ァレンスとする発現プロファイルデータを蓄積した。特に、本研究班では、RNA シーケンシングを全エクソンシーケンシングデータの補完のためにも使うため、RNA シーケンシングデータからリファレンス配列との塩基置換を検出するための解析システムも立ち上げた。その予備的な実験結果から、同じ次世代シーケンシングのプラットフォームを用いても、RNA シーケンシングにおいては低頻度アリルとして全エクソーム解析よりも多数の塩基置換が検出されてしまう事が分かった。この原因の特定は未だできていないが、RNA シーケンシングで得られた配列解析には注意を要することが明らかとなった。

更に、RNA シーケンシングによるアプローチの検証実験として、理化学研究所で行われた ENU 突然変異スクリーニングから見出された T 細胞分化に影響を与える Themis 遺伝子の既知の点突然変異をもつマウスを用いて、分画された T 細胞の RNA シーケンシングにより原因遺伝子変異が特定できるかどうかを確かめた（Kakugawa et. a., Mol. Cell Biol., 2009, 29(18):5128-35)。その結果、T 細胞の RNA シーケンシングからの変異検出により、ホモ変異として Themis 遺伝子内の同定された原因変異が見出された。この結果は、機能的に変化が見出されている細胞の RNA シーケンシングを行う事で、従来のような遺伝学的な手法によるゲノム領域の絞り込みを経ることなく、また全エクソンシーケンシングなどの網羅的な解析によらずとも、発現している RNA 情報の解析からだけでも免疫細胞の機能異常をもたらしている原因変異を決定できる事を実証した。

D. 考察

1）今年度は、新たな倫理審査が終了するまでの間に、市販されているヒト検体を用いて次世代シーケンシングによる免疫不全症候群の病態解析のためのプラットフォーム開発を行った。

2）既に技術的に確立した全エクソームシーケンスの実施だけでなく、免疫不全状態にある血球細胞の全転写産物解析を行う事で、それぞれの血球細胞の機能的な状態と同時に、発現している遺伝子のアリル別の配列解析が可能であることを実証した。

3）本邦のように、集められる希少疾患の症例数に限りがある状況下では、分子レベルの機能解析情報を与える RNA シーケンシングによる転写産物解析と全エクソン配列解析によるゲノム構造解析の併用が現実的な有効なアプローチとなる事が示された。

E. 結論

1）次年度以降の大規模な先天性免疫不全症候群の病態解明のために、次世代シーケンシングを活用した解析プラットフォームを確立した。

2）関係施設での倫理審査を終え、今年度末から本格的に系統的な先天性免疫不全症候群の病態解析のためのゲノミクス解析を実施する体制を整えられた。

F. 健康危険情報　　なし

G. 研究発表

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2．第3回関東甲越免疫不全症研究会「PIDJネットワークを介したPID患者の遺伝子解析（2007～2012年）」満生　紀子、大嶋　宏一、今井　耕輔、小原　收、森尾　友宏、水谷　修紀　東京、2012年9月22日

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syndrome" K. Izawa, R. Nishikomori, H. Oda, K. Nakagawa, E. Hiejima, K. Yoshioka, J. Abe, T. Kawai, T. Yasumi, T. Heike, A. Hijikata, O. Ohara, M. Saito, T. Nakahata, T. Kawai, S. Takei. Florence, Italy, October 3-6 2012

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10. 第 6 回日本免疫不全症研究会「新規B因子昨日獲得型変異を有する非典型的溶血性尿毒症症候群の１家系」大西秀典、船戸道典、近藤直美、小原收、上村治　東京 2013 年 1 月 26 日

11. 第 6 回日本免疫不全症研究会「単一細胞イムノアッセイによる NLRP3 体細胞モザイクの機能的解析の試み」白崎善隆、志村七子、山岸舞、井澤和司、中川権史、西小森隆太、平家俊男、小原收　東京 2013 年 1 月 26 日

