Combined Immunodeficiency Associated with DOCK8 Mutations

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Background - Immune deficiency

Severe Combine Immune Deficiency (SCID)

- Impaired T-cell function
- Severe T cell deficiency
- Decreased or dysfunctional B cells and NK cells
- Less severe forms – skin diseases with elevated IgE and hypereosinophilia
- Multiple mutations identified

Background (cont'd)

Hyper-IgE Syndrome (HIES)

- Autosomal dominant form 60-70% due to STAT3 mutation → decreased differentiation of Th17 cells
  - Severe eczema
  - Recurrent skin infections, often staph aureus
  - Mucocutaneous candidiasis
  - Recurrent sinopulmonary infections
  - Elevated serum IgE and eosinophilia
  - Skeletal abnormalities (ex: scoliosis)

Background (cont'd)

Hyper-IgE Syndrome (HIES)

- Autosomal recessive form
  - Recurrent sinopulmonary infections
  - Eczema
  - Elevated serum IgE
  - Recurrent cutaneous viral infections
  - No skeletal abnormalities
  - Vasculitis, central nervous system abnormalities
  - Loss of function mutation in tyr kinase 2 gene implicated

HIES Scoring System

Clinical Findings	0	1	2	3	4	5	6	7	8	10
Highest IgE (IU/mL)	<200	200-500			501-1000				1001-2000	>2000
Total # skin abcesses/ boils	None		1-2		3-4				>4	
Total # pneumonias	None		1		2		3		>3	
Parenchymal Lung Abnormalities	None						Bronchi-etasis		Pneuma-tocele	
Other serious infection	None				Present					
Fatal Infection	None				Present					
Highest Eosinophils/ uL	<700			701-800			>800			
Newborn Rash	None				Present					
Eczema (worst stage)	None	Mild	Moderate		Severe					
Sinusitis/Otitis (# in worst year)	1-2	3	4-6		>6					
Candidiasis	None	Oral, vaginal	Fingernail		Systemic					
Retained primary teeth	None	1	2		3				>3	
Scoliosis (max. curvature)	<10		10-14		15-20				>20	
Minimal trauma fractures	None				1-2				>2	
Hyperextensibility	None				Present					
Characteristic Face	None		Mild			Present				
Increased Interalar Distance	<1 SD	1-2 SD		>2 SD						
High Palate	None		Present							
Congenital Anomaly	None					Present				

Grimbacher B, Schaffer A, Holland S, et al. Genetic Linkage of Hyper-IgE Syndrome to Chromosome 4. American Journal of Human Genetics. 1999 65:735-744

Study Overview

- Describe 11 patients with loss-of-function mutations in the dedicator of cytokinesis 8 (DOCK8) gene
- Patients had immunodeficiency with low numbers of T, B, and NK cells
- Extensive cutaneous viral infections
- Susceptibility to malignancy

Methods

- All protocols were approved by the IRB of the NIAID
- T-cell subgroups were isolated by negative selection then stimulated with anti-CD3 and anti-CD28 antibodies and cultured in IL-2
- B-cells immortalized with EBV
- T cells immortalized with herpesvirus saimiri
- Comparative genomic hybridization analyses performed with 244K arrays
- DNA sequencing performed after PCR amplification
- Immunoblotting performed with polyclonal rabbit anti-DOCK8 antibodies
- Novel variants identified in the 11 index patients were sought in other groups
  - 6 pts with autosomal dominant hyperIgE syndrome (HIES)
  - 32 pts with other immunologic diseases
  - 15 healthy blood donors
  - 100 healthy white controls

