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TABLE 1

Country of Origin, Genotype, Infectious Phenotype, and Outcome in the Cohort of IRAK-4- and MyD88-Deficient Patients

Kindred	P	Age/Sex (yr)	Mutation of IRAK4	Country of Origin	Outco me Status	Invasive Infections With Gram-Positive Bacteria	Invasive Infections With Gram-Negative Bacteria	Other	Ref.
A	1	7/M	821delT/821delT	Saudi Arabia	†	*S. pneumoniae, S. aureus*			(38)
B	2	17/M	Q293X/BAC210N13del	Portugal	Alive	*S. pneumoniae, S. aureus*			(25,38)
C	3	14/F	Q293X/Q293X	USA	Alive	*S. pneumoniae, S. aureus*		*Enterovirus, Curvularia* species	(15,19,25,38)
D	4	27/F	Q293X/620-621delAC	USA	Alive	*S. pneumoniae, C. septicum*	*N. meningitidis*		(25,26,32)
E	5	16 mo/M	ND	Turkey	†	*S. pneumoniae, S. parasanguis*			(18)
E	6	2 mo/F	523delA/523delA	Turkey	†	*S. pneumoniae,*			(18)
F	7	35/F	Q293X/Q293X	UK	Alive	*S. pneumoniae,*	*S. sonnei*		(12,25)
G	8	11/M	1189-1G>T/1188+520A>G	Hungary	Alive	*S. pneumoniae*			(24,25)
H	9	5. 5/M	Q293X/Q293X	Canada	†	*S. pneumoniae*	*P. aeruginosa*		(14,25)
H	10	10/M	Q293X/Q293X	Canada	Alive	*S. pneumoniae*			(14,25)
I	11	2. 5/M	E402X/del (?)	Spain	†	*S. aureus*	*P. aeruginosa*		(8,25)
I	12	8 mo/F	E402X/del (?)	Spain	†	*S. pneumoniae*	*P. aeruginosa*		(8,25)
I	13	12/M	E402X/ E402X	Spain	Alive	*S. pneumoniae*			(8,25)
J	14	3 mo/F	ND	Israel	†	*S. milleri*			(25)
J	15	12/M	1-1096_40+23del/1- 1096_40+23del	Israel	Alive	*S. pneumoniae*			(25)
K	16	5 mo/F	ND	Canada	†	*S. aureus*			(25,27)
K	17	30/M	Q293X/Q293X	Canada	Alive	*S. pneumoniae*			(25,27)
K	18	30/M	Q293X/Q293X	Canada	Alive	*S. pneumoniae*		*M. avium*	(25,27)
L	19	2. 5/M	118insA/118insA	Japan	†	*S. pneumoniae*			(25,44)
L	20	4/M	118insA/118insA	Japan	Alive				(25,44)
M	21	4 mo/F	ND	USA	†			Undocumented meningitis	(25)
M	22	3/M	Q293X/620-621delAC	USA	Alive	*S. pneumoniae*			(25)

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Kindred	P	Age/Sex (yr)	Mutation of IRAK4	Country of Origin	Outco me Status	Invasive Infections With Gram-Positive Bacteria	Invasive Infections With Gram-Negative Bacteria	Other	Ref.
N	23	5/F	Y48X/631delG	Canada	Alive				(25)
O	24	19/M	1240insA/942- 1481_1125+547del	Canada	Alive	*S. pneumoniae, S. aureus*			(25)
P	25	4 mo/F	ND	Australia	†	*S. pneumoniae*			(25)
[illegible]	26	6 mo/F	Q293X/Q293X	Australia	†	*S. pneumoniae, S. aureus*			(25)
Q	27	14/F	Q293X/Q293X	USA	Alive	*S. pneumoniae*			(25)
R	28	10/M	Q293X/Q293X	USA	Alive	*S. pneumoniae, S. aureus*	*P. aeruginosa*	*Enterovirus*	(25,30)
N	29	2/M	Y48X/631delG	Canada	Alive				PR
S	30	4/F	M1V/1188+520A>G	Slovenia	Alive		*P. aeruginosa*		(16)
T	31	18/M	R12C/831+5G>T	France	Alive	*S. aureus*	*P. aeruginosa*		(20)
U	32	15 mo/M	Q293X/Q293X	UK	†	*S. pneumoniae, S. aureus*			PR
U	33	6 mo/M	Q293X/Q293X	UK	Alive				PR
V	34	6/F	Q293X/G298D	UK	Alive	*S. pneumoniae*			(6)
W	35	13/M	Y430X/1126-1 G>T	El Salvador	Alive			Bacterial infec.	(23)
W	36	4/F	Y430X/1126-1 G>T	El Salvador	Alive	*S. agalactiae, S. pneumoniae*	*S. sonnei*		(23)
X	37	14/F	Q293X/Q293X	UK	Alive	*S. pneumoniae, S. pyogenes*	*P. aeruginosa, H. influenzae* type b		(6)
X	38	18 mo/M	Q293X /593delG	UK	†	*S. pneumoniae*	*P. aeruginosa*		(6)
Y	39	2/M	Q293X /593delG	UK	Alive				(6)
Z	40	6 mo/F	Q293X/Q293X	France	Alive		*P. aeruginosa*		PR
A1	41	14 mo/M	ND	UK	†		*P. aeruginosa*		PR
A1	42	4/F	Q293X/897_900delCAAT	UK	Alive	*S. pneumoniae*	*N. meningitidis*		PR
L	43	3/F	118insA/118insA	Japan	Alive				PR
B1	44	2/M	118insA /R183X	Japan	Alive	*S. pneumoniae*			PR
C1	45	3/F	118insA/118insA	Japan	†	*S. pneumoniae*	*P. aeruginosa*		PR

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Kindred	P	Age/Sex (yr)	Mutation of IRAK4	Country of Origin	Outco me Status	Invasive Infections With Gram-Positive Bacteria	Invasive Infections With Gram-Negative Bacteria	Other	Ref.
C1	46	9 mo/F	118insA/118insA	Japan	†	*S. pneumoniae*			PR
D1	47	12 mo/F	Q293X/831+5G>T	USA	†	*S. pneumoniae*			PR
E1	48	12/M	Q293X/Q293X	France	Alive	*S. pneumoniae*			PR
Kindred	**P**	**Age/Sex (yr)**	**Mutation of MyD88**	**Country of Origin**	**Outco me Status**	**Invasive Infections With Gram-Positive Bacteria**	**Invasive Infections With Gram-Negative Bacteria**	**Other**	**Ref.**
	1	11 mo/M	E52del/E52del	France	†	*S. pneumoniae*		Adenovirus Rotavirus	(49)
	2	5/F	R196C/L93P	Turkey	Alive	*S. pneumoniae*			(49)
	3	17/F	R196C/R196C	Portugal	Alive	*S. pneumoniae*	*Salmonella* spp.		(49)
	4	11/M	R196C/R196C	Portugal	Alive	*S. pneumoniae, S. aureus*, β-hemolytic *Streptococci*	*S. enteriditis*		(49)
	5	11 mo/F	E52del/E52del	Spain	†	*S. pneumoniae*		RSV	(49)
	6	4/M	E52del/E52del	Spain	†	*S. pneumoniae, S. aureus*			(49)
	7	2 mo/M	E52del/E52del	Spain	†	*S. pneumoniae*			(49)
	8	8/M	E52del/E52del	Spain	Alive	β-hemolytic *Streptococci*			(49)
	9	5/F	E52del/E52del	Spain	Alive	*S. pneumoniae, S. aureus*		*C. albicans*	(49)
	10	2/F	E52del/E52del	France	†	*S. aureus, S. pneumoniae*	*H. influenzae* type e	*M. catarrhalis*	PR
	11	5 mo/M	ND	Serbia	†		*P. aeruginosa*		PR
F	12	1/M	E52del/E52del	Serbia	Alive		*P. aeruginosa*		PR

Abbreviations:

† = dead, ND = no data, P = patient, PR = present report, RSV = respiratory syncytial virus.