H. 知的所有権の出願・取得状況（予定も含む）なし

高IgE症候群に関与する遺伝要因の探索

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研究要旨
高IgE症候群はアトピー性皮膚炎、高IgE血症などのアレルギー病態を伴う先天性免疫不全症である。近年、その原因としてSTAT3の遺伝子変異が同定されたが、その臨床経過は様々であり、さらなる遺伝的要因の解明が待たれている。またしばしば高IgE症候群は重症アトピー性皮膚炎との鑑別が困難であり、両者に共通の遺伝要因が存在する可能性がある。本研究は高IgE血症の遺伝要因を詳細に明らかにするとともに、アトピー性皮膚炎に関連する遺伝子群を同定することを目的とする。本年度は重症アトピー性皮膚炎症例(血清IgE値>10000IU/ml)に着目して関連解析を行い、CCR4近傍のSNPとの間に強い関連 ($P=2.5 \times 10^{-7}$) があることを認めた。今後、STAT3変異高IgE症候群症例においてCCR4近傍のSNPの検討を行う。

A．研究目的

近年、先天性疾患の遺伝要因の解析からCommon diseaseの遺伝要因が明らかとなっている。尋常性魚鱗癬で同定されたフィラグリン遺伝子変異がアトピー性皮膚炎の発症要因として重要であることが示されている。本研究はアトピー性皮膚炎や高IgE血症などを呈する高IgE症候群の原因遺伝子を詳細に解明するとともに、それを手がかりとしてアトピー性皮膚炎の病態に関連する遺伝要因も同定することを目的とする。

B．研究方法

本年度は重症アトピー性皮膚炎症例に着目し、関連解析を行った。これまでゲノムワイド関連解析（ＧＷＡＳ）により計15カ所のゲノムワイド水準($P<5\times10^{-8}$)をみたす疾患関連領域(IL1RL1/IL18R1/IL18RAP, MHC領域, OR10A3/NLRP10, GLB1[CCR4近傍], CCDC80, CARD11, ZNF365, CYP24A1/PFDN4, FLG, C11orf30/LRRC32, TMEM232/SLC25A46, TNFRSF6B/ZGPAT, OVOL1, ACTL9, KIF3A/IL13領域)が同定されている。我々はこの領域のSNPsについて、アトピー性皮膚炎で高IgE(>10000IU/ml)血症を伴う症例(119例)とコントロール（1460例）で関連解析を行なった。タイピングはTaqMan法およびInvader法を用いた。

（倫理面への配慮）

本研究は三省合同「ヒトゲノム・遺伝子解析研究に関する倫理指針」に準拠して行い、当該実施機関の倫理委員会の承認を受けたうえで研究を行っている。

C．研究結果

これまでGWASで同定されたアトピー性皮膚炎の15箇所の関連領域のうち、今回、高IgE血症(>10000IU/ml)を呈するアトピー性皮膚炎とCCR4近傍のSNPで$P=2.5\times10^{-4}$と強い関連を認めた（多重比較の有意水準P=0.0033)。

D．考察

アトピー性皮膚炎患者のIgE値は重症例で高く、重症化のメカニズムの解明が待たれている。また、乳幼児期から重症アトピー性皮膚炎として治療を受けていた患者が、高IgE症候群と診断される例も多い。今回、高IgE（>10000IU/ml)を示すアトピー性皮膚炎と関連を認めたSNPはCCR4近傍であった。CCR4はTARC, MDCの受容体である。血清TARC

値はアトピー性皮膚炎の病勢と相関し、重症度評価の一助として有用であることから、この関連が認められたことは興味深い。

今後、これまで高IgE症候群の原因遺伝子として同定されているSTAT3、Tyk2の遺伝子内および遺伝子周囲に存在するvariantを、次世代シークエンサー（Ion PGMシステム）を用いて同定していく。またそれらの頻度について、アトピー性皮膚炎およびコントロール集団で検討していく。また、高ＩｇＥ症候群の症例についてはエクソーム解析を行い、新規の遺伝子変異の探索を行っていく。

