STARWARS OF THE BODY BESEIGED

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Immunodeficiency in Training Physicians

- We teach about all of the parasites, viruses, fungi, & bacteria in great detail.
- We teach little, however, about how the body responds to each of these types of pathogens.
- The responses of the host determines the degree, severity, and type of symptomatology and the outcome of infection in all instances, i.e. HIV!!

EVALUATION OF IMMUNITY: An Overview and Review

Harry R. Hill, M.D.

Objectives:

To review the major portions of the immune system and relate the components to "Star Wars" of the body

- To describe how patients with defects in different portions of host defense present clinically
- To describe the laboratory tests utilized in defining defects in the immune system

CASE PRESENTATION

The patient was a 16 month old male infant who was brought in by his mother who complained that he was always sick. The patient had suffered from one to two upper respiratory infections per month since 4 months of age. There had also been two middle ear infections and a number of "sore throats." The patient had never been hospitalized and had grown normally. Because of the recurrent infections, the patient received numerous courses of antibiotics and was currently receiving 0.2cc of gammaglobulin per month.

Immunologic Deficiencies in Physician Training

This editorial appeals for the repair of a major deficiency in the training of physicians (an immune deficiency), namely an appraisal of immunodeficiency as a cause of specific infections. A primarily "parasite oriented workup of an infection and especially recurrent infections will no longer suffice as the patient's host response clearly determines the clinical presentation and outcome of specific infections.
Gene H. Stollerman, M.D. J. Chronic Diseases

CAUSES OF RECURRENT INFECTIONS

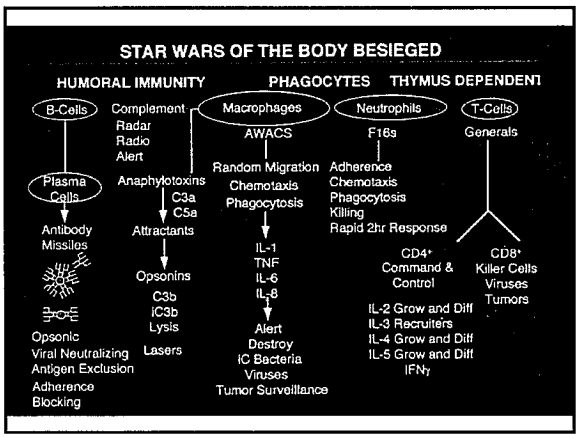
- The normal child may suffer 6-12 infections per year. Day Care Centers increase this.
- Structural and anatomic defects must be ruled out
- Immune deficiency is a possibility

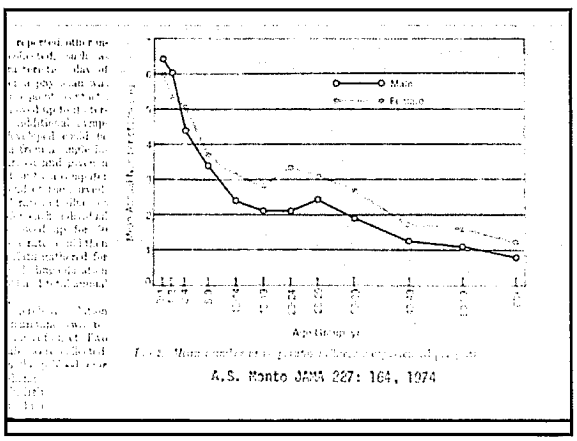
Physical and Anatomic Defects

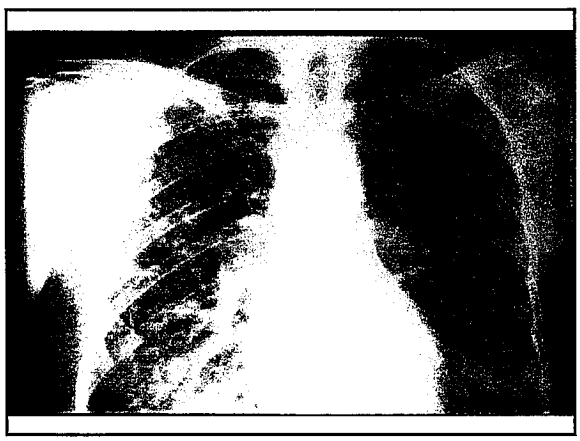
Foreign Bodies:	**Pulmonary - peanuts, carrots, bacon, portacaths, vascular lines. catheters,**
Breakdown Barriers:	**Skin, mucous membrane, fractures, burns, eczema**
Anatomic Problems:	**Eustation tubes, ureters, sinuses, CF, dermal sinuses, basilar skull fx**

The Normal Child with Too Many Infections

- **6 infections per year average in preschool, early school-aged child**
- **Normal to go as high as 12, however**
- **Daycare average is 9/year**
- **Infections generally last 2 weeks with prodrome, acute and convalescent phases**
- **12 infections X 2 weeks = 24 weeks = 6 months/yr!!!!!!! Oh No!!!!!!**







Case History CVID

- 29 year old female who had 5 episodes of pneumonia over the past 2 years, hospitalized for 3 of these.
- Diffuse infiltrate on X-ray; ? Pigeon breeders hypersensitivity pneumonia??
- IgG 60 mg %; IgA < 6 mg%; IgM 25 mg %; (an IgG of ≤ 250 mg% called as a critical value!)
- B cells had normal surface immunoglobulin and were present in normal percentages

CLINICAL FEATURES OF HYPOGAMMAGLOBULINEMIA

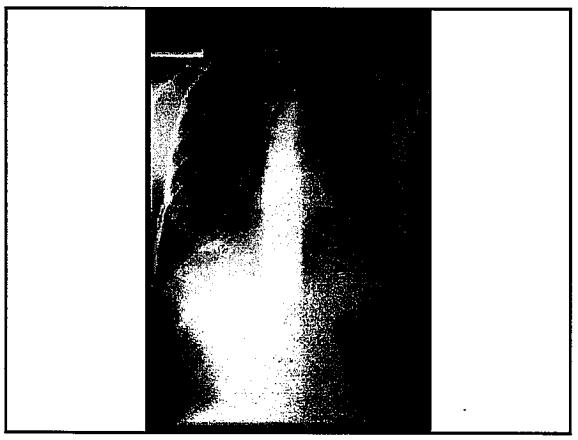
INFECTION	%	INFECTION	%
Sinopulmonary	100	Empyema	4
Sinusitis	66	Meningitis	4
Otitis	32	Bacteremia	5
Pneumonia	86	Giardiasis	34
1-10 episodes	68	UTI	4
10 or more	18		
Bronchiectasis	28		

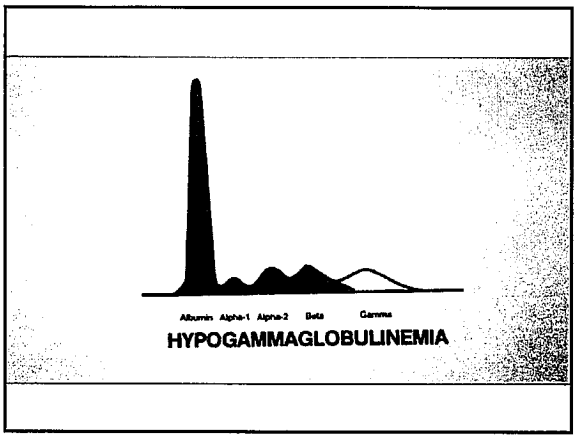
H. flu, S. pneumoniae, S. pyogenes, S. aureus