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TABLE 2

Immunologic Investigation: Blood Lymphocyte Subsets and T-Cell Proliferation in IRAK-4-Deficient Patients*

Patients	P40	P26	P46	P10	P23	P30	P38	P43	P11	P19	Normal Values	P36	P13	P34	P37	P1	P15	P28	P8	Normal Values
(age)	3 mo	6 mo	9 mo	1 yr	1 yr	1 yr	1 yr	1 yr	2 yr	2 yr	3 mo-2 yr	3 yr	4 yr	4 yr	4 yr	5 yr	5 yr	5 yr	6 yr	2–6 yr
Lymph., $10^9/\mu L$	7.5	4.2		5.97	6.2	5.2	5.25	6.38	5.0		(3.4–9)	2.95	2.8	3.6	3.82	3.3	4.8	3.2	**1.9**	(2.3–5.4)
T cells, %																				
CD3	68	62		59	77	66	45	77	71	73	(53–84)	79	58	63	60	75	86	57	73	(56–75)
CD4	42	35	36	45	48	50	33	57	48		(31–64)	49	35	39	33	55	63	35	46	(28–47)
CD8	24	21	29	11	26	14	10	18	29		(12–30)	29	19	19	27	32	19	24	22	(16–30)
CD45RA/CD4											(64–95)									(53–86)
CD45RO/CD4																				
NK cells, %	12	15			5	12	7	8			(4–18)	6	20	9	8		3	3	5	(4–17)
B cells, %	18	22		34	10	20	45	14	7	17	(6–41)	13	19	27	26	23	6	38	18	(14–33)
Prolif. × 10 3cpm																				
CD3											(>30)									(>30)
PHA		64		44	59			40.7	169		(>50)				50		242	387		(>50)
PPD											(>10)						7			(>10)
Candidin											(>10)				10		7			(>10)
Tetanus					35						(>10)									(>10)
S. aureus				237							(>10)									(>10)

Patients	P3	P31	P24	P2	Normal Values	P17	P18	Normal Values
(age)	7 yr	11 yr	13 yr	14 yr	7–14 yr	27 yr	27 yr	Adult
Lymph., $10^9/\mu L$	5.0	2.4	2.1	2.6	(1.9–3.7)	1.3	1.3	(1.4–3.3)
T cells, %								
CD3	68	85	76	75	(56–84)	72	76	(63–84)
CD4	52	48	48	54	(31–52)	56	46	(34–62)
CD8	14	28	24	22	(18–35)	13	20	(14–41)

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Patients	P3	P31	P24	P2	Normal Values	P17	P18	Normal Values
(age)	**7 yr**	**11 yr**	**13 yr**	**14 yr**	**7–14 yr**	**27 yr**	**27 yr**	**Adult**
CD45RA/CD4				58	(46–77)			
CD45RO/CD4				45				
NK cells, %	2	13	2	12	(3–22)	11	8	(5–20)
B cells, %	28	10	10	16	(6–23)	15	12	(6–17)
Prolif. × 10 3cpm								
CD3				80	(>30)	26	48	(>30)
PHA			235	238	(>50)	112	135	(>50)
PPD				94	(>10)	1.7	10	(>10)
Candidin				16	(>10)	7	19	(>10)
Tetanus				123	(>10)	0.5	1	(>10)
S. aureus					(>10)			(>10)

[*] Data given as total lymphocyte counts and percentages of T cells, NK cells, and B cells. Age-specific normal values are shown in parentheses. Proliferative responses to OKT3 (50 ng/mL) ("CD3"), the mitogen PHA, and various antigens (PPD, candidin, tetanus) are listed.

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Table 3

Immunologic Investigation: Blood Lymphocyte Subsets and T-Cell Proliferation in MyD88-Deficient Patients*

Patients	**P1**	**P10**	**P2**	**Normal Values**	**P6**	**P9**	**Normal Values**	**P8**	**P4**	**Normal Values**
(age)	**10 mo**	**15 mo**	**2 yr**	**3 mo-2 yr**	**2.5 yr**	**3 yr**	**2–6 yr**	**7 yr**	**8 yr**	**6–12 yr**
Lymph., $10^9/\mu L$		5.5	**2.4**	(3.4–9)		3.32	(2.3–5.4)	2.43		(1.9–3.7)
T cells, %										
CD3		69	64	(53–84)	68	64	(56–76)	47	77	(60–76)
CD4		39	39	(31–64)	35	39	(28–47)	25	43	(31–47)
CD8		22	27	(12–30)	33	25	(16–30)	22	27	(18–35)
CD45RA/CD4		79		(64–95)			(53–86)		46	
CD45RO/CD4		25							59	
NK cells, %		5	20	(4–18)	7	11.5	(4–17)	26	4	(4–17)
B cells, %	27	24	18	(6–41)	23	17.5	(14–33)	17	18	(13–27)
Prolif. × 10 3cpm										
CD3			46	(>30)					56	(>30)
PHA			175.5	(>50)		50			113	(>50)
PPD			4.2	(>10)					10.3	(>10)
Candidin			1.0	(>10)					15.2	(>10)
Tetanus			26.0	(>10)					21	(>10)

*Data given as total lymphocyte counts and percentages of T cells, NK cells, and B cells. Age- specific normal values are shown in parentheses. Proliferative responses to OKT3 (50 ng/mL) (“CD3”), the mitogen PHA, and various antigens (PPD, candidin, tetanus) are listed.

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Table 4

Immunologic Investigation: Ig Levels and Humoral Responses to Recall Antigens and to Glycans in IRAK-4-Deficient Patients*