Ｅ．結論

日本人の高IgE血症（>10000IU/ml）を伴うアトピー性皮膚炎とCCR4近傍のSNPとの間に$P=2.5 \times 10^{-7}$と強い関連をみとめた。このSNPが高IgE症候群においてどのような影響を有するかを今後検討していく。

Ｆ．健康危険情報

なし

G. 研究発表

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H．知的財産権の出願・登録状況
（予定を含む。）
1．特許取得
一塩基多型に基づくアトピー性皮膚炎の検査方法（アトピー性皮膚炎の罹患リスク検査方法）2013.8.31
玉利真由美、広田朝光、久保充明　理化学研究所　特願2012-192247
2．実用新案登録
なし
3.その他
なし

Ⅳ　研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
峯岸克行	高 IgE 症候群	近藤直実/平家俊男	自己炎症性疾患・自然免疫不全症とその近縁疾患	診断と治療社	東京	2012	193-196
峯岸克行	発症に免疫不全が関与する症候群	門脇孝/永井良三	内科学	西村書店	東京	2012	1327-1329
峯岸克行	原発性免疫不全症	矢崎義男	内科学第10版	朝倉書店	東京	2013	印刷中
高田英俊	Myddsome 異常症（IRAK4 および MyD88 欠損症）	近藤直実/平家俊男	自己炎症性疾患・自然免疫不全症とその近縁疾患	診断と治療社	東京	2012	111-114
高田英俊	Mendel 遺伝型マイコバクテリア易感染症(IL-12、IFN-γ系の異常	近藤直実/平家俊男	自己炎症性疾患・自然免疫不全症とその近縁疾患	診断と治療社	東京	2012	142-147
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V　代表的な研究成果の刊行物

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Functional STAT3 deficiency compromises the generation of human T follicular helper cells

Cindy S. Ma, Danielle T. Avery, Anna Chan, Marcel Batten, Jacinta Bustamante, Stephanie Boisson-Dupuis, Peter D. Arkwright, Alexandra Y. Kreins, Diana Averbuch, Dan Engelhard, Klaus Magdorf, Sara S. Kilic, Yoshiyuki Minegishi, Shigeaki Nonoyama, Martyn A. French, Sharon Choo, Joanne M. Smart, Jane Peake, Melanie Wong, Paul Gray, Matthew C. Cook, David A. Fulcher, Jean-Laurent Casanova, Elissa K. Deenick and Stuart G. Tangye

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Functional STAT3 deficiency compromises the generation of human T follicular helper cells

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T follicular helper (Tfh) cells are critical for providing the necessary signals to induce differentiation of B cells into memory and Ab-secreting cells. Accordingly, it is important to identify the molecular requirements for Tfh cell development and function. We previously found that IL-12 mediates the differentiation of human CD4$^+$ T cells to the Tfh lineage, because IL-12 induces naive human CD4$^+$ T cells to acquire expression of IL-21, BCL6, ICOS, and CXCR5, which typify Tfh cells. We have now examined CD4$^+$ T cells from patients deficient in IL-12Rβ1, TYK2, STAT1, and STAT3 to further explore the pathways involved in human Tfh cell differentiation. Although STAT1 was dispensable, mutations in *IL12RB1*, *TYK2*, or *STAT3* compromised IL-12–induced expression of IL-21 by human CD4$^+$ T cells. Defective expression of IL-21 by STAT3-deficient CD4$^+$ T cells resulted in diminished B-cell helper activity in vitro. Importantly, mutations in *STAT3*, but not *IL12RB1* or *TYK2*, also reduced Tfh cell generation in vivo, evidenced by decreased circulating CD4$^+$CXCR5$^+$ T cells. These results highlight the nonredundant role of STAT3 in human Tfh cell differentiation and suggest that defective Tfh cell development and/or function contributes to the humoral defects observed in STAT3-deficient patients. (*Blood*. 2012;119(17):3997-4008)