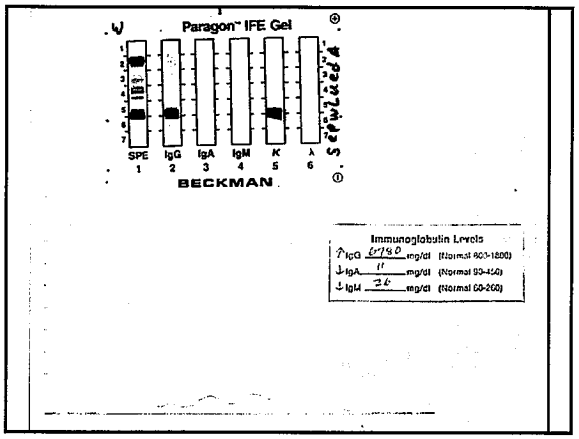
ASSOCIATED FINDINGS IN ACQUIRED HYPOGAMMAGLOBULINEMIA*

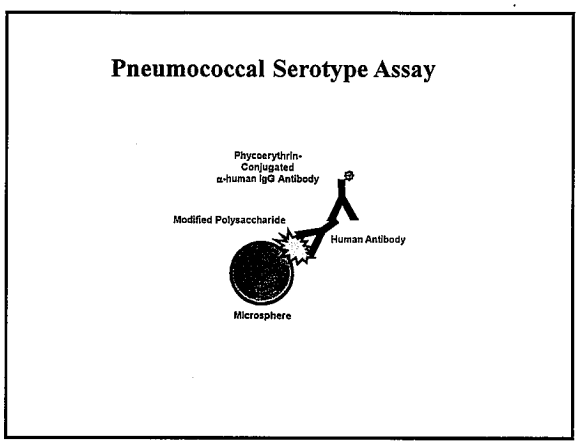
FINDING	%	FINDING	%
Diarrhea	60	Arthritis	8
Malabsorption	60	Allergy	40
Achlorhydria	53	Malignancy	24
Giardia	64	Stomach CA	
X-ray NLH Thymoma	28	Lymphoma	
Splenomegaly	28		
Conjunctivitis	6		

**Amer. J. Med.*





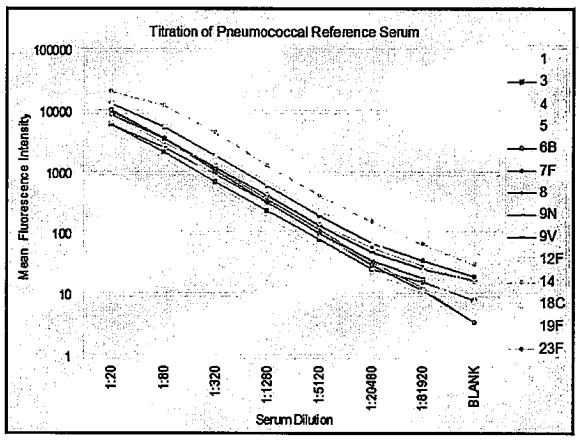


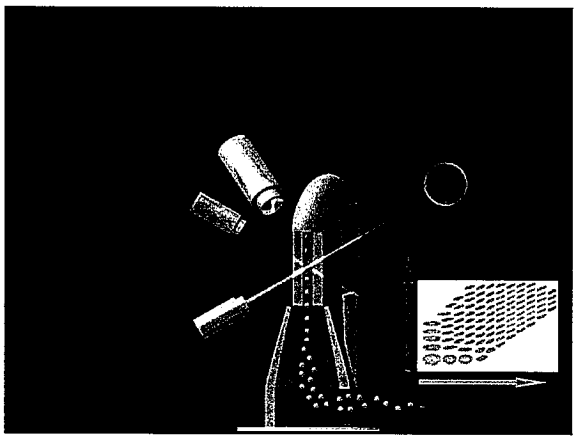


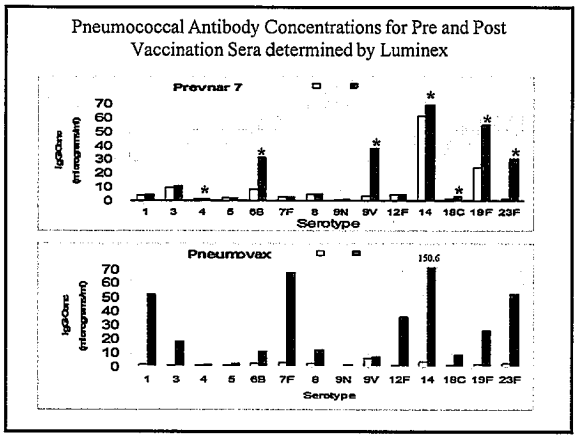
IMMUNOGLOBULIN G SUBCLASSES*

Characteristic	IgG1	IgG2	IgG3	IgG4
% in Serum	70	21	5	4
Half-Life Days	23	23	11	23
C1q Binding	++++	++	++++	-
Sensitize Cells	-	-	-	+
Polysaccharide AB	-	+++	-	-
Protein Ab (D,T)	++++	-	++	-
Viral Protein AB	++	-	++++	-

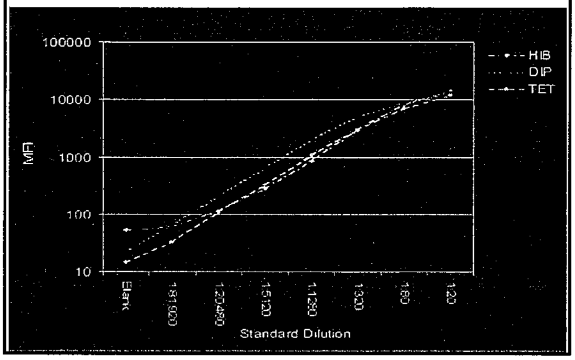
*Based on antigenic and structural differences of heavy chains.