Patients	**P6**	**P22**	**P40**	**P26**	**P46**	**Normal Values**	**P5**	**P10**	**P23**	**P30**	**P32**	**P38**	**P43**	**P11**	**P39**	**Normal Values**
(age)	**2 mo**	**3 mo**	**3 mo**	**6 mo**	**9 mo**	**(3–9 mo)**	**1 yr**	**1 yr**	**1 yr**	**1 yr**	**1 yr**	**1 yr**	**1 yr**	**2 yr**	**2 yr**	**(1–3 yr)**
Serum Ig (g/L)																
IgG	5.7	2.45	**7.38**	5.68	3.84	(2.35–5.49)	5.4	**11.4**	8.9	**16.9**	**2.21**	4.55	**13.8**	**17**	6.31	(3.35–8.96)
IgG1					1.37	NA		7.2		13.05			8.17			(>3)
IgG2					0.30	NA		1.26		2.09			2.01			(>0.30)
IgG3					0.07	NA		0.20		0.68			0.9			(>0.12)
IgG4					0.03	NA		0.28		0.41			<0.3			(<1)
IgA	0.6	0.16	0.25	0.14	0.3	(0.12–0.62)	0.2	0.34	0.47	1.09	0.26	0.34	0.86	0.9	0.25	(0.27–1.22)
IgM	0.6	0.51	0.63	0.40	0.33	(0.34–0.95)	1.1	1.02	1.11	0.98	0.43	0.33	2.27	2.0	0.52	(0.58–1.53)
IgE (kU/L)				**19**	**203**	(<15)		**129**	**801**	**257**		13.8	38.2	**198**		(<40)
Specific antibodies																
Antitetanus				0.21		(>0.1 IU/mL)			0.12	1.15		0.32		>0.1		(>0.1 IU/mL)
Poliovirus						(>40)										(>40)
Diphtheria				0.12		(>0.1 IU/mL)			0.26	0.7						(>0.1 IU/mL)
S. pneumoniae						(>0.3 μg/mL)			**<0.3**	2.04						(>0.3 μg/mL)
H. influenzae b				0.31		(>0.15 μg/mL)				>9	0.56	0.16				(>0.15 μg/mL)
Allohemagglutinin						NA								1/16		(>1/8)

Patients	**P36**	**P13**	**P37**	**P1**	**P15**	**P28**	**P34**	**P8**	**P3**	**Normal Values**	**P31**	**P24**	**P2**	**P17**	**P18**	**P7**	**Normal Values**
(age)	**3 yr**	**4 yr**	**4 yr**	**5 yr**	**5 yr**	**5 yr**	**5 yr**	**6 yr**	**7 yr**	**(3–7 yr)**	**11 yr**	**13 yr**	**14 yr**	**27 yr**	**27 yr**	**32 yr**	**(11 yr-Adult)**
Serum Ig (g/L)																	
IgG	8.82	**13.6**	9.29	11.7	10.3	**18.99**	11.1	**13.6**		(5.49–11.54)	12.8	7.6	**14.4**	**15**	**13**	**16.7**	(6.55–12.78)
IgG1	11.2	10.2		6.85	6.88	8.44		8.41		(>4)	10.6	7.16	8.62				(>4)
IgG2	0.65	2.63		0.4	1.18	5.37		3.12		(>0.40)	2.5	0.95	0.71				(>0.60)
IgG3	0.36	0.13		0.19	0.45	0.29		0.34		(>0.16)	0.72	0.38	0.47				(>0.17)
IgG4	0.29	**1.85**		**1.41**	0.5	**4.89**		**3.54**		(<1)	0.65	0.06	**1.46**				(<1)

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Patients	P36	P13	P37	P1	P15	P28	P34	P8	P3	Normal Values	P31	P24	P2	P17	P18	P7	Normal Values
(age)	**3 yr**	**4 yr**	**4 yr**	**5 yr**	**5 yr**	**5 yr**	**5 yr**	**6 yr**	**7 yr**	**(3–7 yr)**	**11 yr**	**13 yr**	**14 yr**	**27 yr**	**27 yr**	**32 yr**	**(11 yr-Adult)**
IgA	1.19	0.64	0.57	0.63	1.31	1.51	0.6	2.42		(0.41–1.57)	1.02	**0.49**	0.88	**0.3**	**0.6**	1.1	(0.70–3.44)
IgM	1.59	**1.65**	0.85	0.72	0.50	0.96	**3.5**	0.52		(0.54–1.55)	1.38	1.91	0.64	1.3	1.5	1.9	(0.50–2.09)
IgE (kU/L)	6	**977**	**106**	**17,400**	**356**			**187**		(<60)	**180**	36.6	11	**255**	96.5	**400**	(<150)
Specific antibodies																	
Antitetanus	0.58	>10			0.06	0.26	0.47	0.59	1,81	(>0.1 IU/mL)		0.47	0.47	0.34	0.46	**0.06**	(>0.1 IU/mL)
Poliovirus					>40					(>40)			40				(>40)
Diphtheria	0.55			2.04	**0.002**			0.10	0.88	(>0.1 IU/mL)		0.72				0,18	(>0.1 IU/mL)
S. pneumoniae		0.2		1.9	>0.6	**<0.3**	>0.3	**<0.3**		(>0.3 μg/mL)		3.59	**<0.3**	**<0.3**	**<0.3**	>0.3	(>0.3 μg/mL)
H. influenzae	>1	5.1	0.84	>1		1.98	12.3	>1	>1	(>0.15 μg/mL)			>1			1.36	(>0.15 μg/mL)
Allohemagglutinin		1/8	**1/1**	1/128	1/16			**1/2**		(>1/16)		1/32		1/16	1/16	**1 /2**	(>1/16)

*Serum immunoglobulin levels and titers for specific antibodies. Age-specific normal values are shown in parentheses.

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Table 5

Immunologic Investigation: Ig Levels and Humoral Responses to Recall Antigens and to Glycans in MyD88-Deficient Patients*

Patients	P5	P10	Normal Values	P2	P6	P9	Normal Values	P8	P4	Normal Values	P3	Normal Values
(age)	8 mo	15 mo	(6–15 mo)	2 yr	2.5 yr	3 yr	(2–4 yr)	7 yr	8 yr	(5–8 yr)	15 yr	(14 yr-Adult)
Serum Ig (g/L)												
IgG	**2.23**	6.2	(2.35–6.23)	**12.4**	**13.6**	**29.2**	(4.82–8.96)	**12.5**	8.31	(5.49–11.54)	12.5	(6.55–12.78)
IgG1		5.0	NA	10.3	10.36	20.4	(>4)	8.0	6.61	(>4)	9.8	(>4)
IgG2		0.88	NA	0.148	2.52	5.19	(>0.3)	3.2	0.96	(>0.40)	0.85	(>0.6)
IgG3		0.78	NA	0.59	0.3	1.7	(>0.15)	0.47	0.24	(>0.16)	0.56	(>0.17)
IgG4		0.07	NA	0.44	0.4	**3.51**	(<1)	**3.7**	0.1	(<1)	0.84	(<1)
IgA	0.36	0.52	(0.12–0.62)	0.5	0.32	**1.6**	(0.33–1.22)	1.32	1.21	(0.41–1.57)	1.36	(0.70–3.44)
IgM	0.41	0.93	(0.34–1.1)	**1.59**	0.61	1.24	(0.50–1.53)	**0.29**	0.9	(0.54–1.55)	0.69	(0.50–2.09)
IgE (U/mL)	**53**		(<30)	24.5	**115**	34	(<40)		**199**	(<60)	10.4	(<150)
Specific antibodies												
Antitetanus			(>0.1 IU/mL)			0.9	(>0.1 IU/mL)		1.3	(>0.1 IU/mL)		(>0.1 IU/mL)
S. pneumoniae		0.9	(>0.3 μg/mL)	17.96		11.5	(>0.3 μg/mL)		2.74	(>0.3 μg/mL)	3.85	(>0.3 μg/mL)
H. influenzae b			(>0.15 μg/mL)			3.4	(>0.15 μg/mL)			(>0.15 μg/mL)		(>0.15 μg/mL)
Allohemagglutinin			NA	1/4			(>1/8)		1/16		1/16	(>1/16)

*Serum immunoglobulin levels and titers for specific antibodies. Age-specific normal values are shown in parentheses.

