38		Normal	2	2	Normal
	57	F	10(W25)					
			33		Discoid LE			
23) M. C. Douglas et al 1976 (36)	24	F	N.R.		Normal	2	0	Normal
	12	M	N.R.		SLE	1	2	Normal
24) E. Tan† 1976 (120)	22	F	N.R.		SLE	1	3	Normal
25) E. Tan† 1976 (120)	26	F	N.R.		Probable SLE			
26) E. Tan† 1976 (120)	16	F	N.R.					

* Presumed to be hereditary deficiency, family studies not possible

N.R. Not reported

† All these patients had positive antinuclear antibodies. Patient 24 had facial erythema, positive LE cells documented in the past, arthritis without deformity and chronic false positive STS. Patient 25 had facial erythema, photosensitivity, arthritis without deformity and leukopenia. Patient 26 had photosensitivity, arthritis without deformity and a chronic false positive STS.

or complement deposited along the epidermal-dermal junction.

Certain disease states also appear to be associated with heterozygote C2 deficiency. The prevalence of affected individuals is much lower, however, than for the homozygote C2 deficiency state (Table 8). Thirteen percent of the heterozygote C2 deficient individuals were found to have associated disease states compared to 61% of homozygote C2 individuals. This figure, however, may be high as a result of sampling bias. If the heterozygotes found in the kindreds listed in Table 4 are analyzed, they consist of approximately equal numbers of females (65) and males (64). There were no clinical abnormalities among this group. Twenty of the 22 affected individuals listed in Table 9 were detected in Gibson's study which involved screening a population with rheumatic diseases. The female to male ratio in this study was approximately 2:1.

The clinical findings among individuals with heterozygote C2 deficiency are shown in Table

9. SLE and juvenile rheumatoid arthritis (JRA) were the most commonly associated disease states. Female to male ratio (Table 10) was approximately 1:1 for the normal group and 6:1 for the affected group. The ratio for the total group was approximately 1:1.

The prevalence of C2 deficiency in rheumatoid arthritis (RA), juvenile rheumatoid arthritis and SLE has recently been studied (49). Two of 134 RA patients (1.4%), 10 of 273 JRA patients (3.7%) and 7 of 142 SLE patients (5%) had heterozygote C2 deficiency compared to 6 of 509 (1.2%) normal blood bank donors. Also noted was that C2D SLE patients had an earlier age of onset and significantly lower titered antinuclear antibodies than non-C2D SLE patients. The JRA patients with C2D were clinically indistinguishable from the non-C2D JRA patients.

The close positive linkage between the C2D gene and the HLA genes, A10 and B18, is now well established (28, 29, 43). In 24 kindreds typed, the C2D gene was associated with HLA 18 in 22 and HLA 10 in 21 (Tables 4 and 8). More recently the HLA D gene, W2, has also been shown to be in strong positive linkage with C2D. In eight of nine kindreds reviewed here the W2 gene was associated with the C2D gene.

While the haplotype HLA 10, B18 C2D is closely associated with lupus disease as shown in Tables 4 and 8, it can occur completely

TABLE 6
Sex Distribution among Affected and Normal
Individuals with Homozygous C₂ Deficiency

	Number	Females	Males
Normal	15	5	10
Affected	23	19	4
Total	38	24	14

TABLE 7
Some Clinical and Serological Findings among Individuals with Homozygous C₂ Deficiency Associated
with SLE and/or DLE

Patient	Family number (Table 4)	Renal dis- ease	ANA	NDNA	Skin Biopsy immunofluores- cence studies
1) SLE with Discoid	5	0	+	+/-	0
2) SLE with Discoid	12	+	+/-	+	IgG
3) SLE with Discoid	6	+	+	+/-	0
4) SLE	16	0	+	NS	NS
5) SLE	22	0	+	0	IgG
6) SLE	24	0	+	0	0
7) SLE	25	0	+	+	0
8) SLE	18	0	+	+	NS
9) Probable SLE	26	0	+	+/-	0
10) DLE	12	0	NS	NS	NS
11) DLE	19	0	+	0	0
12) DLE	8	0	+	0	IgG, IgM, C1q, C4
13) DLE	23	0	0	0	0
14) DLE	20	0	0	0	0

0 negative, none + positive, present +/- weakly positive NS—not studied.

FIG. 2
from far

1) S. F.
2) S. F.
3) D. J.

4) P. M.
5) N. I.

* No
N.R.

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pedig
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of a fa
familia
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10, B1
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carrie



FIG. 2. Three patients with homozygous C2 deficiency and DLE lesions. From left to right, probands from families 5, 19, 20 (Table 4).

TABLE 8
Additional Kindreds—Heterozygous C₂D Probands

	Heterozygous individuals		HLA			Clinical findings
	F	M	A	B	D	
1) S. Fu et al 1975 (45)	1	2	10	18	W2	Normal
2) S. Fu et al 1975 (44)	1		10	18	?*	SLE
			1	8	?	
3) D. J. Gibson et al 1976 (49) 10 kindreds	18	10	10	W40	N.R.	10 JRA
			10	18		8 SLE
			2	18		2 RA
			2	5		8 Normal
4) P.M. Blatt et al 1976 (14)	1	1	10	18	N.R.	Normal
5) N. Day et al (25)		1	10	18	W2	Chronic Glomerulonephritis

* Not W2

N.R. Not reported.

dissociated from the disease as shown in the pedigree in Figure 3.

This pedigree was originally studied because of a factor XI deficiency which appeared to be familial. Rheumatic diseases occur in multiple generations and the proband had JRA in childhood and SLE later in life. There are two individuals in the family that carry the HLA 10, B18, C2D haplotype, neither of which have any disease manifestation while the proband carries none of these genes.

C3 Deficiency

There are four cases of C3 absence associated with marked increased susceptibility to infection with encapsulated bacteria (9, 10, 13, 52). In three cases the probands have homozygous C3 deficiency on the basis of pedigree analysis. The fourth is an orphan child (13). In one case the susceptibility to infection could not unequivocally be attributed to the C3 deficiency since C1q deficiency and decreased IgG levels were also present (52).

arthritis (JRA)
associated disease
Table 10) was
total group and 6:1
ratio for the total

ency in rheuma-
rheumatoid arthri-
been studied (49).
10 of 273 JRA
SLE patients (5%)
compared to 6
bank donors. Also
patients had an ear-
tantly lower titered
non-C2D SLE pa-
C2D were clini-
the non-C2D JRA

between the C2D
10 and B18, is now
D. In 24 kindreds
associated with HLA
(Tables 4 and 8).
gene, W2, has also
positive linkage
kindreds reviewed
associated with the C2D

LA 10, B18 C2D is
disease as shown
can occur completely

Deficiency Associated

Biopsy immunofluores-
cence studies

0
IgG
0
NS
IgG
0
0
NS
0
NS
0
IgG, IgM, C1q, C4
0
0

In a fifth case with homozygous C3 deficiency, a 3-year-old male presented with fever, rash and arthralgias but no history of recurrent bacterial infections (89). The symptoms were abated by whole blood transfusion. Of interest

is that in the first case reported, rash, diagnosed as erythema gyratum perstans, accompanied the recurrent infections.

The chemotactic deficiency in the five cases reported was variable. One, with one-thousandth normal C3 levels, had no neutrophilia accompanying infection (9). Another with complete absence of C3 had a normal neutrophilic reaction to infection but delayed polymorphonuclear migration (13). A third with 3-4% of normal C3 levels had some neutrophilic response to infection (10). The fourth case who died with pneumococcal meningitis was noted to have many polymorphonuclear cells in the cerebrospinal fluid and a leukocytosis (52). The fifth case, the only one without a history of recurrent bacterial infections, had a slight neutrophilia with the rash but had deficient generation of neutrophil chemotactic factors *in vitro*.

The heterozygous C3 deficient individual does not appear to have increased susceptibility to infections or any other associated clinical abnormalities (10).

TABLE 9
Clinical Findings among Individuals with Heterozygous C₂ Deficiency

None	142
Systemic Lupus Erythematosus	9
Juvenile Rheumatoid Arthritis	10
Rheumatoid Arthritis	2
Chronic Glomerular Nephritis	1
Total	164

TABLE 10
Sex Distribution among Affected and Normal Individuals with Heterozygous C₂ Deficiency

	Number	Females	Males
Normal	142	67	75
Affected	22	19	3
Total	164	86	78

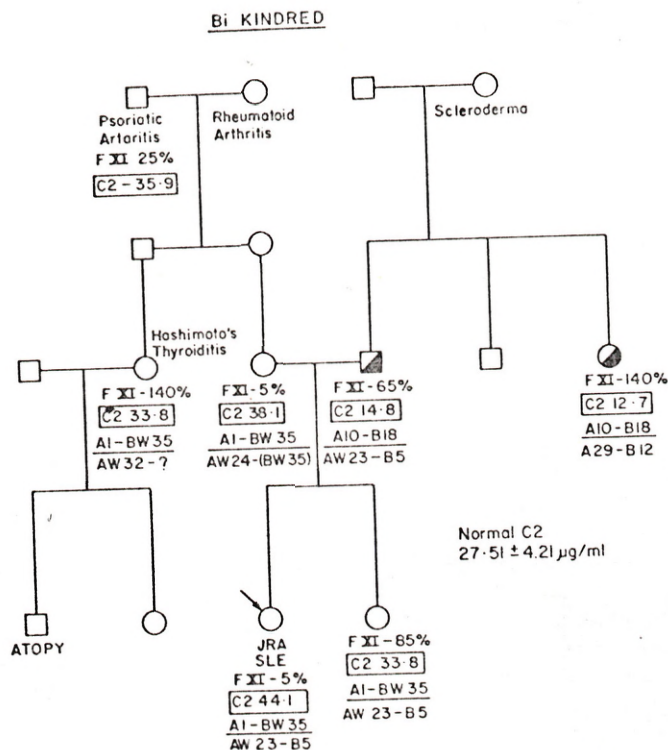


FIG. 3. Bi kindred which has been previously studied (14). Arrow shows proband. Half shaded box and circle show two healthy individuals heterozygous for C2D and HLA 10-B18.