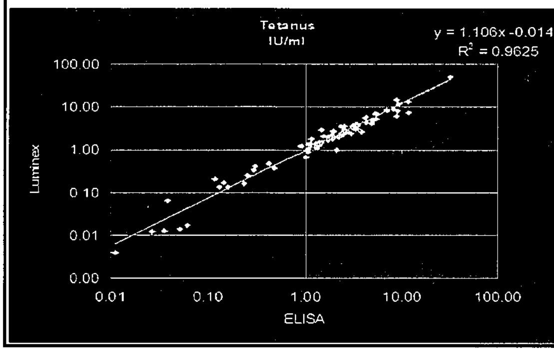




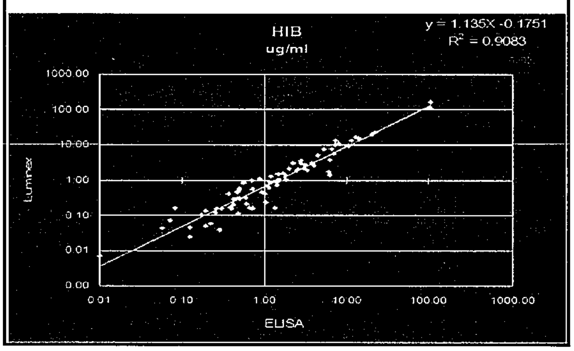
Standard Curve for Multiplexed Luminex Assay for Tetanus, Diphtheria and *Haemophilus influenza* type b



Comparison of the Multiplexed Luminex Assay to an In-House ELISA for IgG Antibodies to Tetanus



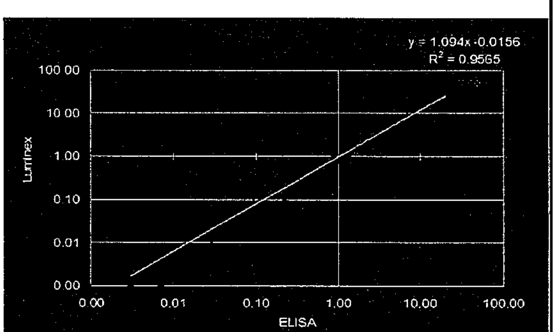
Comparison of the Multiplexed Luminex Assay to an In-House ELISA for IgG Antibodies to *Haemophilus influenza* type b



Summary of IgG Concentrations for Pre and Post-Vaccine Samples for Dip, Tet and Hib determined by the Luminex Multiplexed Assay

		[illegible]	[illegible]	[illegible]	[illegible]
[illegible]					
	Mean	[illegible]	[illegible]	[illegible]	[illegible]
	Range	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]					
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Range	[illegible]	[illegible]	[illegible]	[illegible]

Comparison of the Multiplexed Luminex Assay to an In-House ELISA for IgG Antibodies to Diphtheria

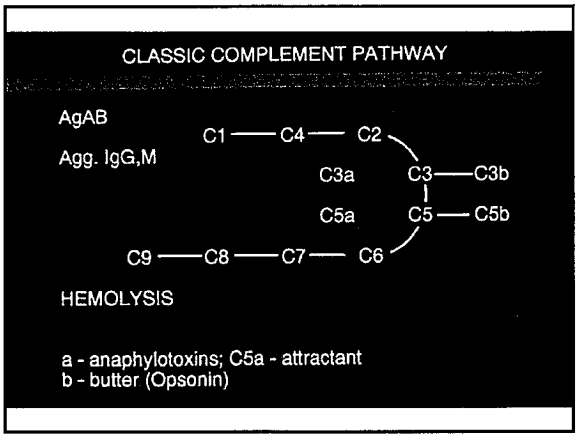


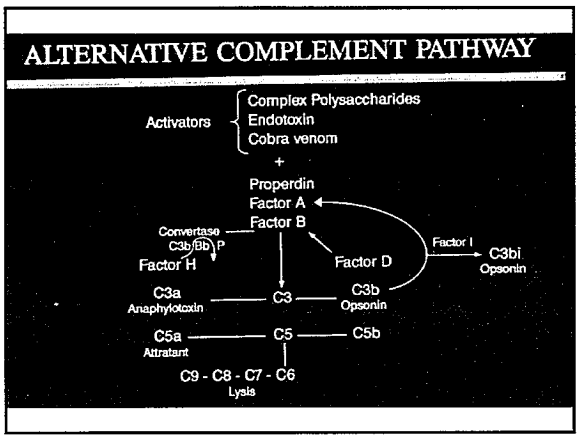
DIAGNOSIS OF ANTIBODY DEFICIENCY

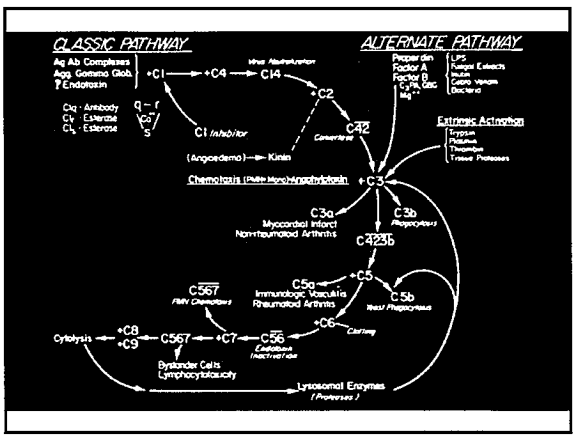
- **Quantitative immunoglobulins or IgG subclasses by Nephelometry; IgA subclasses**
  Specific antibody production by multianalyte or ELISA
  - **Diphtheria and tetanus titers, Hib - IgG1**
  - **Pneumococcal antibody titers, - IgG2**
  - **Influenza titers - IgG3**
- **B cell numbers with CD19, or CD27 or surface IgM, IgD, IgG, IgA – B cell immunodeficiency profile, CD40, CD40L**
- **T-helper and suppressor, memory, naïve, NK & B cell numbers by flow cytometry – T cell Immunodeficiency Profile Extended**

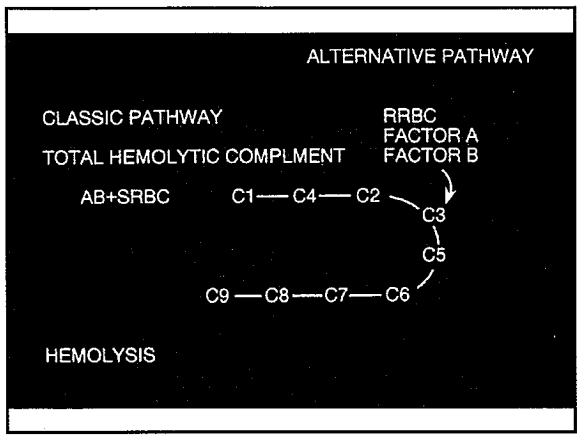
Allergen Specific IgE

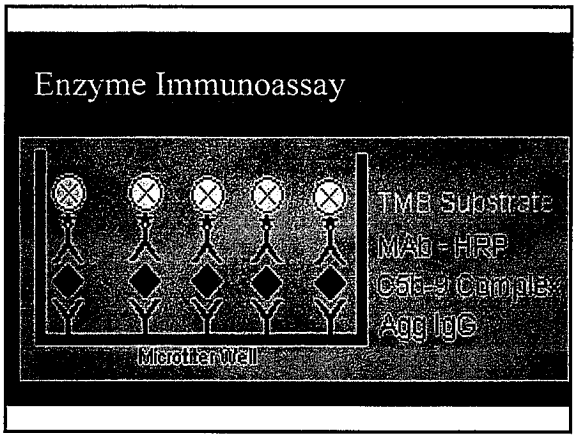
- Individual allergens (240) + 58 Mostly Client Requested Panels (We do what you tell us to do;19-20 pages of allergens and 4 pages of panels for everything and everywhere!!!! Help me simplify them, please!!!!!)
- IgG4s in development (now to IBT)
- Allergen Screens – Jerry Gleich now at Utah - More Screens- Agreed, "Allergens, Inhalent Multiallergen Assay (#0055368) is all I use + !!!

