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Investigação de imunodeficiências primárias em pacientes com lúpus eritematoso sistêmico juvenil

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Programa de: Pediatria

Orientadora: Prof. Dra. Magda Maria Sales Carneiro-Sampaio

São Paulo
2011

Dados Internacionais de Catalogação na Publicação (CIP)

Preparada pela Biblioteca da
Faculdade de Medicina da Universidade de São Paulo

Jesus, Adriana Almeida de
Investigação de imunodeficiências primárias em pacientes com lúpus eritematoso sistêmico juvenil / Adriana Almeida de Jesus. -- São Paulo, 2011.
Tese(doutorado)--Faculdade de Medicina da Universidade de São Paulo. Programa de Pediatria.
Orientadora: Magda Maria Sales Carneiro-Sampaio.

Descritores: 1.Lúpus eritematoso sistêmico juvenil 2.Síndromes de imunodeficiência 3.Via clássica do complemento 4.Imunoglobulinas 5.Complemento C1q 6.Anticorpos antinucleares

USP/FM/DBD-086/11

“Não sejas o de hoje. Não suspires por ontens. Não queiras ser o amanhã. Faz-te sem limites no tempo.”

Cecília Meireles

DEDICATÓRIA

Aos meus pais, Lourdes e Adelson, e aos meus irmãos, Bruno e Fabio, minha gratidão pelo exemplo de ética e perseverança na busca pelo alcance dos meus sonhos.

AGRADECIMENTOS

Aos pacientes portadores de lúpus eritematoso sistêmico juvenil e suas famílias.

À Prof. Dra. Magda Carneiro-Sampaio por todos os incontáveis ensinamentos durante a construção deste trabalho e na minha vida acadêmica.

À Dra. Bernadete Liphaus pela importante co-orientação na concretização deste trabalho.

Ao Prof. Dr. Clovis Artur Almeida da Silva, pelo apoio fundamental na realização deste trabalho, por me trazer desafios a cada dia, pelo incentivo e confiança e pelo exemplo de dedicação e generosidade.

Aos meus tios Dra. Amélia Maria Ribeiro de Jesus e Dr. Roque Pacheco de Almeida, pelo incentivo contínuo desde a iniciação científica.

Às colegas da Unidade de Reumatologia Pediátrica do Instituto da Criança: Dra. Adriana Sallum, Dra. Lúcia Campos e Dra. Nádia Aikawa, com quem tenho o prazer de conviver diariamente, pela compreensão e apoio em todos os momentos.

Aos pós-graduandos e residentes da Unidade de Reumatologia Pediátrica do Instituto da Criança, pelo companheirismo na aprendizagem da reumatologia.

Ao Dr. Luis Eduardo Coelho Andrade, pelas valiosas contribuições na elaboração deste estudo, por viabilizar a dosagem de C1q e C2 e a determinação do número de cópias de *C4*.

Ao Prof. António Coutinho pelas importantes contribuições na idealização deste trabalho.

À Dra. Kaline Medeiros pela realização da determinação do número de cópias de *C4*.

A Rogério Amaral pela dosagem de C2 por imunohemólise radial.

À Mariana Acenjo pela dosagem de C1q e C2 por imunodifusão radial.

A Sílvia Bando e Natália Umetsu pela realização da pesquisa mutacional em *C2*.

À Leuridan Torres pela determinação de C1r e C1s.

Ao Dr. Morton Sheinberg e ao Dr. Cristóvão Mangueira por viabilizarem a dosagem do anticorpo anti-C1q.

À bibliotecária do Instituto da Criança, Mariza Yoshikawa, pela amizade e pela ajuda na pesquisa bibliográfica.

A Nivaldo Rocha e Milene Aparecida Rocha, pela ajuda na confecção da tese.

Às secretárias da pós-graduação Adriana Bezerra e Mônica Souza, por todo o auxílio.

Aos colegas da equipe de Imunologia Pediátrica do Instituto da Criança, pela consultoria e pelo seguimento dos pacientes com imunodeficiências primárias.

A todos os funcionários do Instituto da Criança que estiveram envolvidos na minha aprendizagem durante os anos de convivência.

Aos verdadeiros amigos que fiz na infância, na adolescência, durante a faculdade e residência, pelo apoio e incentivo.

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São Paulo: Serviço de Biblioteca e Documentação; 2005.