Patient Characteristics

Supplemental Table 2. Characteristics of Patients

Variable	Patient 1-1	Patient 2-1	Patient 3-1	Patient 4-1	Patient 4-2	Patient 5-1	Patient 5-2	Patient 6-1	Patient 7-1	Patient 8-1	Patient 8-2
Age (yr)	6	21†	18†	17	14	21	14	14	13†	18†	16
Sex	female	male	female	male	female	female	female	male	female	female	male
Ethnicity	Yemeni	Lebanese	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Mexican	Mexican
Atopy											
Atopic dermatitis	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Allergies	Food - beef, cow's milk, egg, sesame; Environmental - Bermuda grass, mountain cedar	Food - avocado, banana, beef, cantaloupe, carrot, cow's milk, cucumber, egg, kiwi, lamb, lentil, mango, mustard, pea, pineapple, pistachio, pomegranate, salmon, sesame, shrimp, watermelon, wheat, yeast*; Environmental - seasonal rhinitis to Alternaria, ragweed, trees	Food- catfish, eggs, peanuts, shellfish*	Drug - cefaclor	Drug - penicillin	Food - apple, corn, cow's milk, egg, fish, legume, peanut, shellfish, soy, tree nuts, wheat; Drug - penicillin, sulfa; Environmental - grass, trees, pets	Food - cow's milk, egg, legumes, peanuts, tree nuts*; Drug - clarithromycin, penicillin, sulfa; Environmental - cat, grass	Food - crab, egg, tree nuts*; Environmental - seasonal rhinitis	Food - bananas, beans, cow's milk, goat's milk, onion, peanut, soy, tuna*; Drug - sulfa	Food - beans, beef, chicken, cow's milk, egg, fish, peanut, pork, tree nuts, tomato*; Drug - Cefipime, Lactinex, Propofol*; Environmental - dust, dog, grasses, mold	Food - beef, chicken, cow's milk, egg, peanut, pork*; Environmental - dust, dog, mold
Asthma	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
Infections											
Skin and soft tissue	Diaper cellulitis	*Staphylococcus aureus* skin abscesses, axillary lymphadenitis, otitis externa	*Staphylococcus aureus* skin abscesses, otitis externa	(-)	(-)	*Staphylococcus aureus* skin abscesses	*Staphylococcus aureus* impetigo and skin infections, otitis externa	Skin abscesses, impetigo	*Staphylococcus aureus* skin abscesses, otitis externa	*Staphylococcus aureus* skin infections, otitis externa	*Staphylococcus aureus* skin infections, *Acinetobacter baumanii* otitis externa
Respiratory tract	Recurrent otitis media, pneumonias¶§	Recurrent otitis media, sinusitis, adenoviral pneumonia¶§	*Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, RSV pneumonia; otitis media	Recurrent otitis media, sinusitis, bronchitis, croup¶§	Recurrent otitis media, sinusitis, pneumonias, bronchitis¶§	Recurrent otitis media, sinusitis, mastoiditis, pneumonias including *Pneumocystis jiroveci*¶§	Recurrent otitis media, sinusitis, *Pneumocystis jiroveci* and *Haemophilus influenzae* pneumonias¶	Recurrent otitis media, sinusitis¶	Recurrent pneumonias, mastoiditis	(-)	Otitis media, sinusitis, pneumonia
Viral infections	Recurrent orolabial HSV+	Diffuse flat warts, herpes zoster	HSV - keratitis, eczema herpeticum, recurrent genital infections; diffuse molluscum contagiosum	Diffuse flat warts, diffuse molluscum contagiosum, severe primary varicella+	Recurrent orolabial HSV, diffuse flat warts, diffuse molluscum contagiosum+	Diffuse molluscum contagiosum, verrucous warts on fingers, recurrent herpes zoster+	Diffuse flat warts, persistent orolabial HSV	Flat and verrucous warts on face, trunk, extremities+	HSV - keratitis, eczema herpeticum, recurrent orolabial, eyelid, ear canal, and genital infections; Verrucous warts on fingers+	HSV keratitis, eczema herpeticum, herpes zoster	HSV keratitis and groin infection, eczema herpeticum, diffuse molluscum contagiosum+
Other	Oral candidiasis, tooth decay	Pericarditis	Salmonella enteritis, giardiasis, *Staphylococcus aureus* osteomyelitis, vaginal candidiasis	(-)	Salmonella enteritis	Oral candidiasis	Oral candidiasis	*Staphylococcus aureus* osteomyelitis, nail candidiasis	Vaginal candidiasis	*Haemophilus influenzae* and cryptococcal meningitis; recurrent *Staphylococcus aureus* and *Acinetobacter baumanii* sepsis	(-)
Malignancies	(-)	Metastatic anal squamous cell carcinoma	Paranasal and vulvar squamous cell carcinomas; cutaneous T-cell lymphoma	(-)	(-)	(-)	(-)	(-)	Vulvar squamous cell carcinoma	(-)	(-)
Additional history	Poor growth, high forehead, thinning hair	Eosinophilic esophagitis, eosinophilic dermatitis, eosinophilic lung disease, bronchiectasis, hypospadias	Poor growth	Cavernous angioma	(-)	Eosinophilic esophagitis, bronchiectasis and lung cyst, high-arched palate, hyperextensibility	High-arched palate, minimal trauma fracture	Bronchiectasis, scoliosis, high-arched palate	Retained primary teeth, pneumatocele, poor growth	Delayed puberty	Retained primary teeth
HIES score*	40	Not done	Not done	27	30	33	62	42	61	54	42

† deceased
* anaphylaxis
¶ tympanostomy tubes
§ sinus surgery
+ IFN-γ treatment
* of maximal score of 111 (without age correction); scoring criteria according to Grimbacher B, Schäffer AA, Holland SM, et al. Genetic linkage of hyper-IgE syndrome to chromosome 4. Am J Hum Genet 1999;65:735-44.

Index Patients

- Three patients from a group with undefined combined immunodeficiencies were found to carry mutations in the DOCK8 gene
- Shared some clinical features with HIES

	Patient 1-1	Patient 2-1	Patient 3-1
Sex/ethnicity	Female/Yemeni	Male/Lebanese	Female/Caucasian
Atopy	Atopic dermatitis, multiple food and environmental allergies	Atopic dermatitis, multiple food and environmental allergies, asthma, EoE	Atopic dermatitis, multiple food allergies, asthma
Skin infections	Diaper cellulitis	Staph skin abscesses, otitis externa, Eo dermatitis	Staph skin abscesses, otitis externa
Respiratory infections	Otitis media, PNA	Otitis media, sinusitis, adenoviral PNA, bronchiectasis, Eo lung disease	PNA, H. flu, RSV PNA otitis media
Viral infections	Recurrent orolabial HSV	Diffuse flat wards, zoster	HSV, diffuse molluscum contagiosum
Other infections	Oral candidiasis, tooth decay	Pericarditis	Salmonella enteritis, giardiasis, Staph osteomyelitis, vaginal candidiasis
Malignancies	None	Metastatic anal squamous cell carcinoma	Paranasal and vulvar squamous cell carcinoma, cutaneous T-cell lymphoma
HIES score	40	N/A	N/A

Patient Characteristics - Atopy

- After identification of the 3 index cases, sought additional patients to total 11 patients from 8 families
- All patients had atopic dermatitis
- 9/11 had severe and extensive food allergies including anaphylaxis
- 6/11 had reactive airway disease or asthma
- 2/11 had EoE or Eo lung disease

Patient Characteristics - Respiratory Infections

- All patients had recurrent respiratory tract infections
- Most had otitis media requiring tympanostomy tube placement
- 2/11 had mastoiditis
- 7/11 had recurrent sinusitis
- 9/11 had recurrent PNA, bronchitis, and/or bronchiectasis
- Pulmonary pathogens included
- Strep pneumo
- H. flu
- PCP
- Respiratory adenovirus
- RSV

Patient Characteristics – Viral Infections

- All had extensive, frequently co-existing, cutaneous viral infections
- 7/11 had HSV manifesting as recurrent orolabial or anogenital HSV, dermatitis, or eczema herpeticum
- 7/11 had persistent flat/verrucous warts
- 5/11 had extensive molluscum contagiosum
- 2/11 had recurrent herpes zoster
- 1/11 had severe primary varicella infection

