る。胎児水腫やTORCHは、新生児FHLの重要な鑑別疾患となる。診断基準の改定（補足項目の必要性）と簡易スクリーニング法の開発が急務である。また、HLH2004不応例に対する移植までの免疫化学療法と移植法について検討中である。

E．結論

新生児HLHは診断と治療がとくに難しく、現在の診断と治療指針の再検討が必要である。多様な臨床像と遺伝子診断の確定は、患児の治療のみならず、細胞傷害性顆粒の新たな生理機能を解明する手がかりになると思われる。

F．健康危険情報

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（発表誌名巻号・頁・発行年等も記入）

H. 知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
なし

2. 実用新案登録
なし

3.その他
なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

血球貪食症候群の病態・診療研究に資する検体保存体制整備

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研究要旨：疾患の病態解明や診断・治療法開発には、患者由来検体の保存と活用が必須である。特に、難病等の希少疾患の場合は、患者由来検体の体系的かつ計画的な保存ならびに分配システムの構築が望まれる。本研究では、血球貪食症候群の病態・診療研究に資する検体保存体制整備として、１）同疾患の診断体制と余剰検体保存の現状把握、２）現状に対応した余剰検体保存システムの提案、を行った。血球貪食症候群の診断は、細胞マーカー解析、ウエスタンブロット解析、細胞培養等多岐にわたり、かつ、それぞれが異なる診断施設で行われていることが判明した。患者の登録は、臨床研究に関連して登録される症例とそれ以外のものがあり、各々により匿名化の方法が異なることが判明した。やや複雑な経路で検体が収集されること、ゲノム解析が行われる場合があること、核酸を含む細胞抽出物のみならず凍結細胞そのものも検体として存在すること等の現状に対応する検体保存方法として、まずは、核酸を含む細胞抽出物について検体保存方法を提案した。すなわち、小児がんの臨床研究グループでの保存方法として運用中のシステムで構築することが望ましいと結論した。凍結細胞についての保存体制については今後の検討課題とした。

A. 研究目的

極めて希少な難病である血球貪食症候群の病態・診療研究に資する検体保存ならびに供給体制の整備を行うことを目的とする。

B. 研究方法

１）血球貪食症候群における診断システムの概要把握

本研究班で対象とする患者は、日本小児白血病リンパ腫研究グループ(JPLSG)が実施する血球貪食症候群に対する治療研究である HLH2004 計画に登録される者が主であるため、HLH2004 計画に記載された中央診断体制を明確にした。

２）血球貪食症候群の診断後に生じる余剰検体の保存と配分システム

小児がんに対する臨床研究グループですでに実施中の余剰検体保存方法を検討し、本研究における検体保存への応用について、その方法と実行可能な範囲を明確にした。

C. 研究結果

１）血球貪食症候群における診断システムの概要把握

A) HLH2004 の特徴と中央診断システム

HLH2004 は国際組織球協会（Histiocyte Society）が作成している血球貪食症候群(HPS)に対する国際臨床試験を指し、我が国も JPLSG が国際共同研究として参加しているものである。HLH2004 が扱う疾患は、家族性血球貪食性リンパ組織球症(familiar hemophagocytic lymphohistiocytosis[HLH], FHL）と二次性 HLH である。FHL と二次性 HLH とでは治療法が異なるため、発症早期に厳密な鑑別診断が行われる。そのためのシステムとして中央診断システムが整備されている。診断項目は大きく、フローサイトメトリー、蛋白発現解析および細胞機能活性によるスクリーニングならびに上記検査異常例に対する遺伝子配列解析である。すなわち、遺伝子配列解析は FHL の確認のために行われるものである。HLH2004 では総登録症例数を 5 年間で 300 例を予定しており、うち FHL はごくわずかと思われるものの、ゲノム解析の研究指針に従った対応が求められる内容となっている。

中央診断は、i) フローサイトメトリー

による perforin 蛋白解析、ii) ウエスタンブロットによる MUNC13-4、 syntaxin 11 蛋白発現解析、iii) 細胞培養による細胞障害性 T 細胞(CTL)活性測定、および、iv) 特定の原因遺伝子に関する塩基配列決定、を担う各々の施設で実施されている。上記の i)〜iii)については JPLSG が発行する JPLSG 番号が検体に添付されるが、iv)については JPLSG 番号とは異なる連結可能ユニーク ID が解析施設で発行され検体に添付される。なお、遺伝子解析は、primary immunodeficiency diseases Japan (PIDJ) の仕組みの中で解析される。PIDJ は理化学研究所、かずさ DNA 研究所と厚生労働省難治性疾患研究事業の原発性免疫不全症調査研究班に参加する 13 大学の小児科によって原発性免疫不全症（PID）のデータベース作成ならびに網羅的遺伝子解析を目的として作られたものである。

B) HLH2004 で保存される検体の状態

上記の i)から iv)の診断項目のうち、i)と iii)については細胞あるいは細胞抽出物の形で、診断後の余剰部分が保存されている。一方、上記の“iii) 細胞培養による細胞障害性 T 細胞(CTL)活性測定“では、患者末血 T 細胞を各種刺激後にサイトカイン依存培養株として樹立したものであり、生細胞に回復できる状態で凍結保存されている。PIDJ の枠組みで解析される検体は解析終了後破棄される。したがって、HLH2004 で保存されている検体は、(1) 細胞、(2) 細胞抽出物および(3)再培養を目的とした凍結細胞の 3 種類である。 なお、HLH2004 で取り扱う患者検体は末血のみである。

C)HLH2004 以外で収集される患者検体

本研究班では、HLH2004 に登録されない患者も解析対象としている。それらの症例に関する匿名化の方法等個人情報の扱いならびに診断項目や保存状態も調査対象とすべきであるが、本年度は調査ができなかった。

2） 血球貪食症候群の診断後に生じる余剰検体の保存と配分システム

A) HLH2004 で保存された検体

上記 1)に記載した状況から、実行可能な余剰検体の保存方法について検討した。その際、すでに小児がんに対する臨床研究グループの中で、検体保存センターとして研究分担者が運用している余剰検体保存システムを参考とした。以下に概略を記す。匿名化番号発行、匿名化シール発行ならびに検体管理を行うシステムとして、遺伝子情報提供者匿名化システム SCTS21（三井情報開発株式会社製）およびライフサイエンス研究支援システム「BIOPRISM（バイオプリズム）」（NEC 製）を併用（一部後者のみ）して使用している。当初は SCTS21 を使用していたが、設計の柔軟性ならびに他の情報との連携等の発展性を確保するため BIOPRISM を併用しつつ切り替え中である。最終的に連結不可能匿名化を行うか連結が可能な状態で保存を継続するかによって検体保存用匿名化 ID の発行時期や方法が異なるが、(1)“保存用 ID”と“配分用 ID”は区別する、(2)“配分用 ID”は研究ごとに新たに発行する、の 2 点を原則としている。この理由は、研究者間の情報交換によって研究の独立性が失われることを防ぐためである。なお、保存用 ID と配分用 ID の対照表は検体保存施設で定めた個人情報管理者が管理している。

現在、上記のシステムが稼働中であり、本研究で行う検体保存についても同じシステムを利用することが最適と考えられる。その際の検討事項としては、以下の 2 点が想定される。第 1 点は中央で保存すべき検体の決定、第 2 点は検体保存センターに収集される検体の種類と匿名化 ID 番号発行・添付状況の検討、である。

前述 1)-A)および-B)のごとく、HLH2004 で保存されている検体は多種類であり、特に、1)-A)の iii)で記載した凍結状態の CTL については中央保存する体制は十分には整っていない。その理由は、生細胞の保存と解凍後の生細胞率についてはある程度担保できる可能性があるが、CTL 活性が保持

されているか否かを検証できるシステムが無いことである。したがって、保存検体のi)と ii)すなわち、フローサイトメトリー解析後の保存細胞と保存された細胞抽出物が中央保存に相応しい検体と考えられる。

第2点目については、より詳細な調査が必要と考えられるが以下の点を検討する必要がある。各診断施設お発行と添付についてのルールが決定されない状態で保存されていると予想すると、特定の症例について、JPLSG 番号、フローサイトメトリー解析施設 A で発行した ID、ウエスタンブロット解析施設で発行した ID の最大 3 個の ID が存在することになり、各々をひも付けする必要があること、各施設で保存用チューブに添付された ID を確認する必要があること、などの問題が想定される。各施設で保存されている検体は凍結状態が予想されるため、中央保存する場合に ID を付け替えることは非現実的である。従って、複数施設で保存されている状態のまま、中央保存施設に搬送し保存を継続することが望ましいと考えられる。今後の保存検体管理システムは BIOPRISM を主体と考えていることより、BIOPRISM では各施設で発行した ID を入力して管理することとし、配分時に新たな配分用 ID を発行することが現実的な方針と考えられた。

