検査

感染症の起炎病原体の同定は診断に重要で，細菌感染症を反復罹患する症例では，好中球減少・抗体欠乏・補体欠損の有無などを検討する．好中球数減少症があるときは，二次性の好中球減少症を否定するために，薬剤性，自己免疫性（抗好中球抗体，ANA，C3/C4，RF，ANCA，Coombs）の可能性を検討し，二次性の好中球減少症が否定できれば骨髄穿刺を行う．抗体欠乏症の場合は，二次性の薬剤性，悪性腫瘍，腎・消化管から喪失の可能性を検討し，3歳以上であれば破傷風や肺炎球菌ワクチンに対する反応性を検討する．これで異常がみられなければIgGサブクラス欠損症の可能性を考慮する．CH50より補体欠損症が疑われるときは，補体の各因子を定量し，血管浮腫がある場合は，C1インヒビター，発作時C4を検討する．これらに異常がない場合は好中球の機能異常症を疑い，好中球の貪食能，遊走能，活性酸素産生能（NBT色素還元テスト，化学発光法，フローサイトメトリー法），細胞表面CD11/CD18，sLeX発現，酵素活性（MPO，G6PDなど）を評価する．

日和見感染症を呈する場合は，血算（リンパ球・好中球・好酸球の絶対数，血小板数と容積），IgG，IgA，IgM，リンパ球サブセット，リンパ球の増殖反応の検討に加えて，HIV感染の可能性を考慮する．日和見感染症を呈する疾患の代表である重症複合免疫不全症は緊急の治療が必要であるので，これが否定されるまでは迅速に診断を進める．

診断

まず原発性免疫不全症が存在するか否かを診断する．免疫不全症を疑うべき症状としては①反復する細菌感染症：1年間で8回以上の中耳炎または，2回以

表10-35-1 原発性免疫不全症の分類とその原因遺伝子　原発性免疫不全症のWHO分類．主要な原因遺伝子を括弧内に示す．

Ⅰ. T細胞とB細胞両者の免疫不全症
1. T−B−重症複合免疫不全症（*RAG1/2, Artemis, Cernunnos, ADA* など）
2. T−B+重症複合免疫不全症（*γc, JAK3, IL−7Rα, CD45, CD3δ/ε/ζ* など）
3. Omenn症候群（*RAG1/2, Artemis, IL−7Rα* の残存機能を有する突然変異）
4. ZAP70欠損症（*ZAP70*）
5. MHCクラスⅠ欠損症（*TAP1, TAP2, TAPBP*）
6. MHCクラスⅡ欠損症（*C2TA, RFX5, RFXAP, RFXANK*）

Ⅱ. 主として抗体産生不全症
1. 無ガンマグロブリン血症（*BTK, 免疫グロブリンμ重鎖, λ5, Igα, Igβ, BLNK*）
2. 分類不能型免疫不全症（*CD19, CD20, CD21, CD81, ICOS, TACI, BAFF−R*）
3. 高IgM症候群（*CD40L, CD40, AID, UNG*）

Ⅲ. その他の明確な症状を呈する免疫不全症
1. Wiskott−Aldrich症候群（*WASP, WIP*）
2. DNA修復異常症（*ATM, MRE11, NBS1, BLM*）
3. DiGeorge症候群（*TBX1*）
4. 高IgE症候群（*STAT3−DN, TYK2*）
5. 慢性皮膚粘膜カンジダ症（*STAT1−CA, CARD9*）

Ⅳ. 免疫調節異常による疾患
1. 低色素沈着を合併する免疫不全症：Chediak−Higashi, Gricelli症候群（*LYST, RAB27A, AP3B1*）
2. 家族性血球貪食性リンパ組織球増殖症（*PRF1, MUNC13D, STX11*）
3. 伴性リンパ増殖症候群（*SH2D1A, XIAP*）
4. 自己免疫性リンパ増殖症候群（*TNFRSF6, TNFSF6, CASP10, CASP8*）
5. APECED（*AIRE*）
6. IPEX（*FOXP3*）

Ⅴ. 好中球の数または機能の異常
1. 先天性重症好中球減少症（*ELA2, GFI1, G−CSFR, HAX1*）
2. 白血球粘着不全症（*ITGB2, FUCT1*）
3. 慢性肉芽腫症（*CYBA, CYBB, NCF1, NCF2*）
4. IL−12/23Rβ1欠損症（*IL−12/23Rβ1*）
5. IL−12p40欠損症（*IL−12p40*）
6. IFNGR1/2欠損症（*IFNGR1/2*）
7. STAT1欠損症（*STAT1*）

Ⅵ. 自然免疫の異常
1. 外胚葉形成不全−免疫不全症（*NEMO, IkBα*）
2. IRAK4欠損症（*IRAK4*）
3. 単純ヘルペス脳炎（*UNC93B1, TLR3*）

Ⅶ. 自己炎症性症候群
（*MEFV, TNFRSF1A, MVK, CIAS1/NALP3, NOD2*）

Ⅷ. 補体欠損症
（*C1q, C1r, C1s, C4, C2, C3, C5, C6, C7, C8a, C8b, C9, Factor I/H/D*）

略語
ADA：adenosine deaminase，AID：activation−induced cytidine deaminase，AIRE：autoimmune regulator，APECED：autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia，ATM：ataxia telangiectasia, mutated，BLM：Bloom's syndrome protein，BLNK：B cell linker protein，BTK：Bruton tyrosine kinase，CASP：caspase，CIAS1：cold−induced autoinflammatory syndrome，ELA：neutrophil elastase，FOXP3：forkhead box P3，FUCT1：GDP−fucose transporter 1，GFI1：growth factor independent 1，HAX1：HSLS1−associated protein X1，ICOS：inducible costimulatory，IPEX：immune dysregulation, polyendocrinopathy, enteropathy, X−lined，ITGB2：integrin β−2，IRAK4：IL−1 receptor associated kinase，JAK3：Janus kinase 3，MEFV：familial Mediterranean fever，MVK：mevalonate kinase，Mre11：meiotic recombination 11，NALP3：NACHT domain−, leucine−rich repeat, and PYD−containing protein 3，NBS1：Nijmegen breakage syndrome 1，NEMO：NF−κB essential modulator，PRF1：perforin1，RAG：recombinase activating gene，RFX：regulatory factor X，SH2D1A：SH2 domain protein 1A，STAT3：signal transducers and activation of transcription 3，STX11：syntaxin 11，TAP：transporter associated with antigen processing，TLR：Toll−like receptor，TYK2：tyrosine kinase 2，UNC93B1：unc−93 homolog B1，UNG：uracil−DNA glycosylase，WASP：Wiskott−Aldrich protein，XIAP：X−linked inhibitor of apoptosis protein．DN：dominant negative，CA：constitutive active

上の副鼻腔炎・肺炎，皮膚や腹腔内臓器の膿瘍・蜂窩織炎・髄膜炎・骨髄炎・敗血症，②日和見感染症:ニューモシスチス肺炎，1歳以降の難治性カンジダ症，BCGによる重症副反応，サイトメガロウイルス感染症など，③原発性免疫不全症に特徴的な感染症：細胞内寄生細菌（マイコバクテリア・サルモネラ）による感染症，単純ヘルペス脳炎，髄膜炎菌による髄膜炎，劇症型EBウイルス感染症などが重要である．また，感染症に関連した家族歴は本症診断の契機となることが多い．易感染性以外にも，自己免疫疾患やアレルギーなど免疫調節の障害にも注意を払う必要がある．起炎病原体に応じた検査を行い診断する．

経過・予後

重症の免疫不全症では造血幹細胞移植による免疫能再建を行わないと，生後1～2年で死亡することがある．抗体欠乏症では慢性気道感染のため肺機能障害が徐々に進行し，定期的な呼吸機能検査が必要である．

治療・予防

原発性免疫不全症では感染症が重症化するため，病原微生物を迅速に同定し，早期に適切な薬剤が必要である．抗菌薬の予防内服が行われることがある．予防接種は細胞性免疫不全症では禁忌であり，不活化ワクチンも液性免疫不全症では無効なことが多い．

1）免疫グロブリン補充療法：無ガンマグロブリン血症などに対して，免疫グロブリン製剤の補充療法が行われる．一般には3～4週間ごとに200～600 mg/kgを投与する．その際に症例ごとに投与前IgG値（トラフレベル）を呼吸機能が低下しない十分な値（＞500 mg/dL）に設定する．

2）造血幹細胞移植：重症複合免疫不全症，Wiskott-Aldrich症候群，CD40L-CD40欠損症，T細胞性免疫不全症などが適応となる．重症複合免疫不全症では，前処置が不要であるが，それ以外では前処置が必要である．同胞にドナーがいない場合には家族からのT細胞除去骨髄移植，非血縁者間骨髄移植，臍帯血移植などさまざまなドナーを用いた移植が行われる．

3）サイトカイン療法：一部の重症複合免疫不全症にインターロイキン2（IL-2）が有効で，IFN-γが慢性肉芽腫症や一部の細胞内寄生菌に有効である．

4）遺伝子治療：ADA欠損症やγc欠損症では，遺伝子治療が行われる．おもに造血幹細胞にレトロ/レンチウイルスを用いて欠損遺伝子を導入する．

（1）重症複合免疫不全症（severe combined immune deficiency：SCID）

概念・病因

T細胞数の減少とヘルパーT細胞機能の低下による免疫グロブリン産生不全を特徴とする．約半数がX染色体上にあるγc鎖の遺伝子異常による．常染色体劣性遺伝の*JAK3*，*IL-7Rα*，*RAG1*，*RAG2*，*Artemis*など15種以上の原因遺伝子が見いだされている．

臨床症状

生後まもなく日和見感染症を含む各種の感染症に反復罹患，重症化する．原因不明の体重増加不良（failure to thrive），難治性下痢，重症の鵞口瘡（がこうそう）をしばしば認める．ニューモシスチス肺炎，サイトメガロウイルスによる間質性肺炎や麻疹ウイルスによる巨細胞性肺炎が重篤化する．