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Table 6

Immunologic Investigation Summary

	IRAK-4- Deficient Patients	MyD88- Deficient Patients
T lymphocytes subset		
Normal pts/tested pts (%)	24/24 (100)	6/6 (100)
B lymphocytes subset		
Normal pts/tested pts (%)	23/23 (100)	7/7 (100)
NK lymphocytes subset		
Normal pts/tested pts (%)	19/19 (100)	6/6 (100)
T cell proliferation		
Normal pts/tested pts (%)	12/12 (100)	3/3 (100)
IgG levels		
Normal pts/tested pts (%)	15/28 (53.6)	3/8 (37.5)
Pts with increased level/tested pts (%)	12/28 (42.9)	4/8 (50)
Pts with decreased level/tested pts (%)	1/28 (3.6)	1/8 (12.5)
IgG1,3,3 levels		
Normal pts/tested pts (%)	13/13 (100)	7/7 (100)
IgG4 levels		
Normal pts/tested pts (%)	8/13 (61.5)	5/7 (71.4)
Pts with increased level/tested pts (%)	5/13 (38.5)	2/7 (28.6)
IgA levels		
Normal pts/tested pts (%)	25/28 (89.3)	7/8 (87.5)
Pts with decreased level/tested pts (%)	3/28 (10.7)	-
Pts with decreased level/tested pts (%)	-	1/8 (12.5)
IgM levels		
Normal pts/tested pts (%)	26/28 (92.9)	6/8 (75)
Pts with increased level/tested pts (%)	2/28 (7.1)	1/8 (12.5)
Pts with decreased level/tested pts (%)	-	1/8 (12.5)
IgE levels		
Normal pts/tested pts (%)	6/20 (30)	3/6 (50)
Pts with increased level/tested pts (%)	14/20 (70)	3/6 (50)
Specific Ab to protein antigens (tetanus, diphtheria, or polio)		
Normal pts/tested pts (%)	17/17 (100)	2/2 (100)
Ab against *H. influenzae*		
Normal pts/tested pts (%)	14/14 (100)	1/1 (100)
Ab against *S. pneumoniae*		

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	IRAK-4- Deficient Patients	MyD88- Deficient Patients
Normal pts/tested pts (%)	6/13 (46.2)	5/5 (100)
Pts with abnormal response/tested pts (%)	7/13 (53.8)	
Ab production after immunization with PNCV23		
Normal pts/tested pts (%)	4/9 (44.4)	
Pts with abnormal response/tested pts (%)	5/9 (55.6)	
Ab production after immunization with PNCV23+PCV7		
Normal pts/tested pts (%)	2/3 (66.7)	
Pts with abnormal response/tested pts (%)	1/3 (33.3)	
Ab production after immunization with PCV7		
Normal pts/tested pts (%)	1/1 (100)	
Allohemagglutinin		
Normal pts/tested pts (%)	7/10 (70)	3/3 (100)
Pts with decreased level/tested pts (%)	3/10 (30)	

Abbreviations: Ab = antibody, PCV7 = 7 valent conjugate vaccine, PNCV23 = 23 valent nonconjugate vaccine, pts = patients.

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Table 7

Humoral Responses to Viruses and *Toxoplasma gondii*

	IRAK-4-Deficient Patients (positive pts/tested pts)	MyD88-Deficient Patients (positive pts/tested pts)
Herpes simplex virus	0/8	2/4
Varicella zoster virus	5/9	2/3
Cytomegalovirus	2/9	3/4
Epstein-Barr virus	4/8	3/5
HHV6	6/6	Not done
HHV8	0/2	Not done
Parvovirus B 19	2/6	Not done
Rubella	5/6	4/4
Measles	Not done	3/4
Mumps	5/6	2/3
Coxsackie virus B1,2,3,4,6	6/7	Not done
RSV	6/6	Not done
Human metapneumovirus	5/6	Not done
Rotavirus	Not done	1/1
Adenovirus	Not done	1/1
HIV	0/3	Not done
VDRL	0/1	Not done
Toxoplasma	0/3	1/3

Abbreviations: See previous tables. HHV = human herpes virus, HIV = human immunodeficiency virus, VDRL = Venereal Disease Research Laboratory test.

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Table 8

Inflammatory Signs at Admission in Patients With IRAK-4 Deficiency Who Had InvBD

Age Group	Age at Onset	No. of Episodes*	Temperature (°C)			CRP (mg/L)			Whole Leukocyte Count (WLC/μL)			Neutrophil Count (NC/μL)		
			Mean	Max	SD	Mean	Max	SD	Mean	Max	SD	Mean	Max	SD
Neonatal period	7 d to 17 d	5 (T) 5 (CRP) 5 (WLC) 3 (NC)	37.2	38.0	0.6	43.6	150	61.2	9,55 (N: 2700–13,000)	18,000	6807	3525	5308	11,500
Infancy	5 wk to 11 mo	16 (T) 12 (CRP) 13 (WLC) 12 (NC)	38.2	39.9	1.4	69	176.4	51.1	8034 (N: 4300–9700)	16,000	4422	4643	13,760	3999
Childhood	1 yr to 14 yr	27 (T) 22 (CRP) 36 (WLC) 31 (NC)	38.3	41.0	1.0	61.5	156	65.3	9875 (N: 4300–9700)	25,200	4894	4731	15,940	3593

*No. of patients for whom the following data were available: T = temperature, CRP = C-reactive protein concentration, WLC = whole leukocyte count, NC = neutrophil count.

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Table 9

Inflammatory Signs at Admission in Patients With Myd88 Deficiency Who Had InvBD

Age Group	Age at Onset	No. of Episodes	Temperature (°C)			CRP (mg/L)			Whole Leukocyte Count (WLC/μL)			Neutrophil Count (NC/μL)		
			Mean	Max	SD	Mean	Max	SD	Mean	Max	SD	Mean	Max	SD
Infancy	5 wk to 11 mo	7 (T) 12 (CRP) 14 (WLC) 12 (NC)	38.1	39.0	1.3	7.2	6.5	21.8	11,691 (N: 4300–9700)	21,300	4964	3783	7680	1998
Childhood	1 yr to 10 yr	17 (T) 14 (CRP) 16 (WLC) 15 (NC)	37.2	39.1	1.1	47.7	153	83.7	9515 (N: 4300–9700)	23,200	4694	5693	16,900	4070

*No. of patients for whom the following data were available: T = temperature, CRP = C-reactive protein concentration, WLC = whole leukocyte count, NC = neutrophil count.