B) HLH2004 以外で収集される患者検体

本研究では、HLH2004 の登録されない患者の検体についても中央診断あるいは遺伝子解析の対象となることが判明した。これらの検体における個人情報の取り扱い、匿名化 ID 発行状況、診断や研究を目的として送付される施設名等の情報把握が必要だが、本年度は実施できなかった。

D. 考察

今年度は特に HLH2004 計画の中で行われている中央診断と検体保存状況の把握に努めた。その結果、複数の診断施設間での検体保存に関する共通ルールが無い状態で保存されていることが予想できた。今後より詳細な現場での調査が必要だが、中央での検体保存と検体の配分までを視野に入れたシステム構築を考慮した場合、可及的速やかな統一ルールの作成と運用が必要であると考えられる。今後、より詳細な情報収集を行う予定である。

HLH2004 の枠組みの外で収集される検体も本研究班では扱う予定である。今年度は、これに該当する検体の調査は進まなかった。来年度以降の課題として残った。

HLH2004 は JPLSG の実施する臨床試験であり、その中で登録、保存された検体は JPLSG のルールに従って配分される。一方、HLH2004 以外で収集された検体の有効活用については規則が存在しない。特に、検体の所属はどこにあるか（すなわち、所有権）に係る考え方を整理し、それを盛り込んだ形で患者・家族側に説明し同意を得る必要がある。今後の検討課題である。

E. 結論

HLH2004 で収集、保存される患者由来検体に係る状況を調査し把握した。それに基づいて、実行可能性のある余剰検体保存システムについて提案した。

F. 健康危険情報

該当なし

G. 研究発表

1．論文発表

該当なし

2．学会発表

該当なし

H. 知的財産権の出願・登録状況（予定を含む。）

1．特許取得

該当なし

2．実用新案登録

該当なし

3．その他

該当なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

家族性血球貪食症候群のゲノム解析研究

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研究要旨：Familial hemophagocytic lymphohistiocytosis(FHL)は、常染色体劣性遺伝で家族性に発症する致死性疾患群である。これまでperforin, MUNC13-4, syntaxin11, MUNC18-2の遺伝子異常が報告されているが、半数近くの症例では未だその原因遺伝子は同定されていない。本研究では、日本のFHLを対象に原因遺伝子を同定し、FHLの病態解明や分子標的治療を確立することを目的とする。本研究の成果として、日本のFHL13家系の検体収集を行った。8家系を対象とした全ゲノムSNP連鎖解析ではLOD値が陽転化する領域を複数箇所検出することができたが有意なLOD値を得ることができなかった。血族を有する1家系（FHL-102）を対象としたホモ接合体マッピングでは、既知の遺伝子座への連鎖を除外でき、新規候補遺伝子座として3つの遺伝子座を同定することができた。現在、3つの候補遺伝子座から候補遺伝子を選別し、直接シークエンス法による変異の検索を行っている。

A．研究目的

Familial hemophagocytic lymphohistiocytosis(FHL)は、常染色体劣性遺伝で家族性に発症する致死性疾患群である。これまでに、欧米のFHLを対象としたゲノム解析から、*PRF1* (perforin), *UNC13D* (MUNC13-4), *STX11*(syntaxin 11), *STXBP2* (MUNC18-2)の遺伝子異常が報告されている。しかし、半数近くの症例では未だその原因遺伝子は同定されておらず、正確な確定診断や、それに基づく適正な治療法は確立されていない。本研究では、日本のFHLを対象に、ポジショナルクローニング法・ホモ接合体マッピング法を適用し、表現型に大きく影響する原因遺伝子を同定することを目的とする。原因遺伝子の同定はFHLの病態解明や分子標的治療を確立するために必須の研究である。

B．研究方法

1. 検体の収集
   HLHのうち、1) 乳幼児期に発症, 2) 遺伝様式は劣性遺伝, 3) 既知の遺伝子異常は除外されていることを診断基準として日本人FHLの検体を収集する。
   （倫理面への配慮）本研究は、徳島大学ゲノム倫理審査委員会の承認を得て実施する。個人情報は匿名化して取り扱う。
2. FHL家系を対象とする全ゲノム連鎖解析
   (a) SNP タイピング；全ゲノム領域を網羅する高密度SNPマッピングアレイを用いて、SNP遺伝子型タイピングを行い、全ゲノム連鎖解析を行う。
   (b) 連鎖妥当性の検証；Merlin programを用いてLOD値を算出し、統計学的に連鎖の妥当性を検証する。
   (c) 候補遺伝子の同定；有意に連鎖を認めた場合、候補領域から候補遺伝子の選定を行い、直接シークエンス法で変異スクリーニングを行う。
3. 血族家系を対象とするホモ接合体マッピング
   (a) SNP タイピング；全ゲノム領域を網羅する高密度SNPマッピングアレイを用いて、SNP遺伝子型タイピングを行い、連続するホモ接合体領域を検出する。
   (b) 候補領域の検出；罹患者が共有し、非罹患者が共有していない、連続ホモ接合領域を検出し、Genespring GT2 programを用いてRegional LOD値を算出し、統計学的に連鎖の妥当性を検証する。
   (c) 既知の遺伝子座への連鎖を除外する。
   (d) 候補遺伝子の同定； ヒトゲノム情報のデータベースを用いて、候補領域内に存在する候補遺伝子を選別し、直接シークエンス法を用いて候補遺伝子の変異の確認を行う。

C．研究結果

1. 日本人FHLの収集
これまでに日本人FHL13家系35名(患者13名、家系内健常人22名)の検体採取を行った。
2. FHL家系を対象とした全ゲノム連鎖解析
8家系28名 (患者8名、家系内健常人20名)を対象に、Illumina 370 quadを用いて全ゲノムSNP遺伝子型タイピングを行った。Merlin program (ver.1.1.2)を用いて、常染色体劣性遺伝、完全浸透率モデルで、多点LOD値を算出した。LOD値が陽転化する領域として複数箇所の陽性領域を検出することができた。しかし、疾患遺伝子座の異質性（Locus Heterogeneity）が考えられ、有意なLOD値を満たすことはできなかった。解析をより確実にするためにはより多くの検体が必要であり、現在家系調査・検体収集を継続している。
3. 血族家系を対象としたホモ接合体マッピング
血族婚を有する1家系(FHL-102)を対象に、Illumina 370 quadを用いて全ゲノムSNP遺伝子型タイピングを行い、有意に連続するホモ接合体領域を検出した。罹患同胞が共有する連続ホモ接合領域を検出し、有意なRegional LOD 値を示す3つの領域を候補領域として同定することに成功した（J-FHL-1, J-FHL-2, J-FHL-3 ）。3つの候補領域はいずれも、FHL1(9q21.3-22)、FHL2（*PRF1*, 10q22） FHL3(*UNC13D*, 17q25.1) FHL4(*STX11*, 6q24) FHL5(*STXBP2*, 19p13.3-p13.2)とは重複しておらず、既知の遺伝子座への連鎖は除外できた。現在、3つの候補領域内に存在する候補遺伝子を選別し、直接シークエンス法を用いて候補遺伝子の変異の確認を行っている。

D．考察
日本のFHL13家系の検体収集をすることができた。8家系を対象とした全ゲノムSNP連鎖解析ではLOD値が陽転化する領域を検出することができたが有意なLOD値を得ることができなかった。疾患遺伝子座の異質性が考えられ、より確実な解析を行うためにはさらなる家系の収集が必要であった。収集した家系のうち血族を有する1家系（FHL-102）を対象にホモ接合体マッピングを行った。既知の遺伝子座への連鎖を除外でき、新規候補遺伝子座として3つの遺伝子座を同定することができた。現在、候補遺伝子を選別し、直接シークエンス法による変異の検索を行っている。候補遺伝子の選別・同定が困難である場合、3つの候補領域の全配列をキャプチャーした後、次世代シークエンサーで迅速に変異の検索を行う予定である。