Introduction

The generation of robust Ab responses is crucial for the correct functioning of the immune system. The importance of this is apparent in diseases that result from dysregulated humoral immune responses. For example, immunodeficient states and autoimmune disorders can develop as a consequence of impaired or exaggerated Ab responses, respectively. Thus, it is imperative to identify factors that control Ab responses. Early studies found that T cells play an important role in initiating Ab responses (reviewed in Tangye et al[1]). This was mediated by instructive signals in the form of cell–cell contacts and secretion of soluble mediators such as cytokines. More recently, a subset of CD4$^+$ T cells with specialized B-cell helper capabilities was identified that is now referred to as T follicular helper (Tfh) cells.[2,3] Tfh cells are identified by several characteristics that also serve functional roles. Thus, Tfh cells express the chemokine receptor CXCR5,[2,3] which facilitates their positioning to B-cell follicles in secondary lymphoid tissues, and the transcription factor Bcl-6,[4] which is required for the commitment of naive CD4$^+$ T cells to the Tfh lineage.[5-7] Tfh cells also express the costimulatory molecules CD40L, ICOS, OX40, and members of the SLAM family, as well as the cytokine IL-21,[2-4,8-12] all of which play important roles in the induction of T cell–dependent (TD) B-cell activation and differentiation.

Because of the importance of Tfh cells in regulating Ab responses, much work has been performed to determine the requirements for their differentiation from naive CD4$^+$ T cells. It was initially found that IL-21 was required for the development of murine Tfh cells.[13,14] This was later expanded to include IL-6 and IL-27.[15-17] However, conflicting findings have been made about the relative importance of IL-6 and IL-21 to murine Tfh cell formation[18,19]; this may reflect redundancy because these cytokines, as

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Table 1. Primary immunodeficient patients

Disease	Patient ID	Mutation/genotype
MSMD (IL-12Rβ1 deficiency)	IL12RB1#1	1745_1746insCA+1483 + 182-1619-1073del
	IL12RB1#2	628_644dup
	IL12RB1#3	R173P
	IL12RB1#4	1791 + 2T > G
	IL12RB1#5	C198R
	IL12RB1#6	1623_1624delinsTT
MSMD + viral infection	STAT1#1	1928insA (homozygous)
(STAT1 deficiency)	STAT1#2	P696S (homozygous)
	STAT1#3	P696S (homozygous)
MSMD only	STAT1#4	Q463H/WT
(STAT1 deficiency)	STAT1#5	L706S/WT
	STAT1#6	L706S/WT
AD-CMC	STAT1gof#1	A267V
	STAT1gof#2	A267V
	STAT1gof#3	A267V
AD-HIES	STAT3#1	R382Q
	STAT3#2	V637M
	STAT3#3	R382Q
	STAT3#4	H437P
	STAT3#5	Q644P
	STAT3#6	S465F
	STAT3#7	Y657N
	STAT3#8	R382W
	STAT3#9	L706M
	STAT3#10	L706M
	STAT3#13	V463del
	STAT3#14	V463del
	STAT3#15	R593P
	STAT3#16	V463del
AR-HIES (incl infection with mycobacteria, viruses and fungi)	TYK2#1	550_553GCTTdel (homozygous)
MSMD + viral infection	TYK2#2	2292-2301del

MSMD indicates Mendelian susceptibility to mycobacterial disease; WT, wild type; AD-CMC, autosomal dominant chronic mucocutaneous candidiasis; AD-HIES, autosomal dominant hyper-IgE syndrome; AR-HIES, autosomal recessive hyper-IgE syndrome; and gof, gain-of-function