検査成績

T細胞数の減少，リンパ球増殖反応の低下，免疫グロブリンの低値を認める．γc鎖，JAK3の遺伝子異常ではT細胞とNK細胞が欠損し，B細胞数は正常だが，RAG1/2の異常ではT細胞とB細胞が減少するがNK細胞数は正常である．*IL-7Rα*の異常ではT細胞のみ欠損する．

診断

出生直後よりの高度の易感染性，検査上細胞性免疫と液性免疫の両者の異常が認められる場合に疑う．末梢血のリンパ球分画で病因を推定し，遺伝子検査で確定診断する．最近，胸腺から出てきたばかりのT細胞に存在するTREC（T cell receptor excision circle）を使った新生児マススクリーニングが米国で開始されている．

合併症

輸血中にT細胞の混入があると移植片対宿主反応（GVHD）が起こり致命的になることがある．輸血への放射線照射で予防する．

予後

造血幹細胞移植による免疫再建が行われない場合，そのほとんどが1歳までに不幸な転機を取る．早期発見し早期に造血幹細胞移植を行えれば，予後を大幅に改善できるので，早期確定診断の重要性が高い．

治療

早期の造血幹細胞移植が必要である．遺伝子治療の成功例がある．

（2）Omenn症候群

概念・病因

常染色体劣性遺伝のまれな免疫不全症で，落屑を伴う紅皮症・リンパ節腫脹・肝脾腫，好酸球増加・高

IgE 血症を特徴とする．原因は SCID と共通で *RAG1*, *RAG2*, *Artemis*, γc鎖, *IL-7Rα*鎖などの変異である．発症メカニズムは完全には明らかではないが，RAG などのミスセンス変異による酵素活性残存と限定されたT細胞レパトアが関与すると考えられる．

臨床症状

生後早期からの全身性の GVHD 様紅皮症，体重増加不良，慢性下痢，肝脾腫，リンパ節腫脹を呈する．

検査成績

好酸球増加，末梢血中B細胞が欠損する．自己由来の活性化T細胞が増加し，IgE が高値，それ以外の免疫グロブリン（IgG，IgA，IgM）は著しい低値である．

診断

特徴的な臨床症状と検査所見で疑い，遺伝子検査で確定する．母親由来のT細胞が児に定着し，類似の症状を呈することがあるので鑑別が必要である．

治療

早期の造血幹細胞移植．

(3) 胸腺低形成（thymus hypoplasia）

概念・病因

胸腺の低形成による細胞性免疫不全症，副甲状腺の低形成による低カルシウム血症，心大血管の異常を呈する．約 90%の症例で染色体 22q11 のハプロイドゲノムの欠損を有する．典型例は DiGeorge 症候群とよばれる．

臨床症状

ウイルス，真菌，結核菌などによる感染症に罹患する．さらにヘルパーT細胞機能の障害のため特異抗体の産生障害，それによる細菌感染症を呈する．低カルシウム血症とテタニー，顔面の形態異常（小顎症，耳介低位），大血管異常を認める．

検査成績

胸部CT で胸腺の欠損がみられる．T細胞数はやや低下，B細胞数は相対的に増加する．

診断

大血管奇形，テタニー，顔貌異常で気づかれることが多い．染色体検査で 22q11 または 10p13 の欠失を認める．

治療

低カルシウム血症，大血管奇形の治療が救命のため必要で，重症の細胞性免疫不全がある場合には胸腺移植が試みられることがある．

(4) 無ガンマグロブリン血症（agammaglobulinemia）

概念・病因

B細胞の分化障害が原因の液性免疫不全症である．主要な原因は，X染色体上の *BTK* 遺伝子の変異であるが，常染色体劣性遺伝の疾患もある．

臨床症状

母親からの移行抗体の減少する生後2カ月頃より，遅くとも5歳までに発症し，中耳炎，副鼻腔炎，肺炎などの細菌感染症を反復する．起炎菌としては，莢膜を有するインフルエンザ桿菌や肺炎球菌が多い．その他の感染症の原因としては，エンテロウイルスが重要である．

検査成績

血清中の免疫グロブリンが著しく減少しており（IgG < 200 mg/dL，IgA < 20 mg/dL 以下，IgM < 20 mg/dL 以下），免疫学的には特異抗体が欠損している．フローサイトメトリーで，末梢血単核球中のB細胞は2%以下と著減している．骨髄のB細胞分化は，プロB細胞からプレB細胞への移行段階で停止している．B細胞系列以外には異常は認めない．

診断

身体所見ではリンパ節を触知せず，口蓋扁桃が小さい．薬剤性や感染性の二次性のB細胞減少症を除外し，分類不能型免疫不全症，高IgM症候群などと鑑別診断する．

合併症

感染症のコントロールが不十分だと慢性閉塞性肺疾患（COPD）を高頻度に合併する．エンテロウイルスによる中枢神経感染症が慢性化することがある．

予後

早期診断・治療開始により予後は改善する．

治療

免疫グロブリン補充療法．

(5) 高IgM症候群（hyper-IgM syndrome）

概念・病因

免疫グロブリン重鎖遺伝子のクラススイッチ再構成に障害があり，IgM，IgD の産生は正常だが，IgG，IgA，IgE の産生が障害される．T細胞からB細胞へのシグナル伝達異常とB細胞の内因性異常がある．X染

色体上のCD40リガンド（CD40L）の遺伝子異常によるものの頻度が高いが，CD40，AID，UNGなど常染色体劣性遺伝もある．

臨床症状

高IgM症候群の臨床症状は，①IgG，IgAの低下による細菌感染症，②好中球減少症による口内炎・歯肉炎，③CD40－CD40Lのシグナル伝達異常に起因し*Pneumocystis jiroveci*などの真菌やクリプトスポリジウムなどの原虫による日和見感染症に罹患する．自己免疫症状として血球減少症，関節炎，炎症性腸疾患を合併する．

検査成績

T細胞数・B細胞数は正常で，正常またはそれ以上の血清IgM，IgDを有しながら，IgG，IgA，IgEの著明な低下を特徴とする．リンパ球増殖反応は正常で，これは乳児期のニューモシスチス肺炎症例では重症複合免疫不全症との鑑別に重要である．CD40Lの異常によるものでは，活性化したT細胞上のCD40Lの発現が低下していることが多く，CD40Lの塩基配列に異常を認め，好中球減少を高頻度で認める．

診断

CD40L－CD40の異常によるものでは，クリプトスポリジウム感染により胆管炎と肝癌を発症する．AIDやUNGの欠損症では，クラススイッチは障害されているが日和見感染症はみられない．

治療

全症例に免疫グロブリン補充療法の適応がある．CD40L異常症の好中球減少症では，G－CSFが有効であることが多い．CD40L－CD40の異常症に対しては，ニューモシスチス肺炎の予防のためST合剤の内服が必要である．CD40L－CD40の異常症の場合，早期の造血幹細胞移植が必要である．

（6）選択的IgA欠損症（selective IgA deficiency）

概念・病因

血清IgAのみが低下する．

臨床症状

無症状のものが多いが，IgG2の欠損を合併すると気道の細菌感染症に罹患する．本症の患児では，輸血などIgAを含む製剤の再投与によりアナフィラキシーを発症するので注意が必要である．

検査成績

血清IgAのみ低値を示す．

治療

一般には無治療，易感染性があれば，免疫グロブリンの補充療法．

（7）分類不能型免疫不全症（common variable immunodeficiency：CVID）

概念・病因

成人で高頻度にみられる低ガンマグロブリン血症である．原因が不明で暫定的に分類された疾患で，多くは10歳以降に発症し同種血球凝集素の欠損・ワクチンへの低反応を呈し，既知の免疫不全症でないものと定義されている．CD19，CD20，CD21，CD81，ICOS，TACI，BAFF－Rの異常によるものが報告されているが，多くがいまだ原因不明である．

臨床症状

細胞外寄生菌に対する易感染性．呼吸器・消化器感染症が多い．

検査成績

血清IgG，IgA，IgMが低下し，蛋白・多糖抗原に対する抗体産生が低下しているが，B細胞数はほとんどで正常である．

診断

無ガンマグロブリン血症，伴性リンパ増殖症候群，高IgM症候群，重症複合免疫不全症の原因遺伝子を除外することが重要である．

合併症

30～50%で自己免疫疾患がみられ，悪性腫瘍の合併が多い．

治療

免疫グロブリン補充療法．

（8）Wiskott－Aldrich症候群

概念・病因

血小板数と血小板サイズの減少，T細胞の機能異常とアトピー性皮膚炎，多糖抗原に対する抗体産生の障害がみられる免疫不全症で，X染色体上の*WASP*遺伝子の異常が原因である．

臨床症状

血小板減少による出血傾向，アトピー性皮膚炎，細菌，真菌，ウイルスに対する易感染性．

検査成績

血小板数減少と血小板サイズの減少が特徴である．

細胞性免疫の異常を呈し，血清IgM低値を認め，IgEは高値をとることが多い．

診断

男児で血小板減少，アトピー性皮膚炎，免疫不全症の3主徴を伴う症例に，WASP蛋白の欠損または遺伝子異常を認める．

合併症

自己免疫疾患や悪性リンパ腫の合併の頻度が高い．軽症型でWASP蛋白が存在するものでは，伴性血小板減少症の表現型をとる．

予後

根治療法が行われないと，頭蓋内出血，悪性腫瘍，重症感染症のため予後不良．

治療

摘脾が血小板減少に有効である．根治療法として造血幹細胞移植がある．

(9) 高IgE症候群（hyper IgE syndorome）

概念・病因

黄色ブドウ球菌による皮膚膿瘍と肺炎，アトピー性皮膚炎，血清IgEの著しい高値を3主徴とする免疫不全症である．免疫系だけではなく骨，軟部組織，歯牙の異常を呈する多系統疾患である．高IgE症候群の責任遺伝子として*TYK2*と*STAT3*がある．