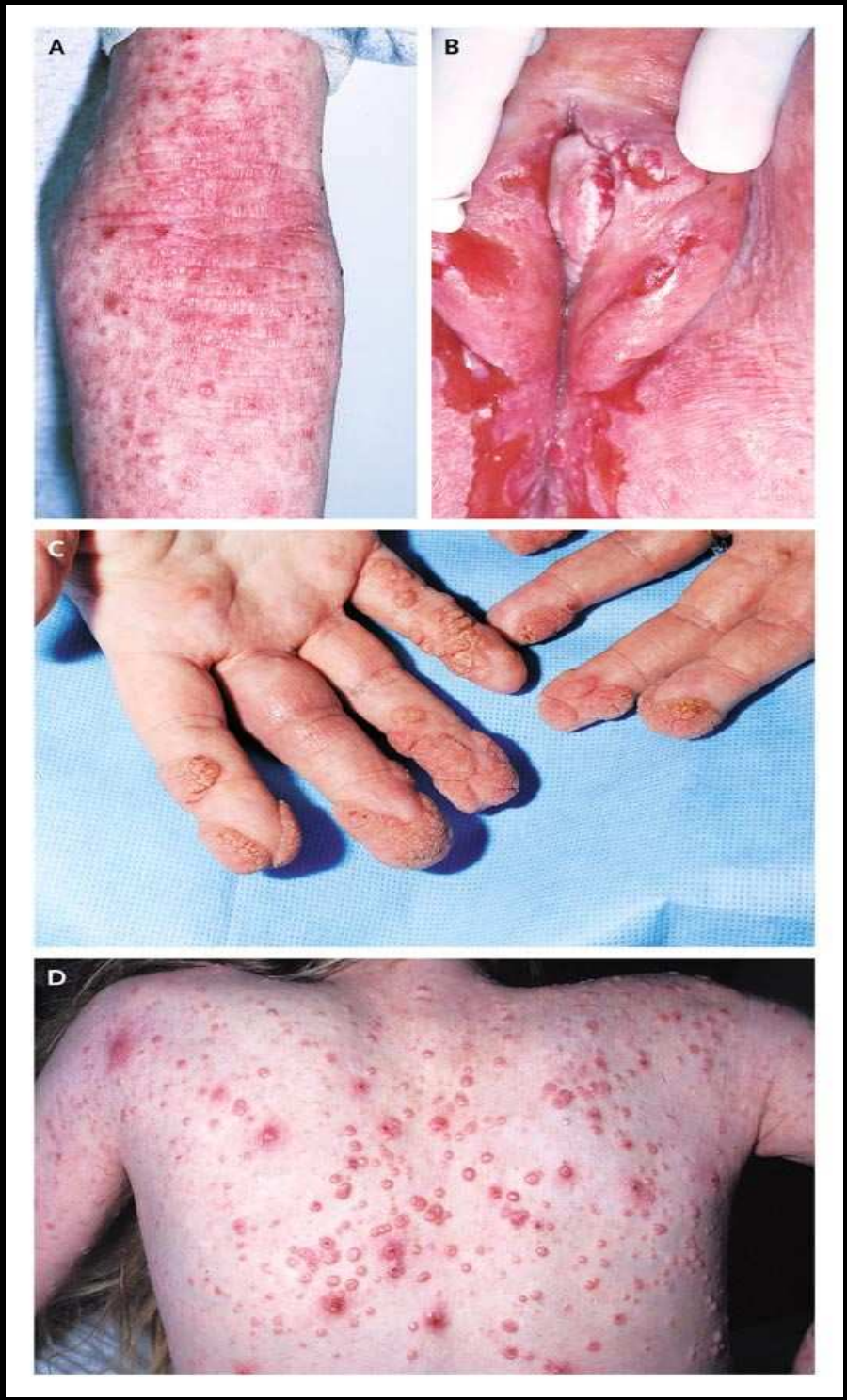
Characteristic Dermatologic Findings

A: Atopic Dermatitis

B: Herpes simplex virus

C: Human papilloma virus

D: Molluscum contagiosum



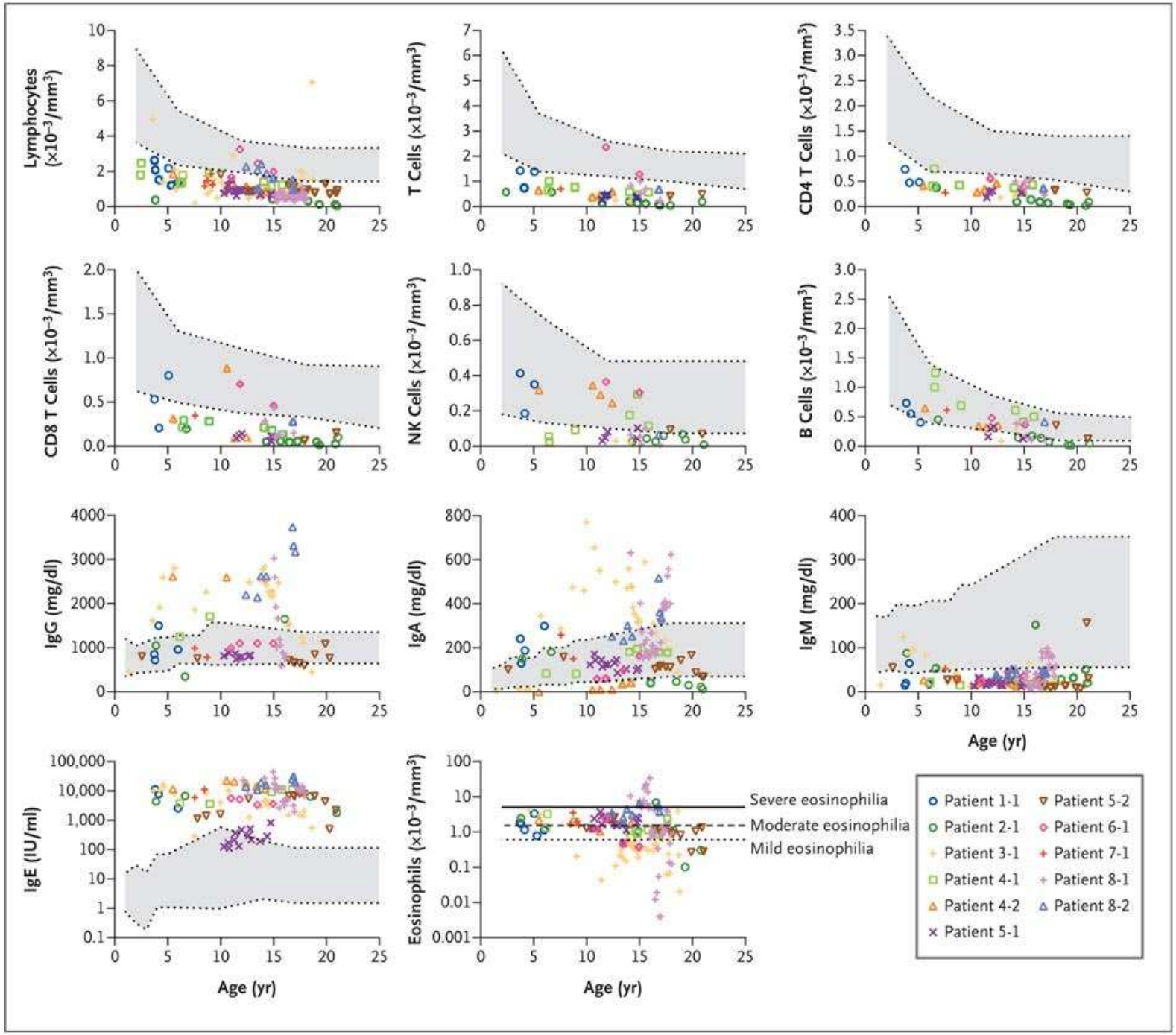
Patient Characteristics – Skin Infections