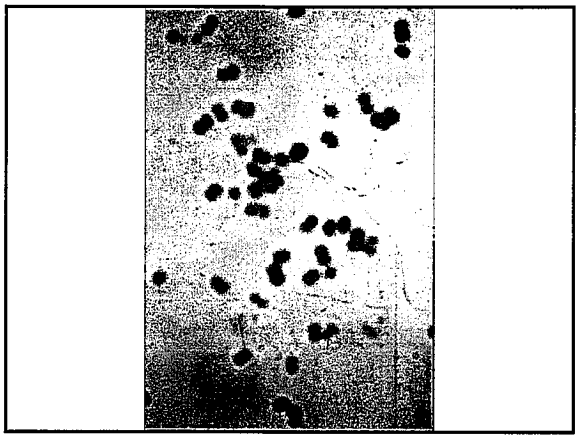






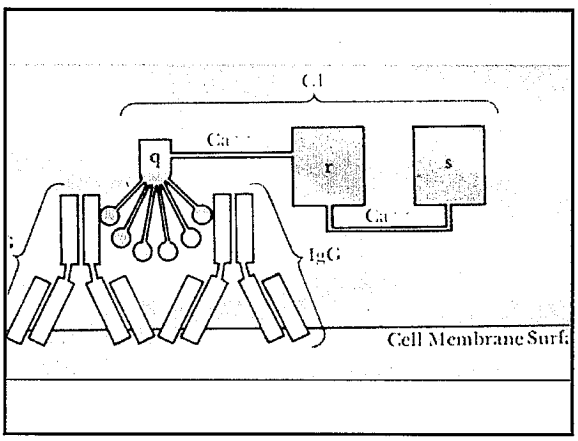


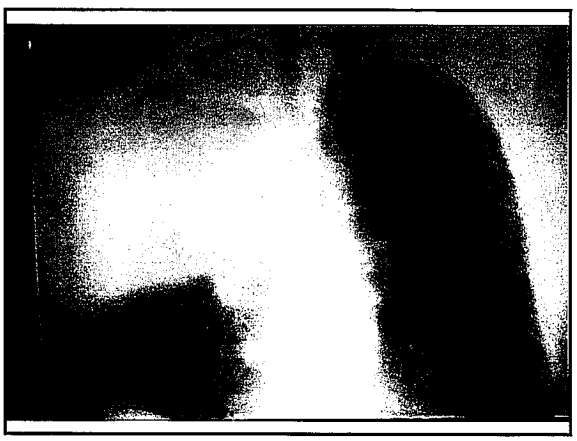




Summary of Complement Reactions

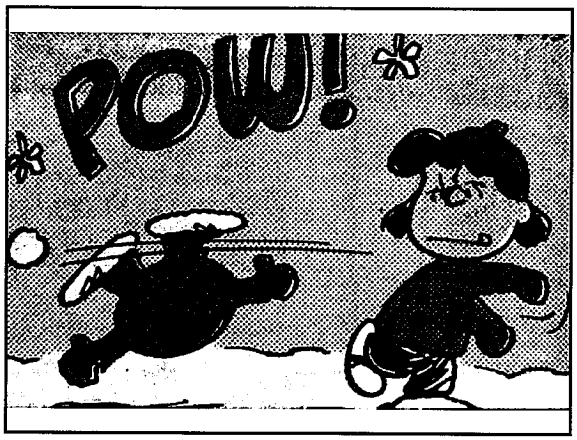
- C1-9 with 4 out of line
- C3a and C5a are anaphylotoxins
- C5a is attractant
- C3b and iC3b act as butter or opsonins
- The terminal membrane attack complex C5-9 lyse bacteria or RBC, etc
- Alternative pathway functions in absences of antibody

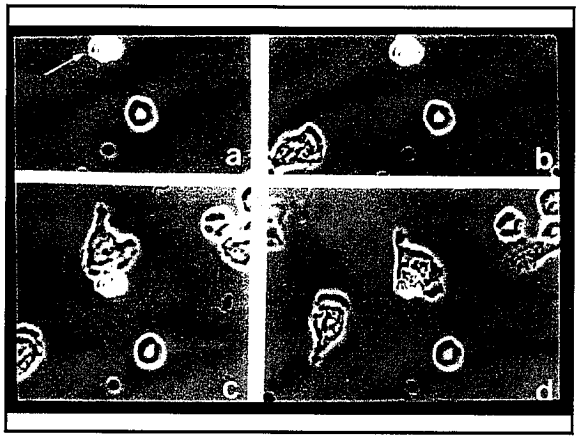


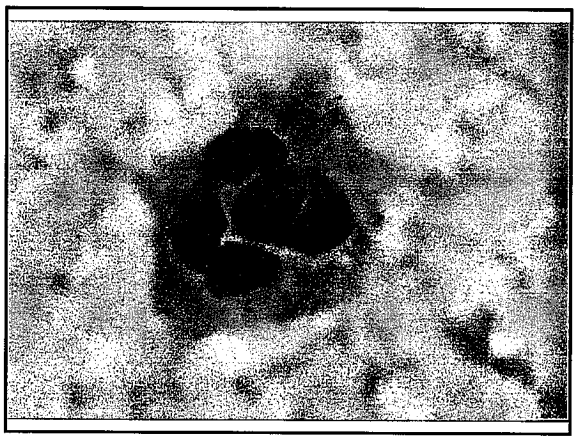


COMPLEMENT ABNORMALITIES

- Clinical Findings:
  - Early component deficiencies - lupus like disease, nephritis, arthritis, C1qrs, C2, C4
  - C3 - severe infections
  - C5,6, 7, 8 - Disseminated *Neisseria* gonorrhea and meningitidis infections
  - Alt. pathway - *S. pneumonia*, *H. flu* sepsis
  - Hereditary and acquired C1 esterase (Quantitative + Functional)