C3 Inactivator Deficiency

A single case of C3 inactivator deficiency has been reported (1, 6, 7, 16). The proband had Klinefelter's syndrome and increased susceptibility to infection similar to the C3 deficient cases. The C3 concentration in this patient was approximately one-third normal levels at all times due to a catabolic rate of C3 that was five times normal. Total hemolytic complement, *in vitro* phagocytosis, and chemotaxis were all markedly reduced. These abnormalities were all corrected by infusion of purified C3 inactivator. The depressed C3 levels in this patient have been shown to be due to unchecked activation of the C3b feedback pathway.

C5 Deficiency

Two apparent homozygous C5 deficient individuals in the same family have been reported (105, 106). The proband, with undetectable C5 levels, had SLE and increased susceptibility to infection. There are no unusual features about the SLE present in this case other than perhaps the lack of progression of the renal disease present. The LE tests were positive, the ANA was positive, and antibodies to native DNA were present. During periods of disease activity, the early components of complement were depressed. The skin lesion which was prominent was typical histologically for lupus and deposits of immunoglobulins and complement were present along the dermal-epidermal junction.

A 10-year-old half-sister of the proband, also an apparent homozygote for C5D, had 1-2% of normal C5 levels. No underlying disease was present, but there was a history of frequent upper respiratory infections and two episodes of pneumococcal pneumonia up to the age of 5. She has been healthy in recent years. Other family members had either normal or approximately half-normal C5 levels consistent with an autosomal codominant pattern of inheritance.

In vitro studies showed marked impairment of (84) cytolytic activities with serum hemolytic activity and bactericidal activity against *Salmonella typhi* absent in the proband. The other homozygote C5D individual with 1-2% C5 levels had approximately 30% of serum hemolytic activity and 12% bactericidal activity (105); chemotactic activities with incubation of

proband serum with aggregated gamma globulin or endotoxin resulted in no chemotaxis for polymorphonuclear cells. The proband serum had normal activity for opsonization of *Saccharomyces cerevisiae* (baker's yeast) or *Candida albicans* for phagocytosis by polymorphonuclear cells, and normal phagocytosis and intracellular killing of *Staphylococcus aureus*.

C5 Dysfunction

Several families have been reported in which multiple members have increased susceptibility to infection associated with deficiency of serum phagocytosis enhancing factor (64, 79, 81). A characteristic syndrome (Leiner's disease) consisting of eczema and secondary infection is present in affected infants. A genetic transmission has not been established. Clinically, these patients have primarily increased susceptibility to infection with gram-negative bacteria. *In vitro*, their plasma shows diminished ability to mediate opsonization of baker's yeast, rice-starch, or *Staphylococcus aureus* particles. The deficiency has been thought to be related to a dysfunction of the C5 component of complement since normal human and mouse serum and isolated C5 correct the phagocytosis defects *in vitro* while C5 deficient mouse sera does not. A selective dysfunction of the molecule is postulated since the C5 in these patients is antigenically and hemolytically normal (79). Similar deficiency in opsonization is not found in the patients with hereditary absence of C5, suggesting that deficiency of some other serum factor may be responsible for the abnormalities in the patients with Leiner's syndrome.

C6 Deficiency

Two cases of hereditary absence of C6 have thus far been described. The first case was an 18-year old black female in normal health (65). Pedigree analysis was consistent with a Mendelian codominant pattern of inheritance. Eight family members were heterozygote for C6 deficiency with approximately 50% of normal C6 levels in the serum. Hemolytic complement levels were within normal limits. All were in good health. *In vitro* studies on the serum of the proband showed absent bactericidal activity against *Salmonella typhi* and normal generation of chemotactic activity in the presence of aggregated IgG and endotoxin and normal immune adherence. In contrast to

the C6 deficient rabbits which have clotting abnormalities related to the absence of C6, there were no clotting abnormalities of the blood in the propositus.

The second case, a six-year-old black male, had recurrent meningococcal meningitis (68). Serum complement hemolytic and bactericidal activity was absent. The complement components other than C6 were normal, as were complement dependent chemotaxis, opsonization, neutrophil function, bactericidal antibody titers against autologous meningococci and the coagulation system. The pedigree in both families was consistent with an autosomal codominant pattern of inheritance.

C7 Deficiency

Two cases of hereditary absence of C7 have been described. One case is similar to the C6 deficiency case reported (129). Biologic function mediated by the early components, opsonic activity, immune adherence, and chemotactic generation are all normal. Functions requiring terminal components bactericidal and hemolytic activities are absent. There is no clinical disease associated.

The second case, a 42-year-old Caucasian female, had Raynaud's phenomenon and sclerodactyly (15). Activation of the classical and alternate pathways and generation of biologic function *in vitro* were normal through C7 except for a deficiency in opsonization of unsensitized yeast particles. There is no increased susceptibility to infection, however, *in vivo*.

C8 Deficiency

There are also two patients with hereditary absence of C8 that have been studied; both are black females. One has a history of recurrent disseminated gonococcal infections (93); the other has SLE with membranous glomerular nephritis, photosensitivity, butterfly rash and positive LE test (56). All complement dependent functions tested were normal in both cases except for bactericidal and hemolytic activities which were absent. Bactericidal activity against *Neisseria gonorrhoea* tested in the first case was absent suggesting that bacterial lysis may be important in host defense against infection with such organisms. Of interest is that the homozygous C6 deficient individual who is in normal health had one bout of disseminated *Neisseria gonorrhoea* infection (65).

Acquired Deficiencies of Complement

There are three acquired complement deficiency states in which persistent complement depression over prolonged periods appears to be associated with certain disease states. These are: 1) C1 esterase inhibitor deficiency associated with SLE, 2) C3 deficiency associated with mesangio-capillary glomerulonephritis, and 3) early complement components depression associated with the "SLE related syndrome."

C1 Inhibitor Deficiency and SLE

Six patients with hereditary deficiency of C1 esterase and SLE or DLE have been reported (two SLE, four DLE). In one kindred reported in detail, identical twin boys with DLE were studied (62). Markedly depressed levels of C1 esterase inhibitor were present which was thought to be congenital since the mother had been diagnosed as having hereditary angioneurotic edema on a clinical basis. In addition, she had SLE by clinical criteria. Serologically, she had a false positive VDRL and a negative LE cell preparation. The twin boys had weakly positive ANA's, false positive serology for syphilis, negative LE cell tests and native DNA antibodies with positive antibodies to single stranded DNA. These C1 inhibitor levels ranged between 0.032 to 0.043, with the normal range being 0.094 and 0.318. C4 levels were chronically depressed and C4 turnover studies showed a marked increase in C4 metabolism. Immunofluorescent studies of skin biopsies showed immunoglobulin and complement deposits in both normal skin and the DLE lesions.

Donaldson has referred to two unrelated patients with hereditary angioneurotic edema who have a biopsy diagnosis of discoid lupus and weakly positive serology. These studies have not as yet been reported in detail (32).

Another set of twins, identical girls with complete absence of C1 inhibitor, have been reported which are of particular interest in that one remains normal while the other has manifested the classical recurring symptoms of hereditary angioneurotic edema since 6 years of age (104). At age 14 she developed classical SLE with positive anti-native DNA antibodies and a profuse proliferative glomerulonephritis. Initially during the period of clinically active HANE, C4 level was less than 4% of normal with normal C3. With active SLE, C3 fell to 50% of normal with C4 remaining low. Follow-

ing treatment with Prednisone and Imuran the C3 returned to normal and the C4 increased to 50% of normal. The twin without clinical HANE has not developed any symptoms of SLE. Her C4 levels are chronically 10-15% of normal levels.

C3 Deficiency and Glomerulonephritis

For some time it has been known that a portion of patients with a form of chronic glomerulonephritis termed mesangio-capillary glomerulonephritis have associated depressed serum levels of the third component of complement (130). Depression of C3 in the presence of normal levels of the early classical components C1, C4, and C2, and the finding that properdin and C3 without immunoglobulins were present in the glomerular deposits, suggest that complement activation in these patients was by the alternate pathway (76, 108). Subsequently, a protein capable of inducing C3 cleavage in normal serum was found in the serum of these patients and termed C3 nephritis factor (C3NEF) (123-125). The relationship of the hypocomplementemia to the glomerulonephritis in these patients is not as yet known. Since immunoglobulins could not be found in the lesions, it was suggested that activated complement could produce glomerular lesions. However, it has been found that chronic activation of the alternate pathway in animal models does not result in nephritis (114, 126).