- 8/11 had staph skin infections or abscesses
- 2/11 had staph osteomyelitis
- 5/11 had mucosal or nail candidiasis
- 6/11 had recurrent otitis externa
- 1/11 had cryptococcal and H. flu meningitis
- Other infections: salmonella enteritis, giardiasis, pericarditis

Patient Characteristics – Malignancy

- 3/11 developed malignancy in late childhood or early adulthood
- Cancers occurred in patients with long-standing HSV, HPV, and molluscum including vulvar, facial, and anal squamous-cell carcinoma
- Two died from metastatic squamous-cell carcinoma
- One died from cutaneous T-cell lymphoma-leukemia

Immunologic Assessment



Immunologic Assessment

- 9/11 had low absolute lymphocyte counts
- 10/11 had low total T cell counts
- 11/11 had low CD4 counts
- 10/11 had low CD8 counts
- CD4/CD8 ratios were normal
- Tregs were assessed by CD4+/CD25+/FOXP3+, decreased due to lymphopenia in 2/11 patients but proportionally normal
- 6/11 had low NK cells
- 5/11 had low B cells
- 10/11 had mild to moderate eosinophilia

Immunologic Assessment (cont'd)

- 6/11 had hypogammaglobulinemia
- 5/11 had normal IgG
- IgA levels varied
- IgM levels were uniformly low (mean 35 +/- 13, normal >49)
- All patients had high IgE (range 818-43,600)
- 6/11 patients received IVIg therapy

Specific Antibody Responses

Supplemental Table 3. Specific antibody function of patients.

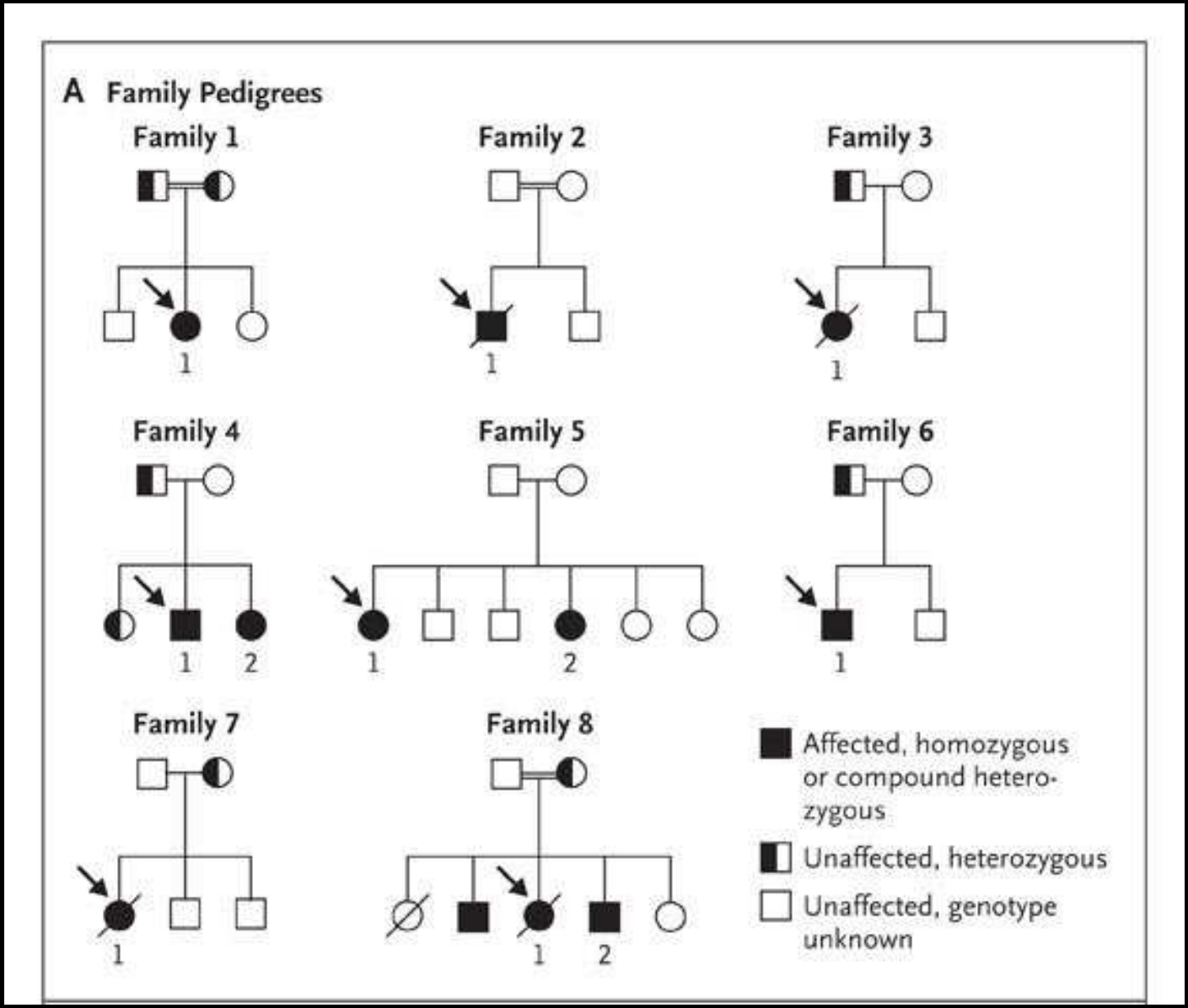
Specific antibodies to	Patient 1-1	Patient 2-1	Patient 3-1	Patient 4-1	Patient 4-2	Patient 5-1	Patient 5-2	Patient 6-1	Patient 7-1	Patient 8-1	Patient 8-2
Tetanus toxoid	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)
Diphtheria		(-)	(-)	(+)	(+)	(-)	(-)	(+)	(-)	(+)	(+)
Pneumococcal†	20/23		1/3	21/23	20/23	1/23	23/23	7/23		5/23	10/23
Rubella	(+)		(+)	(+)	(+)	(+)	(+)	(+)			(+)
Haemophilus influenzae type B	(+)	(-)		(+)	(+)	(-)	(+)	(-)		(-)	(+/-)
Varicella-zoster virus	(+)		(+)			(+)	(+)				
Measles			(-)							(-)	
Poliovirus	(-)		(-)								
Bacteriophage øX174				(-)	(-)						
Mumps			(-)								
Hepatitis B virus									(+)		(-)
Started on IVIG	no	yes	no	yes*	yes*	yes	yes	no	no	yes	no