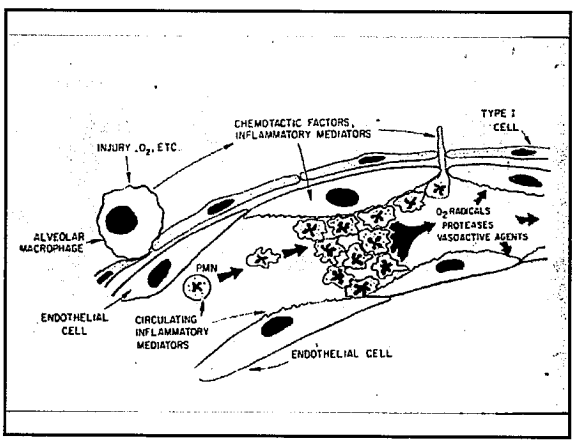


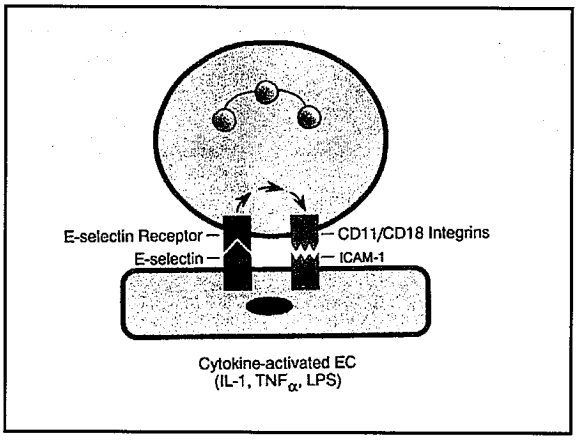


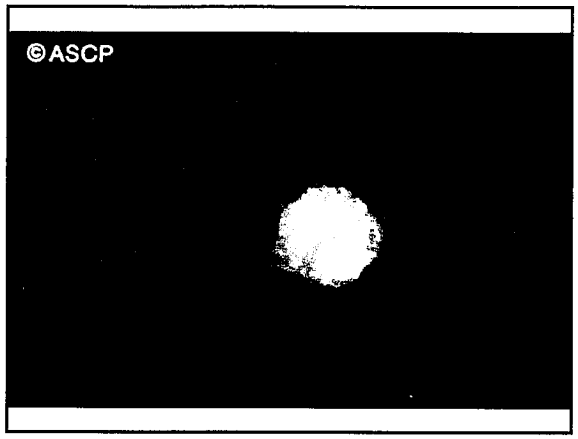
PHAGOCYTE ABNORMALITIES

Clinical Findings

Abscesses
Chronic cutaneous candidiasis
Pulmonary infections
Otitis
Candida
Aspergillous

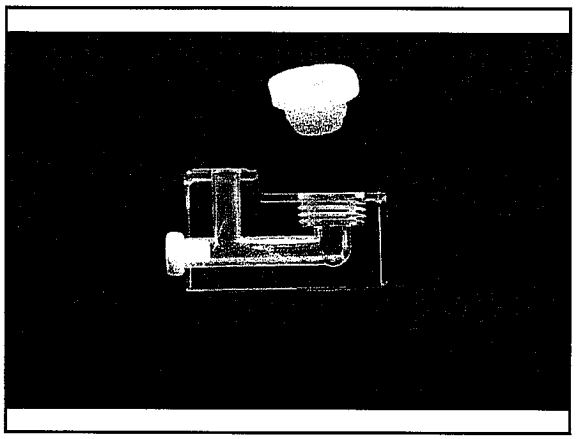




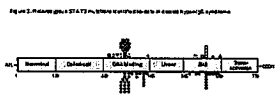


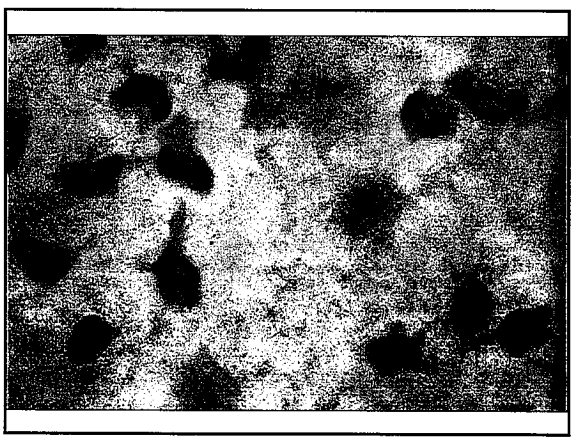
Clinical Features of the Hyper IgE (Job) Syndrome

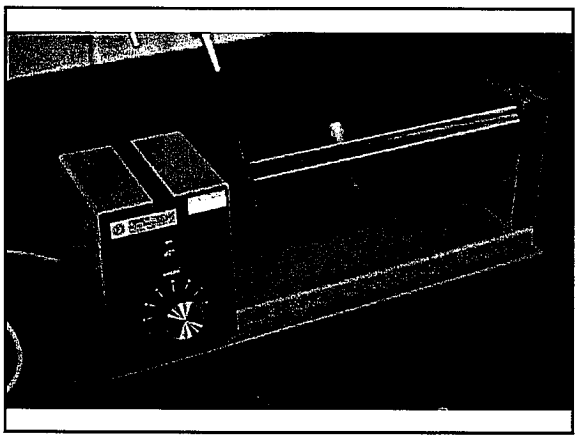
- Early Onset eczema
- Extreme elevation IgE
- Sinopulmonary Infections
- Staphylococcal abscesses
- Candida mucocutaneous infections
- Job facies – broad nose and nasal bridge Prominent forehead, doughy skin
- Bone fractures
- Decreased interferon gamma
- Defective chemotaxis, elevated IgE
- Mutations in STAT 3 gene

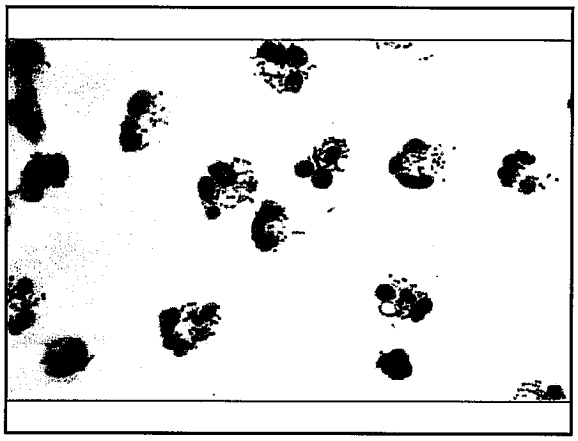


Mutations in STAT 3 in Jobs





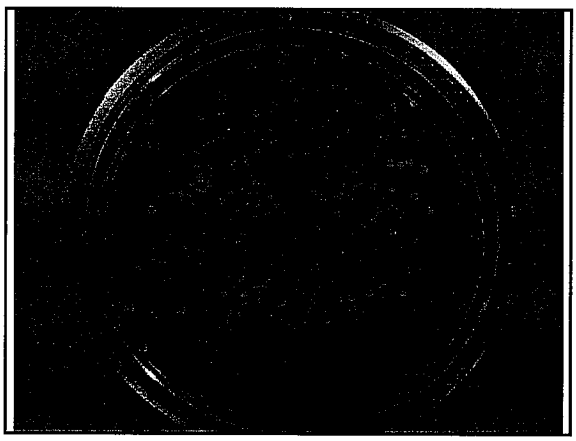




Functional Screening Methods

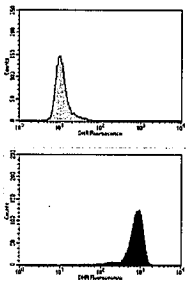
Neutrophil Oxidative Burst Assay (DHR)

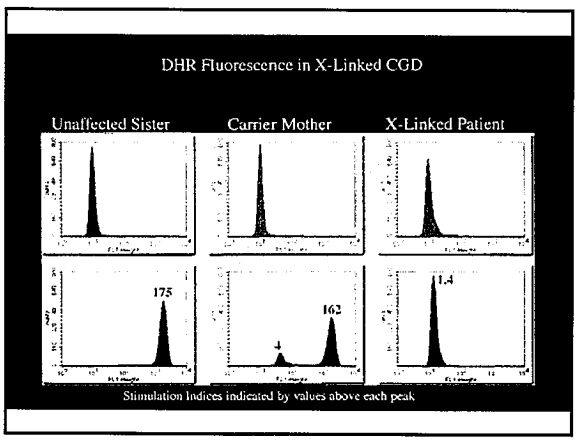
White blood cells, after lysis of rbc's in whole blood, are incubated with DHR123 and catalase, then stimulated with Phorbol 12-myristate 13-acetate (PMA). Dihydrorhodamine oxidation to fluorescent rhodamine by the respiratory burst of the cell is assessed by flow cytometry by determining the increase in mean channel fluorescence.