well as IL-27, can operate through STAT3.[20,21] We and others previously showed that IL-12 is the key cytokine implicated in the differentiation of human Tfh cells in vitro.[11,22] IL-6, IL-21, IL-23, and IL-27 also induce human Tfh-like cells in vitro, albeit to a much lesser extent than IL-12.[11,17,22] We have now extended these observations by investigating the molecular requirements for the differentiation of naive human $CD4^+$ T cells into Tfh cells. This was achieved by studying patients with primary immunodeficiencies resulting from mutations in *IL12RB1*, *STAT1*, *STAT3*, and *TYK2*. IL-12–mediated induction of human Tfh-like cells was abolished in the absence of IL-12Rβ1 or TYK2, and significantly reduced in $CD4^+$ T cells deficient in STAT3 function. In contrast to the effects of IL-12, induction of Tfh cells by IL-6, IL-21, IL-23, and IL-27 was completely dependent on STAT3. These studies indicate that multiple cytokine pathways are involved in the differentiation of human Tfh cells, and IL-12 most efficiently induces human Tfh cells predominantly in a STAT3-dependent manner. This defect in generating Tfh cells from STAT3 mutant ($STAT3_{MUT}$) $CD4^+$ T cells would contribute to impaired TD humoral immune responses observed in patients with *STAT3* mutations. In contrast, the ability of non–IL-12 cytokines to induce Tfh cell function is sufficient to elicit intact Ab responses in persons with impaired IL-12R signaling.

Methods

Human patient samples

Patients with mutations in *IL12RB1*, *STAT1*, *TYK2*, and *STAT3* have been previously described (Table 1[23-28]). PBMCs were isolated from these patients and healthy donors (Australian Red Cross). Tonsils were obtained from St Vincent's Hospital, Sydney. All studies were approved by Institutional Human Research Ethics Committees, and all participants gave written informed consent in accordance with the Declaration of Helsinki.

Antibodies

Alexa-647–conjugated anti–IL-21, biotinylated anti-ICOS, PE–anti-CD4, Pacific Blue–anti-CD4, peridinin chlorophyll protein complex (PerCP)/cyanine 5.5–anti-CD45RA, anti-IFNγ, and FITC–anti-CD45RA were purchased from eBiosciences. Alexa-647–anti-CXCR5, allophycocyanin–anti-CD38, FITC–anti-CD20, PE–anti-CD4, anti-CD27, PerCP–anti-CD3 mAb, and streptavidin–PerCP were purchased from Becton Dickinson. Allophycocyanin–anti-CD4 was purchased from Caltag, and FITC–anti-CCR7 was purchased from R&D Systems.

$CD4^+$ T-cell isolation

$CD4^+$ T cells were isolated from healthy donors or immunodeficient patients with the use of Dynal beads.[23] Peripheral blood (PB) $CD4^+$ T cells were labeled with anti-CD4, anti-CD45RA, and anti-CCR7, and naive

Table 2. Primers for qPCR

Gene	Primers	UPL probe	Amplicon size, bp
BCL6	fwd: gagctctgttgattcttagaactgg rev: gccttgcttcacagtccaa	9	110
TBX21	fwd: tgtggtccaagtttaatcagca	9	77
IL21	rev: tgacaggaatgggaacatcc fwd: aggaaaccaccttccacaaa rev: gaatcacatgaagggcatgtt	7	68
IFNG	fwd: ggcattttgaagaattggaaag rev: tttggatgctctggtcatctt	21	112
GAPDH	fwd: ctctgctcctcctgttcgac rev: acgaccaaatccgttgactc	60	112

$CD45RA^+CCR7^+$ $CD4^+$ T cells were isolated (> 98% purity) with the use of a FACSAria (BD Biosciences).

Cell cultures

Naive PB $CD4^+$ T cells were labeled with CFSE (Molecular Probes) and cultured with T-cell activation and expansion beads (anti-CD2/CD3/CD28; Miltenyi Biotec) alone (nil culture) or under Th1 (IL-12 [20 ng/ml; R&D systems]), Th2 (IL-4 [100 U/ml]), or Th17 (IL-1β [20 ng/ml; Peprotech]), IL-6 (50 ng/mL; PeproTech), IL-21 (50 ng/mL; PeproTech), IL-23 (20 ng/mL; eBioscience), anti–IL-4 (5 μg/mL), and anti-IFNγ (5 μg/mL; eBioscience)[23,29] polarizing conditions, or with IL-6, IL-21, IL-23, or IL-27 (50 ng/mL; eBioscience) alone. After 4 or 5 days, expression of intracellular cytokines, transcription factors, and surface phenotype of cells determined.