臨床症状

皮膚膿瘍が寒冷膿瘍（cold abscess）となることが特徴である．免疫異常以外に特有の顔貌，骨粗鬆症，病的骨折，脊椎側弯，関節の過伸展，乳歯の脱落遅延などを合併する．

検査成績

高IgE血症（2000 IU/mL以上），好酸球増加（700/μL以上）．

合併症

肺炎罹患後の肺嚢胞は，本症に特徴的である．

予後

肺嚢胞が存在する症例ではアスペルギルス，多剤耐性緑膿菌感染症の治療に難渋することがある．

診断

血清IgE値や好酸球数，肺炎・皮膚膿瘍の罹患回数，脊椎側弯症，病的骨折，乳歯の脱落遅延，特徴的顔貌，肺の器質的病変の有無などをスコア化し，高得点のものを高IgE症候群と臨床診断する．遺伝子検査により確定する．

治療

スキンケアと感染症に対する早期の治療が重要である．本症の患児では感染症に罹患していても重症感に乏しく，CRPの上昇も軽度で感染症の早期発見が困難である．予防的抗菌薬（ST合剤など）と抗真菌薬の投与を行う．

(10) 制御性T細胞の異常症（IPEX症候群）

概念・病因

本症の原因が制御性T細胞の異常であることから，*Foxp3*遺伝子がヒトの制御性T細胞の生成に必須であることが明らかにされた．

臨床症状

生後早期から，重症の水溶性・血性下痢，1型糖尿病と甲状腺機能低下症など多臓器の内分泌障害，アトピー性皮膚炎，自己免疫性溶血性貧血・好中球減少症・血小板減少症を呈する．

検査成績

リンパ球分画・増殖反応，血清IgG，IgMは正常であるが，血清IgE，IgAの高値と好酸球増加を認める．各種の自己抗体を認め，末梢血中の$CD4^+CD25^+$の細胞は存在するが，Foxp3蛋白を欠損する．

診断

乳児期の男児がIgE上昇を伴う難治性の重症下痢を呈し，1型糖尿病を合併した場合に疑い，制御性T細胞中のFoxp3蛋白の欠損と遺伝子検査で確定する．

予後

適切な治療が行われなければ乳児期に不幸な転帰をとる．

治療

対症的に輸血，ホルモン補充療法，経静脈栄養が必要なことがある．治療に対する重症のアレルギー反応に注意が必要である．免疫抑制薬が有効なことがある．根治には，早期の造血幹細胞移植が必要であるが，生着不全の報告が多い．

(11) 伴性リンパ増殖症候群（X-linked lymphoproliferative syndrome）

概念・病因

EBウイルス（EBV）はほとんどのヒトが感染しており，

B細胞内に潜伏感染しキラーT細胞により制御されている．本症ではこのEBV感染が重症化する．原因は，*Sap/SH2D1A*（1型），*XIAP*（2型）の遺伝子異常である．

臨床症状

EBVの感染前は特に異常がなく，初回感染後に発症する．2～3歳で初感染すると劇症型伝染性単核球症，4～6歳で初感染すると悪性リンパ腫を発症し，6～9歳では免疫グロブリン異常症を発症することが多い．

検査成績

T細胞とB細胞の数と増殖反応は正常だが，抗体産生能は著減している．末梢血中のメモリーB細胞数は著減，ヘルパーT細胞の機能も障害されている．患児のT細胞・NK細胞は自己のEBV感染B細胞に対するIFN-γの産生が低下し，NKT細胞は完全に欠損している．

診断

家族歴・臨床症状より疑い，免疫学的検査，遺伝子検査で確定する．

合併症・予後

悪性リンパ腫を高頻度で合併する．免疫グロブリン異常症で発症したものは，ほかの病型と比較すると予後がよい．

治療

EBVに対して高力化の免疫グロブリンの補充療法が有効なことがある．早期の造血幹細胞移植が必要である．

(12) 毛細血管拡張性失調症（ataxia telangiectasia）

概念・病因

上下気道の易感染性に眼球結膜・皮膚の毛細血管拡張と小脳性運動失調を合併する．DNA二重鎖切断修復に関与する*ATM*遺伝子の異常による．

臨床症状

上下気道の感染が反復し遷延する．1歳頃より毛細血管拡張や運動失調が出現する．

検査成績

T細胞数の減少，リンパ球増殖反応の低下を認め，血清IgG，IgE，IgAも低値．α-フェトプロテインの増加や染色体の異常も認める．

診断

副鼻腔炎，中耳炎，肺炎などの上下気道の感染に加えて，毛細血管拡張と運動失調を合併していれば診断可能であるが，乳児期の易感染性のみでは診断は困難である．確定診断は*ATM*蛋白発現の検討と遺伝子検査による．

合併症

悪性腫瘍の合併が高率である．急性リンパ性白血病，悪性リンパ腫の頻度が高く，化学療法に対する副作用の頻度が高い点に注意が必要である．気管支拡張症もしばしば合併する．

予後

悪性腫瘍や気道感染症により小児期に不幸な転機をとることが多い．

治療

抗菌薬の予防投与や免疫グロブリン補充療法による感染予防が行われるが，根治療法は存在しない．

(13) 慢性肉芽腫症（chronic granulomatous disease：CGD）

概念・病因

食細胞の活性酸素産生障害のために貪食した細菌・真菌を殺菌できず，また消化管・気道・尿路への肉芽腫形成を特徴とする．活性酸素の産生にかかわるNADPHオキシダーゼの異常による．伴性劣性のgp91-phox欠損症と常染色体劣性遺伝のp22-phox，p47-phox，p67-phox，rac2の遺伝子異常により発症する．伴性劣性の遺伝形式をとるものが全体の約2/3を占める．

臨床症状

カタラーゼ陽性の化膿菌（黄色ブドウ球菌など）による皮膚，リンパ節，肺，肝の感染症に罹患し，膿瘍形成や肉芽腫形成を伴いやすい．カンジダやアスペルギルスによる真菌感染症も高頻度でみられ，非定型抗酸菌などの細胞内寄生細菌に対しても易感染性である．

検査成績

好中球のNBT色素還元能陰性で，化学発光が欠如し，活性酸素の産生能低下がみられる．

診断

臨床症状より疑い，gp91-phox，p22-phox，p47-phox，p67-phox，rac2の蛋白の欠損遺伝子異常を同定することにより確定診断する．

予後

軽症例では成人まで生存するが，アスペルギルスによる真菌感染症が致命的なことがある．

治療

ST合剤，抗真菌薬の予防内服．IFNγの投与も一部の症例では有効である．根治療法として造血幹細胞移植がある．

(14) 白血球粘着異常症（leukocyte adhesion deficiency：LAD）

概念・病因

白血球上の接着分子が欠損症し，好中球・単球の遊走能や貪食能の低下とNK細胞活性の低下がみられる．β_2インテグリンの*CD18*遺伝子異常によるもの（1型），CD15欠損によるもの（2型）などがある．

臨床症状

生下時臍帯脱落遅延を認め，反復性の皮膚の重症感染症，創傷治癒の遷延，持続性の歯肉炎などを認める．

検査成績

著明な末梢血白血球数増加．

治療

ST合剤の投与が有効である．根治には造血幹細胞移植．

(15) Chediak-Higashi症候群

概念・病因

反復感染症，部分的白子症，白血球・血小板・メラノサイト内の巨大顆粒を特徴とする．好中球の機能不全，キラーT細胞やNK細胞活性の低下がみられ，易感染性と高率のリンパ系悪性腫瘍を合併する．*LYST*遺伝子の異常による．

臨床症状

反復性の細菌感染症，貧血，肝脾腫，白血球減少，血小板減少，皮膚や毛髪の色素異常，日光過敏症，中枢神経異常など多彩な症状を呈する．

検査成績

末梢血の塗抹標本で巨大顆粒を認める．

治療

造血幹細胞移植．

(16) 原発性補体欠損症（primary complement deficiency）

概念・病因

補体各成分をコードする遺伝子の異常により，補体蛋白が欠損する．

臨床症状

細菌に対する易感染性がみられる．C5～C9の欠損では髄膜炎菌などのナイセリアに対する易感染性がみられる．C1～C3欠損症では全身性エリテマトーデスや腎炎の自己免疫症状を合併することがある．

診断

CH50の低下でスクリーニングし，補体の各因子を定量する．〔峯岸克行〕

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10-36 後天性免疫不全症候群

【⇨ 4-4-3）】

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ORIGINAL ARTICLE

B-cell function after unrelated umbilical cord blood transplantation using a minimal-intensity conditioning regimen in patients with X-SCID

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Abstract Patients with X-linked severe combined immunodeficiency (X-SCID) suffer from severe and persistent infections, and usually die early in life unless treated by hematopoietic stem cell transplantation. If a patient has an HLA-identical sibling donor, preparative conditioning is not necessary for T-cell engraftment and B-cell function. However, in the absence of such a donor, long-term reconstitution of full B-cell function is often problematic, leading in many cases to a lifetime requirement for immunoglobulin replacement therapy. Preparative myeloablative conditioning has been shown to improve long-term B-cell function, but may aggravate pre-existing infection and transplant-related toxicity. It is thus important to determine the minimum intensity of conditioning that assures immunoglobulin production. In the present study, we performed reduced-intensity conditioning (RIC), consisting of fludarabine 125 mg/m^2 and melphalan 80 mg/m^2, prior to unrelated umbilical cord blood transplantation (UCBT) for five patients with X-SCID, none of them had an HLA-identical donor. Four patients survived more than 4 years without sequelae, and none required long-term immunoglobulin replacement therapy. One patient succumbed to sepsis in conjunction with severe GVHD. Our result demonstrates that the RIC regimen described above in combination with UCBT is an effective and less toxic conditioning to correct B-cell function in patients with X-SCID.