Recent studies on a group of patients with partial lipodystrophy and hypocomplementemia have suggested that chronic C3 depression may predispose to glomerulonephritis by increasing the host's susceptibility to infection (8, 91, 92, 121). In the most recent report, 21 patients with partial lipodystrophy were studied (115). Seventeen had low serum C3 with normal C4 and C2 concentration. Fourteen had a serum C3 splitting factor which appeared to be C3 NEF. These abnormalities occurred in 10 patients without clinically overt disease. However, seven patients had nephritis and renal biopsy demonstrated mesangio-capillary type lesions. In all those with nephritis, C3 NEF was present and hypocomplementemia and lipodystrophy preceded the nephritis by prolonged periods. The authors suggest that a high rate of mesangio-capillary nephritis in these patients indicates complement activation via the alternate pathway which predisposes

to the development of this form of glomerular disease, and that this may be true in general for the association of depressed C3 and mesangio-capillary nephritis. It is postulated that the complement deficiency predisposes to infection which in some way mediates the glomerulonephritis. There has been one reported case of increased susceptibility to recurrent infections in a patient with hypocomplementemia and partial lipodystrophy (8), and in the recent study, 3 of the 21 patients also had evidence of some increased susceptibility to infection. However, among patients with mesangio-capillary glomerulonephritis and hypocomplementemia, there has not been an increased susceptibility to infection noted (91). The relationship of hypocomplementemia to the partial lipodystrophy which is also acquired is unclear.

Early Complement Component Depression Associated with the "SLE-Related Syndrome"

In recent years a group of patients have been identified whose features are similar to those of SLE in some respects, but who do not meet the diagnostic criteria for SLE (5, 70, 74, 88). They have in common with SLE patients several distinctive clinical and serologic findings (Table 11). Recurrent rash as shown in Figure 4 was the most prominent feature. Arthralgias are a frequent symptom. Angioneurotic edema of the face and small bowel occur less frequently but severe bouts can occur (Figs. 5a and b). This syndrome, for lack of a better name, has been called the SLE-related syndrome.

The striking serologic features are marked hypocomplementemia and presence of a substance which precipitated with C1q (Fig. 6). Complement component studies showed marked depression of the early components C1 through C3 with normal or elevated levels of the terminal components (Table 12). The C1 esterase inhibitor was normal or elevated and C3 Proactivator (factor B) levels were essentially normal.

The size of the C1q reactant as determined by sucrose density gradient analysis was approximately 7S in contrast to the much more rapidly sedimenting C1q reactive material from SLE patients with circulating complexes (4).

Characterization of antinuclear antibodies in this group of patients and additional patients with a similar syndrome from Dr. McDuffie's,

TABLE 11

Four Patients with Marked Hypocomplementemia, Cutaneous Rash and Serum C1q Precipitins: Clinical Data (5)

	PUR	BLO	CIC	SPI
Sex	Female	Female	Female	Female
Age	32 years	32 years	39 years	32 years
Duration of symptoms	6 years	3 years	3 years	3 years
Rash	++++	+++	++	+++
Arthralgia	+++	++	+	++
"Angioneurotic" edema	+	++	+++	++
ANF	0→1+	1+	1+	0
LE cells	0	0	0	0
Serum λ globulin	elevated	normal	elevated	elevated
CH ₅₀ *	<30	<30	<30	<56
Serum C1q precipitins	+++	++	+	++

* Normal range 150-250



FIG. 4. Picture of patient PUR. Similar rashes occurred in all patients in Table 11 in association with C1q precipitins and marked complement depression in the serum (2).

Dr. Gewurz's, and Dr. Oishi's laboratories are shown in Table 13. In contrast to the high titers found in patients with SLE antibodies, antibody levels to native DNA in these patients are low. However, significant titers to SSDNA were present in all but one, and high titered Sm antibodies was present in three. Patient HUR, who had the typical clinical syndrome, differed from the rest in that high titered antinuclear antibodies were present. The pattern of the ANA was speckled and this was most likely the result of the high titered Sm antibody in this serum. Also of interest is that the ANA in patient PUR did not turn positive until several years after onset of the syndrome.

DISCUSSION

The presence of multiple systems mediating host defense has made the evaluation of the specific role of any single system difficult. This has been particularly true in assessing the role of complement. In spite of such difficulties it is now apparent from the studies of patients with hereditary deficiencies of individual complement components that complement does have specific roles in host defense in man. From this review the following may be concluded:

1. Deficiencies of C1, C4 and C2 are associated mainly with diseases resembling SLE but with other connective tissue diseases as well.

ipitins: Clinical

SPI
Female
32 years
3 years
+++
++
++
0
0
elevated
<56
++

laboratories are contrast to the high SLE antibodies, A in these patients titers to SSDNA, and high titers in three. Patient clinical syndrome, that high titers are present. The patient attacked and this was the high titers Sm. So of interest is that did not turn positive set of the syndrome.

ON
e systems mediating the evaluation of the system difficult. This in assessing the role of such difficulties it is studies of patients with of individual complement does have use in man. From this be concluded:

C4 and C2 are associated with diseases resembling SLE and connective tissue diseases

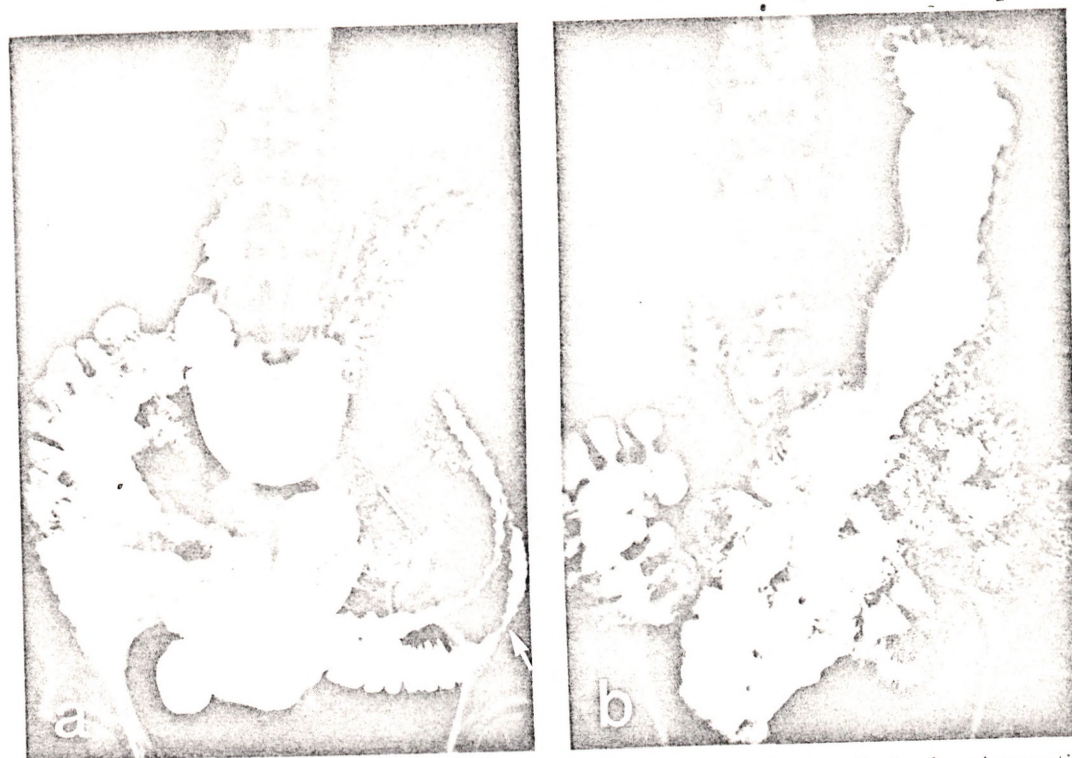


FIG. 5. Upper gastrointestinal x-ray examination of patient CIC. a) during attack of angioneurotic edema. Film shows edema of wall (large arrow) and narrowing of lumen of small bowel (small arrow). b) similar study one week after the attack. Attack was associated with C1q precipitins and marked complement depression in the serum (2).

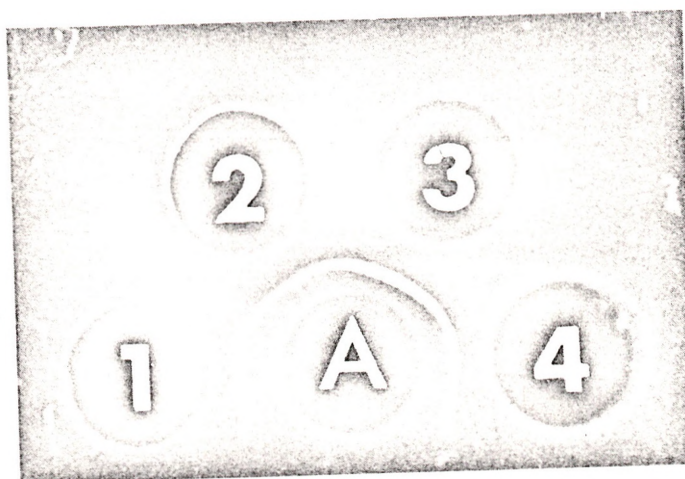


FIG. 6. Gel diffusion plate showing the precipitation of isolated C1q (well A) with CIC serum undiluted (well 1), BLO serum diluted 1:2 (well 2) and PUR serum diluted 1:4 (well 4). Anti-C1q in well 3 is present to show the specificity of the reactors (5).