E．結論
日本のFHL13家系の検体収集をすることができた。8家系を対象とした全ゲノムSNP連鎖解析ではLOD値が陽転化する領域を検出することができたが有意なLOD値を得ることができなかった。さらなる家系の収集が必要であった。血族を有する1家系（FHL-102）を対象としたホモ接合体マッピングでは、既知の遺伝子座への連鎖を除外でき、新規候補遺伝子座として3つの遺伝子座を同定することができた。現在、候補遺伝子を選別し、直接シークエンス法による変異の検索を行っている。

G．研究発表
1. 論文発表：該当なし
2. 学会発表：該当なし
（発表誌名巻号・頁・発行年等も記入）

H．知的財産権の出願・登録状況
該当なし

［ III ］研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

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［ IV ］研究成果の論文

Jagged1 Suppresses Collagen-Induced Arthritis by Indirectly Providing a Negative Signal in CD8$^+$ T Cells[1]

Mika Kijima,[2]* Akiko Iwata,[2]*† Yoichi Maekawa,* Hisanori Uehara,‡ Keisuke Izumi,‡ Akiko Kitamura,* Hideo Yagita,§ Shigeru Chiba,¶ Hiroshi Shiota,† and Koji Yasutomo[3]*

Distinct Notch ligands possess a characteristic ability in terms of functional T cell differentiation. However, the precise role or the therapeutic potential of each Notch ligand in autoimmune diseases is largely unknown. In this study, we examined whether Jagged1 modulates a collagen-induced rheumatoid arthritis (CIA) model by altering T cell responses. The injection of a soluble Jagged1-encoding plasmid, sJag1-P, before or even after initial type II collagen (CII) immunization suppressed the disease severity of CIA. However, this treatment did not suppress CII-specific CD4$^+$ T cell proliferation and CII-specific Ab production. Depletion of either CD4$^+$ or CD8$^+$ T cells ameliorated CIA severity and sJag1-P further improved CIA in CD4$^+$ but not CD8$^+$ T cell-depleted mice. Injection of OVA and Jagged1-encoding plasmids inhibited proliferation of OVA-specific granzyme B-producing CD8$^+$ T cells, although Jagged1 could not directly inhibit CD8$^+$ T cell proliferation in vitro. The blockade of Jagged1 by an anti-Jagged1 Ab exacerbated CIA, whereas this effect was not observed in the absence of CD8$^+$ T cells. These data indicate that Jagged1 is able to deliver an indirect negative signal into CD8$^+$ T cells in vivo, which suggests its therapeutic potential in the treatment of CD8$^+$ T cell-mediated diseases, including rheumatoid arthritis. *The Journal of Immunology,* **2009, 182: 3566–3572.**

Notch is a transmembrane receptor that generally regulates cell fate or differentiation (1, 2). Several articles have demonstrated that Notch regulates the functional differentiation of mature CD4$^+$ T cells (2–8). There are two Notch ligand families, Delta-like (DL)[4] 1, 3, and 4 and Jagged 1 and 2, and each ligand has distinct roles in CD4$^+$ T cell activation (3, 7, 9, 10). One of the ligands, Jagged1, has CD4$^+$ T cell suppressive ability (9, 10) and also controls type II Th cell (Th2) differentiation (7). As for the contribution of Notch signaling to CD8$^+$ T cell activation, one article reported that overexpression of DL1 renders CD8$^+$ T cells unresponsive (11), whereas another article demonstrated that blocking Notch signaling using a γ-secretase inhibitor suppressed CD8$^+$ T cell responses (12). Furthermore, we have recently demonstrated that Notch2 signaling directly controls the expression of effector molecules in cytotoxic CD8$^+$ T cells (13). Regarding the involvement of Notch signaling in autoimmune diseases, one report demonstrated that a γ-secretase inhibitor blocked Notch signaling, thereby suppressing the progression of experimental autoimmune encephalomyelitis (EAE) (6). Another report demonstrated that the administration of anti-Jagged1 mAbs exacerbated the clinical features of EAE, whereas administering anti-DL1 mAb reduced disease severity (14). Although these articles do not demonstrate which Notch and Notch ligands are interacting to regulate each phenotype, these data suggest that modulation of Notch signaling has an alternative beneficial way to treat autoimmune diseases.

In this study, we investigated whether Jagged1 was able to suppress development of collagen-induced arthritis (CIA), where both cellular and humoral mechanisms are involved in the pathogenic process (15, 16). We found that injection of a soluble Jagged1-encoding plasmid (sJag1-P) in DBA/1J mice that were previously immunized with bovine collagen type II (CII) suppressed CIA progression. Treatment with sJag1-P inhibited CD8$^+$ but not CD4$^+$ T cell activation, which contributes to the suppression of CIA development. These results indicate that modulation of Jagged1 could provide a new strategy for treating T cell-mediated autoimmune diseases, including rheumatoid arthritis.

Materials and Methods

Mice

Six- to 8-wk-old DBA/1J and C57BL/6 mice were purchased from Japan SLC and all mice were maintained under specific pathogen-free conditions in the animal research center of the University of Tokushima, Tokushima, Japan. All animal studies were approved by the animal research committee of the University of Tokushima.

Flow cytometry

FITC-conjugated anti-CD4, allophycocyanin-conjugated anti-CD25, or FITC-conjugated anti-CD8 mAbs (eBioscience) and PE-conjugated H-2K^b OVA tetramer (MBL International) were used for cell staining. In some experiments, cells were fixed with 4% paraformaldehyde and stained with allophycocyanin-conjugated anti-granzyme B (Caltag Laboratories) or PE-conjugated anti-Foxp3 (eBioscience) mAbs in saponin-containing buffer. Fluorescence intensity of ~10^5 cells was examined using a FACSCalibur cytometer (BD Biosciences).

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[2] M.K. and A.I. contributed equally to this study.

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[4] Abbreviations used in this paper: DL, Delta-like; CIA, collagen-induced arthritis; CII, collagen type II; c-P, control plasmid; EAE, experimental autoimmune encephalomyelitis; sJag1-P, soluble Jagged1-encoding plasmid.

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ELISA

Five μg/ml bovine CII in PBS was coated on a 96-well plate overnight at 4°C. After washing with PBS plus 0.05% Tween 20 (PBST), serially diluted serum samples were applied to each well and incubated at room temperature for 1 h. Wells were washed with PBST, followed by the addition of alkaline phosphatase-conjugated goat anti-mouse IgG, IgG1, and IgG2a (Southern Biotechnology Associates). Alkaline phosphatase activity was measured using 4-nitrophenyl phosphate disodium salt hexahydrate (Sigma-Aldrich) as a substrate. To determine the titer of soluble Jagged1, 1 μg/ml anti-FLAG-specific mAb (Sigma-Aldrich) was coated on a plate and 1/10 diluted sera was added to the wells. Biotin-conjugated anti-Jagged1 Ab (Santa Cruz Biotechnology) followed by streptavidin-HRP (eBioscience) was then incubated. After the addition of tetramethylbenzidine (eBioscience), the absorbance of each well was measured at 415 nm. To detect IL-17, IL-4, and IFN- γ in cell culture supernatants, we used the mouse IFN-γ, IL-10 (Endogen), or IL-17 ELISA kit (R&D Systems).

Induction of CIA and DNA vaccine

Male DBA/1J mice were injected intradermally at the base of the tail with 200 μg of bovine CII (Collagen Research Center, Tokyo, Japan) emulsified in 100 μl of CFA (Difco). This was followed by a booster injection at 3 wk after the primary immunization. Mice were monitored for signs of arthritis by clinical scoring and by measuring the width of the forelimbs at each of the time points indicated. For assessment of disease severity, the following clinical scoring scale was used: 0, no evidence of erythema or swelling; 1, erythema and mild swelling confined to the midfoot or ankle joint; 2, erythema and mild swelling extending to the ankle and the midfoot; 3, erythema and moderate swelling extending from the ankle to the metatarsal joints; and 4, erythema and severe swelling encompassing the ankle, foot, and digits. Total disease severity scores were recorded as the sum of clinical scores for all four limbs. A 3× FLAG tag was fused to the C terminus of the full-length mouse Jagged1 gene or to the extracellular domain (aa 1–1607), and cloned into pcDNA3.1. The OVA gene (provided from Dr. T. Sakai, University of Tokushima) was also cloned into pcDNA3.1. Fifty μg of DNA in PBS was injected into the gastrocnemius muscle after bupibacaine treatment. The injection schedule for CIA was as follows: days −23, −15, and −7 or days −14 and −7, where the day of the second CII immunization was designated as day 0. As for the Ab treatment experiments, purified anti-CD4 (GK1.5) mAb, anti-CD8 (53-6.7) mAb (150 μg) or anti-Jagged1 mAb (100 μg) (14) was injected into mice every other day starting on the day of the second CII booster injection. In some experiments, both OVA and full-length Jagged1-encoding plasmids (each 100 μg) were injected once every 7 days (days 0, 7, and 14) into the gastrocnemius muscle two days after bupibacaine treatment.