Keywords X-SCID · Reduced-intensity conditioning · Umbilical cord blood transplantation · Fludarabine/melphalan

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Introduction

X-linked severe combined immunodeficiency (X-SCID), which accounts for approximately half the cases of SCID, is caused by mutations of the γc chain. Immunological characteristics of this disease include profound impairment of both cellular and humoral immunity due to the absence or diminished numbers of T cells and natural killer (NK) cells, and abnormal B-cell function in spite of normal or elevated numbers of B cells. Therefore, patients with X-SCID suffer from severe and persistent infections, including opportunistic pathogens, and usually die early in life unless treated by hematopoietic stem cell transplantation (HSCT) or gene therapy [1]. Previous reports demonstrated excellent results of HLA-identical BMT with

survival rate over 90 %, and full restoration of T- and B-cell function [2, 3]. Since most patients do not have an HLA-identical sibling donor, HLA-haploidentical bone marrow transplantation (BMT) was developed in the early 1980s, when better T-cell depletion methods became available. However, the survival rate was lower, at around 60–80 %, and about half of the patients required life-long immunoglobulin replacement therapy despite normal T-cell immunity with or without pre-transplant conditioning [2–4]. These results suggested that T-cell-depleted, HLA-haploidentical bone marrow cells might not be a suitable source of HSCT for correcting B-cell function. Another strategy to treat this condition is to use unrelated donor HSCT with conventional myeloablative conditioning regimens, which leads to stable reconstitution of T- and B-cell function [5]. However, this approach has been associated with significant treatment-related toxicities and aggravation of pre-existing infections. To avoid these problems, reduced-intensity conditioning (RIC) regimens have been developed. Recently, Rao et al. [6] reported that a RIC regimen using a total dose of 150 mg/m^2 of fludarabine, 140 mg/m^2 of melphalan, and Campath 1H or ATG resulted in an improved survival and reduced transplantation-related mortality, compared with myeloablative conditioning, in children with primary immunodeficiency (PID) undergoing unrelated BMT. They used the same regimen for patients with X-SCID who would be expected to need less intensive conditioning because their immune system is already profoundly impaired. Based on their report, we performed unrelated umbilical cord blood transplantation (UCBT) with pre-transplant conditioning using a further reduced dosage of fludarabine and melphalan, in the absence of Campath 1H or ATG, to investigate whether a minimally intensive conditioning regimen could assure correction of B-cell function in X-SCID patients.

Patients and methods

Patients

Five patients with typical X-SCID received unrelated UCBT because they had no HLA-matched sibling donor. As shown in Table 1, mutations in the γc chain gene were detected in all patients. Patient 3 suffered from pneumonia caused by *Pneumocystis jiroveci* at the time of diagnosis of X-SCID. All patients except for patient 3 were diagnosed with X-SCID at birth because their brothers had the same disease. Immunoglobulin replacement therapy was initiated once hypogammaglobulinemia was confirmed, and IgG trough levels were maintained over 500 mg/dL. This study was performed with the approval of Institutional Review Board at each university and with the written informed consents of the parents.

Conditioning regimen and GVHD prophylaxis

Pre-transplant conditioning for all patients consisted of fludarabine (25 mg/m^2 per day) from day −7 to day −3

Table 1 Patient characteristics

Patient	1	2	3	4	5
Age at diagnosis (months)	0	0	4	0	0
Age at UCBT (months)	3	3	10	3	3
Mutations in the γc chain	868 G > A	691 G > A	c.735 741*	IVS4 + 2 T > A	568A > G
HLA identity	6/6	5/6	6/6	5/6	5/6
Nucleated cell dose (×10^7/kg)	7.1	10.0	5.0	9.5	11.2
CD34+ cell dose (×10^5/kg)	1.09	3.65	ND	3.50	1.68
Hematological recovery					
Nt > 500/μL	30	20	36	20	12
Plt > 5 × 10^4/μL	10	16	95	17	16
Ret > 1 %	18	16	38	20	15
Complications at UCBT	None	None	Pneumonia	None	None
Additional infections during UCBT	None	None	Sepsis	CMV	Sepsis
GVHD					
Prophylaxis	CyA	FK + sMTX	FK	FK + sMTX	CyA + PSL
Acute (grade)	0	0	II	II	III
Chronic	–	–	Extensive	–	Extensive
Therapy	–	–	FK + mPSL	FK + PSL	FK + MMF + PSL

*c.735 741** c.735_741delAGCCACC→insGGGAGCAATACTT, *ND* not determined, *Nt* neutrophils, *Plt* platelets, *Ret* reticulocytes, *sMTX* short-term methotrexate

(total dose 125 mg/m^2) and melphalan (40 mg/m^2 per day) from day −4 to day −3 (total dose 80 mg/m^2). Neither ATG nor Campath 1H was included in the conditioning regimen.

Prophylaxis for acute GVHD was performed with either cyclosporine A (CyA) with/without prednisolone or FK506 with/without short-term methotrexate as shown in Table 1.

Graft characteristics

As shown in Table 1, UCB units were either serologically full-matched or one locus mismatched at 6/6 (A, B, DR) HLA loci. Infused nucleated cell doses were 5.0 × 10^7/kg–11.2 × 10^7/kg (mean 8.6 × 10^7/kg), which contained CD34+ stem cells, ranging from 1.09 × 10^5/kg to 3.65 x 10^5/kg (mean 2.48 × 10^5/kg) except for patient 3, whose information on CD34+ cells was not available.

Chimerism studies

Hematological recovery was defined as achievement of absolute neutrophil count (ANC) >500/μL for 3 consecutive days and a platelet count >5.0 × 10^4/μL for 7 consecutive days without need for further transfusion. Chimerism was analyzed at Human Leukocyte Antigen Laboratory (Kyoto, Japan) as described previously [7]. Briefly, T cells, B cells and NK cells were separated by anti-CD3, anti-CD19 and anti-CD56 microbeads (Invitrogen Dyanl AS, Oslo, Norway), respectively. Donor- and recipient-specific polymorphic short tandem repeats (STR) were amplified by PCR, and subsequently analyzed by SDS-PAGE.

Immunological reconstitution studies

Immunological reconstitution status after transplantation was monitored by serum immunoglobulin levels (IgG, IgA, IgM and IgE), isohemagglutinin, and specific antibodies, and by flow cytometry analyses of peripheral mononuclear cells for CD3, CD4, CD8, CD19, CD16 and CD56.

Results

The age at transplantation was 3 months in four patients and 10 months in one patient (Table 1). All patients received UCBT using fludarabine (125 mg/m^2) and melphalan (80 mg/m^2) as a pre-transplant conditioning. They all achieved engraftment of ANC > 500 μL and platelets > 5.0 × 10^4/μL at a mean of 23.6 days (range 12–36 days) and 30.8 days (range 10–95 days), respectively. All but one survived more than 4 years without complication. One patient, patient 5, succumbed to sepsis in conjunction with severe GVHD.

Infections

Patient 3 suffered pneumonia due to *P. jiroveci* infection prior to admission and intravenous trimethoprim/sulfamethoxazole therapy was initiated. The pneumonia resolved with the engraftment of donor cells. He also experienced an episode of sepsis due to enterococci after UCBT, which was cured by appropriate antibiotics. Patients 1, 2, 4, and 5 were diagnosed with X-SCID at birth by sequencing of the γc chain because their brothers had the same disease. They had been protected in a clean environment soon after birth and they did not experience any infection until UCBT. Patient 5 developed sepsis due to a catheter infection, which was the cause of death at day 491 after UCBT.

Regimen-related toxicity and GVHD

Mild mucositis and myelosuppression were observed with this reduced-intensity conditioning, and no other regimen-related toxicity was noted.

Patient 3 developed acute GVHD grade II (skin stage 3) and extensive chronic GVHD, while patient 4 developed acute GVHD grade II (skin stage 3, liver stage 1 and gut stage 1). Symptoms in both cases resolved on prednisolone and FK506. Patient 5 developed acute GVHD grade III (skin grade 1, liver grade 3 and gut stage 3), followed by extensive chronic GVHD. He succumbed to sepsis in conjunction with uncontrolled GVHD, although he was treated with prednisolone, FK506 and mycophenolate mofetil (MMF).

Chimerism

Median follow-up was 68 months (range 48–73 months). As shown in Table 2, all survivors had complete donor T-cell chimerism. One survivor, patient 3, also had complete lymphocyte and granulocyte chimerism, which was confirmed by day 52. The others demonstrated mixed chimerism in these cell lineages. The percentage of the donor cells in each cell lineage had been stable since day 168 after UCBT in patient 1. In patients 2 and 4, detailed chimerism using fractionated cells was analyzed only the date indicated in Table 2. Donor cells of patient 5 constituted only 5 % of his peripheral blood nucleated cells at day 420 after UCBT, although T cells were 100 % of donor origin.