TABLE 12
Serum Complement Component Studies on Three Patients with Marked Hypocomplementemia, Cutaneous Rash, and Serum C1q Precipitins (5)

	PUR	BLO	CIC	Normal range
C1 SFU/ml*	3,450	53,000	99,000	140,000-290,000
C1q μ g/ml	10	36	57	158-208
C1s μ g/ml	102	135	125	62-78
C4 SFU/ml*	6,600	19,600	15,100	145,000-380,000
C4 μ g/ml	<25	92	29	211-633
C2 SFU/ml*	2,000	10,000	4,200	15,400-27,400
C2 μ g/ml	668	1,787	952	10,600-26,200
C3 % Normal SFU*	370	540	200	950-1800
C3 μ g/ml	148	273	213	153-323
C3 PA μ g/ml	76	80	65	69-81
C5 μ g/ml	163	220	163	66-82
C6 μ g/ml	102	114	147	102-134
C8 μ g/ml	175,300	273,200	139,100	136,000-240,000
C9 SFU/ml*	274	394	321	143-175
C9 μ g/ml	19.4	40	28	12-30
C1 inhibitor μ g/ml				

* Hemolytic titrations, all other determinations by radial immunodiffusion.

TABLE 13
Characterization of Antinuclear Antibodies

	ANA	NDNA μ g/ml	SSDNA μ g/ml	Sm	RNA protein
PUR		1.25	5.43	0**	0**
Jan 1970	0	0.85	5.38	0	0
Nov 1971	4	1.24	5.78	0	0
March 1972	5	2.50	3.21	8	0
C1C	4	0.59	3.31	6	0
BLO	0	1.56	0.10	0	0
SP1	0	0.84	3.77	0	0
EAS	0	0	9.36	0	0
COD	0	2.10	3.16	15	0
HUR	12	<2.50	<2.50	0	0
Normal	0				

* Titer (log base 2) immunofluorescent test.

** Titer (log base 2) hemagglutination tests.

2. Deficiencies of C3 are associated with life-threatening bacterial infections.

3. Deficiencies of terminal components C5-C8 may be associated with increased susceptibility to infections where bacterial lysis is important in elimination of the organism. However, this observation is based on only a few patients.

It is now well established that C3 plays a major role in host defense against bacterial infections. Not only does hereditary absence of C3 increase susceptibility to bacterial infections, but deficiency of other components of the complement system that prevent the efficient

activation of C3, such as C2 or C3bINA deficiencies, also appears to be associated with increased susceptibility to such infections. While patients with C2 deficiency do not have life-threatening bacterial infections of the type seen in the homozygous C3 deficient patient, they often have histories of frequent infections, especially the younger patients as demonstrated by the studies of Glovsky et al (50). This is understandable since the alternate pathway in the C2 deficient patients is intact so that activation of C3 can occur. The increased susceptibility to infection then may be a relative one which may have greater impor-

tance in childhood when other host defense systems such as humoral immunity have not fully developed.

Increased susceptibility to bacterial infections occurs in only a small percentage of patients with acquired deficiency of C3, which may relate to lesser degrees of C3 depression present in this group. Nonetheless, Peters has postulated that increased susceptibility to infection and subsequent immune complex formation may be the cause of the glomerular nephritis which occurs in a portion of these patients (92). The lack of evidence for glomerular immune complex deposition in these cases is against this hypothesis. It is known that the alternate pathway is activated by nonimmune substances. Another hypothesis consistent with the finding of alternate pathway activation and the glomerular C3 and properdin deposits found in these patients is that the hypocomplementemia predisposes to infection with yet uncharacterized agents that directly activate the alternate pathway. The chronic depression of C3 that is present in these cases may be analogous to the SLE-related syndrome where a low molecular weight substance which does not appear to involve immunoglobulins is associated with chronic depression of the early components of the classical complement system.

Along the same line, equally speculative, but potentially productive hypotheses can be advanced for the association of deficiencies of the early components of complement with lupus-like disease. One possibility is that deficiency of the early components of complement predispose the host to infections with certain organisms that are involved in the production of connective tissue diseases such as C3 absence predisposes to specific bacterial infections. The possibility that SLE and other connective tissue diseases have an infectious basis, particularly viral, is a widely held concept (94, 110). Viral-like particles have been described in SLE and dermatomyositis (18, 53, 71). In addition C-type viral antigens have been found on lymphocytes and in glomerular deposits in patients with SLE (67, 78).

One hypothesis which has been put forth is that with deficiencies of early complement component the likelihood of proliferation of such an agent may be enhanced and result in the clinical picture of SLE, or other syndromes of connective tissue disease, in some (particularly

females) but not necessarily all such deficient individuals (5). Of interest in this regard is the recent work of Cooper and his colleagues which has shown that antibody-independent lysis of oncornaviruses which occurs with human serum does not occur with C2 or C4 deficient sera.

The studies on acquired complement deficiency states, particularly the SLE-related syndrome, are of interest because of the clinical and serologic abnormalities they present which may relate to similar findings in classic SLE on one hand and lupus disease seen in C2 deficient patients on the other. The depression of early complement components in these patients is similar to the selective depression of early complement components during disease activity in SLE (109). This is in contrast to certain other hypocomplementemic states, for example, acute glomerulonephritis and chronic glomerulonephritis which are characterized by greater depression of the terminal component. The low C1q levels in these patients appear to relate to the presence of circulating low molecular weight C1q precipitins which also occur in some SLE patients. While the exact nature of these substances is not known, there is some evidence to suggest that they may be of foreign origin. It is notable that in Cooper's study C1q could be demonstrated to react directly with viral particles. If such agents were involved in the etiology of SLE, then the marked depression of early complement components in SLE and the SLE-related syndrome may indicate a mechanism of host defense to such organisms or similar ones rather than just a reflection of immune complex formation and disease activity as has been generally thought.

Another major possibility is that the association of C2D genes with lupus disease is not a causal relationship, but is rather a result of linkage to some other gene which determines the functional predisposition. It is known from mouse studies that genes determining complement levels are linked to both histocompatibility and immune response genes. The HLA region of chromosome 6 in man is remarkably similar to the H-2 region in the mouse as shown in Figure 7. The HLA genes linked to the C2D genes may then be of more primary importance. The lack of increased incidence of HLA 10 or B18 among the classic SLE group of patients, however, rules out an absolute causative relationship of these genes. The incidence

a, Cutaneous

al range

-290,000

-208

-78

-380,000

-633

-27,400

-26,200

-1800

-3-323

-9-81

-6-82

-2-134

-0-240,000

-3-175

-2-30

RNA protein

0**

0

0

0

0

0

0

0

0

0

2 or C3bINA deficiency associated with such infections. deficiency do not have infections of the type 3 deficient patient, frequent infections, patients as demonstrated by Glovsky et al (50). Since the alternate pathway in these patients is intact, infection can occur. The infection then may be more severe than in patients with greater impor-

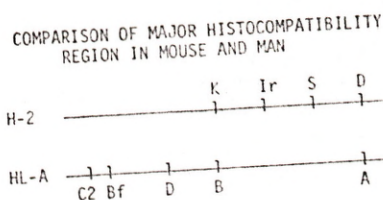


FIG. 7. The A and B loci appear to be analogous to the D and K loci of H-2. The systems differ in that the D locus which determines the mixed lymphocyte reaction lies outside of B whereas the analogous gene in the mouse is part of the Ir region. S has been shown to determine C4 levels in the mouse (22, 116). In man C4 has also been shown to be closely linked to the HLA genes but its precise location in the complex has not been determined. The C2D locus which is also very closely linked to the locus determining factor B synthesis (Bf) is outside of both D and B loci. The location of the C2 locus in the H-2 system has not been determined.

of HLADW2 in classic SLE patients is not known, so that its importance remains to be determined. The possibility also exists that a yet unidentified closely linked gene, most likely an Ir gene, is more directly related to the disease than the C2D or HLA genes thus far studied. The family in the pedigree in Figure 3 would be consistent with such a possibility.

Clinically, the patients with C2 deficiency and lupus disease appear to differ from the group of patients with classic SLE in (1) the increased frequency of DLE lesions; (2) the low incidence of renal disease; (3) the low incidence and titers of antibodies to native DNA; and (4) the infrequent occurrence of immunoglobulin and complement deposits in the skin. Another difference is that, thus far, C2D genes have not been found in blacks whereas SLE may in fact have a higher incidence among blacks (113). Of interest is that the four individuals with C6D or C8D are black and one with C8D has SLE. The C8D and HLA genes are not linked in this patient.

The differences in clinical and serological manifestations of classic SLE, the lupus disease seen in C2 deficient patients which appears to be a variant of SLE, and the SLE-related syndrome may reflect multi-genetic factors involved in the pathogenesis of these disorders. Datta and Schwartz have recently shown that independent genetic factors determine the expression of virus in autoimmunity in certain strains of mice (24).

The absence of DNA antibodies in most of the patients with the C2 variant of SLE and SLE-related syndrome suggests that the gene or genes determining this immune response that is characteristic of classic SLE is lacking in these patients. Of interest in this regard is that the proposita in family 20 has a selective deficiency of IgG1. This has not been previously reported and further studies are in progress.

The infrequent finding of immunoglobulins and complement in the DLE lesions in patients with C2D contrasts markedly with the 90% occurrence of these deposits in "classic" DLE lesions (122). This questions the importance of immune complexes in the pathogenesis of DLE and suggests that a more primary process, possibly related to the genetic finding in the C2D patients with DLE, may be present.