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Table 10

Prophylaxis

	IRAK-4-Deficient Patients	MyD88-Deficient Patients
Antibiotic prophylaxis	28/48	6/12
Penicillins	6	1
Cotrimoxazole	10	1
Penicillins plus cotrimoxazole	8	4
Cephalosporin	1	-
Azythromycin	1	-
Quinolone	2	-
IgG treatment	15/48	4/12
Antibiotic prophylaxis plus IgG treatment	13/48	4/12
No prophylaxis	18/48	6/12

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Article

Defective IL-10 signaling in hyper-IgE syndrome results in impaired generation of tolerogenic dendritic cells and induced regulatory T cells

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The Journal of Experimental Medicine

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Hyper-IgE syndrome (HIES) is a primary immunodeficiency characterized by recurrent staphylococcal infections and atopic dermatitis associated with elevated serum IgE levels. Although defective differentiation of IL-17–producing CD4+ T cells (Th17) partly accounts for the susceptibility to staphylococcal skin abscesses and pneumonia, the pathogenesis of atopic manifestations in HIES still remains an enigma. In this study, we examined the differentiation and function of Th1, Th2, regulatory T cells (T_{reg} cells), and dendritic cells (DCs) in HIES patients carrying either *STAT3* or *TYK2* mutations. Although the in vitro differentiation of Th1 and Th2 cells and the number and function of T_{reg} cells in the peripheral blood were normal in HIES patients with *STAT3* mutations, primary and monocyte-derived DCs showed defective responses to IL-10 and thus failed to become tolerogenic. When treated with IL-10, patient DCs showed impaired up-regulation of inhibitory molecules on their surface, including PD-L1 and ILT-4, compared with control DCs. Moreover, IL-10–treated DCs from patients displayed impaired ability to induce the differentiation of naive CD4+ T cells to FOXP3+ induced T_{reg} cells (iT_{reg} cells). These results suggest that the defective generation of IL-10–induced tolerogenic DCs and iT_{reg} cells may contribute to inflammatory changes in HIES.

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Abbreviations used: cDC, conventional DC; DN, dominant-negative; HIES, hyper-IgE syndrome; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; iT_{reg} cell, induced T_{reg} cell; MoDC, monocyte-derived DC; mRNA, messenger RNA; nT_{reg} cell, natural T_{reg} cell; pDC, plasmacytoid DC; Q-PCR, quantitative RT-PCR.

Hyper-IgE syndrome (HIES) is a rare complex primary immunodeficiency, characterized by atopic dermatitis, extremely high serum IgE levels, staphylococcal skin abscesses, and pneumonia associated with disproportionately mild inflammatory responses (Grimbacher et al., 2005; Minegishi, 2009). Treatments so far are symptomatic, including the prevention of bacterial and fungal infections and management of eczema. Previous studies suggested the benefit from bone marrow transplantation, Ig replacement, and IFN and G-CSF administration (Grimbacher et al., 2005), but a general role for immune reconstitution and modulation in HIES is unproven. To improve the long-term quality of life of HIES patients, it is necessary to develop a new treatment strategy based on a better understanding of molecular mechanisms of this syndrome. We recently demonstrated that

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most cases of HIES are caused by dominant-negative (DN) mutations of the *STAT3* gene (Holland et al., 2007; Minegishi et al., 2007). However, the pathogenesis of this syndrome remains unclear. In particular, the molecular mechanisms underlying the allergic manifestations, including atopic dermatitis and extremely high serum IgE levels, remain one of the great enigmas in the pathogenesis of this syndrome.

STAT3 is a transcription factor that binds to the promoter regions of various genes, including those encoding acute-phase proteins. STAT3 plays a critical role in signal transduction for many cytokines, including those of the γc family (IL-2, IL-7, IL-9, IL-15, and IL-21), the gp130 family (IL-6, IL-11, IL-27, and IL-31), the IL-10 family (IL-10 and IL-22), and receptor-type tyrosine kinases. The systemic deletion of STAT3 in mice is lethal, but studies involving the tissue-specific deletion of STAT3 have demonstrated that STAT3 plays a critical role in cell migration, survival, proliferation, apoptosis, inflammation, and tumorigenesis in many tissues (Akira, 2000). Furthermore, recent data unanimously demonstrated that STAT3 plays an essential role for Th17 cell development in humans (de Beaucoudrey et al., 2008; Ma et al., 2008; Milner et al., 2008; Renner et al., 2008; Minegishi et al., 2009), which could explain, at least in part, why HIES patients suffer from recurrent staphylococcal infections confined to the skin and lung (Minegishi et al., 2009).

Allergic diseases may result from an inappropriate balance between effector Th2 cells and T_{reg} cells (Umetsu and DeKruyff, 2006; Akdis and Akdis, 2009; Lloyd and Hawrylowicz, 2009). Th2 cells respond to allergens and produce IL-4, IL-5, IL-9, and IL-13. Th2 cytokines induce changes in blood vessels that lead to the up-regulation of intercellular adhesion molecule 1 and vascular cell-adhesion molecule 1, in turn leading to the recruitment of very late antigen 4–expressing eosinophils. These factors also induce the survival and activation of eosinophils. In addition, IL-4 and IL-13 are responsible for promoting Ig class switching to IgE (Hammad and Lambrecht, 2008). Newly identified cytokines such as IL-25, IL-31, and IL-33 also participate in Th2 cell–mediated inflammation (Dillon et al., 2004; Wang et al., 2007; Kakkar and Lee, 2008). Th1 cells may also contribute to allergic inflammation by inducing the apoptosis of epithelial cells in atopic dermatitis (Trautmann et al., 2000).

T_{reg} cells are key mediators of peripheral tolerance that actively suppress effector T cells and inhibit immune response–mediated tissue damage. Both FOXP3$^+$ T_{reg} cells and IL-10–producing FOXP3$^-$ T_{reg} cells play an essential role in the regulation of allergic inflammation (Curotto de Lafaille et al., 2001; Zheng and Rudensky, 2007; Sakaguchi et al., 2008). There are two types of FOXP3$^+$ T_{reg} cells: natural T_{reg} cells (nT_{reg} cells) and induced T_{reg} cells (iT_{reg} cells). nT_{reg} cells develop in the thymus, whereas iT_{reg} cells develop in the periphery. In the presence of TGF-β1, naive FOXP3$^-$ CD4$^+$ T cells are converted into FOXP3$^+$ iT_{reg} cells (Chen et al., 2003; Coombes et al., 2007; Rubtsov and Rudensky, 2007; Zheng et al., 2007). Mutations in the human *FOXP3* gene result in immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome (Bennett et al., 2001; Wildin et al., 2001). Patients with IPEX syndrome suffer from enteropathy, autoimmune diabetes and thyroiditis, food allergy, and atopic dermatitis with extremely high serum IgE levels. FOXP3 deficiency in mice also leads to atopic manifestations (Fontenot et al., 2003; Lin et al., 2005).

DCs are central to the orchestration of the various types of immunity and tolerance (Banchereau et al., 2000; Kapsenberg, 2003; Steinman et al., 2003). Immature DCs function as sentinels in the periphery, undergoing terminal differentiation in response to various danger signals. Maturing DCs migrate to the lymph nodes, where they acquire potent antigen-presenting capacity and induce vigorous T cell responses by expressing co-stimulatory molecules and secreting large amounts of proinflammatory cytokines. The interaction between DCs and naive CD4$^+$ T cells is considered to determine the fate of CD4$^+$ T cells. Cytokines produced by DCs, such as IL-12 and IFN-α, may bias CD4$^+$ T cell priming toward the Th1 pathway (Schulz et al., 2000). Notch ligands, such as Jagged 1, expressed by DCs may promote CD4$^+$ T cells toward the Th2 pathway (Amsen et al., 2009). In addition, DCs play a key role in the induction and maintenance of peripheral T cell tolerance (Steinman et al., 2003; Rutella et al., 2006).