Immune reconstitution

Table 3 shows the results of immunologic evaluation at the most recent follow-up after UBCT in all survivors. Absolute numbers of lymphocytes were normal after

Table 2 Leukocyte chimerism

Patient	1		2		3		4		5
Days after UCBT[a]	168	1620	60	1098	52	2021	90	2078	420
T cell (donor %)	100	>95	90	94		100	100	92	100
B cell (donor %)	24	20	20	8		100	70	50	ND
NK cell (donor %)	55	69	15	33		100	90	84	ND
Granulocyte (donor %)	65	59	18	48	>95	100	20	13	ND
Lymphocyte (donor %)					>95				

ND not determined

[a] Days after UCBT when chimerism was determined

Table 3 Immune reconstitution

Patient	1	2	3	4	5 (at day 470)
WBC (/μL)	7700	7400	4710	9200	1000
Lymphocyte (/μL)	3700	4370	4120	4100	400
CD3 (%)	82.2	60.4	63.7	81.3	38.6
CD4 (%)	48.4	24.4	35.3	42.0	33.0
CD8 (%)	27.8	28.7	25.7	32.2	11.5
CD19 (%)	13.4	37.9	32.0	15.1	0.0
CD16/56 (%)	1.8	0.6	4.0	2.7	13.0
B-cell function					
IgG (mg/dL)	937	531	692	1157	660 (under i.v.Ig)
IgA (mg/dL)	58	32	55	101	89
IgM (mg/dL)	117	77	115	231	112
IgE (IU/mL)	37	<3	4.2	1	ND
Isohemagglutinin	+	+	−	+	−
Specific antibody	−	+	+	+	ND
T-cell function					
PHA stimulation (SI)	164.4	243.6	220.9	1213.4	1.1
ConA stimulation (SI)	897.7	322.1	225.5	713.1	1.1
NK activity (%)	15	4	10	19	ND

Normal values; PHA stimulation (SI) >100, ConA stimulation (SI) >75, NK activity 18–40 %

ND not determined, *SI* stimulation index

transplantation (Table 3). Numbers of CD3+, CD4+, CD8+ T cells and CD19+ B cells were within normal ranges, and T-cell function was normal by assessment with PHA and ConA stimulation. Immunoglobulin serum levels were within normal ranges of age-matched controls in all four patients, and none requires IgG substitution (Tables 3, 4). Also each patient had a positive antibody response. NK activity was lower than normal in all but patient 4.

Growth and psychomotor development

As shown in Table 4, all survivors have shown normal height, body mass index (BMI), psychomotor development and performance status to date.

Discussion

We report the outcome of unrelated UCBT in five patients with X-SCID using a RIC regimen. The most important result of this study is all four survivors are free from immunoglobulin replacement therapy.

Previous studies showed that about two-thirds of SCID patients required immunoglobulin replacement therapy after T-cell-depleted, HLA-haploidentical BMT from related donors without pre-transplant conditioning [2, 8]. In Europe, about half of SCID patients who received HLA-haploidentical related marrow cells were conditioned mostly with busulfan (8 mg/kg) and cyclophosphamide (200 mg/kg) [3]. However, the mortality rate for this type of conditioning was higher than that of patients without conditioning. Further, pre-transplant conditioning in combination with HLA-haploidentical related marrow cells did not always result in correction of B-cell function, and about one-third of the SCID patients continue to require immunoglobulin replacement therapy. In contrast, all surviving SCID patients, who had received bone marrow cells from unrelated donors after conventional conditioning with busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg), did not require immunoglobulin replacement therapy [5, 6]. This conventional conditioning regimen, however, has been associated with a significant mortality rate due to treatment-related toxicities such as profound pancytopenia, severe organ toxicity, and exacerbation of pre-existing infections. In addition, children treated with myeloablative regimens often suffer from delayed effects such as infertility, hormonal dysfunction, growth failure and secondary malignancies [9]. Recently, Rao et al. [6] reported the outcome of 33 patients with primary immunodeficiency (PID) [SCID ($n = 6$) and non-SCID ($n = 27$)] who received unmodified unrelated donor marrow grafts following reduced-intensity conditioning consisting of fludarabine (150 mg/m^2), melphalan (140 mg/m^2), and alemtuzumab (Campath 1H) or anti-thymocyte globulin (ATG). All patients had primary engraftment, and most patients achieved normal immunoglobulin production and B-cell function, although it is not clear whether patients with SCID were on immunoglobulin replacement therapy or not. From these

Table 4 Current status

Patient	1	2	3	4	5
Clinical status	Alive	Alive	Alive	Alive	Dead (at 17 months)
Follow-up (months)	68	48	73	69	17 months
Last i.v.Ig (months)	44	32	8	3	17 months
i.v.Ig at present	Off	Off	Off	Off	NA
Height	−1.0 SD	+1.92 SD	−1.0 SD	−0.2 SD	Short stature
Body mass index	15.9	14.5	14.5	15.2	BW 6 kg
Mental status	Normal	Normal	Normal	Normal	Normal
Karnofsky performance status	100 %	100 %	100 %	100 %	30 %

i.v.Ig intravenous immunoglobulin, *NA* not applicable, *SD* standard deviation, *BW* body weight

results, we speculated that T-cell depletion might interfere with B-cell engraftment and function. In this context, it is interesting to note that patients in our study who had acute GVHD complications showed higher B-cell chimerism and early immunoglobulin production after UCBT. However, one of our patients succumbed to sepsis in conjunction with severe GVHD. Unlike patients with hematologic malignancies, who benefit from the graft-vs-leukemia effect of donor cells, there is no such benefit from GVHD in patients with PID [10]. Thus, it is inevitable to use immunosuppressive drugs to prevent GVHD, and modifications such as the addition of ATG to our protocol to reduce the risk of GVHD will need to be evaluated in a future study [11]. Of note, two of our patients who did not develop acute GVHD gradually corrected their B-cell function, and immunoglobulin replacement therapy could be discontinued 32 and 44 months after UCBT. These results suggest that the RIC regimen described here may provide a minimal-intensity conditioning regimen in combination with UCB, which can assure sufficient production of immunoglobulin.

Some reports have raised concern about cardiac toxicity associated with high-dose melphalan and fludarabine used in combination [12, 13]. However, patients with this adverse event had been suffering from advanced hematologic malignancies and had been heavily treated with cytotoxic drugs including anthracyclines prior to pre-transplantation conditioning, and the total dosage of fludarabine (150 mg/m^2) and melphalan (140 mg/m^2) used for conditioning was much higher than the present study. In addition, reduction of melphalan from 140 to 80 mg/m^2 is expected to result in a lower frequency of cardiac toxicity. We only observed mild myelosuppression and mucositis as adverse events of the RIC regimen. Engraftment of unrelated cord blood cells, which might not be achieved with lower concentration of melphalan, was observed in all patients in our study. To date, none of our patients has shown any delay in growth or mental development. Long-term follow-up is necessary to validate the efficacy and safety of this RIC regimen.

Regarding B-cell engraftment and function, T-cell depletion from related donor bone marrow cells may not be a suitable source of HSCT for PID patients who do not have an HLA-identical sibling donor as described above. Recently, it was reported that UCB from unrelated donors could be used successfully for patients with PID [14, 15]. As UCB contains T cells, faster emergence of donor T cells is expected even though the infused T cells are functionally naïve. UCB recipients were able to discontinue immunoglobulin replacement therapy sooner and more frequently compared with T-cell-depleted bone marrow recipients although the estimated 5-year over all survival rates were comparable when UCB recipients received a myeloablative conditioning regimen [15]. In addition, UCBT is more tolerant of HLA disparity because the incidence and severity of GVHD is lower than for unrelated BMT. These results together with ours support the application of UCBT for patients with X-SCID who do not have an HLA-identical sibling donor.

Another risk factor for a poor outcome using HSCT for SCID is a pre-existing infection [8]. In our patients, all but one were diagnosed with X-SCID at birth from their family histories, and they had been kept in a protective environment for 3 months until they received UCBT. There are two reasons why we performed UCBT at the age of 3 months. One is to minimize regimen-related toxicities because infants are more susceptible to cytotoxic drugs, and the other is to expect higher survival rate after transplantation in the first 3.5 months of life as described previously [2, 16]. Early diagnosis before any infectious episodes is necessary for safe HSCT in the patients with SCID. Recently, screening of newborns for SCID has been recommended [17], and the RIC regimen described above in combination with UCBT is an alternative to HLA-haploidentical BMT for such patients.

In conclusion, our regimen in combination with UCBT is well tolerated and resulted in normal immunoglobulin production and B-cell function in our patients. Future studies with a modification of GVHD prophylaxis for patients with X-SCID who do not have an HLA-matched

sibling donor will be needed to further improve the outcome.

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Conflict of interest The authors declare no conflict of interest.

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ORIGINAL RESEARCH

A Non-invasive Diagnosis of Histiocytic Necrotizing Lymphadenitis by Means of Gene Expression Profile Analysis of Peripheral Blood Mononuclear Cells

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Abstract Histiocytic necrotizing lymphadenitis (HNL), also called Kikuchi-Fujimoto disease, is a benign, self-limiting inflammatory disease with fever and painful cervical lymphadenopathy of unknown etiology. A lymph node biopsy is required for the definitive diagnosis because of no specific symptoms or laboratory findings for HNL. To establish the rapid non-invasive diagnostic method for this disease, we investigated genes specifically expressed in the patients by analyzing whole transcriptome using microarray analysis of peripheral blood mononuclear cells (PBMC). The top five up-regulated genes (*IFI44L*, *CXCL10*, *GBP1*, *EPSTI1* and *IFI27*) in HNL were interferon-induced genes (ISGs). The expression levels of the up-regulated genes by microarray were verified by quantitative PCR. High levels of serum CXCL10 concentration were confirmed at the symptomatic phase of HNL patients. The expression levels of these 5 genes positively correlated with each other (r^2=0.28–0.60). The genes were also highly expressed in HNL lymph nodes. The discriminant analysis using the expression levels of these five genes distinguished HNL with 84 % accuracy. The combination of up-regulated ISGs in HNL seemed to be a specific response induced by viral infections or autoantigens. An analysis of the gene expression profile of PBMC may provide a rapid non-invasive diagnosis of HNL.