(-), (+), (+/-) indicate whether protective levels of antibodies were absent, present, or indeterminate, respectively

†Number of serotypes showing positive response of those tested

*IVIG trial not continued

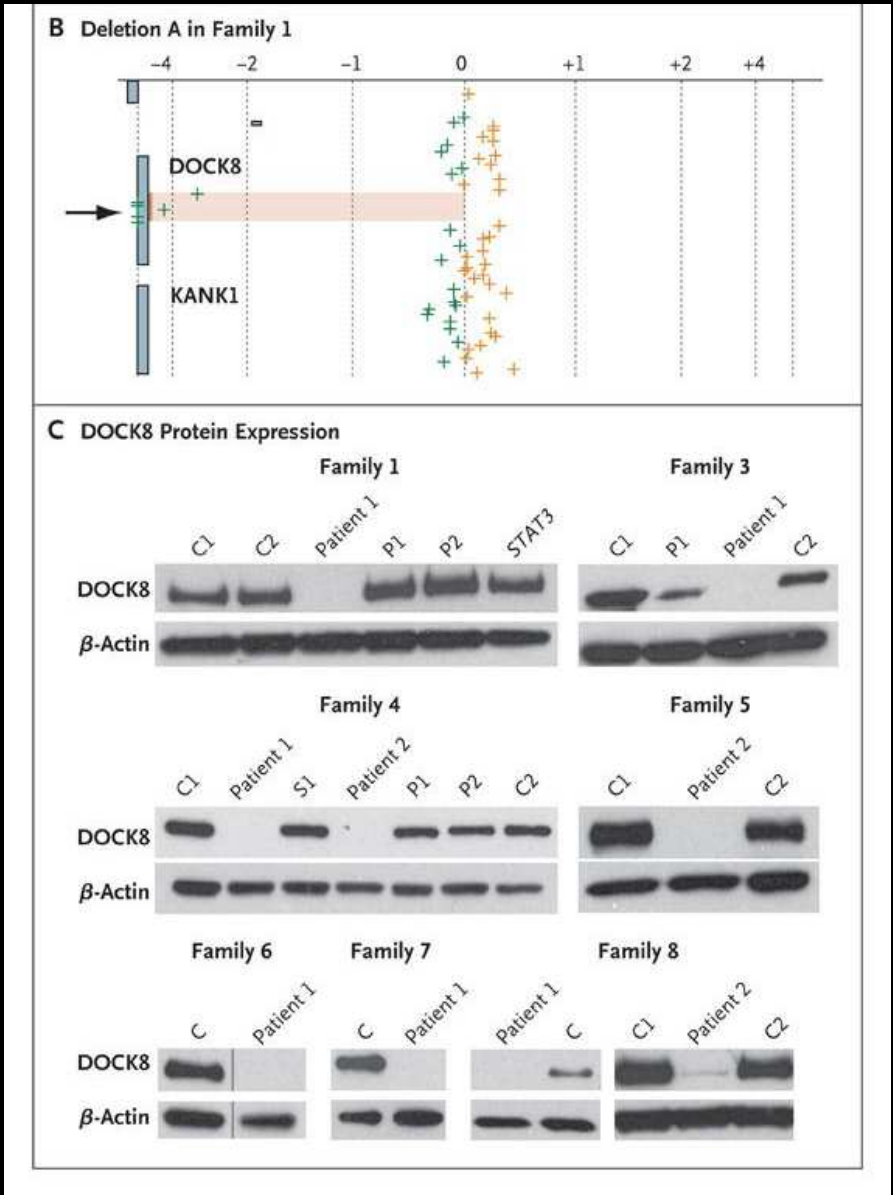
Pedigree Analysis



DOCK8 Mutation Analysis

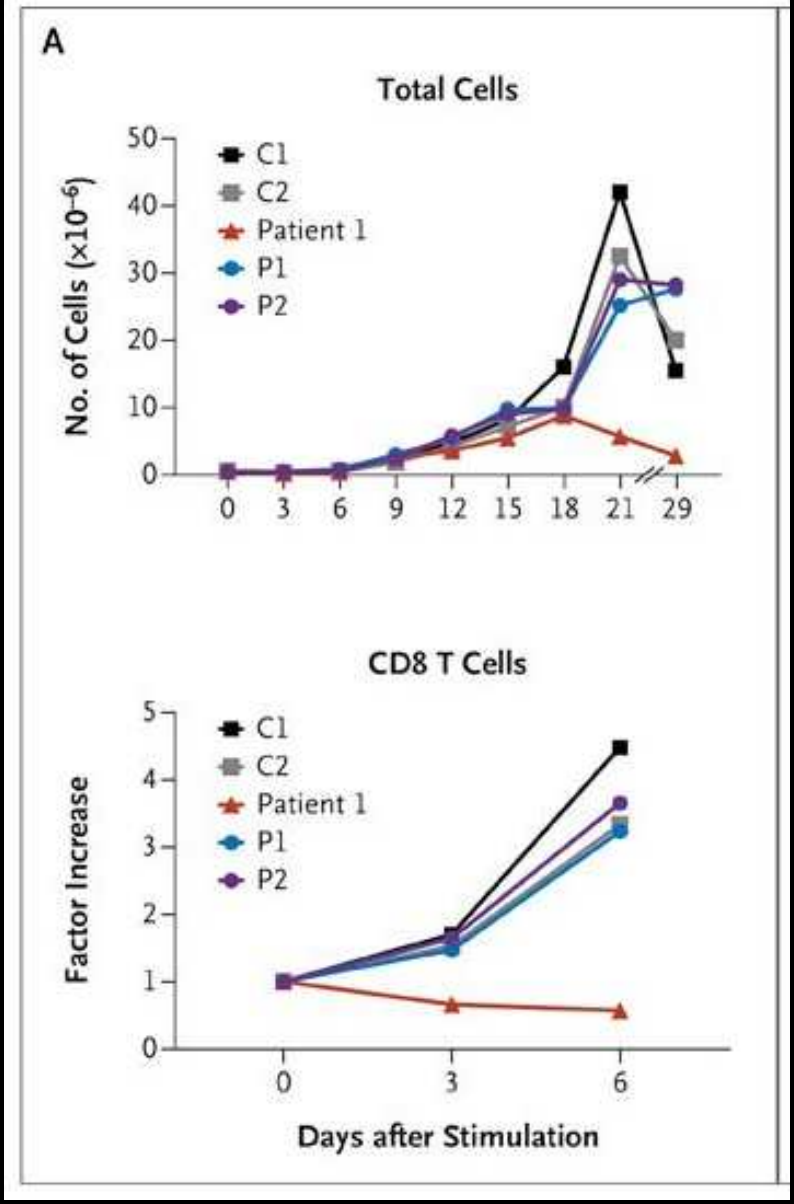
- Families 1 and 2 had homozygous deletions in the DOCK8 gene
- Deletions were confirmed by the failure of PCR to amplify deleted exons
- Deletions were not detected in any of the 38 patients with other immune disorders (including 6 HIES) or 115 controls
- Families 3,4,5, and 6 had heterozygous deletions in the DOCK8 gene
- Heterozygous deletions resulted in apparent homozygosity of SNPs within the corresponding sequenced regions

DOCK8 Molecular Analyses