We investigated the molecular mechanism underlying the atopic manifestations in HIES by studying Th1–Th2–T_{reg} cell balance and the development and function of primary and monocyte-derived DCs (MoDCs). The results suggest that IL-10 signaling by DCs may be crucial for the generation of tolerogenic DCs and iT_{reg} cells for the maintenance of an appropriate Th1–Th2–T_{reg} cell balance in vivo in humans.

RESULTS

Normal Th1 and Th2 differentiation from naive CD4$^+$ T cells but increased Th2 cytokine production from activated T cells in PBMCs of *STAT3* patients

We first evaluated Th1 and Th2 cell development of naive CD4$^+$ T cells in *STAT3* patients. Naive CD4$^+$ T cells were unstimulated or stimulated with anti-CD3 and anti-CD28 (anti-CD3/CD28) mAbs under neutral, Th1, and Th2 differentiation conditions, and the development of IFN-γ– and IL-4–producing cells was evaluated by cytoplasmic staining and flow cytometry. The development of Th1 and Th2 cells was similar in control subjects and *STAT3* patients (Fig. S1 A). This observation was confirmed by ELISA of the culture supernatants of naive CD4$^+$ T cells, showing similar levels of IFN-γ, IL-5, and IL-13 secretion for control subjects and *STAT3* patients (Fig. S1 B). We next evaluated Th1 and Th2 cytokine production from PBMCs after stimulation with anti-CD3/CD28 mAbs. The production of IFN-γ was equivalent between control subjects and *STAT3* patients, but the production of IL-5 and IL-13 was increased in *STAT3* patients compared with control subjects (Fig. S1 C). These results suggest that cells in PBMCs other than naive CD4$^+$ T cells are likely to be responsible for increased Th2 cytokine production in *STAT3* patients.

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The number and suppressive activity of T_{reg} cells in the peripheral blood are normal in *STAT3* patients

We next evaluated the number of FOXP3$^+$ T_{reg} cells among PBMCs because STAT3 is involved in the transduction of IL-6 and IL-21 signals, which may influence the balance between nT_{reg} cell and Th17 cell differentiation (Harrington et al., 2005; Bettelli et al., 2006; Ivanov et al., 2006; Veldhoen et al., 2006). Similar numbers of PBMCs were obtained from control subjects and *STAT3* patients, and these cells were stained for extracellular CD4 and CD25 and intracellular FOXP3 and evaluated by flow cytometry. The percentages of CD4$^+$CD25$^+$ cells and CD4$^+$FOXP3$^+$ cells did not differ significantly between control subjects and *STAT3* patients (Fig. 1 A).

We then investigated the function of T_{reg} cells in the peripheral blood ex vivo. CD4$^+$CD25$^+$CD62L$^+$ T_{reg} cells were obtained from the peripheral blood of control subjects and *STAT3* patients at a purity of >99% and were co-cultured with autologous CD4$^+$CD25$^-$CD62L$^+$ responder T cells in the presence or absence of anti-CD3/CD28 mAbs. The addition of 1.25×10^3 control T_{reg} cells to the 1.25×10^4 control responder T cells resulted in levels of [^{3}H]thymidine incorporation 55% lower than those obtained after the addition of 1.25×10^3 control responder T cells. Levels of [^{3}H]thymidine incorporation were similarly lowered by the addition of 1.25×10^3 patient T_{reg} cells to the 1.25×10^4 patient responder T cells (Fig. 1 B). Modification of the ratio of T_{reg} cells to responder T cells from 1:1 (T_{reg} cell/responder T cell) to 1:100 resulted in no significant difference in the percent suppression of [^{3}H]thymidine incorporation between control subjects and *STAT3* patients (Fig. 1 C). These results indicate that the in vivo generation and ex vivo function of T_{reg} cells were normal in *STAT3* patients.

Defective IL-10 signaling in MoDCs from *STAT3* patients

We next evaluated the generation of MoDCs in vitro. Isolated CD14$^+$ monocytes from the PBMCs of control subjects and *STAT3* patients were cultured with GM-CSF and IL-4 for 5 d and then allowed to mature in the presence of LPS for 2 d. MoDC differentiation was normal, as shown by evaluations of the forward and side light scatter of the cells (Fig. S2 A), the expression levels of CD1a (Fig. S2 B), CD80, CD83, and CD86 (Fig. S2 C), and FITC-dextran uptake (Fig. S2 D). Of note, levels of CD86 expression before LPS stimulation were slightly higher in *STAT3* patients than in control subjects (Fig. S2 C), suggesting that autocrine IL-10 may regulate the expression of DC maturation markers in control subjects but not in *STAT3* patients (Corinti et al., 2001).

We have previously demonstrated that *STAT3* plays an important role in IL-10 signal transduction in human monocyte-derived macrophages (Minegishi et al., 2007). We therefore investigated IL-10 signal transduction in MoDCs. Consistent with our previous findings, the transcriptional