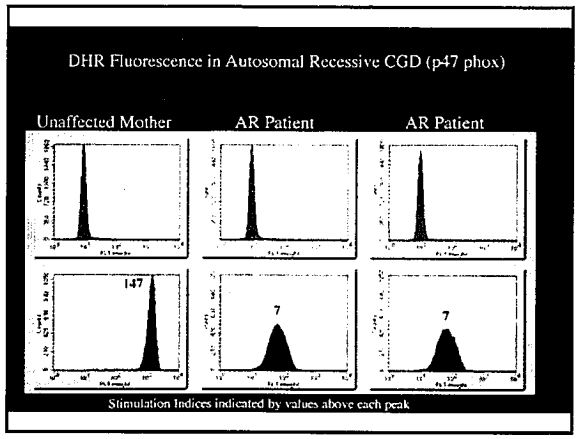


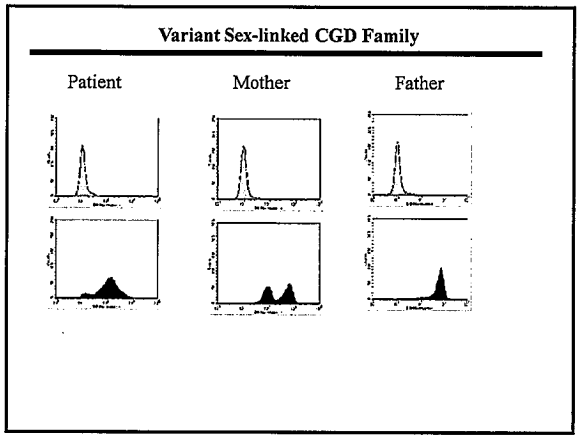
Neutrophil Oxidative Burst Assay

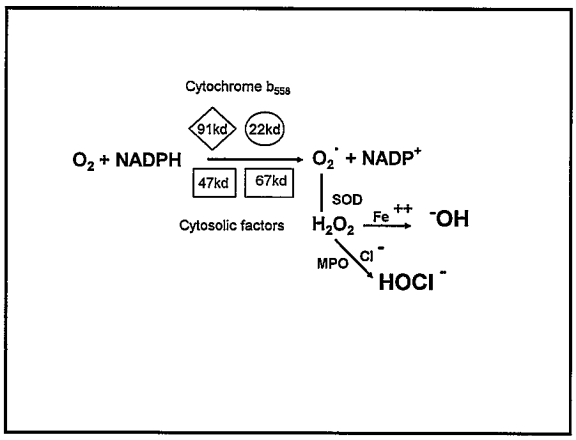
Normal

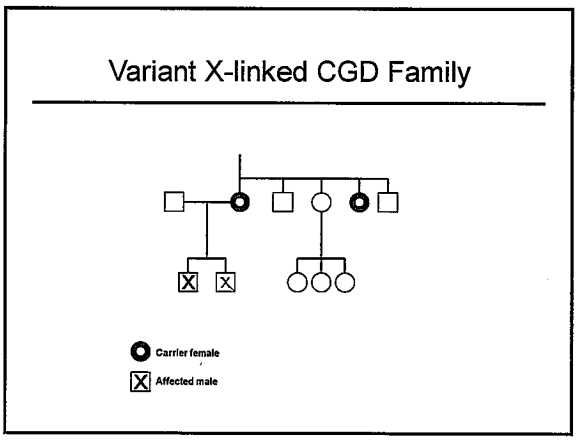






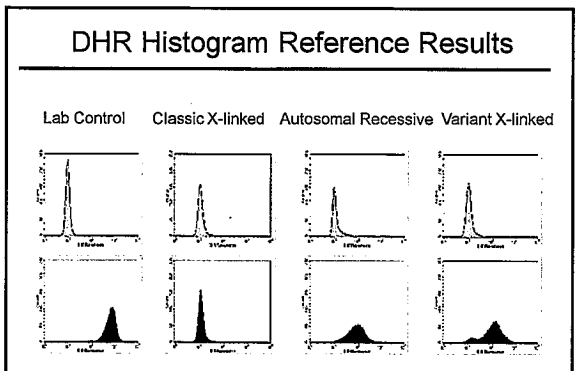






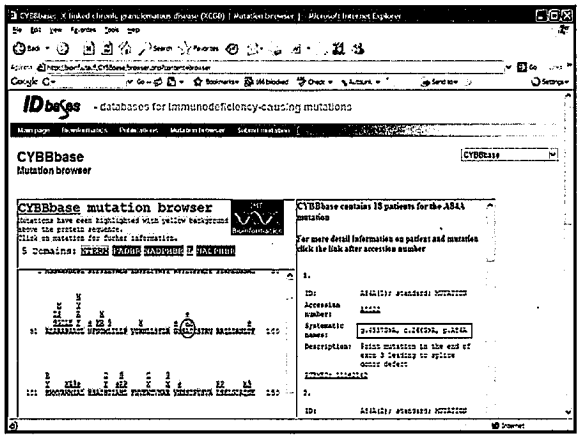
Chronic Granulomatous Disease

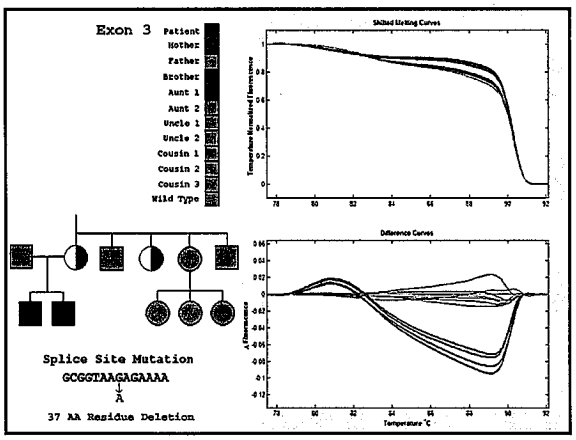
- Severe infections become apparent early in most cases and include:
  - pneumonia
  - lymphadenitis
  - skin and visceral abscesses
  - Osteomyelitis
  - Granulomas forming at sites of infection
- Sixty-five percent are inherited in an x-linked recessive manner due to an absence of the heavy chain of cytochrome b_{558} (gp 91) due to mutations in the 13 exons encoding the CYBB gene
- Thirty percent are inherited in an autosomal recessive manner due to the absence of phox 47 encoded on chromosome 7 complicated by the presence of a pseudogene that can interfere
- An additional 5% are due to phox 67 and the gp22 light chain of cytochrome b_{558} on chromosomes 1 and 16, respectively