The present data suggest that the combination of C2D and HLA genes or a yet unidentified link gene predisposes to a form of SLE which lacks some of the immune responses characteristically seen in patients with classic SLE. The features of the patients with the C2 variant of SLE and the SLE syndrome that are similar to those in classic SLE may be determined by other genes or factors that they have in common. The careful study of these subgroups of patients may help delineate some of these factors, and possibly provide new insights into the biologic activities of the complement system.

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EVIDENCE FOR DEFECTIVE IMMUNOREGULATION IN THE SYNDROME OF FAMILIAL CANDIDIASIS ENDOCRINOPATHY

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Abstract We studied three children with candidiasis endocrinopathy syndrome, together with their parents and five siblings, to explore the possibility that defective immunoregulation allows autoimmune phenomena to be involved in the pathogenesis of this syndrome. Inheritance of the syndrome appeared to be autosomal recessive. Immunologic abnormalities in this family included hypergammaglobulinemia, selective IgA deficiency, anergy, autoimmune endocrinopathies and active chronic hepatitis. Defective

suppressor T-cell function was noted in the two surviving children with clinically apparent disease and in a clinically normal sibling.

Analysis of the immunologic abnormalities in the family suggests that defective immunoregulation rather than disordered effector mechanisms may explain the large number of immunologic defects noted. These defects, in turn, may result in the clinical manifestation of the syndrome. (N Engl J Med 300:164-168, 1979)

THE association of chronic mucocutaneous candidiasis with hypofunction of one or more endocrine organs is referred to as the syndrome of candidiasis endocrinopathy. A case reported in 1929¹ featured candidiasis and hypoparathyroidism, which, together with adrenal insufficiency, were noted in 1956.² Subsequent reports have documented the association of a wide spectrum of endocrine and non-endocrine organ dysfunction with chronic mucocutaneous candidiasis.³⁻⁹ The genetic pattern of inheritance and the pathogenetic mechanisms involved have not been established, but most speculations have focused on an immunologic defect.^{3,10-16}

Detailed investigations of the immune and endocrine functions of a family, three of whose members had the syndrome of candidiasis endocrinopathy, are here reported. Analysis of the heterogeneous abnormalities found in the immune system of family members suggests that defective immunoregulation at the level of the suppressor T lymphocyte is demonstrable in some cases and may be involved in immunopathogenic mechanisms that express themselves as the syndrome.

CASE REPORTS

The father of this family (Subject I₁) died at the age of 38 years of carcinoma of the petrous temporal bone; the mother (Subject I₂) is clinically well. The oldest child of this non-consanguineous marriage (Subject II₁) had a history compatible with an immunodeficiency state and died at the age of eight years of measles complicated by bronchopneumonia. Four siblings remain well at the time of this report; in three children the syndrome of candidiasis endocrinopathy has been diagnosed, and the clinical features follow.

SUBJECT II₁. A 12-year-old boy had an unusually severe measles infection when he was three years old. After the infection there was a progressive and permanent loss of all his body hair. At seven years

of age repeated seizures and tetanic attacks developed. At this time he was noted to have moniliasis affecting his nail beds, alopecia totalis and positive Trousseau and Chvostek signs. The serum calcium level was 3.3 mg per 100 ml of serum, phosphate 10.6 mg per 100 ml of serum and serum alkaline phosphatase 192 IU. An Ellsworth-Howard test was positive, with a calcemic and phosphaturic response to administration of parathyroid extract that confirmed the diagnosis of idiopathic hypoparathyroidism. Calcium levels were corrected with calcium and vitamin D therapy but have been difficult to stabilize. His condition is complicated by malabsorption of fat.

SUBJECT II₂. A six-year-old girl also suffered from idiopathic hypoparathyroidism and experienced her first tetanic spasms at the age of 4½ years, when her calcium was 5.7 mg per 100 ml of serum with a phosphate level of 11 mg per 100 ml of serum. An ACTH infusion test revealed adrenal insufficiency. Adrenal antibodies were present in her serum. Oral and nail-bed candidiasis was first noted 11 months later.

SUBJECT II₃. At the age of two years and eight months a girl in hepatic failure was noted to have florid oral and nail-bed candidiasis. She was jaundiced and had gross ascites, and positive findings included a high titer of smooth-muscle and parietal-cell antibodies. Liver biopsy revealed severe chronic active hepatitis, with plasma cell disruption of the limiting plate, rosette formation and areas of post-necrotic cirrhosis. No contact with infectious hepatitis was found; she had neither hepatitis B antigen nor antibody in her serum, and her liver disease was considered autoimmune in nature. Calcium metabolism was normal, but impaired cortisol levels after ACTH infusion were compatible with adrenal insufficiency. Other abnormalities included a selective IgA deficiency, an IgE level of 4200 ng per milliliter and absent delayed hypersensitivity reactions to candida and phytohemagglutinin. Treatment with steroids and azathioprine was begun, and liver function improved rapidly. However, the fungal disease became impossible to control, and she died, apparently as the result of an overwhelming viral pneumonitis. At autopsy, thymic tissue was not detected, and extensive esophageal candidiasis was noted.

Table 1 summarizes these cases and includes the tissue-typing data for this family. Figure 1, a pedigree chart, notes the distribution of the clinical and immunologic abnormalities within the family.

MATERIALS AND METHODS

The studies on Subject II₃ were done before immunosuppressive therapy was begun.

Skin Tests

Delayed hypersensitivity reactions were sought with intradermal injections of 0.1 ml of 1:1000 dilution of *Candida albicans* (Hollister-Stier, Canada) and 10 µg of phytohemagglutinin (Wellcome Reagents, Research Triangle, North Carolina).

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Table 1. Clinical and Histocompatibility Profile.

SUBJECT	AGE yr	SEX	CLINICAL MANIFESTATIONS	ANTIGENS CODED AT HISTOCOMPATIBILITY LOCI		
				A	B	C
I ₁	38	M	Carcinoma of petrous temporal bone	A-3, Aw-33	B-22, B-40	W4
I ₂	40	F	None	A-9	B-17	W3
II ₁	—	F	Death at age of 8 yr; measles & pneumonia.	NT*	NT	NT
II ₂	16	M	None	A-3, A-9	B-22, Bw-17	W3
II ₃	13	F	None	A-3, A-9	B-22, Bw-17	W3
II ₄	12	M	Hypoparathyroidism; alopecia; chronic mucocutaneous candidiasis; malabsorption.	A-9, Aw-33	B-40, Bw-17	W3/4
II ₅	11	F	None	A-9, Aw-33	B-40, Bw-17	W3
II ₆	6	F	Hypoparathyroidism; chronic mucocutaneous candidiasis; adrenal failure.	A-3, A-9	B-22, Bw-17	W3
II ₇	4	M	None	A-9, Aw-33	B-40, Bw-17	W3/4
II ₈	3	F	Active chronic hepatitis; chronic mucocutaneous candidiasis; adrenal failure; death at age of 3 yr; absent thymus.	NT	NT	NT

*Not tested.

Humoral Immunity

Isohemagglutinins, antinuclear factor, rheumatoid factor, anti-DNA binding and mitochondrial antibodies were measured or sought in each case, but no abnormality was found. Antibodies to thyroglobulin, thyroid microsomal antigen, kidney mitochondria, smooth muscle, parietal cells and ovarian cytoplasm were sought using standard techniques by Dr. A. Forbes, Massachusetts General Hospital, and in our own laboratories; adrenal antibodies were measured by Dr. R. Blizzard, University of Virginia School of Medicine. The histocompatibility profile was prepared by Dr. R. Cone at Yale.

Enumeration of T and B Lymphocytes

B cells were detected by a standardized technic using fluorescent polyvalent antihuman immunoglobulin serum.¹⁷ T cells were detected by their ability to form rosettes with sheep red blood cells.¹⁸

Lymphocyte Stimulation and Suppressor T-Cell Activity

The method used in our laboratory to assess DNA activity in lymphocytes exposed to antigens (candida and allogeneic lymphocytes) and the mitogens phytohemagglutinin and concanavalin A are standard and have been reported in detail.¹⁹ Suppressor T-cell activity was measured as follows: lymphocytes from the subjects to be tested were obtained from a Ficoll-Hypaque gradient and stimulated with 100 µg of concanavalin A per 10⁶ cells for three and four days. It has previously been shown both in animals and in human subjects that concanavalin A can activate immunoregulatory functions in a subpopulation of T lymphocytes.²⁰ The stimulated cells were treated with mitomycin C and then prompted to suppress the response to phytohemagglutinin (0.5 µg per 10⁶ cells) of allogeneic control lymphocytes; 1 × 10⁵ responder cells and putative suppressor cells were placed in the micro well of a culture plate and the response of the admixture to phytohemagglutinin was assessed after three and four days in culture. Control wells contained responder cells from the same control subject and 1 × 10⁵ cells from the patient being tested, but these mitomycin-

treated cells, though cultured for three days, had not been exposed to concanavalin A. Each set of suppressor cells was tested on cells from two control subjects. The latter cells were exposed to the suppressor cells for both three and four days because we have found that cells from normal people occasionally fail to exhibit suppressor activity on the third and fourth days but always do so on one of these days. To be confident of a lack of normal suppressor T-cell activity after stimulation with concanavalin A, poor suppression of responsiveness to phytohemagglutinin was required by cells from both generation periods at three and four days. As compared to the control cultures, reductions of less than 35 per cent in the response to phytohemagglutinin was considered abnormal.