T- and B-cell coculture assays

Naive $CD4^+$ T cells were activated for 5 days (see previous section), treated with mitomycin C (100 μg/mL; Sigma-Aldrich) and then cocultured at a 1:1 ratio (50×10^3/200 μL/well) with sort-purified allogeneic naive ($CD20^+CD27^-CD38^{inter}$) tonsillar B cells.[11,29] After 7 days Ig secretion was determined by ELISA.[29]

Cytokine and transcription factor expressions

Activated $CD4^+$ T cells were restimulated with phorbol 12-myristate 13-acetate (100 ng/mL) and ionomycin (750 ng/mL) for 6 hours, with Brefeldin A (10 μg/mL) added after 2 hours. Cells were then fixed with formaldehyde, and expression of cytokines was detected by intracellular staining.[23,29] RNA was extracted with the use of RNeasy kit (QIAGEN) and transcribed into cDNA with the use of random hexamers and Superscript III (Invitrogen). All quantitative PCR (qPCR) primers (Integrated DNA Technologies) were designed with Roche UPL Primer Design Program. Primer sequences, Roche UPL probes, and size of each amplicon are listed in Table 2. qPCR was performed with Roche LightCycler 480 Probe Master Mix and Roche Lightcycler 480 System with the following conditions: denaturation at 95°C for 10 minutes; amplification for 45 cycles at 95°C for 10 seconds, 65°C for 30 seconds, and 72°C for 5 seconds; and cooling at 40°C for 30 seconds. All reactions were standardized to *GAPDH*.

Results

Patients deficient for IL-12Rβ1 have altered differentiation of $CD4^+$ T cells in vivo

IL-12 can induce human naive $CD4^+$ T cells to differentiate into IL-21–expressing cells that resemble Tfh cells in vitro.[11,22] To investigate this function of IL-12 further, we examined patients with homozygous or compound heterozygous null mutations in *IL12RB1*.[26] We first determined the frequency of total $CD4^+$ T cells and $CD4^+$ T cells with a naive ($CD45RA^+CCR7^+$), memory ($CD45RA^-CCR7^{+/-}$), or Tfh ($CXCR5^+$) phenotype in healthy donors (age range, 16-64 years) and patients deficient for IL-12Rβ1 (Figure 1A-D,F). Patients deficient for IL-12Rβ1 had a normal frequency of $CD4^+$ T cells (Figure 1; Table 3). In contrast, they had a significant increase in the frequency of naive and a corresponding significant decrease in memory $CD4^+$ T cells (Figure 1A-E; Table 3). When the phenotype of $CXCR5^+$ T cells was analyzed, ~ 90% were found within the $CD45RA^-$ (ie, memory) subset (Table 3[2,3,11]). Therefore, we analyzed the frequency of both $CXCR5^+CD45RA^-$ and $CD45RA^+$ T cells in healthy donors and in patients deficient for IL-12Rβ1. No significant difference was observed for the frequency of circulating $CD4^+$ $CXCR5^+CD45RA^-$ or $CD45RA^+$ Tfh-like cells in healthy donors and patients deficient for IL-12Rβ1 (Figure 1A-B,F; Table 3).

Naive $CD4^+$ T cells from patients deficient for IL-12Rβ1 are unable to differentiate into IL-21–expressing cells in response to IL-12