Cell culture

$CD4^+$ or $CD8^+$ T cells were purified from total spleen or lymph node cells by incubating cells with anti-CD4 mAb (GK1.5) or anti-CD8 mAb (53-6.72), followed by positive selection of $CD4^+$ or $CD8^+$ cells with anti-rat IgG micro beads (Miltenyi Biotec). Full-length cDNA of mouse Jagged1 was cloned into the pKE004 retroviral vector (3) and the retrovirus was constructed by transfecting vectors into Plat-E cells as previously reported (17, 18). DCEK cells (19) were infected with retrovirus one time and GFP^+ cells were sorted by cell sorting. Purified $CD8^+$ T cells from C57BL/6 mice were incubated with 5 μM CFSE (Molecular Probes) for 10 min at 37°C. After three washes, labeled cells were incubated with DCEK cells for three days and the dilution of CFSE was measured by flow cytometry. $CD4^+$ T cells purified from DBA/1J mice 7 days after CIA induction were stimulated with CII protein (30 μg/ml) in the presence of irradiated spleen cells from DBA/1J mice for 3 days. Proliferation was measured in triplicate by incorporation of [^{3}H]thymidine (1 μCi/well; PerkinElmer Life Sciences) during the last 8 h of culture.

Real-time PCR

RNA was isolated from T cells by TRIzol (Invitrogen) and cDNA was generated using Omniscript reverse transcription (Qiagen) according to the manufacturer's instructions. mRNA expression levels were quantified using the ABI 7500 real-time PCR System (Applied Biosystems). cDNAs were amplified with TaqMan probes using SYBR Premix Ex *Taq* (Takara). mRNA expression levels were normalized relative to GAPDH gene expression levels. Fold differences in mRNA levels were determined using SDS system software version 1.4.0 (Applied Biosystems). Primer sequences are as follows: GAPDH, 5′-TCCACCACCCTGTTGCTGTA-3′ (forward primer) and 5′-ACCACAGTCCATGCCATCAC-3′ (reverse primer); Deltex1, 5′-CACTGGCCCTGTCCACCCAGCCTTGGCAGG-3′ (forward primer) and 5′- CTCATAGCCAGATGCTGTGACCAG-3′ (reverse primer).

Luciferase assay

293T cells were transfected with soluble Jagged1-encoding pcDNA3.1 using FuGENE6 transfection reagents (Roche Diagnostics), and cell supernatants were collected 3 days after transfection. Jurkat cells were transfected with TP-1 reporter genes using FuGENE6 transfection reagents (Roche Diagnostics) in the presence or absence of soluble Jagged1-containing supernatant (dilution 1/10).

Twenty-four hours after transfection, luciferase activity was measured by the Dual luciferase assay system (Promega) according to the manufacturer's protocol. Firefly luciferase activity was normalized to the *Renilla* luciferase activity of pRL-TK. All values were obtained from experiments conducted in triplicate and repeated at least four times.

Histology

Paws and knee joints were fixed in 10% formalin, decalcified by 10% EDTA in PBS for 14 days, embedded in paraffin, and sectioned. Tissue sections from knee joints were stained with H&E.

Statistical analysis

The Mann-Whitney *U* test was used to analyze the clinical scores. The unpaired *t* test was used to analyze the other results. Values of $p < 0.05$ were considered to be significant.

Results

Soluble Jagged1 suppresses CIA

To investigate whether Jagged1 prevents CIA, we first examined whether overproduction of Jagged1 in vivo using a soluble Jagged1-encoding plasmid has preventive effects for CIA. Soluble mouse Jagged1 (aa 1–1607) was cloned into pcDNA3.1 (sJag1-P) and injected into the gastrocnemius muscle 1 day before and 7 and 14 days after the first immunization of bovine CII. After the second immunization of CII (designated as day 0), clinical scores were compared in sJag1-P and control plasmid (c-P)-treated mice (Fig. 1*a*). The c-P-treated mice began to show clinical arthritic symptoms 5 days after the second immunization (Fig. 1*a*). CIA symptoms in sJag1-P-treated mice were delayed ~9–10 days compared with c-P mice. Overall clinical scores were lower in sJag1-P than in c-P-treated mice (Fig. 1*a*). Histological analysis revealed little infiltration of cells or bone erosion in sJag1-P-treated mice compared with marked cell infiltration and bone erosion in control mice (Fig. 1*b*).

We next tested whether Jag1-DNA injection had a therapeutic effect on CIA by starting sJag1-P treatment 7 and 14 days after the first CII immunization (Fig. 1*c*). sJag1-P treatment, even after the initial CII immunization, significantly suppressed clinical CIA scores compared with c-P-treated mice (Fig. 1*c*), suggesting that Jagged1 also has a therapeutic effect on CIA. We could not see any therapeutic effect when sJag1-P was injected after the second CII immunization (data not shown).

Soluble Jagged1 stimulates Notch receptors

Several papers demonstrated that soluble Jagged1 has an inhibitory activity for Notch and Notch ligand interactions (20, 21). Therefore, we investigated whether soluble Jagged1 generated by our method had stimulatory activity for the Notch receptor. We first tested whether Jagged1 protein is really present in sera after sJag1-P injection. Jagged1 protein was found in sera from Jag1-P-treated mice that received CII and in controls that did not receive CII (Fig. 2*a*). The level of Jagged1 protein was relatively higher in CII-immunized mice than in nonimmunized mice (Fig. 2*a*), suggesting that CII-induced inflammatory responses help generate Jagged1 protein in vivo. We next tested whether soluble Jagged1 could stimulate Notch using a reporter gene assay. 293T cells were

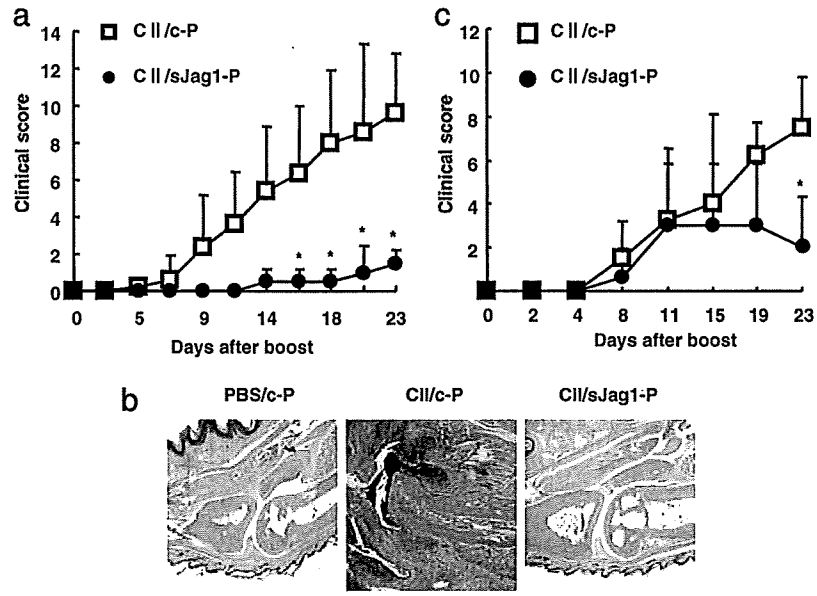

FIGURE 1. Jagged1 DNA vaccination prevents development of CIA. *a*, DBA/1J mice immunized by CII received sJag1-P (●) or c-P (□). The plasmid was injected 2 days before and 6 or 14 days after the first CII immunization. After the second CII immunization (day 0), CIA clinical scores were monitored. Results are shown as mean ± SE. The asterisk (*) indicates statistically significant differences ($p < 0.01$). Five to seven mice per group were used for each experiment and the results are representative of four independent experiments. *b*, Histological evaluation was conducted 25 days after the second CII immunization. At this time, joint samples were stained with H&E. Data are shown in mice immunized by PBS and c-P (PBS/c-P), CII, and c-P (CII/c-P), or CII and sJag1-P (CII/sJag1-P). The results are representative of four independent experiments. *c*, DBA/1J mice that were immunized by CII received sJag1-P (●) or c-P (□). Plasmids were injected 7 and 14 days after the first CII immunization. After the second CII immunization (day 0), clinical scores of CIA were evaluated. Results are shown as mean ± SE. The asterisk (*) indicates statistically significant differences ($p < 0.05$). Five to seven mice per group were used for each experiment, and these results are representative of three independent experiments.