- Genetic variants consisted of large missing portions of the DOCK8 coding sequence
- Included a conserved DOCK homology region 1 (DHR1) domain
- DOCK8 mRNA present in tissues from lung, kidney, pancreas, and placenta
- Monocytes, B cells, and T cells from healthy controls contain DOCK8 mRNA
- DOCK8 proteins were detected in lymphocytes from unrelated patients with HIES
- DOCK8 proteins were not detected in T cell cultures from the 11 patients in this study
- Two patients had truncated protein generated from remaining exons

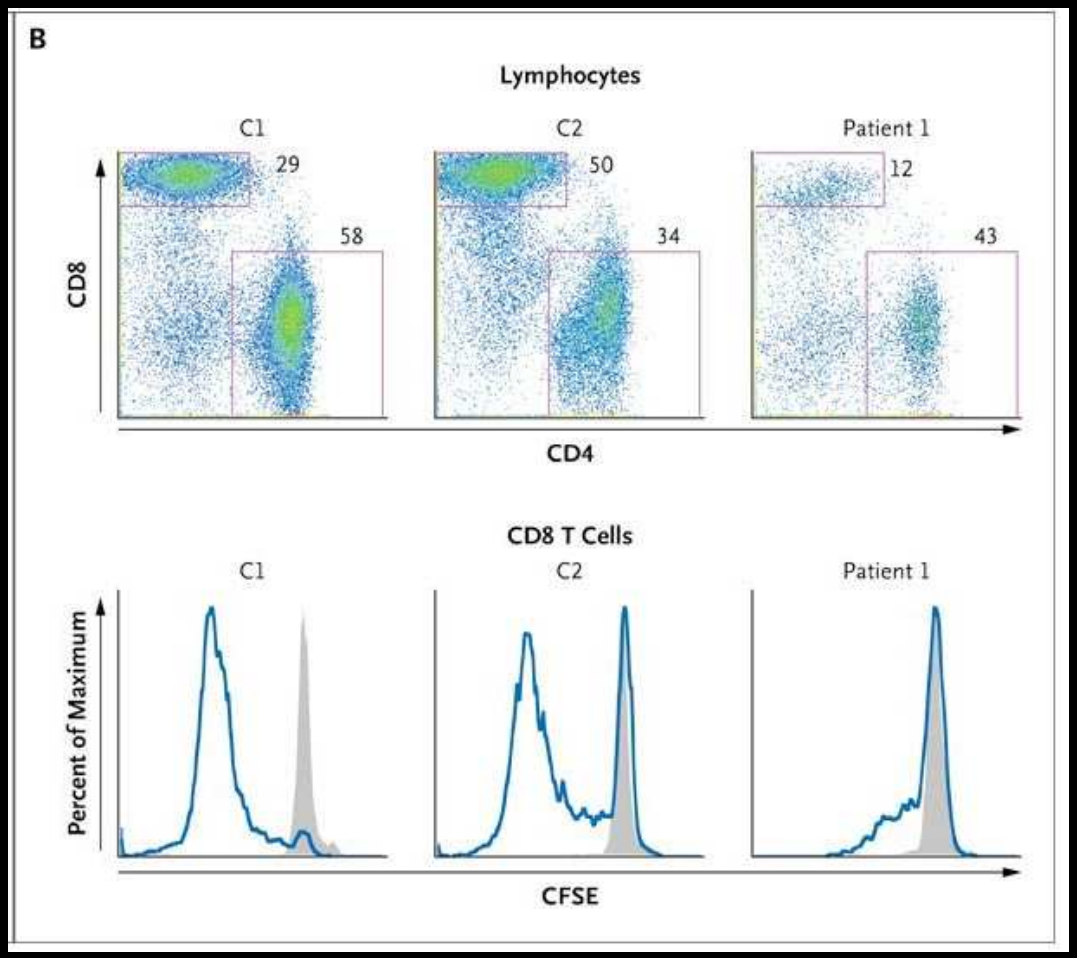
Function of CD8 Cells



- PBMCs activated with anti-CD3 and anti-CD28 antibodies for 3 days, T cells were expanded in culture with IL-2
- Absolute numbers of CD8 T cells calculated on the basis of flow
- CD8 cells did not expand well from activated DOCK8-deficient patients