To assess the potential of $CD4^+$ T cells deficient for IL-12Rβ1 to differentiate into Tfh-like cells, we examined the ability of naive cells to express IL-21 in vitro. Naive $CD4^+$ T cells were cultured with T-cell activation and expansion beads alone (nil) or with IL-12 (Th1). After 5 days, cells were restimulated with phorbol 12-myristate 13-acetate/ionomycin, and the expression of IL-21 and IFNγ was then determined. Naive $CD4^+$ T cells from either healthy donors or patients deficient for IL-12Rβ1 expressed little IL-21 or IFNγ when cultured under neutral (nil) conditions (Figure 1G-J). However, when normal naive $CD4^+$ T cells were cultured under Th1-polarizing conditions (ie, with IL-12), IL-21– and IFNγ–expressing cells were readily detectable (Figure 1G,I,J). In contrast, IL-12 failed to induce IL-21 or IFNγ in naive $CD4^+$ T cells deficient for IL-12Rβ1 (Figure 1H-J). Next, we questioned whether naive $CD4^+$ T cells deficient for IL-12Rβ1 could differentiate into IL-21–expressing cells in response to other cytokines and signaling pathways. Accordingly, naive $CD4^+$ T cells from healthy donors and patients deficient for IL-12Rβ1 were subjected to Th2 (IL-4) and Th17 (IL-1β, IL-6, IL-21, IL-23) polarizing conditions or were cultured in the presence of IL-6, IL-21, IL-23, or IL-27. A small frequency of IL-21–expressing cells could be generated from both normal and IL-12Rβ1–deficient naive $CD4^+$ T cells activated with IL-21 or IL-27 (Figure 1I). Similarly, although IL-12 could not induce IFNγ in naive $CD4^+$ T cells deficient for IL-12Rβ1, the ability of IL-27 to induce IFNγ was unaffected by *IL12RB1* mutations (Figure 1J). Taken together these results indicate that, although IL-12–induced IL-21 expression is abrogated by *IL12RB1* mutations, other cytokines and their associated signaling pathways that induce IL-21 (eg, IL-21 and IL-27, albeit to a lesser extent than IL-12) are intact, which is consistent with normal Ab responses to infection and vaccinations in these patients.[30,31]