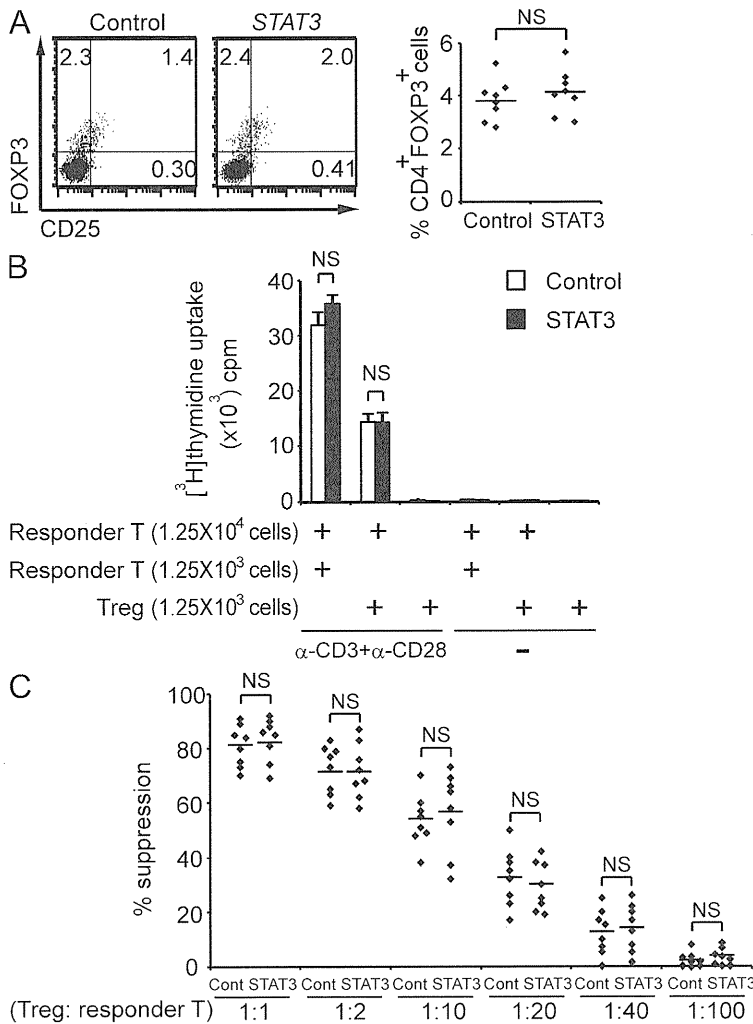

Figure 1. The number and suppressive activity of T_{reg} cells in the peripheral blood are normal in *STAT3* patients. (A, left) Representative dot plots gated on CD4$^+$ peripheral blood T cells from a control subject and a *STAT3* patient. (right) Summary data from eight control subjects and eight *STAT3* patients showing percentages of CD4$^+$FOXP3$^+$ cells. Data are representative of at least two independent experiments. (B) CD4$^+$CD25$^-$CD62L$^+$ responder T cells and CD4$^+$CD25$^+$CD62L$^+$ T_{reg} cells were isolated by cell sorting. Responder T cells and T_{reg} cells were co-cultured as indicated for 5 d with or without a 1:100 (vol/vol) dilution of anti-CD3 + anti-CD28 mAb–coated beads. For the evaluation of proliferation, 1 µCi (37 kBq) [^{3}H]thymidine was added to the culture medium for the last 18 h. Graph shows mean ± SD. (C) CD4$^+$CD25$^-$CD62L$^+$ responder T cells and CD4$^+$CD25$^+$CD62L$^+$ T_{reg} cells were isolated and cultured as in B at the indicated ratio. Summary data (n = 8 each) are shown. Data are representative of at least two independent experiments performed in triplicate. (A and C) Horizontal bars indicate mean values.

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up-regulation of *SOCS3* and *VCAN* (*CSPG2*), two genes responsive to IL-10, was impaired in MoDCs from *STAT3* patients, as demonstrated by comparison with control subjects (Fig. 2 A). Intact signal transduction was observed for TGF-β1, the other critical inhibitory cytokine, in the MoDCs of *STAT3* patients (Fig. S2 E). We evaluated the effect of prior treatment with IL-10 on the phenotypic maturation of MoDCs. IL-10 was added to the culture medium on day 3 of DC differentiation. The prior treatment with IL-10 did not block the differentiation of MoDCs in response to GM-CSF and IL-4 (Fig. S3, A and B) but inhibited the LPS-induced maturation of MoDCs, with inhibition of the up-regulation of co-stimulatory molecules CD80 and CD86 and defective up-regulation of the DC maturation marker CD83 in a control subject. In contrast, the maturation of MoDCs derived from *STAT3* patients showed little sign of inhibition by prior treatment with IL-10 (Fig. S3 C). Up-regulation of CD80, CD83, and CD86 expression by LPS was inhibited by IL-10 pretreatment in control subjects, but the IL-10 pretreatment failed to inhibit the up-regulation of CD80, CD83, and CD86 by LPS in *STAT3* patients (Fig. 2 B). Consistent with this observation, the production of inflammatory cytokines, including TNF, IL-6, and IL-12p40, was suppressed by prior treatment with IL-10 in control subjects. The suppression by IL-10 was severely impaired in the MoDCs from *STAT3* patients (Fig. 2 C). Untreated and IL-10–treated MoDCs were harvested, extensively washed, and co-cultured with third-party allogeneic naive $CD4^+$ T cells from control subjects. LPS-stimulated mature MoDCs induced a significant increase in the uptake of $[^3H]$thymidine by naive $CD4^+$ T cells, with IL-10–treated DCs (IL-10–DCs) from a control subject markedly inhibiting the incorporation of $[^3H]$thymidine. In contrast, the down-regulation was very modest in the IL-10–treated MoDCs from *STAT3* patients. In the absence of MoDCs or naive $CD4^+$ T cells, almost no incorporation of $[^3H]$thymidine was detected (Fig. S4 A). Production of IFN-γ, IL-5, and IL-13 followed a very similar pattern, with prior IL-10 treatment inducing significant down-regulation in control subjects and barely detectable down-regulation in *STAT3* patients (Fig. 2 D). Thus, IL-10 was defective in MoDCs from *STAT3* patients, impairing suppression of (a) the up-regulation of co-stimulatory molecules on MoDCs, (b) the up-regulation of cytokine production by MoDCs, (c) the proliferation of co-cultured naive $CD4^+$ T cells, and (d) cytokine production by co-cultured naive $CD4^+$ T cells.

IL-10 signaling defect in MoDCs leads to the defective generation of tolerogenic DCs and iT_{reg} cells

Control MoDCs up-regulated the expression of inhibitory molecules, including PD-L1, PD-L2, ILT-3, and ILT-4 but not ICOS-L by IL-10 treatment. The up-regulation of these inhibitory molecules was severely impaired in the MoDCs of *STAT3* patients (Fig. 3 A). We then investigated the functional consequences of the defective up-regulation of inhibitory molecules for MoDCs by co-culturing untreated and IL-10–DCs with third-party allogeneic naive $CD4^+$ T cells from control subjects. *FOXP3* messenger RNA (mRNA) levels in $CD4^+$ T cells co-cultured with control IL-10–DCs were approximately four times higher than those for cells co-cultured with untreated control MoDCs. However, up-regulation was severely impaired when naive $CD4^+$ T cells were co-cultured with IL-10–DCs from *STAT3* patients (Fig. 3 B). This observation was confirmed by the cytoplasmic staining of FOXP3 protein and flow cytometric analysis of the $CD4^+$ T cells (Fig. 3 C). This up-regulation of FOXP3 was not likely to be mediated by simple T cell activation because naive $CD4^+$ T cells cultured with control IL-10–DCs proliferated less vigorously compared with those with control DCs and because naive $CD4^+$ T cells cultured with patient DCs proliferated more vigorously compared with those with control DCs in the absence or presence of pretreatment with IL-10 (Fig. S4 B). iT_{reg} cells from control subjects and *STAT3* patients expressed equivalent amount of CD25 on their surface, but the expression levels of CTLA-4 and GITR (glucocorticoid-induced TNFR-related) were up-regulated by the co-culture with control IL-10–DCs, but the up-regulation was impaired by the co-culture with patient IL-10–DCs (Fig. S5).

We further evaluated the consequences of defective FOXP3 up-regulation by investigating iT_{reg} cell activity. Purified $CD4^+CD25^+$ T cells from the co-culture were added to autologous $CD4^+CD25^-$ responder T cells, and the mixture was stimulated with anti-CD3/CD28 mAbs. $CD4^+CD25^+$ T cells cultured with control IL-10–DCs efficiently suppressed the proliferation of CFSE-labeled autologous responder T cells (Fig. 3 D, left, second panel). The suppression of proliferation was severely impaired by $CD4^+CD25^+$ T cells cultured with patient IL-10–DCs (Fig. 3 D, left, third panel). We further evaluated cytokine production by a co-culture of responder T cells and $CD4^+CD25^+$ T cells. The production of IFN-γ, IL-5, and IL-13 was suppressed by co-culture with $CD4^+CD25^+$ cells cultured with control IL-10–DCs (Fig. 3 E). The cytokine production was rather increased by the addition of $CD4^+CD25^+$ T cells cultured with patient IL-10–DCs, which might be caused by decreased iT_{reg} cells and increased activated T cells in this $CD4^+CD25^+$ T cell population from *STAT3* patients. Thus, the IL-10 signaling defect in MoDCs results in the impaired generation of tolerogenic DCs and iT_{reg} cells.