Keywords Histiocytic necrotizing lymphadenitis · Kikuchi-Fujimoto disease · interferon-stimulated genes · gene expression · discriminate analysis

Abbreviations

ACTB	Beta actin
ADV	Adenovirus
AUC	Area under the curve
cDNA	Complementary DNA
C_T	Threshold cycle
CXCL10	Chemokine (C-X-C motif) ligand 10
EPSTI1	Epithelial stromal interaction 1 (breast)
FluA	Influenza type A virus
GBP1	Guanylate binding protein 1 interferon-inducible
HNL	Histiocytic necrotizing lymphadenitis
IFN	Interferon
IFI27	Interferon alpha-inducible protein 27
IFI44L	Interferon-induced protein 44-like
IL	Interleukin
IM	Infectious mononucleosis

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ISG	Interferon-stimulated gene
KD	Kawasaki disease
LNitis	Purulent lymphadenitis
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
SLE	Systemic lupus erythematosus
SoJIA	Systemic onset juvenile idiopathic arthritis

Introduction

Histiocytic necrotizing lymphadenitis (HNL), also called Kikuchi-Fujimoto disease, is a benign, self-limiting inflammatory disease with fever and tender cervical lymphadenopathy of unknown etiology [1, 2]. Severe HNL patients with hemophagocytic syndrome or prolonged fever need immunosuppressive therapy [3]. It is necessary to distinguish it from other febrile diseases with lymphadenopathy including leukemia, malignant lymphoma, infectious mononucleosis (IM), purulent/tuberculous lymphadenitis, Kawasaki disease (KD), systemic juvenile idiopathic arthritis (SoJIA), and systemic lupus erythematosus (SLE). Lymph node biopsies are required for the definitive diagnosis because of no specific symptoms or laboratory findings including imaging tests for HNL.

Histologic findings of involved lymph nodes include paracortical areas of coagulative necrosis with abundant karyorrhectic debris. Karyorrhectic foci consist of histiocytes, plasmacytoid dendritic cells, immunoblasts, and lymphocytes [1, 2]. Neutrophils and plasma cells are absent or scarce. Assessed by immunohistochemical analysis, histiocytes are positive for myeloperoxidase and CD68 antigen, and lymphocytes are predominantly $CD8^+$ cytotoxic T cells [1, 2, 4]. In the earlier stages, histiocytes and plasmacytoid dendritic cells (pDCs) are usually prominent in the lesions, suggesting that these cells have a close correlation with the pathogenesis of HNL [1, 2, 5]. By immunohistochemical analyses, it has been suggested that perforin and Fas pathways play important roles in the induction of apoptosis and necrotizing lesions [6, 7]. It was supported by the findings of microarray analysis which showed up-regulation of apoptosis- and cell cycle-associated genes in lymphnodes of HNL patients [8].

Some HNL cases were reported to occur in association with viral infections (e.g. Epstein-Barr virus, human herpes virus type 6, and human T-lymphotropic virus type 1) or autoimmune disease (SLE) [1, 9]. Increased serum concentrations of interferon (IFN)-γ, interleukin (IL)-6, and 2',5'-oligoadenylate synthetase as well as pyrexia and extranodal involvement of skin, bone marrow, and liver in the patients suggest the exaggerated systemic inflammatory response to viral pathogens or to autoantigens in the pathophysiology of HNL [10, 11].

To establish a rapid non-invasive diagnostic method for HNL, and to characterize the molecular pathophysiology of the disease, we investigated the gene expression profile of peripheral blood mononuclear cells (PBMC) by microarray analysis and performed discriminate analysis.

Materials and Methods

Patients

Twenty-four patients with HNL participated in this study. The clinical features of the patients are shown in Table I. The specimens were obtained from the patients before or without the treatment with steroids or immunosuppressive drugs etc. In addition, 93 disease controls and 34 healthy donors were included in this study (Table II). Cervical lymph node samples of patients with HNL (n=9) and reactive lymphadenopathy (n=4) were provided from Department of Pathology, Faculty of Medicine, Fukuoka University, Japan. For the analysis of serum concentrations of CXCL10, blood samples were obtained from 12 patients with HNL, 5 with KD, 4 with IM, and 5 with bacterial lymphadenitis. All patients were diagnosed by trained pediatricians and pathologists, according to the clinical manifestations, laboratory findings, and histological examinations. Informed consent was obtained from all participants for this study, according to the process approved by the Ethical Committee of Kyushu University, Fukuoka, Japan.

Total RNA Extraction and RNA Amplification

PBMC were separated from peripheral blood by density gradient centrifugation using Lymphocyte Separation Medium (MP Biomedicals LLC, CA, USA). Total RNA was extracted from PBMC or lymph nodes cells using an RNA extraction kit (Isogen) (Nippon Gene, Osaka, Japan), according to the manufacturer's instructions. An Amino Allyl MessageAmp aRNA Kit (Life Technologies, CA, USA) was used to amplify the total RNA.

Microarray Analysis

In order to characterize the gene expression profile of PBMC in HNL patients, microarray analysis of PBMC was performed using an AceGene Human Oligo Chip 30 K (Hitachi Solutions, Tokyo, Japan) that contains approximately 30,000 genes. The arrays were scanned by FLA-8000 (Fujifilm, Tokyo, Japan), and changed to the numerical values by ArrayVision Software (GE Healthcare, Buckinghamshire, UK). The numerical data were normalized using the LOWESS method, as described previously [12]. In the microarray analysis of PBMC, data from 2 patients with HNL, 5 with SoJIA, 3 with KD and a healthy donor were compared. Genes that were consistently up-regulated in PBMC of HNL patients, compared with patients with SoJIA or KD and a healthy donor, with more than

Table I Clinical manifestations and laboratory findings of HNL patients

Number of HNL patients	24
Gender male:female	11:13
Age at onset (years)	11.7 (3.5–14.3)*
Family history of HNL	0
Fever	24
Cervical lymphadenopathy	24
Biopsy of lymph node	8
Relapse of HNL	7
Association with autoimmune disease	1
WBC (/μL)	2840 (1340–6010)*
Platelet count (x10^9/L)	158 (86–308)*
LDH (IU/L)	625 (211–1179)*
CRP (mg/dL)	0.7 (0–3.7)*
Ferritin (ng/mL) (n=17)	262 (74–8123)
ESR (mm/1 h) (n=19)	31 (8–60)

*The variables expressed as median (range)

CRP C-reactive protein, *ESR* erythrocyte sedimentation rate, *LDH* lactate dehydrogenase, *WBC* white blood cell count

two-fold differences in the mean expression levels were selected. The data with low signal-to-noise ratios (S/N<2) were not used for further analysis. The data were analyzed using GeneSpring Software (Agilent Technologies, CA, USA).

Real-Time Quantitative PCR

First-Strand cDNA Synthesis Kit (GE Healthcare) with random hexamers was used to prepare the first-strand cDNA.

Table II The number of subjects in each group for discriminate analysis

Group	Number of subjects
1. Histiocytic necrotizing lymphadenitis (HNL)	24
2. Disease control (DC)	93
Kawasaki disease (KD)	11
Systemic onset juvenile idiopathic arthritis (SoJIA)	14
Systemic lupus erythematosus (SLE)	4
Measles	18
Varicella	5
Infectious mononucleosis (IM)	15
Adenovirus infection (ADV)	5
Influenza type A virus infection (FluA)	5
Purulent lymphadenitis (LNitis)	5
Sepsis	7
Lymphoid malignancy	4
3. Normal control (NC)	34

IFI44L, *CXCL10*, *GBP1*, *EPSTI1*, and *IFI27* mRNA expression levels were analyzed by TaqMan® gene expression assays Hs00199115_m1, Hs00171042_m1, Hs0026671_m1, Hs01566789_m1, and Hs00271467_m1, respectively (Life Technologies), and TaqMan Gene Expression Master Mix (Life Technologies). A TaqMan human *ACTB* (beta actin) endogenous control (Life Technologies) was used as an internal control. The mRNA expression levels of the interested and *ACTB* genes were quantified by a StepOnePlus™ Real Time PCR System and analyzed by using StepOne™ Software Version 2.1 (Life Technologies), as manufacturer's instruction. A comparative threshold cycle (C_T) method was used to determine the gene expression levels [13], by comparing the values of PBMC and lymph nodes from NHL patients with the median gene expression levels in 10 healthy donors and 4 patients with reactive lymphadenopathy, respectively. All experiments were carried out in triplicate.

Serum Concentration of CXCL10

Serum concentrations of CXCL10 (also known as IP-10) were measured by BD™ Human Chemokine Cytometric Bead Array Kit (BD Biosciences, NJ, USA) and an EPICS XL flow cytometer (Beckman Coulter, CA, USA) as manufacturers' instructions.

Statistical Analysis

Computation was carried out by using a statistical software; JMP version 8.0 (SAS Institute), according to JMP 8 Statistics and Graphics Guide, Second Edition. When we analyzed the data obtained by RT-PCR, logarithmically transformed values were used, because they showed log-normal distribution. Correlation coefficient was determined by Pearson's method. Statistical differences of the values of gene expression levels of PBMC and lymph nodes, and serum CXCL10 concentrations between HNL and controls were analyzed by Dunnett's test. Statistical difference of the serum CXCL10 concentrations between symptomatic and convalescent phases of HNL patients was analyzed by paired t-test. Canonical discrimination analysis was performed to distinguish HNL from disease controls and healthy donors by using the log-value of relative gene expression levels. The model was determined by stepwise procedure.

Results

Microarray Analysis

By microarray analysis of the mRNAs from PBMC in HNL patients, disease controls and healthy controls, one hundred and thirty seven up-regulated genes in HNL patients were

Table III Microarray analysis of peripheral blood mononuclear cells from patients with HNL, SoJIA, KD, and a healthy donor

The difference of mean gene expression levels between 2 HNL patients, 5 SoJIA patients, 3 KD patients and a healthy control donor in microarray analysis is given. One hundred and thirty-seven genes that showed more than two-fold expressional differences between HNL patients and SoJIA patients, KD patients as well as a healthy donor were selected, and the top 10 genes are listed

Gene name	Synonyms	Fold differences between a healthy donor		
		HNL	SoJIA	KD
Interferon-induced protein 44-like	*IFI44L*	12.99	3.32	0.39
Chemokine (C-X-C motif) ligand 10	*CXCL10*	12.04	2.21	0.56
Guanylate binding protein 1, interferon-inducible, 67 kDa	*GBP1*	8.30	2.51	1.12
Epithelial stromal interaction 1 (breast)	*EPSTI1*	7.10	3.01	1.05
Interferon, alpha-inducible protein 27	*IFI27*	6.90	2.14	1.05
Tumor necrosis factor (ligand) superfamily, member 10	*TNFSF10*	6.36	2.84	1.87
Immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides	*IGJ*	6.26	2.08	2.29
Interferon-induced protein 44	*IFI44*	5.59	1.97	0.96
Interferon-induced protein with tetratricopeptide repeats 3	*IFIT3*	5.29	2.31	0.40
Proteasome (prosome, macropain) activator subunit 2 (PA28 beta)	*PSME2*	4.87	2.32	0.85

identified (data not shown), and the top 10 genes are shown in Table III. Nine of these genes, other than *IGJ*, were interferon-stimulated genes (ISGs) [14].