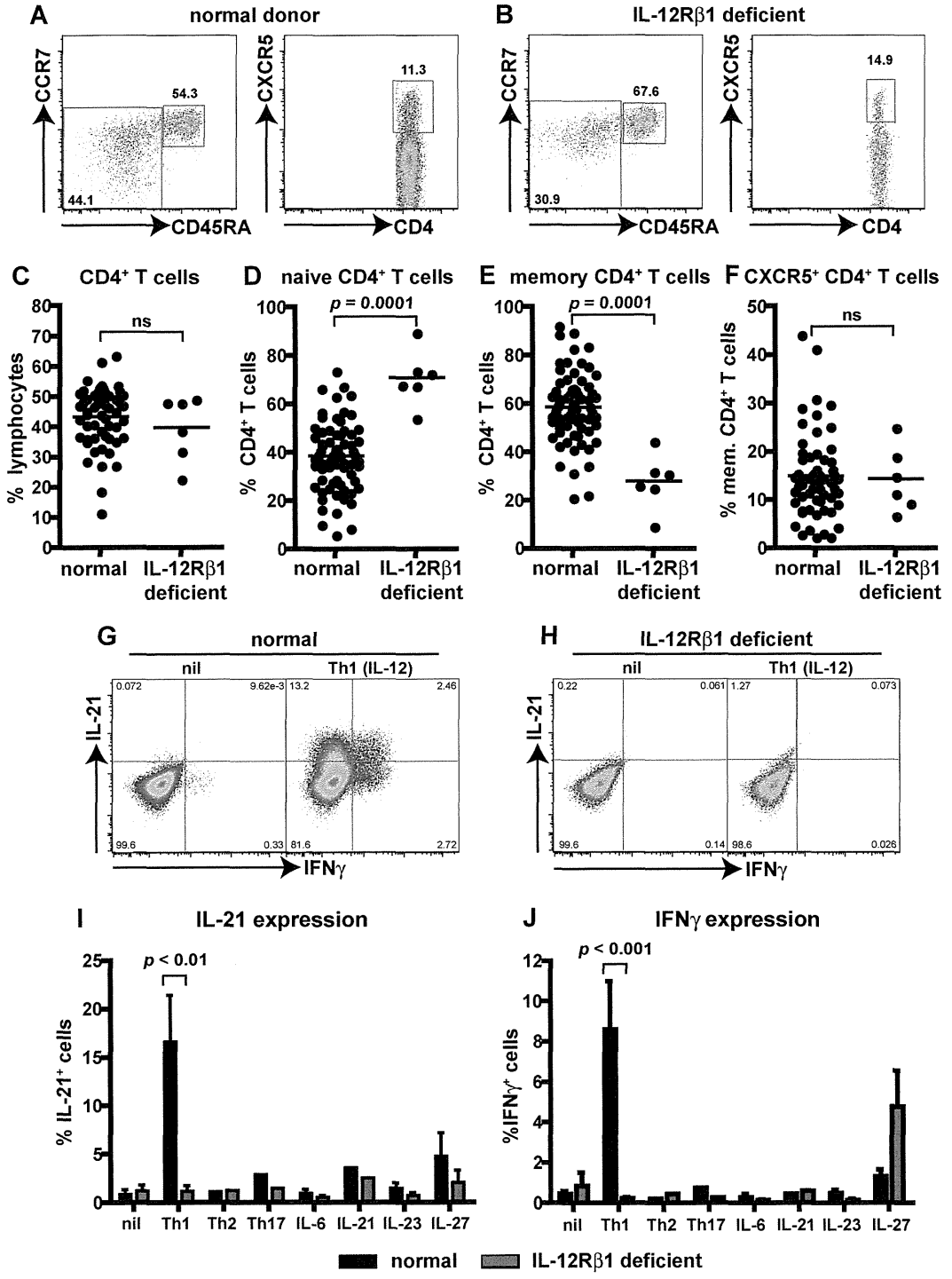

Figure 1. Naive CD4+ T cells deficient for IL-12Rβ1 fail to differentiate into IL-21–expressing cells in response to IL-12. (A-F) The frequency of total CD4+ T cells, and naive (CD45RA+CCR7+), memory (CD45RA+CCR7−/+), and CXCR5+CD45RA− CD4+ T cells, in PBMCs was determined for healthy donors and patients deficient for IL-12Rβ1. (A-B) Representative dot plots from 1 donor and 1 patient. (C-F) The frequency of (C) total, (D) naive (CD45RA+CCR7+), (E) memory (CD45RA+CCR7−/+), and (F) CXCR5+CD45RA− CD4+ T cells from all healthy donors (total CD4+ T cells, n = 54; naive CD4+ T cells, n = 70; memory CD4+ T cells, n = 70; CXCR5+CD45RA− CD4+ T cells, n = 61) and patients deficient for IL-12Rβ1 examined (n = 6). (G-J) Naive CD4+ T cells isolated from healthy donors (n = 5) and patients deficient for IL-12Rβ1 (n = 5) were cultured for 5 days under neutral (nil); polarizing Th1, Th2, or Th17 conditions; or in the presence of IL-6, IL-21, IL-23, or IL-27, and intracellular expression of IL-21 and IFNγ were then determined. (G-H) Representative dot plots of IL-21 and IFNγ expression by activated naive CD4+ T cells from 1 donor and 1 patient deficient for IL-12Rβ1. (I-J) Percentage of activated normal and IL-12Rβ1–deficient naive CD4+ T cells induced to express (I) IL-21 or (J) IFNγ in response to the indicated culture. The values represent the mean ± SEM.

Analysis of cytokine responsiveness in STAT-deficient human CD4+ T cells

The cytokines that induce IL-21 in human naive CD4+ T cells (IL-12, IL-6, IL-21, IL-23, IL-27)[11,22] function by activating JAK/STAT signaling pathways. These cytokines phosphorylate STAT1 (IL-6, IL-12, IL-21, IL-23, IL-27), STAT3 (IL-6, IL-12, IL-21, IL-23, IL-27), STAT4 (IL-12, IL-23), and STAT5 (IL-12).[20,21,24,32-35] We confirmed these studies by showing that these