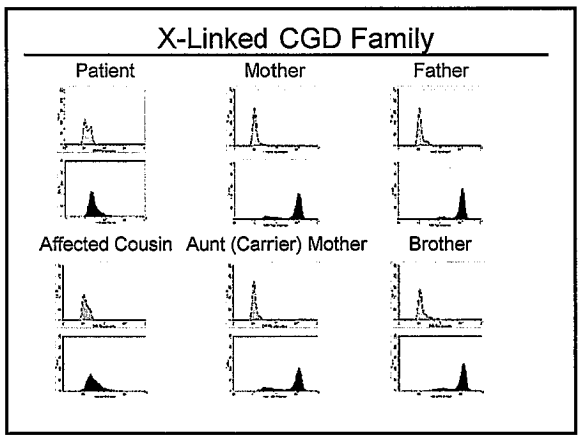


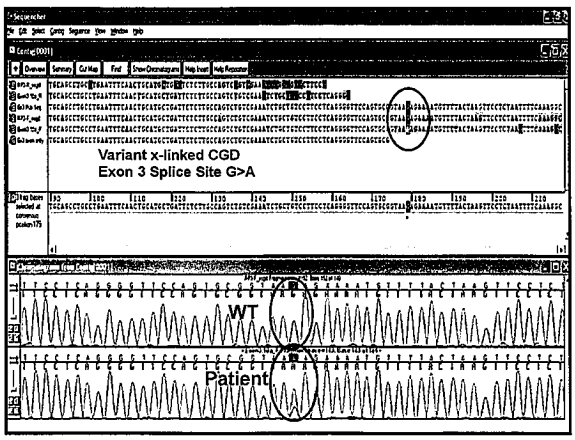
Molecular Methods

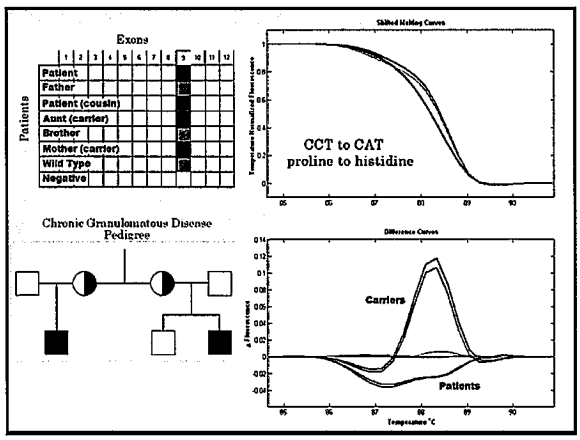
- PCR followed by high-resolution melting analysis
  - DNA extracted from whole blood
  - Primers for all 13 exons encoding the heavy chain of cytochrome b_{558} are plated onto 96 well plates
  - PCR amplification on MJ Research Block thermocycler
  - High resolution melting performed on LightScanner™
  - Analysis of melting data utilizing LightScanner™ software
  - Involved exons identified were sequenced for suspected mutations