transfected with sJag1-P and supernatants were collected after 72 h in culture. Jurkat cells transfected with the TP1 reporter gene were stimulated with soluble Jagged1-containing medium for 24, 48, or 72 h. This augmented TP1 reporter activity 72 h after stimulation (Fig. 2*b*), indicating that soluble Jagged1 was able to stimulate Notch receptors in vitro. We then examined the expression of a Notch target gene, *Deltex1*, in $CD4^+$ or $CD8^+$ T cells of sJag1-P and c-P-treated mice (Fig. 2*c*). Immunization of OVA in mice up-regulated *Deltex1* in both $CD4^+$ and $CD8^+$ T cells, and treatment with sJag1-P further up-regulated the expression in both types of cells (Fig. 2*c*). Taken together, these data strongly suggest that soluble Jagged1 generated by our method stimulates Notch receptors but does not inhibit endogenous Notch signaling in our experimental system.

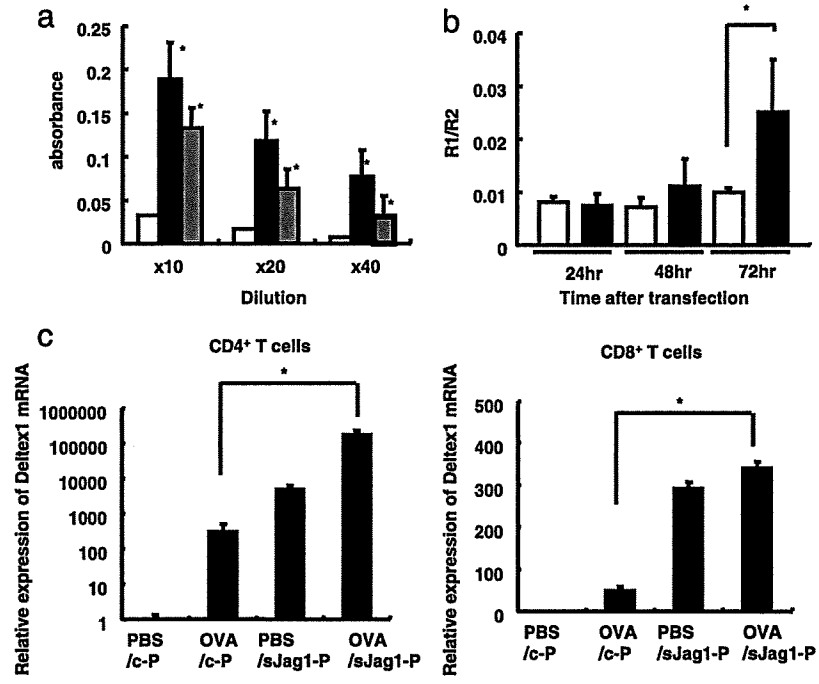

FIGURE 2. Soluble Jagged1 stimulates Notch receptors. *a*, Jagged1 protein was measured in the serially diluted sera from mice immunized with CII and sJag1-P (filled bar) or PBS and sJag1-P (gray bar) (day 27). The titer of mice immunized by CII alone was used as control (open bar). Results are shown as mean ± SD; an asterisk (*) indicates statistically significant differences ($p < 0.05$) against control. *b*, Soluble Jagged1 protein was produced by transfecting c-P or sJag1-P into 293T cells. Soluble Jagged1 protein-containing medium (filled bar) or control medium (open bar) was added to Jurkat cells previously transfected with TP-1 reporter genes. Luciferase activity was measured 24, 48, or 72 h after TP-1 transfection. Firefly luciferase activity (R1) was normalized to *Renilla* luciferase activity (R2) of pRL-TK. Results are representative of four independent experiments. Results are shown as mean ± SE. The asterisk (*) indicates statistically significant differences ($p < 0.05$). *c*, The sJag1-P or c-P plasmid was injected two times into DBA1/J mice immunized by OVA, and $CD4^+$ or $CD8^+$ T cells from lymph nodes were isolated 2 days after plasmid injection. Expression of *Delex1* mRNA in $CD4^+$ (*left*) or $CD8^+$ (*right*) T cells was measured by real-time PCR. The value obtained from *Deltex1* expression in T cells from unimmunized mice was set as 1. Data are shown as the mean ± SE. The asterisk (*) indicates statistical significance ($p < 0.05$).

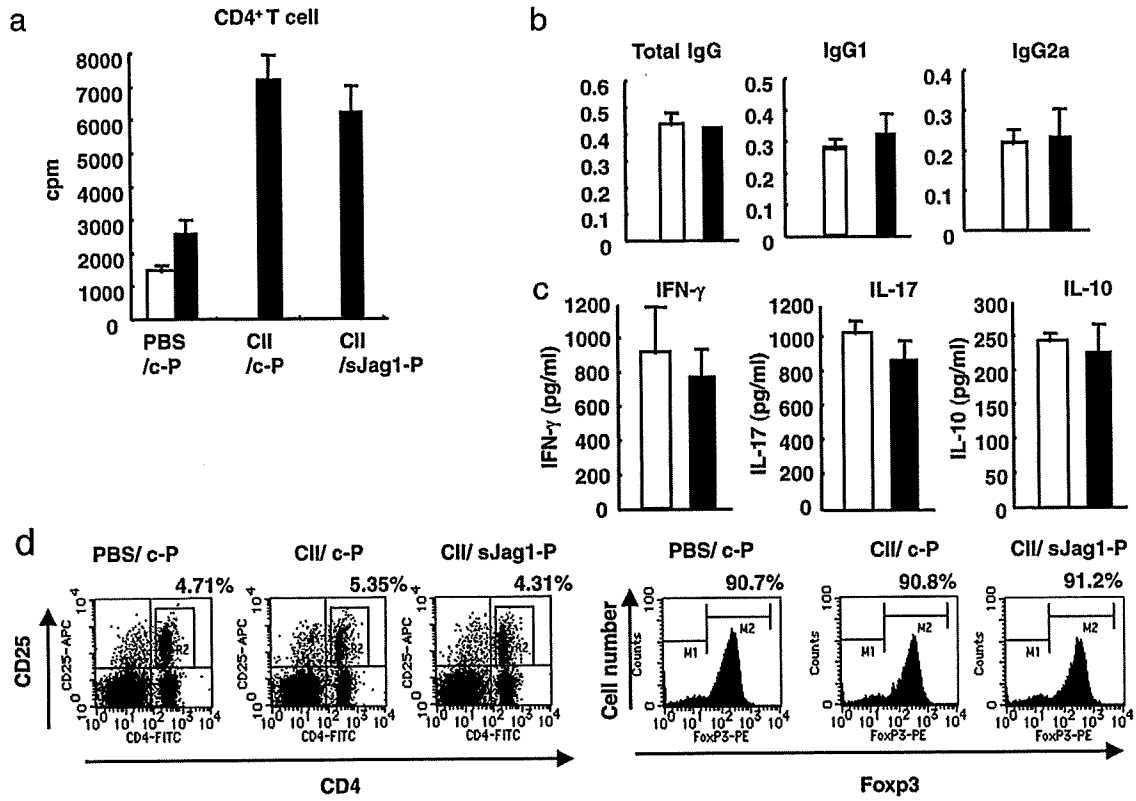

FIGURE 3. sJag1-P does not affect $CD4^+$ T cell activation in vivo. *a*, DBA/1J mice were immunized by CII and treated with c-P (CII/c-P) or sJag1-P (CII/sJag1-P). Spleen cells were isolated 8 days after the second CII immunization and $CD4^+$ T cells were stimulated with CII (30 μg/ml) in the presence of irradiated spleen cells for 3 days. As a negative control, $CD8^+$ T cell-depleted spleen cells from PBS-immunized and c-P-treated mice were stimulated with CII (PBS/c-P; open bar). [^{3}H]Thymidine incorporation for the final 7 h was counted. Data are shown as the mean ± SE. *b*, The titer of anti-CII IgG, IgG1, or IgG2a in sera from sJag1-P-treated (filled bars) or c-P-treated (open bars) mice 10 days after the second CII immunization was evaluated by ELISA. Results are shown as mean ± SE. *c*, Isolated $CD4^+$ T cells from c-P-treated (open bars) or sJag1-P-treated (filled bars) mice were stimulated with CII (30 μg/ml) in the presence of irradiated spleen cells for 3 days. The IFN-γ, IL-17, and IL-10 levels in the culture supernatants (1×10^6 cells) were measured by ELISA. The value of each cytokine from unstimulated $CD4^+$ T cells from CII immunized mice was below detection limits (data not shown). All results are representative of at least three experiments. Data are averages ± SE from three independent experiments. *d*, Frequency of $CD4^+Foxp3^+$ cells in lymph nodes of PBS and c-P-treated (PBS/c-P), CII and c-P-treated (CII/c-P), or CII and sJag1-P-treated (CII/sJag1-P) mice 7 days after the second CII immunization was evaluated by flow cytometry. The number indicates the percentage of $CD25^+$ cells in $CD4^+$ cells or $Foxp3^+$ cells in $CD4^+CD25^+$ cells.