The generation of $FOXP3^+$ iT_{reg} cells is dependent on TGF-β1 (Chen et al., 2003; Coombes et al., 2007; Rubtsov and Rudensky, 2007; Zheng et al., 2007). We therefore investigated the relationship between IL-10–DCs and TGF-β1 in the generation of $FOXP3^+$ iT_{reg} cells. TGF-β1 in the culture medium efficiently up-regulated FOXP3 expression in naive $CD4^+$ T cells in the presence of untreated immature DCs (Fig. 3 F). Control IL-10–DCs up-regulated FOXP3 expression, equivalent to TGF-β1 (Fig. 3 F, fifth dataset vs. third dataset), and a combination of control IL-10–DCs and TGF-β1 (Fig. 3 F, seventh dataset) further up-regulated FOXP3 expression. TGF-β1 effectively up-regulated FOXP3 expression in naive $CD4^+$ T cells when co-cultured with patient DCs, but patient IL-10–DCs were inefficient for the up-regulation

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of FOXP3 expression (Fig. 3 F, sixth dataset). Thus, in addition to TGF-β1, IL-10–DCs play a crucial role in the generation of FOXP3$^+$ iT_{reg} cells. Moreover, these results demonstrated that the defect in FOXP3 up-regulation was not caused by the lack of TGF-β1 in IL-10–DCs from *STAT3* patients because the addition of exogenous TGF-β1 did not rescue this

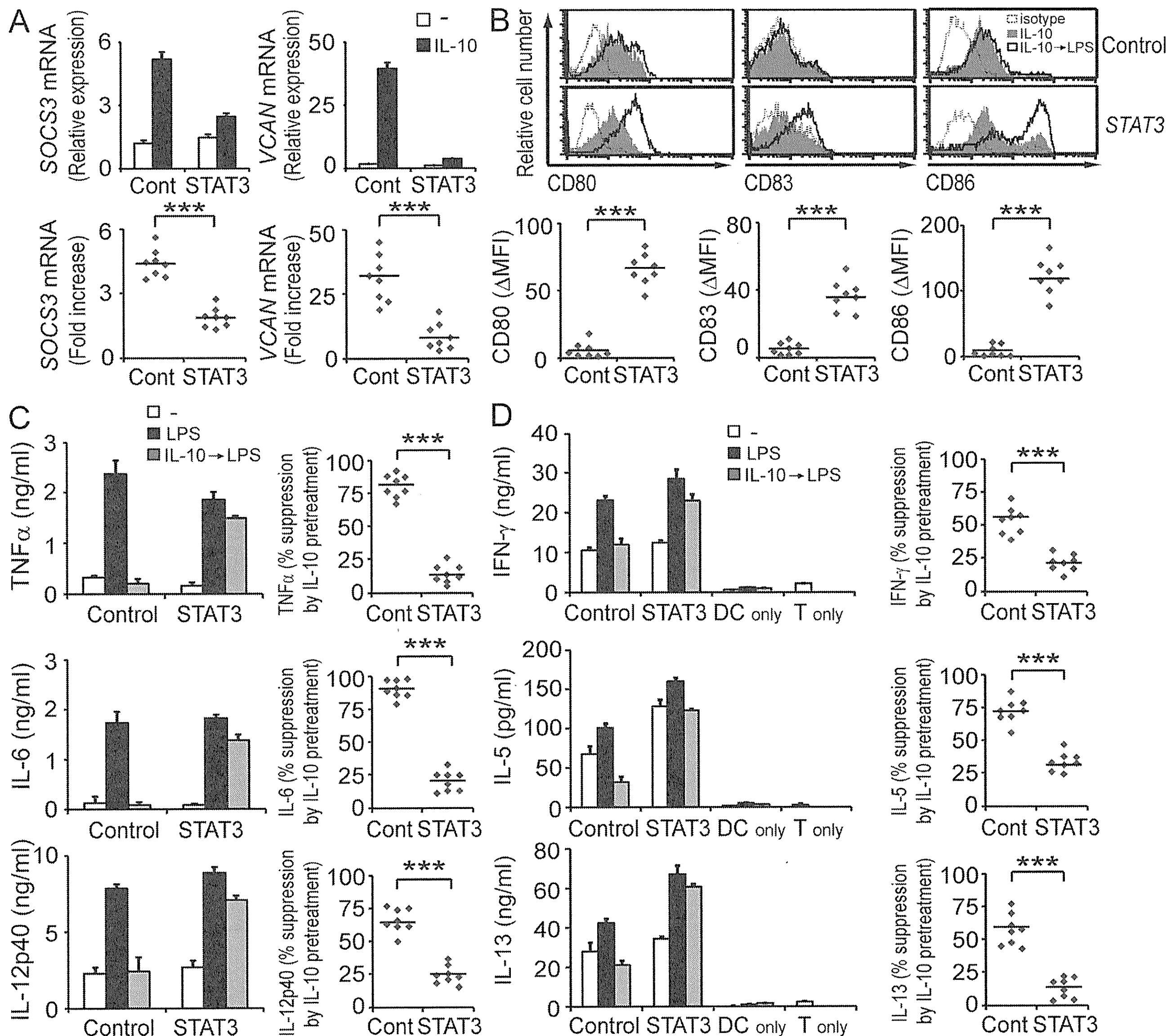

Figure 2. Defective IL-10 signaling in MoDCs from *STAT3* patients. (A) Immature DCs from a control subject and a *STAT3* patient were stimulated with or without IL-10 for 2 h, and the amounts of *SOCS3* and *VCAN* (*CSPG2*) mRNAs were analyzed by Q-PCR. The representative data are shown at the top, normalized to *HPRT* levels, with the level of unstimulated control cells defined as 1.0. Summary data (n = 8 each) showing fold increase are at the bottom. Data are representative of at least two independent experiments performed in triplicate. (B) IL-10–treated MoDCs (IL-10–DCs) were generated from CD14$^+$ monocytes by culturing with GM-CSF and IL-4 in the presence of IL-10 from day 3 of the culture. Representative histograms of CD80, CD83, and CD86 expression of IL-10–DCs (IL-10) and LPS-matured MoDCs after prior treatment with IL-10 (IL-10 → LPS) from a control subject and a *STAT3* patient are at the top. Dashed lines indicate staining with isotype-matched control mAbs. Summary data (n = 8 each) showing delta values of mean fluorescence intensity (ΔMFI), LPS-matured IL-10–DCs minus IL-10–DCs, of CD80, CD83, and CD86 are at the bottom. (C) Representative cytokine levels in the culture supernatants of unstimulated immature MoDCs, LPS-matured MoDCs (LPS), and LPS-matured MoDCs after prior treatment with IL-10 (IL-10 → LPS) from a control subject and a *STAT3* patient are on the left. Summary data (n = 8 each) showing percent suppression are on the right. All samples were evaluated in triplicate. (D) Third-party allogeneic naive CD4$^+$ T cells from control subjects were co-cultured with immature DCs (—), LPS-matured DCs (LPS), or LPS-matured DCs after prior treatment with IL-10 (IL-10 → LPS), and the levels of the cytokines were evaluated by ELISA in triplicate. Representative data are on the left, and summary data of percent suppression by IL-10 pretreatment (n = 8 each) are on the right. Data are representative of at least two independent experiments. (A, C, and D) Graphs show mean ± SD. (A–D) Horizontal bars indicate mean values. ***, $P < 0.001$.

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