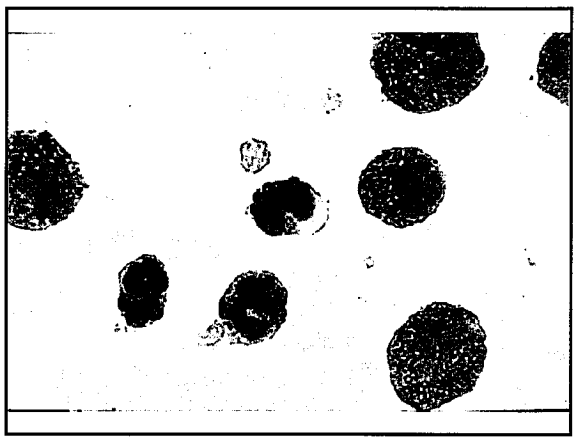


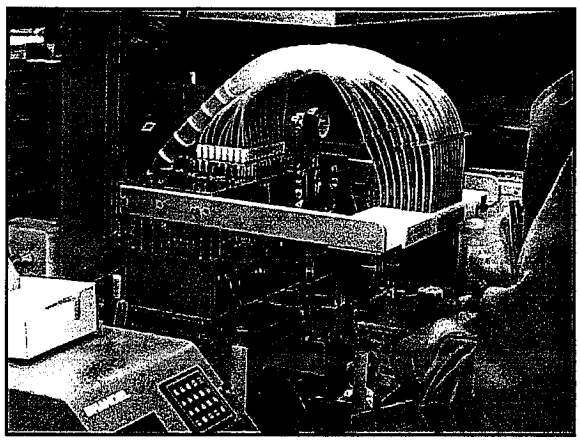












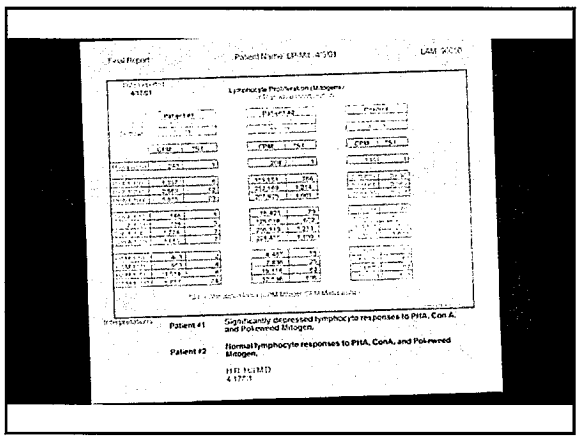
CELL MEDIATED IMMUNE DEFECTS

Clinical Findings

Severe viral infections

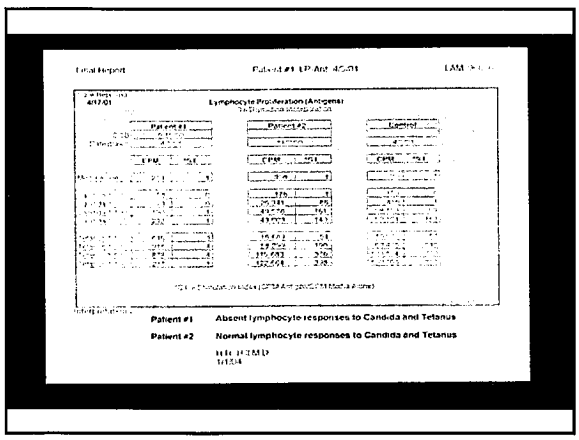
Fungal infections

Intracellular bacterial infections

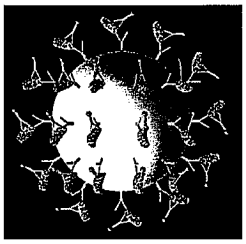


WHO NOMENCLATURE FOR DIFFERENTIATION ANTIGENS

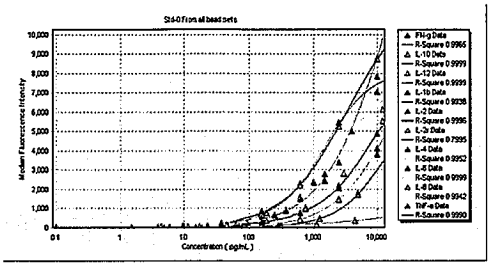
ANTIGEN	Mab & CELL TYPE
CD 1	Thrombocytes
CD2	E-Rosette Receptor, T & NK
CD3	Mature T Cells
CD4	Helper-Inducer T Cells, Monos
CD5	CLL, PLL Cells, T & B
CD7	T ALL & NK
CD8	Cytotoxic T Cells
CD25	Suppressor T Cells
CD45 Ra	Naïve T Cell
CD45Ro	Memory T Cell
CD16/56	Natural Killer Cells

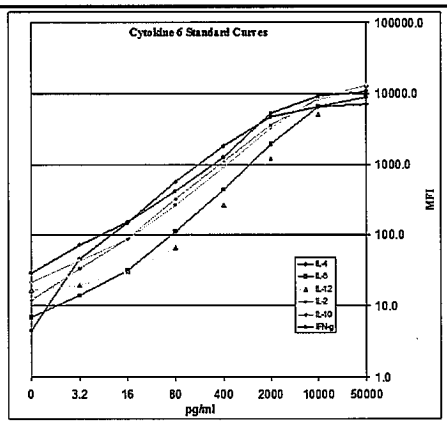


Cytokine Capture/Sandwich



10 Multiplexed Cytokine Panel for Serum

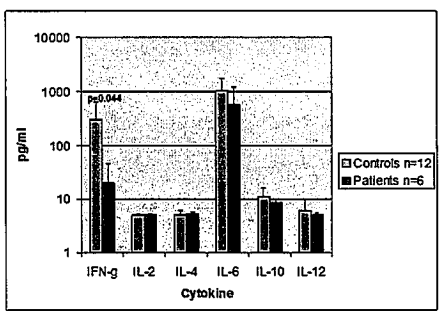




Initial Evaluation of Patients with Recurrent Infections

- Document nature, severity and timing of infections
- Obtain a good family history
- Careful physical examine looking for evidence of past infections
- Check humoral immunity with IgG, IgM, IgA, IgE, (IFE if adult). Check vaccine responses to proteins (Hib, DT, influ) and polysaccharides (pneumovax) by luminex multianalyte assay
- If abscesses check PMNs with CBC & diff, DHR, MPO stain, maybe chemotaxis and IgE or staph or candida IgE or PMN receptor profile for CD11/18 expression
- Cell mediated immunity check DTH skin tests to candida and DT. T cell immunodeficiency profile, LAM, ? Cytokine profile by multianalyte assay

Mean Cytokine Production by PBMCs of Jobs Patients and Normal Controls in Response to *S. aureus*



Notes from a Traveling Philosopher

Every patient with severe recurrent infections most likely has some defect in their host defense mechanism. The problem may be structural, anatomic, metabolic, infectious, allergic, immunologic, or even neurohumoral (physical, mental stress). We may not even be able to detect some of these subtle defects yet. We should continue to strive to decipher these at the cellular, immunologic, and molecular level and devise new ways to treat them.

Harry R. Hill, M.D.
Grand Teton Mountain Park, July 2004.

References

- Shyur, S.D. and Hill, H.R.: Immuno-deficiencies in the 1990s. Pediat. Infect. Dis. J. 10:595-611, 1991.
- Shyur, S.D. and Hill, H.R.: Recent advances in the genetics of primary immuno- deficiency syndromes. J. Pediat. 129:8-24, 1996.
- Fleisher, T.A. and Torner, R.H.: Introduction to diagnostic laboratory immunology. JAMA 278: 1823-1834, 1997.
- Puck, J.M.: Primary immunodeficiency diseases. JAMA 10:1835-1841, 1997.
- Ochs, H.D., Smith, C.I.E. and Puck, J.M.: Primary Immunodeficiency Diseases Oxford University Press. Oxford and New York. 1999.
- Stiehm, E.R., Ochs,H.D., and Winklestein, J.A.: Immunologic Disorders of Infants and Children. Elsevier, Saunders. Philadelphia. 2004.

References

- Renner ED, Rylaarsdam S, Añover-Sombke S, Rack AL, Reichenbach J, Carey JC, Zhu Q, Jansson AF, Barboza J, Schimke LF, Leppert MF, Getz MM, Seger RA, Hill HR, Belohradsky BH, Torgerson TR, Ochs HD: Novel signal transducer and activator of transcription 3 (STAT3) mutations, reduced TH17 cell numbers, and variably defective STAT3 phosphorylation in hyper-IgE syndrome. J. Allergy Clin Immunol. 122:(1)181-187, 2008
- Bender JM, Rand TH, Ampofo K, Pavia AT, Schober M, Tebo A, Pasi B, Augustine NH, Pryor RJ, Wittwer CT, and Hill HR: Family clusters of variant X-linked chronic granulomatous disease. Pediatr. Inf. Dis. 28:529-533, 2009.
- Kumánovics A, Perkins SL, Gilbert H, Cessna MH, Augustine NH, Hill HR: Diffuse large B cell lymphoma in Hyper-IgE syndrome due to STAT3 mutation. J Clin. Immunol. 30:886-893, 2010

References

- Kumánovics A, Wittwer CT, Pryor RJ, Augustine NH, Leppert MF, Carey JC, Ochs HD, Wedgwood RJ, Faville Jr. RJ, Quie PG, and Hill HR: Rapid molecular analysis of the STAT3 gene in Job syndrome of Hyper IgE and recurrent infectious diseases. J. Molec. Diagn. 12:213-219, 2010
- Roos D, Kuhns DB, Maddalena A, Roesler J, Lopez JA, Ariga T, Avcin T, de Boer M, Bustamante J, Condino-Neto A, Di Matteo G, He J, Hill HR, Holland SM, Kannengiesser C, Köker MY, Kondratenko I, van Leeuwen K, Malech HL, Marodi L, Nunoi H, Stasia MJ, Ventura AM, Wittwer CT, Wolach B, and Gallin JI: Hematologically important mutations: X-linked chronic granulomatous disease (third update). Blood Cells Mol. and Dis. 45:246-265, 2010
- Hill HR, Augustine NH, Pryor RJ, Reed GH, Bagnato JD, Tebo AE, Bender JM, Pasi BM, Chinen JI, Hanson C, de Boer M, Roos D, and Wittwer CT: Rapid genetic analysis of X-linked chronic granulomatous disease by high-resolution melting. J. Molec. Diagn. 12:368-376, 2010