Soluble Jagged1 does not inhibit $CD4^+$ T cell proliferation

$CD4^+$ T cell activation is involved in CIA progression (22). Therefore, to examine whether sJag1-P treatment affects $CD4^+$ T cell proliferation, we stimulated $CD8^+$-depleted spleen cells from sJag1-P or c-P-treated and CII immunized mice. $CD4^+$ T cell activation was comparable between sJag1-P-treated mice and c-P-treated mice (Fig. 3*a*), although previous reports demonstrated that Jagged1 was able to suppress $CD4^+$ T cell proliferation (14).

We next tested whether sJag1-P treatment affects CII Ab production to evaluate the contribution of $CD4^+$ T cells in sJag1-mediated CIA suppression. The titer of anti-CII specific total IgG, IgG1, and IgG2a in the serum of sJag1-P treated mice was comparable to that of c-P treated mice (Fig. 3*b*), suggesting that sJag1-P treatment has little inhibitory effect on $CD4^+$ T cells in terms of helper functions against B cells. We next tested cytokine production from $CD4^+$ T cells. The production of IFN-γ, IL-10, and IL-17 was measured in $CD4^+$ T cells 7 days after the second CII immunization of mice. The production of IFN-γ, IL-10, and IL-17 from $CD4^+$ T cells from sJag1-P-treated mice was comparable to that from c-P treated mice (Fig. 3*c*).

Recent evidence has demonstrated that naturally occurring regulatory T cells or Ag stimulation-driven regulatory T cells have important roles in the suppression of autoimmune responses (23, 24). Therefore, we investigated whether sJag1-P treatment preferentially activates $CD4^+Foxp3^+$ regulatory T cells. We examined the percentage of $CD4^+Foxp3^+$ regulatory T cells 4 days after the second injection of sJag1-P or c-P (Fig. 3*d*), but no significant difference in percentage or total cell number of $CD4^+Foxp3^+$ regulatory T cells between the two groups was observed (Fig. 3*d*). Taken together, these results suggest that the effect of sJag1-P in CIA is independent of either the inhibition of $CD4^+$ T cells or the specific activation of $Foxp3^+$- or IL-10-producing regulatory T cells.

Treatment with sJag1-P reduced $CD8^+$ T cell activation in vivo

$CD8^+$ T cells are also involved in CIA induction (16). Because crucial epitopes of CII for $CD8^+$ T cells have not been identified, it is difficult to analyze $CD8^+$ T cell responses against CII by an in vitro T cell stimulation assay. Indeed, we could not detect $CD8^+$ T cell proliferation against CII by coculturing $CD8^+$ T cells from CII-immunized mice with irradiated spleen cells and CII protein (data not shown). To evaluate whether sJag1-P was able to suppress $CD8^+$ T cell responses, we coimmunized mice with two plasmids encoding Jagged1 and OVA and monitored the number of OVA-specific $CD8^+$ T cells and granzyme B expression in such cells. Vaccination of Jagged1 inhibited the proliferation of OVA-specific $CD8^+$ T cells (Fig. 4*a*) and also inhibited the expression of granzyme B in $CD8^+$ T cells (Fig. 4*a*). These results indicate

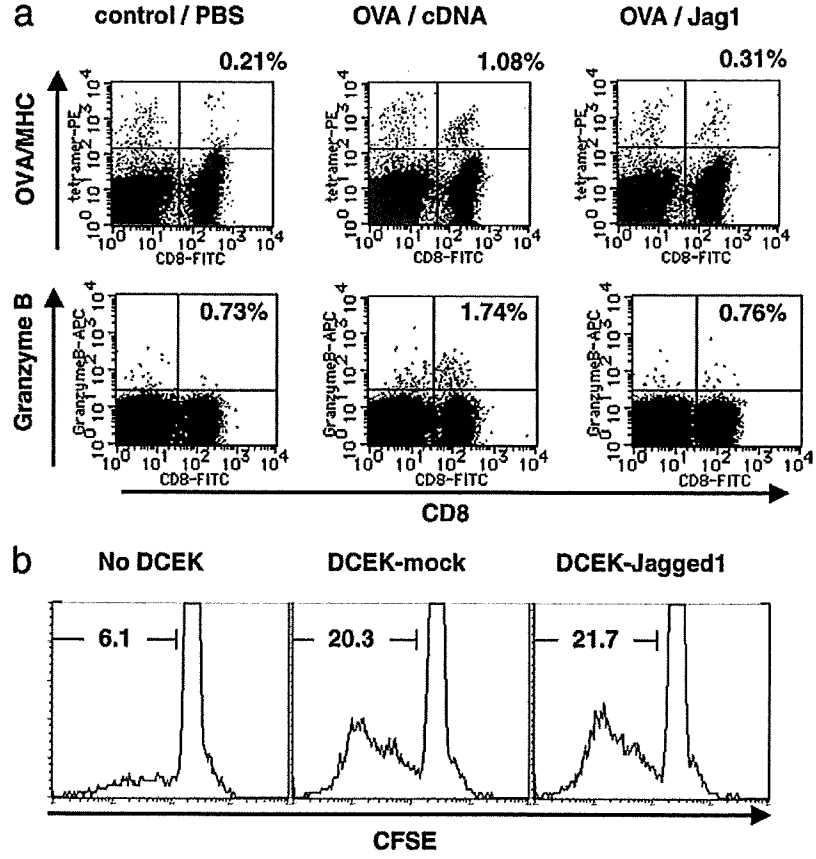

FIGURE 4. Jagged1 inhibits $CD8^+$ T cell activation and functional differentiation. *a*, OVA-specific T cells (OVA/MHC) or granzyme B-producing $CD8^+$ T cells (Granzyme B) 5 days after the third injection of C57BL/6 mice with pcDNA3.1 and PBS (control/PBS), pcDNA3.1 encoding OVA (OVA/cDNA), or pcDNA3.1 encoding OVA and Jagged1 (OVA/Jag1) were evaluated by flow cytometry. The number indicates the percentage of OVA-specific T cells in total $CD8^+$ cells, or granzyme B^+ cells in $CD8^+$ cells. Five to seven mice per group were used for each experiment, and these results are representative of three independent experiments. *b*, Purified $CD8^+$ T cells from C57BL/6 mice were labeled with CFSE. Such cells (1×10^7) were stimulated with DCEK cells (1×10^6) transduced with either a control vector (DCEK-mock) or Jagged1 (DCEK-Jagged1) for 3 days. As a negative control, cells were not stimulated with DCEK (No DCEK). CFSE intensity on $CD8^+$ T cells was analyzed by flow cytometry. The number indicates the percentage of $CFSE^{low}$ cells in $CD8^+$ T cells. Results are representative of four independent experiments.

that Jag1 is able to inhibit the activation and functional differentiation of $CD8^+$ T cells.

We next tested whether overstimulation of $CD8^+$ T cells by Jagged1 affects T cell proliferation in vitro. We stimulated purified $CD8^+$ T cells from BALB/c mice with DCEK cells transduced with control vector or Jagged1. Because DCEK cells express K^k and D^k molecules, BALB/c-derived $CD8^+$ T cells should exhibit an allo-MHC specific response. $CD8^+$ T cells from BALB/c mice were labeled by CFSE and cocultured with DCEK cells for 3 days (Fig. 4*b*). $CD8^+$ T cells from BALB/c mice proliferated against DCEK cells, and Jagged1 overexpression did not affect proliferation or IFN-γ secretion (data not shown). Taken together, these data suggest that Jagged1 has no direct inhibitory effect on $CD8^+$ T cell activation in vitro.