RESULTS

Abnormalities were found in the humoral immune system of the family. Many members of the family had polyclonal increases in serum immunoglobulins, and these elevated levels were found in both the clinically affected and the healthy members. Serum and

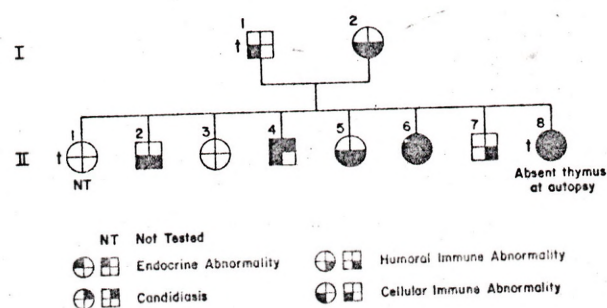


Figure 1. Family Pedigree Showing the Association of Immunologic Abnormalities with Endocrine Dysfunction and Mucocutaneous Candidiasis.

Table 2. Humoral Immune Profile.

SUBJECT	B LYMPHOCYTES (11-23)*	SERUM IgM (80-250)	IgG (600-1,400)	IgA (60-400)	IgE (<100)	ORGAN-SPECIFIC AUTOANTIBODIES
	%		mg/100 ml			
I ₁	14	343	1,440	259	NT‡	None
I ₂	23	84	2,080	300	80	Thyroglobulin
II ₂	27	112	1,920	175	1,250	Parietal cell; adrenal.
II ₃	11	300	1,560	231	NT	None
II ₄ †	41	140	1,280	140	50	None
II ₅	10	256	1,840	Absent	400	Smooth muscle
II ₆ †	42	480	2,680	238	120	Parietal cell; adrenal.
II ₇	20	120	1,200	175	40	Thyroglobulin
II ₈ †	59	280	5,800	Absent	4,200	Smooth muscle; parietal cell.

*Figures in parentheses represent normal values for our laboratory.

†Subjects with syndrome of candidiasis endocrinopathy.

‡Not tested.

secretory IgA were absent in II₅ and II₈. Two of the nine subjects tested had antithyroid antibodies, three had parietal-cell antibodies, two had smooth-muscle antibodies, and two had adrenal antibodies. Table 2 details the results of investigations of the humoral immune status of the family.

In the lymphocyte stimulation studies the absolute increase in counts per minute above background counts (Δ cpm) obtained with cells from control subjects on the day of the study was averaged and given a value of one. The results in the patients are presented as they pertain to this normal value — e.g., a phytohemagglutinin stimulation of 0.29 would indicate that the Δ cpm measured in that culture was only 29 per cent of that seen with cells from the control subjects. The mean ($\pm$ S.E.M.) counts per minute for control cells in each test were: for candida stimulation, $15,232 \pm 1264$; for mixed lymphocyte reaction, $26,481 \pm 3640$; for phytohemagglutinin stim-

ulation, $53,726 \pm 4720$; and for concanavalin A stimulation, $38,899 \pm 2722$. Three members of the family had abnormal suppressor T-cell function, for after exposure to concanavalin A for three and four days, their lymphocytes failed to manifest suppressor activity sufficient to cause at least a 35 per cent reduction in the response to phytohemagglutinin of the allogeneic responder cells; two of these subjects had the syndrome of candidiasis endocrinopathy, and the third, who is only four years of age, is normal to date except for the presence of autoantibodies to thyroglobulin. Table 3 details the results related to competence within the cell-mediated immune system.

DISCUSSION

Chronic mucocutaneous candidiasis, selective IgA deficiency and the thyrogastric cluster of autoimmune diseases usually occur independently but may group together, as in this family, and present the syndrome of candidiasis endocrinopathy. Chronic mucocutaneous candidiasis and IgA deficiency can coexist, possibly causally, and a high rate of autoimmune disease has been reported by many investigators in IgA deficiency. The thyrogastric cluster includes autoimmune diseases featuring endocrine organs as targets, as the name implies, as well as pernicious anemia, vitiligo, premature graying of the hair and myasthenia gravis. Such disease clustering often includes diabetes. This group of diseases is characterized by the presence of organ-specific autoantibodies, in contrast to the "lupus" group of autoimmune diseases, in which antinuclear antibodies predominate. Occasionally, diseases from this latter cluster associate with the thyrogastric group (e.g., idiopathic thrombocytopenic purpura and thyroiditis); in our study autoimmune active chronic hepatitis, a disease of the lupus cluster, was associated with the autoimmune endocrinopathies.

Table 3. Cellular Immune Profile.

SUBJECT	DELAYED HYPERSENSITIVITY*		T LYMPHOCYTES (55-80)†	LYMPHOCYTE STIMULATION TESTS‡				SUPPRESSOR T-CELL ACTIVITY (>35)
	CANDIDA	PHYTOHEMAGGLUTININ		CANDIDA	MIXED LYMPHOCYTE	PHYTOHEMAGGLUTININ	CONCANAVALIN A	
			%					%
I ₁	+	+	73	0.11	1.46	1.06	1.7	NT¶
I ₂	+	+	66	0.06	1.5	0.29	0.16	46
II ₂	+	+	78	0.13	0.07	0.22	0.16	55
II ₃	+	+	62	2.2	2.06	1.9	1.9	73
II ₄ §	+	+	61	0.16	0.92	0.03	0.06	13
II ₅	+	+	79	0.26	1.7	1.1	1.2	37
II ₆ §	+++	+	57	5.8	1.7	0.34	0.2	19
II ₇	+	+	61	0.76	0.82	1.5	1.2	29
II ₈ §	-	-	18	0.10	0.12	0.06	0.07	NT

*Assessed as mm of induration measured in the skin 24 hr (phytohemagglutinin) & 48 hr (candida) after injection. + = >10-20 mm of induration, ++ = >20-30 mm of induration, & +++ = >30 mm of induration.

†Figures in parentheses represent normal values for our laboratory.

‡Expressed in relation to results from normal subjects acting as controls, which were given a value of 1.

§Subjects with syndrome of candidiasis endocrinopathy.

¶Not tested.

In one study,² eight out of 12 patients had evidence of hepatitis, as indicated by the findings of fibrosis and scarring at autopsy. Of 31 cases with candidiasis and an associated abnormality reported in another series,³ pernicious anemia was present in seven, alopecia totalis in six, and juvenile cirrhosis in three cases. If the presence of circulating autoantibodies can be interpreted as evidence for subclinical disease, 16 of the 31 patients also had autoantibodies against kidney tissue. Other non-endocrine findings reported have been pulmonary fibrosis,^{1,2,5} enamel hypoplasia and keratoconjunctivitis.⁵ Endocrine dysfunction, however, is more common than the non-endocrine organ dysfunction.^{3,4,21} Hypoparathyroidism is the commonest, followed by adrenal failure.^{5,21} Other associations reported are chronic lymphocytic thyroiditis,^{22,23} diabetes mellitus, ovarian failure³ and ACTH deficiency.⁶ The reported spectrum of abnormalities in an individual patient has been very variable, not only in the combination of organs affected but also in the timing and sequence.

In the family described above, the profile of histocompatibility antigens was not remarkable. It is of interest that both parents had immunologic abnormalities and that the disease is probably inherited in an autorecessive fashion.

With the development of data to establish the concept that a distinctive subpopulation of T cells has regulatory control of both B lymphocytes and the effector T lymphocytes that produce delayed hypersensitivity reactions, it became clear that many diseases suspected of having an immunopathogenesis might be caused by defects in the "regulatory" cells. Although cells other than T cells may have an immunoregulatory influence there is excellent evidence from animal models that the major influence necessary to maintaining tolerance to self-antigens, and the appropriateness of the immunologic response to foreign antigens, is supplied by an immunoregulatory T cell. The syndrome of candidiasis endocrinopathy, with its clustering of autoimmune and inappropriate immunologic responses, could well represent a defect at this level. Already, we have evidence for excessive and inadequate suppressor activity leading to disease in human beings. An example of the former is acquired hypogammaglobulinemia,²⁴ and one of the latter, systemic lupus erythematosus.²⁵ Abnormal functioning of suppressor T cells can prevent the secretion of IgA and markedly increase the secretion of IgE. Both phenomena are present in this family.

Current technology for assessing immunoregulatory T-cell functions is crude and nonspecific. We are no doubt unable to detect subtle defects, but the assessment of suppressor capacity after concanavalin A stimulation has been useful in a number of investigations and it seems likely that consistent failure to develop suppressor functions after exposure to this mitogen does indicate a defect in this cell.^{24,25} Nevertheless, the data can only be interpreted as suggestive and worthy of further study, and no assumption

relating defective immunoregulation to the cause of this disease can be made at this stage. No doubt, many suppressor cells that do respond to this polyclonal activator have subtle or specific defects not recognizable in this assay. Of the two children with candidiasis endocrinopathy syndrome who were able to be examined, both had defective suppressor cell activity by the above criteria. We have observed this phenomenon before but have not noted it in patients with uncomplicated chronic mucocutaneous candidiasis. Disordered immunoregulation in the syndrome of candidiasis endocrinopathy could explain the autoimmune disease, the absent IgA and elevated IgE levels, the polyclonal hypergammaglobulinemia and possibly the pronounced failure of the lymphocytes from so many members of this family to proliferate normally after *in vitro* challenge. The latter association would require that elaborate "cross-talk" between regulator and effector cells was necessary for proliferation, a concept for which there is already evidence. It should be noted that there is also evidence that immunoregulatory cells provide an amplifying as well as a suppressor influence.²⁶ Failure of this aspect of immunoregulation may lead to the failure of proliferation noted here.