Quantitative RT–PCR Analysis

The 5 most up-regulated genes (*IFI44L*, *CXCL10*, *GBP1*, *EPSTI1*, and *IFI27*) in HNL patients were analyzed by RT-PCR to confirm the microarray data. As shown in Fig. 1, the expression levels of the 5 genes in HNL patients were significantly higher than those in normal controls. Gene expression profile of the relapsing patients was not significantly different from that of patients without relapse (data not shown). Interestingly, positive correlations were observed in the expression levels among the 5 genes (r^2=0.28–0.60 : Fig. 2), suggesting the up-regulation of these 5 genes by some common mechanism, possibly through the stimulation of type 1 IFNs. Although the expression levels of these genes in HNL patients were higher than those in normal controls, bacterial infections, and lymphoid malignancy, the expression levels of each gene were not specific for HNL: these were equivalent to or less than those of viral infections and SLE (Fig. 1).

Analysis with Lymph Node and Serum Samples of HNL Patients

We then investigated the expression levels of these ISGs in involved lymph nodes by quantitative PCR. The genes were expressed significantly higher in HNL than in reactive lymphadenopathy (Fig. 3). Next, we analyzed the serum concentration of CXCL10. Although serum CXCL10 concentrations of HNL patients were not significantly different from those of KD, IM, and purulent lymphadenitis patients (Fig. 4a), the CXCL10 levels at symptomatic phase diminished at convalescent phase in HNL patients (Fig. 4b).

Discriminate Analysis

For the purpose of classifying HNL based on the gene expression profile, we performed canonical discrimination analysis by using the log-value of relative gene expression levels of the 5 ISGs. The subjects were separated into 3 groups as shown in Table II for discriminate analysis. In the stepwise procedure, all 5 genes were selected as parameter. The canonical discriminate analysis was able to classify the subjects into 3 groups with 84.2 % accuracy (misclassified number: 24). The area under the curve (AUC) of HNL group was 0.975 (Fig. 5a and Table IV). The scoring coefficients in canonical plot were as follows:

$$\text{Canonical 1} = -0.1947\text{IFI44L} - 0.2058\text{CXCL10} + 0.4870\text{GBP1} - 0.1620\text{EPSTI1} + 0.2197\text{IFI27},$$

$$\text{Canonical 2} = 0.2485\text{IFI44L} + 0.2488\text{CXCL10} - 0.2387\text{GBP1} - 0.2802\text{EPSTI1} + 0.1598\text{IFI27}$$

In the 2 group model (HNL and disease control), top 3 genes (*IFI44L*, *CXCL10*, and *GBP1*) were selected as parameters and the statistical power was equal to 3 group model; the accuracy and AUC were 82.2 % (misclassified number: 21) and 0.942, respectively (Fig. 5b and Table IV). The scoring coefficients in canonical plot were as follows:

$$\text{Canonical 1} = -0.2632\text{IFI44L} - 0.3061\text{CXCL10} + 0.5101\text{GBP1}$$

Discussion

In this study, we observed that ISGs (*IFI44L*, *CXCL10*, *GBP1*, *EPSTI1*, and *IFI27*) were up-regulated in PMBC as well as in involved lymph nodes of HNL patients (Table III, Fig. 1). The discriminate analysis showed that the expression levels of these genes were specific for HNL patients (Fig. 5).

The type I IFN response protects a host against the invasion of viral pathogens. The cellular factors mediating this defense are the products of the ISGs [14]. The involved lymph nodes of HNL are primarily composed of pDCs, histiocytes and T lymphocytes, and pDCs are known to be one of the major producers of type I IFNs [1, 2]. It is reported that CXCL10 and IL-18 were expressed in histiocytes, and CXCR3 and IFN γ were expressed in T lymphocytes by immunohistochemical staining, which suggested that the cytokine and chemokine pathways play important roles in the pathophysiology of HNL [15]. Elevated serum levels of CXCL10 (Figs. 1 and 4), as well as IFN-γ and IL-6 [11] suggest a systemic inflammation in HNL, which possibly contributed to the gene expression profile of PBMC. It is supposed that pDCs were the major producer of the type 1 IFNs which induced ISGs

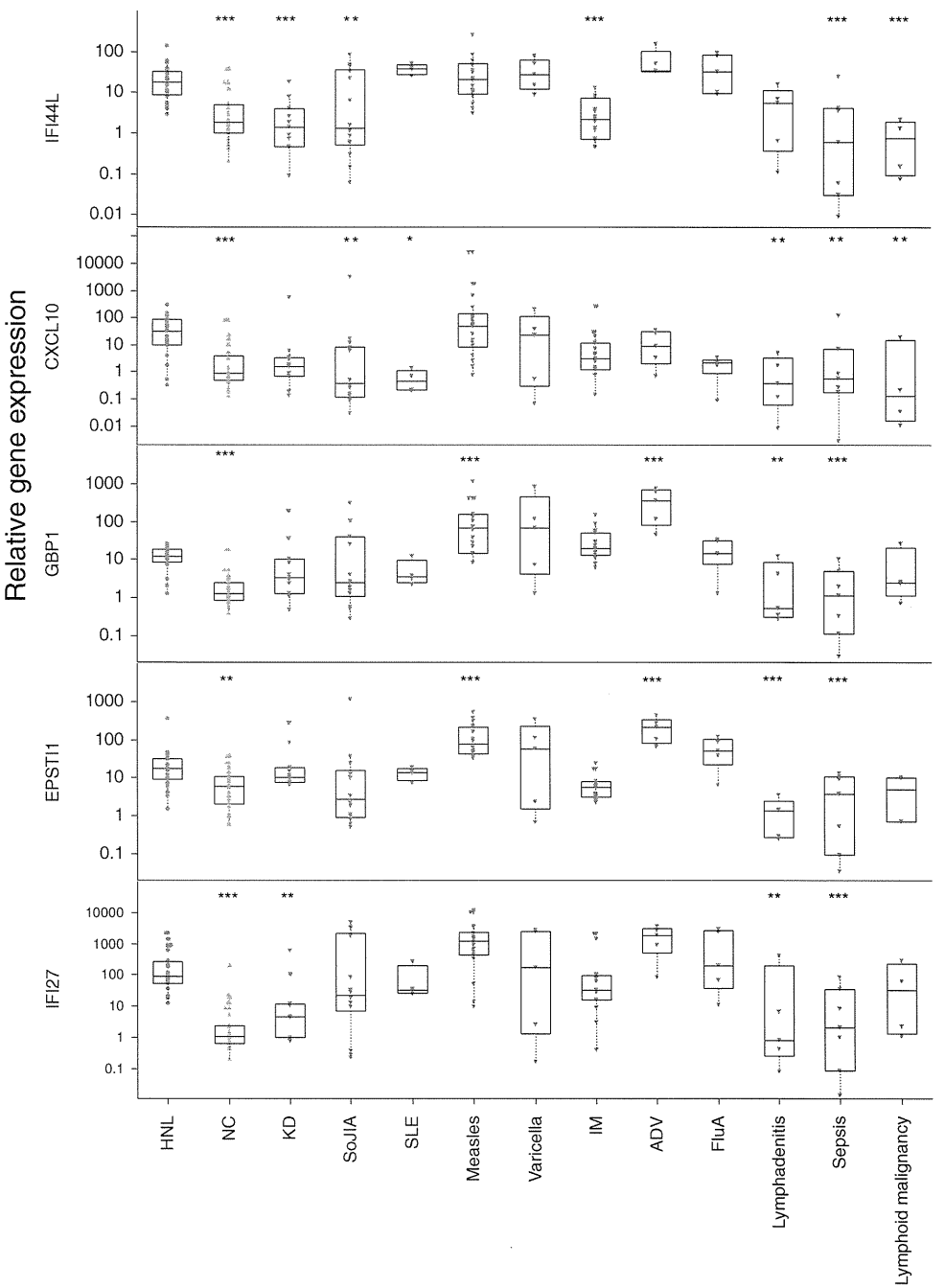

Fig. 1 Relative gene expression levels of mRNA from PBMC. The form of boxplot is as follows. The *bottom* and *top* of the box are the 25th percentile and the 75th percentile points, respectively. The *line* inside the box is the median. Each whisker extends up to 1.5 interquartile ranges from the end of the box. *$p<0.05$, **$p<0.01$, ***$p<0.001$