Depletion of $CD8^+$, but not $CD4^+$ T cells, canceled the effect of sJag1-P

To evaluate the effect of sJag1-P on the $CD8^+$ T cell response during CIA, we depleted $CD4^+$ or $CD8^+$ T cells in CII-immunized mice that were treated with sJag1-P (Fig. 5). Such an approach allows us to evaluate whether $CD8^+$ T cells actually contribute to CIA suppression by sJag1-P. The depletion of $CD8^+$ T cells alone suppressed CIA induction (Fig. 5*a*). Treatment of CII-immunized DBA/1J mice depleted of $CD8^+$ T cells with sJag1-P did not further suppress clinical scores of CIA (Fig. 5*a*). Conversely, depletion of $CD4^+$ T cells also suppressed CIA induction, whereas treatment with sJag1-P further suppressed CIA (Fig. 5*b*). These data strongly suggest that

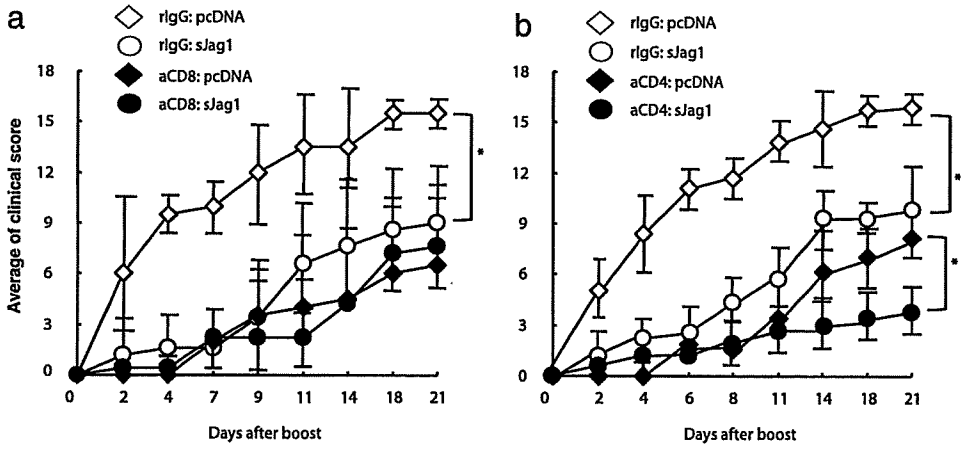

FIGURE 5. sJag1 suppresses $CD8^+$ T cell activation. The anti-CD8 (αCD8) mAb (filled symbols in *a*) and anti-CD4 (αCD4) mAb (filled symbols in *b*) or control rat IgG (open symbols in both *a* and *b*) was injected into DBA/1J mice every other day starting on the day of the second CII immunization. sJag1-P (circles) or c-P (pcDNA; diamonds) was injected into the mice according to the same protocol for treating CIA. Clinical scores of each mouse were monitored. Five to seven mice per group were used for each experiment and these results are representative of three independent experiments. An asterisk (*) indicates statistically significant differences ($p < 0.05$).

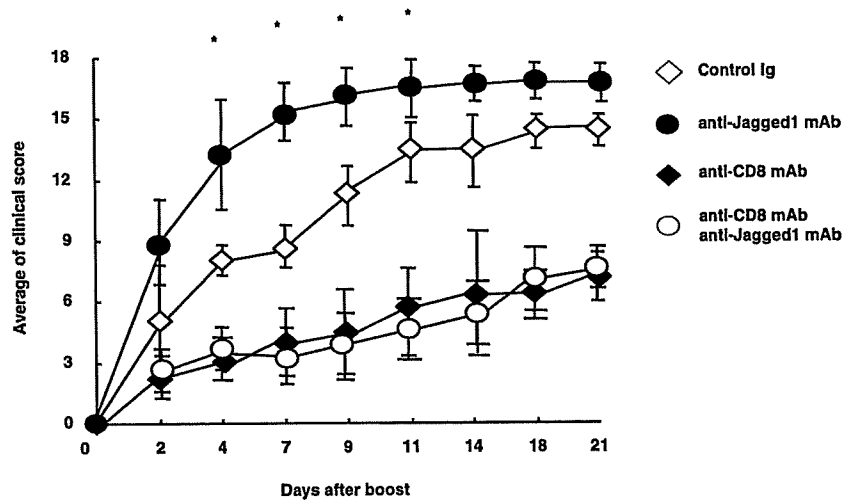

FIGURE 6. Treatment of anti-Jagged1 mAb exacerbated CIA. The anti-CD8 mAb (♦), anti-Jagged1 mAb (●), both anti-CD8 and anti-Jagged1 mAbs (○) or control rat IgG (◇) were injected into DBA/1J mice every other day starting on the day of the second CII immunization. Clinical scores of each mouse were monitored. Five to seven mice per group were used for each experiment and these results are representative of three independent experiments. An asterisk (*) indicates statistically significant differences between control rat IgG and anti-Jagged1 treated groups ($p < 0.05$).

$CD8^+$ T cell activation is suppressed by sJag1-P, which is responsible for CIA suppression by sJag1.

Blocking Jagged1 exacerbated CIA

To examine the physiological roles of Jagged1 during CIA induction, we injected a blocking Ab against Jagged1 in the CIA model. We found a significant increase in the mean clinical score in mice treated with anti-Jagged1 mAb (Fig. 6). Overall disease incidence was similar in all groups (100%) (data not shown).

We next examined whether anti-Jagged1 mAb still had an effect on the CIA clinical course in the absence of $CD8^+$ T cells. $CD8^+$ T cell-depleted mice immunized by CII to induce CIA were further treated by anti-Jagged1 mAb (Fig. 6). Treatment of anti-Jagged1 mAb did not exacerbate the clinical course of CIA, indicating the importance of $CD8^+$ T cells to exert the effect of the anti-Jagged1 mAb.

Discussion

Recent studies have provided evidence that Notch signaling controls mature T cell differentiation and activation (2, 5, 13). For the contribution of Notch signaling in T cell-mediated autoimmune disease, two reports have shown that the inhibition of Notch signaling by a γ-secretase inhibitor or an anti-DL1 mAb was able to suppress EAE by modulating $CD4^+$ T cell responses (6, 14). In contrast, treatment with an anti-Jagged1 mAb in mice exacerbates EAE, and Jagged1 has a suppressive ability for $CD4^+$ T cell activation in vitro (14). Although these data suggest that Jagged1-mediated Notch signaling suppressed $CD4^+$ T cell responses, it remains unclear whether Jagged1 modulated $CD8^+$ T cell activation. The results presented here demonstrate that overexpression of soluble Jagged1 by injecting sJag1-P is able to suppress CIA induction and progression. The major effect of sJag1-P on the suppression of CIA was to indirectly inhibit $CD8^+$ T cell activation. These data suggest that modulation of Jagged1 could be a new strategy for suppressing $CD8^+$ T cell-mediated autoimmune and infectious disease.

CIA is a model for human rheumatoid arthritis and both $CD4^+$ and $CD8^+$ T cells are responsible for the progression of CIA. One report indicated that a perforin deficiency ameliorates the clinical score of CIA, suggesting that the killing activity of $CD8^+$ T cells was responsible for CIA progression (16). Our data showed that injection of sJag1 did not improve the CIA clinical score in the absence of $CD8^+$ T cells. Furthermore, the treatment with anti-Jagged1 mAb exacerbated the CIA clinical score, whereas the same treatment could not affect the CIA clinical score in the absence of $CD8^+$ T cells. Although we have not tested whether the injection of sJag1 decreases the killing activity of $CD8^+$ T cells, coinjection of OVA plasmids with Jag1 in mice disturbed the proliferation and granzyme B production of OVA-specific $CD8^+$ T cells. These data strongly suggest that injection of sJag1 disturbs the proliferation and functional differentiation of CII-specific $CD8^+$ T cells, which would be involved in the amelioration of CIA. Furthermore, the data showing that treatment with anti-Jagged1 Ab exacerbated CIA suggest that Jagged1-Notch interaction has physiological roles in suppressing inflammatory responses induced by CII.