The most difficult phenomenon to explain remains the candidiasis itself. Clinically important infection was present in one subject despite a good *in vitro* response of her lymphocytes to candida, normal IgA levels and delayed hypersensitivity to the antigen. It appears that an effective host response to this fungus requires optimal integrity and co-operation among the various participants of the immune response.

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SPECIAL ARTICLE

GUIDELINES FOR THE ETHICAL, SOCIAL AND LEGAL ISSUES IN PRENATAL DIAGNOSIS

A Report from the Genetics Research Group of the Hastings Center,
Institute of Society, Ethics and the Life Sciences

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MEDICINE'S ability to collect information about the fetus has increased dramatically in the last few years. A variety of technics — collectively known as prenatal diagnosis — have made it possible to learn much about a fetus's genetic and metabolic state, chromosomal constitution (including gender), bone structure and other information, and, moreover, to learn it earlier and earlier in gestation.

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Many of the technics yield this information at a time that makes the selective abortion of a fetus legally possible. Selective abortion is morally unacceptable to many people. This report does not discuss this question in detail. It recognizes that selective abortion raises a variety of deeply troubling moral, social and legal questions that must always be kept in mind for the exercise of responsible decision making.

Since the release in the United States of a prospective study of mid-trimester amniocentesis in the fall of 1975 and its subsequent publication, the comparative safety and accuracy of this form of prenatal diagnosis has been widely accepted.¹ A study carried out in Canada and published shortly after the United States study disclosed similar findings.² As a result, it has been predicted that amniocentesis will soon become much more common, and ultimately part of standard obstetric practice, where indicated.

Rapid development of other forms of prenatal diagnosis is proceeding along several fronts. Ultrasound, often used as an adjunct of amniocentesis, is also used as a separate form of fetal diagnosis, not only for the detection of birth defects but also to establish gestational age, or to detect multiple pregnancy early.³ This technic has been expanded to include real-time scanning, which yields a moving picture of the fetus.⁴

JOB'S SYNDROME

Recurrent, "Cold", Staphylococcal Abscesses

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"So went Satan forth from the presence of the Lord, and smote Job with sore boils from the sole of his foot unto his crown"—
Job, II, 7.

We have examined two girls who have had recurrent, "cold", staphylococcal abscesses since birth. The staphylococci do not seem to be unusually virulent, and neither child has diabetes or any other condition known to predispose to infection. Since we are not aware that any similar cases have been described previously, we report these cases in detail.

Case-reports

FIRST CASE

The patient is a nine-year-old girl who was originally referred, aged seven, to the University of Washington Hospital. She was normal at birth but at three weeks she had eczema which became infected. She later had recurrent ear, nose, and sinus infections which were treated many times with antibiotics. At three years a tonsillectomy was done because of chronic otitis media. During the next few months she had furuncles of the buttocks, abdomen, and extremities. At forty-five months she had severe left-cervical adenitis which was treated with antibiotics and incision and drainage. Culture of pus from the cervical abscess grew staphylococci which were resistant to penicillin, tetracycline, erythromycin, and streptomycin (P., T., E., and S.). Three weeks later she had right-lower-lobe pneumonia which responded to treatment with oxacillin. At forty-seven months she had furuncles on the face and cellulitis about the right eye. She was referred for evaluation because of the persistent skin, ear, sinus, and mastoid infections.

On clinical examination she was noted to have red hair, fair skin, seborrhoea of the scalp, many scars from old abscesses, atrophic skin over the cheeks, weeping lesions about both ears, perforated tympanic membranes, chronic conjunctivitis, drainage and crusting about the nose, dental caries, and atrophic changes about the fingernails. Her height and weight were at the 10th percentile for age (Nelson 1964). There was no lymphadenopathy or hepatosplenomegaly.

Hæmatocrit, white-blood-cell count, platelets, and urinalysis were normal. The sweat chloride was 6.8 mEq. per litre. Examination of the bone marrow showed normal cellular elements and decreased iron stores. Intermediate strength purified protein derivative of tuberculin and fungus skin-tests were negative. A battery of scratch tests for allergies was negative. X-rays showed maxillary and ethmoid sinusitis, bilateral mastoiditis, and a persistent infiltrate in the lower lobe of the right lung. Tests of immunological function are summarised in the accompanying table.

She was started on intermittent oxacillin, prophylactically. Infections continued to recur, and continuous therapy was started with 500 mg. of oxacillin three times a day.

At age seven years and four months, while on oxacillin prophylaxis, she had a 6 cm. × 10 cm. mass in the anterior midline of the neck which extended along the left mandible. There was no fever or cervical lymphadenopathy. The mass was only slightly tender and did not feel warmer than the rest of the body. The hospital surgeons thought that the mass was an enlarged, thyroglossal-duct cyst which was not infected. The white-blood-cell count was 12,000 per c.mm. with 66% neutrophils, 25% lymphocytes, 5% monocytes, and 4%

ts have emphasised that it may arise either in patients active leprosy or, more commonly, in those who had leprosy (Muir 1948, Browne 1965). Schulz found normal vitamin-A levels in the blood of leprosy patients with ichthyosiform changes in the

our patients hydration alone relieved the condition, dry skin reappeared when daily hydration was discontinued. This suggests strongly that the important factor is the water content of the skin, and that loss of water from the surface of the skin should be prevented, hydration, by means of a hydrophobic barrier. This suggestion is supported by the observation that dry skin occurs in those situations where there is an irreversible deficiency of endogenous hydration.

Blank (1952) found that human skin becomes brittle as its water content falls below 10 mg. per 100 mg. dry skin. It seems that as long as the relative humidity of the atmosphere is 60% or over, an equilibrium exists between the skin and the atmosphere, and excessive skin drying does not take place. Blank and Underwood (1952) also found a direct association between air moisture and smoothness and suppleness of the skin. In the patients described by us the dryness of the tropical atmosphere was an important contributory factor in the occurrence of dry skin, in addition to the cutaneous anaesthesia and diminished sweating already referred to.

In leprosy patients, other advantages follow hydration of the skin besides the prevention of fissuring with its risk of deep inflammation. The softening of localised hard areas at the sites of pressure on the soles of the feet reduces the liability to plantar ulceration. Skin texture, which is improved noticeably, and this enhances the degree of tactile and thermal appreciation remaining. Hydration of the skin reduces the risk of burning. Leprosy patients frequently burn themselves by holding utensils that are too hot for the normal person to touch. The patient with leprosy has impairment of peripheral sensation discovers—as does the Indian fire-walker who immerses his feet in fire for hours before performing—that hydrated skin is a less efficient conductor of heat than hard, horny skin.

Although this report mainly concerns leprosy patients, the findings presented seem to be applicable to the dryness of other conditions, to judge from our investigations made in Africa and India on patients who do not have leprosy.

Summary

Dry skin is a frequent and serious complication of leprosy. The low water content of the skin is associated with diminished sweating and peripheral neuropathy, and aggravated by low atmospheric humidity. Dry skin in leprosy, and probably in other conditions, may be relieved, and fissuring prevented, by daily soaking of the affected part in water, followed by an application of soft paraffin.

We thank Prof. P. W. Brand and Prof. H. H. Gass (of Vellore), Dr. Elizabeth Spiess, the very cooperative patients of the Chevayur Leprosarium, Kerala, India, and Dr. R. M. B. MacKenna for helpful advice.

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IMMUNOLOGICAL STUDIES

Test	Case 1	Case 2
Serum-protein immunoelectrophoresis	Normal immunoglobulin pattern	Normal immunoglobulin pattern
Blood-type	A	O
Isohaemagglutinin titre	Anti-B (1/128)	Anti-A (1/16) Anti-B (1/4)
Schick	Negative	Negative
Typhoid-H titre	>1/160 (3 immunisations)	1/320 (4 immunisations)
Staphylococcal agglutinin titre* (normal $1/10$ to $1/100$)	1/320	1/640
Bone-marrow plasma-cells	Normal	Normal
Inflammatory cycle†	Normal	Normal
Phagocytosis in vitro‡	Normal	Normal

* By the method of Boger et al. (1960).

† By the method of Rebeck and Crowley (1955).

‡ By the method of Ecker and Lopez-Castro (1947) with *Staph. aureus* as the object of phagocytosis.

eosinophils. The mass was incised and 200 ml. of purulent material was obtained. Culture of the pus grew staphylococci resistant to P., T., E., and S. Pus drained intermittently and, two months later, a necrotic lymph-node was excised from the neck. Culture of this abscess also grew out staphylococci resistant to P., T., E., and S. Three weeks later she had another mass in the neck which was only slightly tender and erythematous. The mass was incised and a culture grew staphylococci resistant to P., T., E., and S. Seven days of intensive therapy with intramuscular oxacillin resulted in decreased drainage from the neck lesion and partial clearing of infected skin lesions about the eyes and ears.

The neck lesion drained occasionally over the next year and twice required incision. Staphylococci, resistant to P., T., E., and S., were cultured both times. At eight years and five months, she had a tooth abscess: the tooth extracted. Culture of this abscess grew out β -haemolytic streptococci which were not group A, B, C, D, or G. Three months later the old abscess site in the neck again drained, and the cultures grew staphylococci resistant to P., T., E., and S.