Impaired CD8 T-Cell Activation and Proliferation in Association with DOCK8 Deficiency in Family 1

Function of CD8 Cells (cont'd)



- PBMCs labeled with CFSE were either unstimulated or stimulated then analyzed
- Location of each dot reflects a cell's intensity for CD4 staining versus CD8 staining
- Samples from Patient 1 had a lower proportion of CD8 T cells (12% of lymphocytes) after activation compared to controls
- CD4 cell proliferation was unaffected in DOCK8 deficient patients (data not shown)
- Induction of CD25 on CD8 cells was impaired in two of three DOCK8 deficient patients tested (data not shown)

Discussion

- DOCK180 superfamily of guanine nucleotide exchange factors interact with Rho GTPases
- DOCK8 is a member of this family, exact function under investigation
- Likely regulates cytoskeletal rearrangements required for cell structure, migration, adhesion
- Experimental model: DOCK2 -/- mice
- Decreased number of T cells
- Reduced responsiveness of T cell antigen receptor
- Allergic disease
- High levels of IgE
- No susceptibility to viral infections

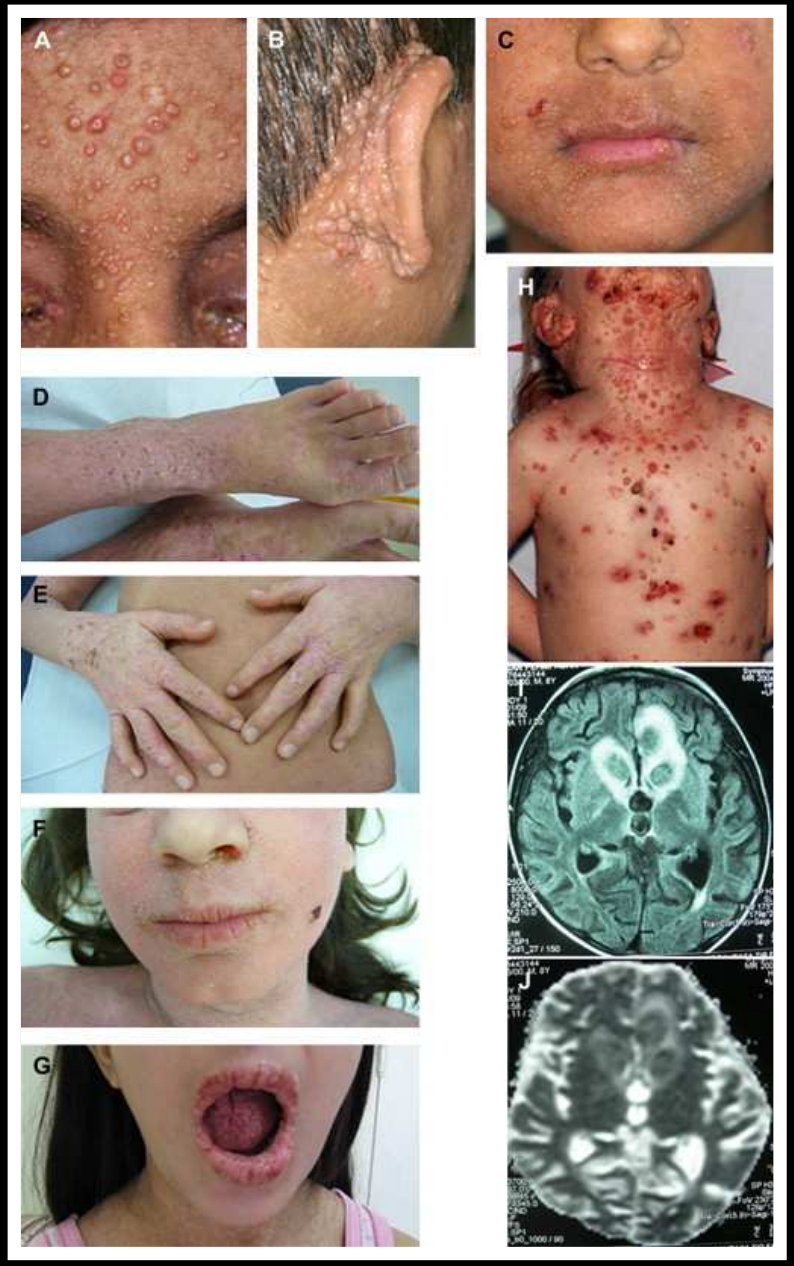
Discussion (cont'd)

- DOCK8 mutation was found in 11 patients with combined immune deficiency, including subgroup of patients previously thought to have autosomal recessive HIES
- Allergic manifestations not typical of primary immunodeficiency or HIES
- Malignancy
- HIES does not show increased incidence of cancers
- DOCK8 deficient patients developed squamous cell carcinomas and cutaneous T cell lymphoma-leukemia
- Impaired CD8 T-cell function suggests impaired tumor surveillance as a possible mechanism for malignancy
- DOCK8 deletions in primary lung cancers, gastric cancers, breast cancers, and gliomas have been reported (Int J Onc 2008, J Neurooncol 2008)
- DOCK8 mutation (heterozygous) also reported in several cases of mental retardation, developmental delay, and autistic-spectrum disorder (Genomics 2008)