Soluble Jagged1 had a suppressive ability on $CD8^+$ T cells but little effect on $CD4^+$ T cells in terms of suppression of CIA. One might argue that soluble Jagged1 competes with DL1 for recognition by Notch receptors, because we have recently found that the stimulation of $CD8^+$ T cells by DL1-Notch2 interaction enhanced $CD8^+$ T cell functional differentiation (13). However, this possibility is unlikely, because the injection of sJag1-P stimulated Notch receptors directly in vitro and further up-regulated the Notch target gene that was induced by endogenous Notch-Notch ligand interaction in vivo. As for the mechanism of suppression of $CD8^+$ T cells by soluble Jagged1, we could not demonstrate a direct effect of Jagged1 on $CD8^+$ T cell activation in vitro. Furthermore, sJag1-P affected $CD8^+$ but not $CD4^+$ T cell proliferation in vivo. Therefore, sJag1 might have selective functions to suppress $CD8^+$ T cells, but not $CD4^+$ T cell activation, by affecting APCs or stromal cells. Of course, it is still possible that higher concentrations of sJag1 may be able to suppress $CD4^+$ T cells by a similar mechanism. In addition, we have examined whether synovial endothelial cells from Jagged1-treated mice have $CD8^+$ T cell-suppressive functions in vitro (data not shown), but we could not observe such an effect, excluding the effect of Jagged1 on synovial endothelial cell functions. Regarding the T cell-suppressive mechanism of Jagged1, we have to consider the contribution of regulatory T cells because several articles reported that Notch signaling controls regulatory T cell functions. For instance, overexpression of Jagged1 in dendritic cells was able to induce peripheral tolerance by supporting the differentiation of regulatory T cells (10). Rutz et al. reported that Jagged1 was able to directly inhibit $CD4^+$ T cell activation (9) and Elyaman et al. reported that Jagged1 is involved in IL-10 production from $CD4^+$ T cells (14). However, our study did not find evidence of an increased number of $Foxp3^+$ regulator T cells or increased IL-10 production from $CD4^+$ T cells, suggesting that these two types of regulatory T cells are not responsible for the effect of soluble Jagged1. Several

articles revealed the presence of $Foxp3^+CD8^+$ regulatory T cells (25–27). However, we could not detect increased $CD8^+$ $Foxp3^+$ cells the after injection of sJag1-P in CIA mice (data not shown), which does not suggest that $CD8^+Foxp3^+$ regulatory T cells contribute to the Jagged1-mediated suppression of CIA. Identification of the cellular target of sJag1, including unidentified regulatory T cells that suppress $CD8^+$ T cells, remains an important issue to be clarified.

We demonstrated that inhibition of Jagged1 in CIA caused deterioration of the clinical symptoms and that this effect was canceled by depleting $CD8^+$ T cells, which suggests that Jagged1 has a protective role in CIA progression by affecting $CD8^+$ T cell responses. As for the clinical application of Jagged1, overexpression or inhibition of Jagged1 might be a useful tool for modulating $CD8^+$ T cells in disease. Aberrant overt activation of $CD8^+$ T cells is responsible for a variety of autoimmune diseases, including rheumatoid arthritis, graft-vs-host diseases, and persistent infectious diseases. Therefore, Jagged1 protein administration or Jagged1 gene expression in situ might become a therapeutic strategy to suppress such diseases. In contrast, inhibition of Jagged1 might be useful to strengthen T cell activation, for instance, for tumor-specific CTLs.

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Disclosures

The authors have no financial conflict of interest.

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Jagged1 on Dendritic Cells and Notch on CD4$^+$ T Cells Initiate Lung Allergic Responsiveness by Inducing IL-4 Production[1,2]

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Jagged1, a Notch ligand, and Notch have been implicated in Th2 differentiation, but their role in initiating IL-4 production and Th2 differentiation in vivo and the development of allergic airway responses has not been defined. In this study, we show that Jagged1 is up-regulated on bone marrow-derived dendritic cells (BMDCs) pulsed with allergen and that the transfer of these BMDCs before allergen challenge induces airway hyperresponsiveness (AHR) and eosinophilic airway inflammation. Treatment of CD4$^+$ T cells with a γ-secretase inhibitor (GSI), which inhibits Notch signaling, resulted in decreased cytokine production when the cells were cocultured with allergen-pulsed, Jagged1-expressing BMDCs and, after the transfer of allergen-pulsed BMDCs, IL-4-deficient (IL-4$^{-/-}$) recipients of GSI-treated naive CD4$^+$ T cells developed lower levels of AHR, reduced numbers of eosinophils, and lower Th2 cytokine levels when challenged with allergen. In vivo treatment of wild-type mice with Jagged1-Fc enhanced AHR and airway inflammation, whereas the transfer of BMDC transfected with *Jagged1* small interfering RNA (siRNA) cells into WT or IL-4$^{-/-}$ mice before transfer of CD4$^+$ T cells resulted in decreased AHR, inflammation, and Th2 cytokines, indicating the critical role for Jagged1 expression on APCs. These data identify the essential role of the interactions between Notch on CD4$^+$ T cells and Jagged1 on APCs in the initiation of IL-4 production and Th2 differentiation for the development of AHR and allergic airway inflammation. *The Journal of Immunology,* 2009, 183: 2995–3003.

Asthma is a complex disease characterized by persistent airway inflammation and airway hyperresponsiveness (AHR)[4] (1) in response to the inhalation of airborne allergens, infectious pathogens, or chemical agents (2). A number of cell types, including Th2 cells, eosinophils, and mast cells are recruited to the lung and activated to release various cytokines and chemokines, enhancing airway inflammation (3). Several clinical and experimental investigations have shown that CD4$^+$ T cells, especially Th2-type cells, play a pivotal role in the development of AHR and eosinophilic inflammation (1, 3–10).

Naive Th cells (Th0) have the potential to differentiate into IFN-γ producing Th1 cells, Th2 cells secreting IL-4, IL-5, and IL-13, or IL-17-producing Th17 cells (11). In the process of differentiation, naive T cells first encounter Ags presented by dendritic cells (DCs) in the T cell zones of secondary lymphoid organs; these DCs have intrinsic capacities to drive either Th1 or Th2 responses. When DCs recognize bacterial and viral products via TLRs, the cells produce IL-12 and induce Th1 polarization (12). Although these TLR-stimulated DCs promote Th1 differentiation, DCs stimulated with agents such as fungal products, parasitic nematodes, or cholera toxin induce Th2 responses (12, 13). Central to initiating Th2 differentiation is IL-4, which synergizes with TCR signals to induce Th2 differentiation through the activation of STAT6, which up-regulates Gata3 (14–16). In animal models of allergic asthma, transfer of Th2-type cells into mice induces airway eosinophilia and AHR following allergen challenge (17, 18). We and others previously demonstrated that IL-4 produced from CD4$^+$ T cells played an essential role in the development of AHR and airway inflammation and that exogenous IL-4 administration restored AHR and allergic airway inflammation by driving Th2 differentiation in the sensitization but not the challenge phase (19–21). Although the critical roles for Th2 cytokines in the pathogenesis of allergic asthma have been well established, the initial events resulting in IL-4 release in vivo and its importance as a Th2-initiating event under physiological conditions is not well defined.

Recently, the differential expression of Notch ligands on APCs in concert with Notch receptors on T cells has been shown to promote Th0 differentiation in response to different Th1- or Th2-promoting stimuli (22–25). In vertebrates, there are four Notch receptors (Notch1–4) and five Notch ligands, the Delta-like families (Delta1, Delta3, and Delta4) and the Jagged families (Jagged1 and Jagged2) (26). Notch receptors and their ligands are expressed on the surface of mature lymphocytes and APCs, respectively. Notch proteins are transcriptional activators expressed as transmembrane heterodimeric surface receptors. Ligand binding releases the Notch intracellular domain by proteolytic cleavage; this allows the Notch intracellular domain to enter the nucleus and transactivate genes through its association with the CSL/RBP-J transcription factor and coactivators of the Mastermind-like family (26–28). γ-Secretase inhibitors (GSI) can effectively prevent enzymatic cleavage of the cytoplasmic domain of Notch receptors, thereby inhibiting the downstream signaling events triggered by

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[4] Abbreviations used in this paper: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; BMDC, bone marrow-derived dendritic cell; DC, dendritic cell; GSI, γ-secretase inhibitor; MCh, methacholine; R_L, lung resistance; siRNA, small interfering RNA; WT, wild type.

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