Through the time of observation at this hospital, the patient has had intermittent drainage from both ears, recurrent skin infections about the nose, eyes, and ears, and occasional furuncles on the face, arms, and extremities. Many cultures for mycobacteria have been negative. The only organisms isolated from closed lesions in this patient have been coagulase-positive *Staphylococcus aureus*, resistant to P., T., E., and S. Similar staphylococci have frequently been cultured from open lesions, although other organisms have also been found occasionally. She is at present much improved, and is receiving oxacillin, 2000 mg. daily, and frequent hexachlorophene baths.

Both parents, an older half-brother, and two younger sisters have been well. None has had staphylococcal infections.

SECOND CASE

The patient is a six-year-old girl who was referred to this hospital, aged two. Her birth weight after a normal-term pregnancy was 2300 g. (5 lb.). The only abnormality noted at birth was hyperextensible joints. At two weeks, eczema appeared on the face and spread to involve the neck and flexor surfaces of the extremities. At four months the eczema became worse, and she was treated for five days in a hospital. The rash improved but then became worse, and she was admitted to the hospital for a second time at five months. During that hospitalisation, she had an abscess over the medial aspect of her right knee which required incision and drainage. She then began having frequent subcutaneous abscesses of the scalp, neck, back, buttocks, and knees. Several observers noted independently that the abscesses had remarkably little local erythema or tenderness. Cultures of these abscesses have always grown *Staph. aureus*. Many cultures for

mycobacteria and fungi have been negative. She continued to have weeping, crusting lesions about the ears, eyelids, and nose. She also had a chronic, purulent, nasal discharge and many infected skin lesions over the flexor surfaces of the arms and legs.

When first seen at this hospital, she was a small, active child with fair skin and bright-red hair. Her height and weight were well below the 3rd percentile for age (Nelson 1964). There were numerous scars from the abscesses that had been drained. On the buttocks were two large non-tender abscesses which were 6 cm. in diameter. There was another large, non-tender abscess on the back of the neck. Weeping, crusting lesions were present about the ears, eyes, and nose. Joints of the extremities were hyperextensible. There was no lymphadenopathy or hepatosplenomegaly.

Hematocrit and urinalysis were normal. The white-blood-cell count was 18,000 per c.mm. with 64% neutrophils, 33% lymphocytes, and 3% monocytes. Platelets were normal. The bone marrow was hypercellular with a normal myeloid-erythroid ratio and a notable eosinophilia. The sweat chloride was 16 mEq. per litre. Intermediate strength PPD and fungus skin-tests were negative. A battery of scratch tests for allergies was negative. X-rays of the skull showed premature closure of the sagittal suture. X-rays of the chest and long bones were normal.

She improved somewhat with occasional antibiotic therapy and hexachlorophene baths, though she still required occasional incisions for drainage of abscesses. At three years she had cough, fever, and large abscesses of the right buttock and left thigh. She was admitted to hospital and was found to have multiple, staphylococcal abscesses, staphylococcal pneumonia, cardiomegaly, and congestive heart-failure. Staphylococci isolated from the abscesses were resistant to penicillin. She was critically ill for two weeks but responded to therapy with methicillin, digitalis, diuretics, and large doses of gamma-globulin.

She improved for the next three months but then infections recurred about the eyes and ears and she had an abscess on the forehead. The abscess was noted to have little warmth, tenderness, or redness. Cultures of the pus from this abscess grew staphylococci, resistant to penicillin. She was started on intermittent cloxacillin therapy, 500 mg. for four days a week. During the next five months she had an abscess of the right thigh and an abscess on the neck.

At four years she began having a persistent cough. Staphylococci and normal flora were isolated from sputum cultures. Chest X-ray showed bilateral bronchiectasis, and postural drainage was begun three times daily.

Over the next year and a half she was relatively asymptomatic and free of severe infection. She continued to have periodic recurrences of infections about the eyes, cheek, and ears. Occasionally, pus drained from both ears, and infected eczematoid lesions appeared on the elbows and knees. She also continued to have a mild, chronic cough. Mild clubbing of the fingers was noted at the age of five and a half.

At age five years and nine months her height was 94 cm. and her weight was 10.5 kg. (23 lb.). She had no new symptoms and was attending school regularly. Chest X-ray showed new extensive, bilateral, pulmonary infiltrates in the right middle lobe and the lingula of the left upper lobe. The infiltrates did not clear with increased postural drainage and antibiotic therapy and, one month later, she was started on a home-care programme of mist and aerosol therapy for her pulmonary disease. She improved remarkably within a week. Her appetite increased greatly and the pulmonary infiltrates partly cleared.

The only organisms isolated from closed lesions in this patient were coagulase positive *Staph. aureus*, resistant to penicillin. Cultures of open infections grew similar staphylococci, although other bacteria were isolated occasionally.

Both parents and three siblings are well. None has had staphylococcal abscesses, skin infections, or eczema. A sister and a brother are of normal height and weight. The other sister physically resembles the patient, having hyperextensible

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joints and very small stature, but has not had frequent infections.

Discussion

The common features of these two patients include fair skin, red hair, and recurrent, "cold", staphylococcal abscesses. Both have chronic, purulent, nasal discharge, sinusitis, chronic otitis media, and infected eczematoid skin lesions. Both have pulmonary disease, although this has been more severe in the case 2 than in case 1. The antibiotic-sensitivity pattern of the staphylococci isolated from each patient has not changed over several years, and all the infections in each patient may have been caused by the same strain. It seems unlikely, however, that the infections have been caused by unusually virulent staphylococci, because the parents and siblings have not had staphylococcal infections.

The manifestations of these patients are clearly distinct from the immunological-deficiency syndromes, which include agammaglobulinemia, Chediak-Higashi syndrome, Aldrich syndrome, and ataxia-telangiectasia (Good et al. 1962). There was no evidence of systemic diseases such as leukemia, lymphoma, or reticulo-endotheliosis. The clinical signs were similar to those of the syndrome of progressive septic granulomatosis reported by Carson et al. (1965). Patients with this syndrome have pigmented, lipid histiocytes, chronic lymphadenitis, chronic dermatitis, chronic pulmonary disease, and fatal infections. In contrast with the cases presented here, patients with progressive septic granulomatosis have been males with hepatosplenomegaly who were infected with bacteria other than staphylococci; they have not been reported as having "cold" abscesses.

We suggest that these patients have a defect which makes them unusually susceptible to staphylococci. Since the mechanisms of resistance to staphylococcal infections are not understood, it is difficult to suggest a specific defect. No abnormality was found in serum-immunoglobulins, antibody formation, inflammatory cycle, or phagocytosis in vitro. Two observations on these patients may be relevant. The first is that despite years of recurrent abscesses, there were few systemic infections. Therefore, the patients probably have normal "systemic" resistance to staphylococcal infections. The second observation is that the abscesses are cold. Miles (1956) has shown that the first few minutes after bacteria have been injected into the skin constitutes the decisive period for inflammation. Local nonspecific factors are probably of primary importance during the decisive period. We suggest that these patients have a defect in local nonspecific mechanisms of resistance. Perhaps there is an abnormality of the mediators of the acute inflammatory response (Spector 1964).

The pitiful appearance of these patients and the history of recurrent abscesses and skin infections makes the name "Job's syndrome" seem suitable.

Summary

Two girls with life-long histories of "cold" staphylococcal abscesses are described. The signs were different from those of the recognised syndromes of recurrent infections. The infections were partially controlled with continuous antibiotic prophylaxis. It is suggested that the patients have a defect in local resistance to staphylococcal infections.

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Preliminary Communication

LOSS OF EJACULATION IN MALE RHESUS MONKEYS AFTER ADMINISTRATION OF PROGESTERONE TO THEIR FEMALE PARTNERS

THE endocrine factors concerned in the control of primate sexual behaviour are imperfectly understood but, although little studied, have special relevance for the human situation. Observations in the field have shown that, at the time of greatest sexual skin swelling in the baboon, which is usually near mid-cycle, the males show most interest in the females.^{1,2} In the laboratory, rhythmic changes in sexual behaviour have been noted to be related to the menstrual cycle of the female.³⁻⁵ We have found that, in ovariectomised rhesus monkeys given hormone-replacement treatments, the sexual interactions between opposite-sexed pairs are strikingly influenced by the administration of oestradiol and progesterone.^{6,7} The most notable finding is that dramatic changes in the sexual behaviour of males result when hormones are administered to their female partners: oestradiol stimulates male copulatory activity and progesterone depresses it. We describe here some further work on the depression of sexual activity in the male after the administration of progesterone to the female.

METHODS

Three adult, sexually active male and three mature, female rhesus monkeys were investigated for a period of 18 months. Two females were paired with each male, making a total of six paired combinations. The females were regularly introduced, by means of a transfer cage, into the home cages of the males for testing sessions of 60 minutes. Between tests, animals were housed in individual cages without access to each other. Experienced observers recorded the behaviour during testing sessions from behind a one-way vision mirror, and components of the behaviour observed were quantified by means of a scoring system in terms of time, sequence, and frequency. After bilateral ovariectomy, the females were allowed 10, 16, and 41 weeks respectively to attain a basal condition, at which time routine testing was commenced. After a further 4 weeks, oestrogen was administered to the females in two different

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