Follow-up Studies - Engelhardt et al, JACI 2009

- Performed genome-wide SNP analysis for 16 patients from 14 families with autosomal recessive HIES (positive NIH HIES score >40) and absence of significant skeletal findings
- A candidate gene was identified and 11 additional patients from 6 additional families were analyzed
- Analysis demonstrated mutations in DOCK8
- Total of 21/27 patients with AR-HIES were found to have DOCK8 mutations
- Clinical phenotype: upper respiratory tract infections, recurrent PNA, bronchiectasis, skin abscesses, severe recurrent viral infections, candidiasis, atopic dermatitis, multiple food and environmental allergies, central nervous system vasculitis, brain infarction
- These patients had more profound CD4 defects, CD8 responses were less affected

Follow-up Studies - Engelhardt et al, JACI 2009



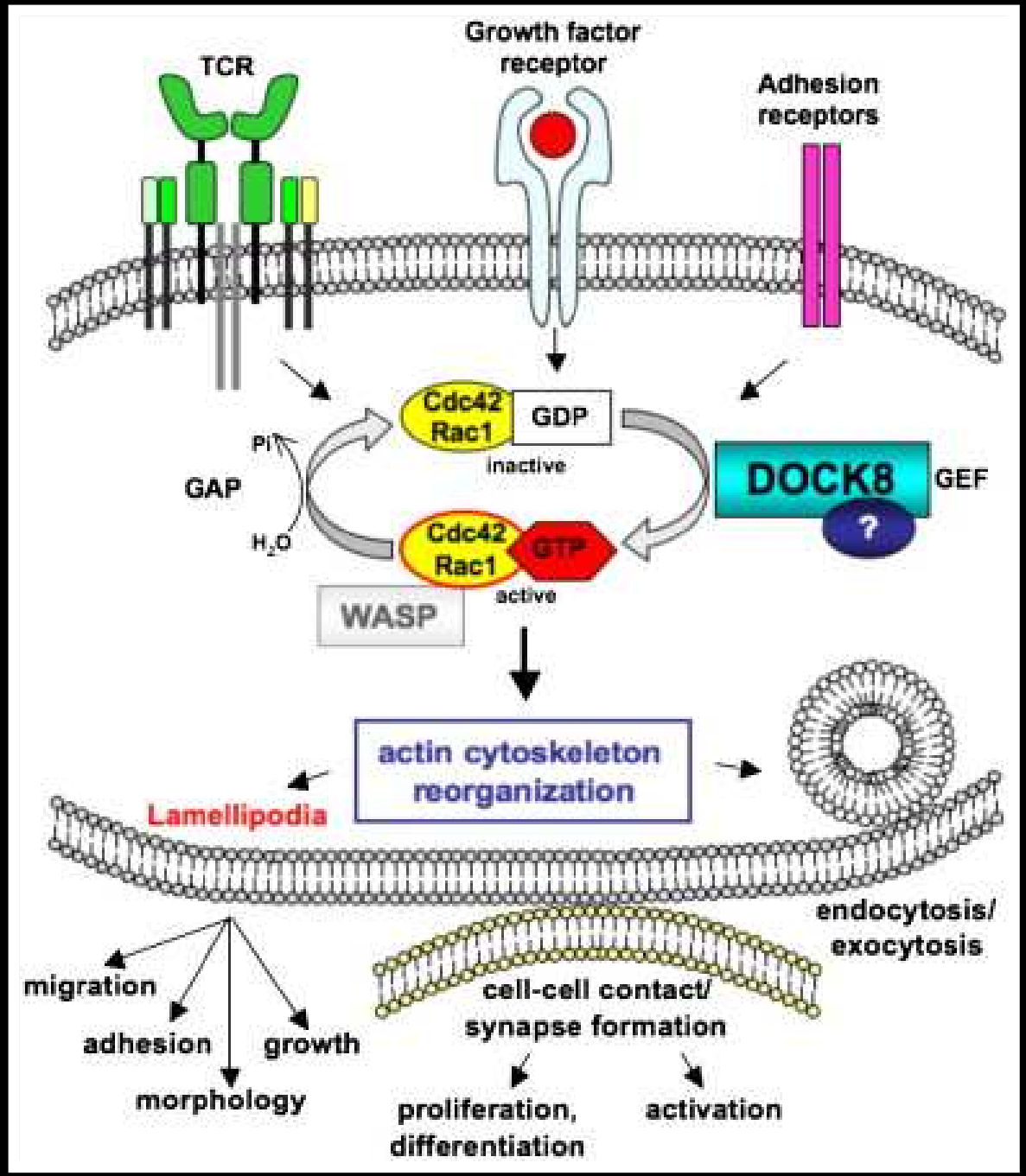
Source: Journal of Allergy and Clinical Immunology 2009; 124:1289-1302.e4

DOCK8 mutations cripple B cell immunologic synapses, germinal centers, and long-lived antibody production – Randall et al, Nature Immunology 2009

- Identified DOCK8 mutations in a mouse genetic screen for mutations that disrupt antibody maturation and persistence
- DOCK8 mutant B cells unable to form marginal zone B cells
- Unable to persist in germinal centers and undergo affinity maturation
- DOCK8 mutations disrupted accumulation of ICAM-1 in the B cell immunological synapse
- Immunization of DOCK8-mutant mice elicited normal early wave of antibody formation but no hypermutation or affinity maturation
- Mutation had no effect on CD40 or LPS-stimulated activation or proliferation
- Mutation had no affect on B cell response chemotactic factors including S1P, CXCL12, and CXCL13

DOCK8 Proposed Mechanism

- DOCK8 activates CDC42 and Rac1
- GTPase activation induces dynamic filamentous actin rearrangement and lamellipodia formation via WASP
- Cell growth, migration, adhesion
- Possible role in the formation of immunologic synapse
- T cell activation, proliferation, differentiation



Source: Journal of Allergy and Clinical Immunology 2009; 124:1289-1302.e4

Conclusions

- Autosomal recessive DOCK8 mutation is associated with a novel variant of combined immunodeficiency
- Clinical features include recurrent sinopulmonary and cutaneous bacterial and viral infections, elevated serum levels of IgE, severe atopy including food and environmental allergies, and malignancy
- DOCK8 mutation was found in a number of patients who met criteria for autosomal recessive HIES
- DOCK8 may play a critical role in formation of the B cell immunologic synapse and well as T cell activation, proliferation, differentiation

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