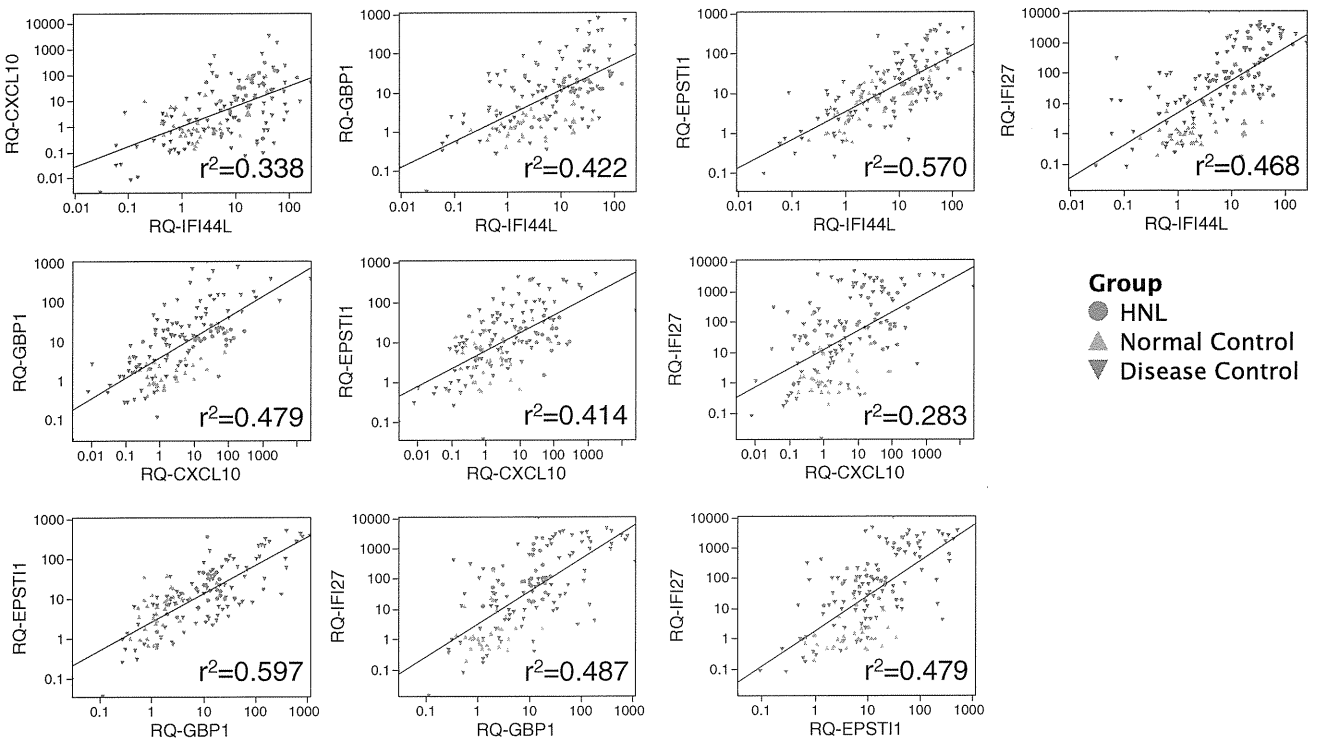

Fig. 2 Scatter plot of gene expression levels of 5 ISGs. The line is least-squares fit to data. *r2* correlation coefficient, *RQ* relative quantitation value

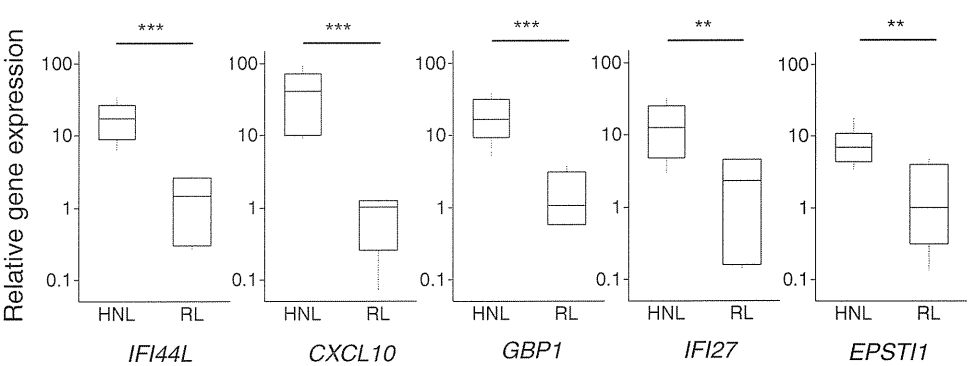

Fig. 3 Relative gene expression levels of mRNA from lymph nodes of patients with HNL and reactive lymphadenopathy. The form of box-plot is the same as Fig. 1. *RL* reactive lymphadenopathy $^{**}p<0.01$, $^{***}p<0.001$

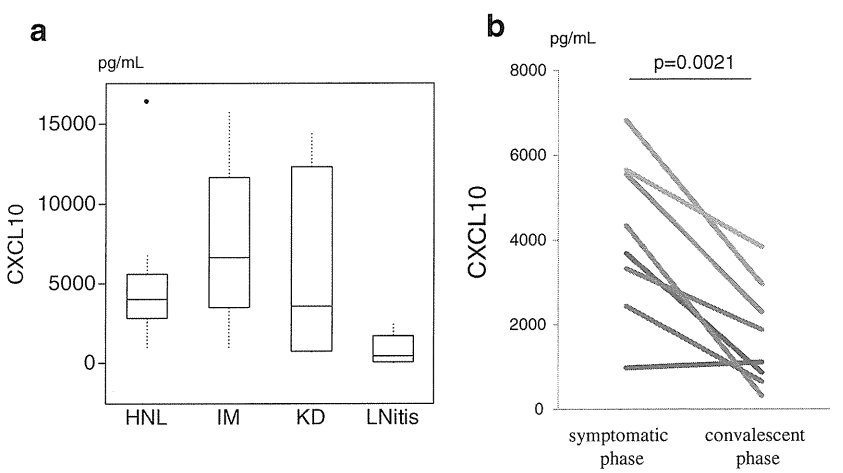

Fig. 4 Serum concentration of CXCL10 in HNL patients. **a** Serum concentration of CXCL10 at acute phase of HNL, IM, KD and LNitis. The form of box-plot is the same as Fig. 1. There was no significant difference between HNL (n=12) and disease control; IM (n=4), KD (n=5), and LNitis (n=5). **b** Diminishing serum CXCL10 concentration at convalescent phase of HNL (n=8)

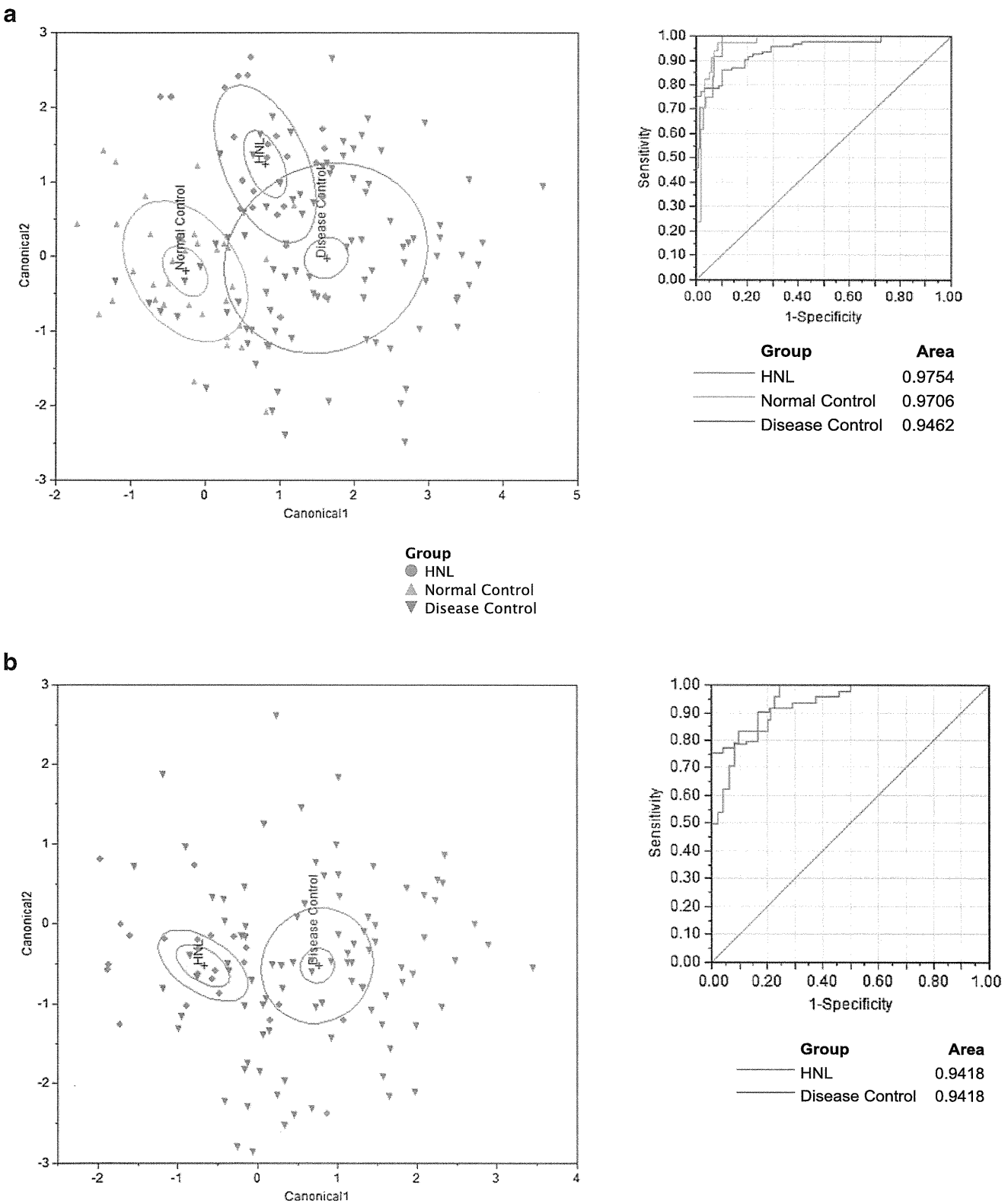

Fig. 5 Canonical plot and ROC curve in 3 groups; HNL (n=24), disease control (n=93), and normal control (n=34) (**a**), and in 2 groups; HNL and disease control (**b**). The inner and outer ellipses show 95 % and 50 % confidence regions, respectively

expression in this disease. On the other hand, these cytokines themselves were not highly up-regulated in PMMC of HNL patients in this study (data not shown). A paucity of pDCs in PBMC would account for it [16].