Abreviaturas dos títulos dos periódicos de acordo com *List of Journals Indexed in Index Medicus.*

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ABREVIATURAS

LES	Lúpus eritematoso sistêmico
LESJ	Lúpus eritematoso sistêmico juvenil
IDP	Imunodeficiência primária
APECED	Autoimmune-Polyendocrinopathy-Candidiasis-Ectodermal-Dystrophy
IPEX	Immunedysregulation-Polyendocrinopathy-Enteropathy X-linked syndrome
ALPS	Síndrome linfoproliferativa autoimune
Ig	Imunoglobulina
DGC	Doença granulomatosa crônica
ACR	Colégio Americano de Reumatologia
DM1	Diabetes mellitus tipo 1
SLEDAI-2K	Systemic Lupus Erythematosus Disease Activity Index 2000
SLICC/ACR-DI	Systemic Lupus International Collaborating Clinics/ACR-Damage Index
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
D	Deficiência
IDCV	Imunodeficiência comum variável
P	Paciente
Treg	Células T regulatórias

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RESUMO

Jesus AA. *Investigação de imunodeficiências primárias em pacientes com lúpus eritematoso sistêmico juvenil* [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2011. 46p.

Objetivos: Os objetivos deste estudo foram: avaliar a frequência de imunodeficiências primárias de anticorpos e Complemento em pacientes com lupus eritematoso sistêmico juvenil (LESJ); avaliar possíveis associações entre a presença de imunodeficiência primária (IDP) e dados demográficos, ocorrência de infecções, manifestações clínicas, atividade da doença, dano cumulativo e terapêutica direcionada ao LESJ; e determinar a frequência do anticorpo anti-C1q, estabelecendo a sua especificidade, sensibilidade e valores preditivos para o diagnóstico de LESJ. **Métodos:** Setenta e dois pacientes com LESJ foram avaliados para a determinação dos níveis séricos de imunoglobulinas (IgG, IgA, IgM e IgE) e subclasses de IgG, e dos componentes iniciais da via clássica do sistema Complemento (C1q, C1r, C1s, C4, C2, C3). Sessenta e sete pacientes e 26 controles saudáveis foram avaliados para a presença do anticorpo anti-C1q. O número de cópias do gene C4 foi determinado por PCR (reação de polimerase em cadeia) em tempo real nos pacientes com deficiência de C4. Setenta pacientes foram avaliados para a presença de deficiência de C2 tipo I. **Resultados:** Evidência de IDP foi identificada em 16 pacientes (22%): 3 com deficiência (D) de C2, 3 com C4D, 2 com C1qD, 4 com IgG2D (<20mg/dL), 3 com IgAD (<7mg/dL), e 3 com IgMD (<35mg/dL); um destes pacientes apresentou deficiência concomitante de IgA, C4 e C2. Quatro dos 13

pacientes do sexo masculino (30%) e 12 das 59 pacientes do sexo feminino (20%) apresentaram diagnóstico de IDP. As características clínicas de LES não diferiram entre os pacientes com e sem IDP. A mediana do SLICC/ACR-DI foi maior entre os pacientes com IDP (p=0,0033), assim como a frequência de SLICC/ACR-DI>1 (p=0,023). Os grupos também foram semelhantes quanto à ocorrência de infecção e terapêutica utilizada para o LESJ. Os únicos dois casos de LESJ com idade de início antes dos 2 anos apresentaram C1qD e IgMD, respectivamente. Para o diagnóstico de LESJ, o anticorpo anti-C1q apresentou especificidade de 100% (IC 86.7-100%), sensibilidade de 19.4% (IC 10.7-30.8%), valor preditivo positivo de 100% (IC 75.3-100%) e valor preditivo negativo de 32,5% (IC 22,4-43,9%). **Conclusões:** Foi observada uma elevada frequência de imunodeficiências de anticorpos e Complemento nos pacientes com LESJ, sugerindo que esses defeitos podem contribuir para a patogênese do lúpus. Esses achados indicam que os dois grupos de IDPs devem ser investigados em pacientes com LES de início precoce e de maior gravidade.

Descritores: 1.Lúpus eritematoso sistêmico juvenil 2.Síndromes de imunodeficiência 3.Via clássica do Complemento 4.Imunoglobulinas 5.Complemento C1q 6.Anticorpos antinucleares

SUMMARY

Jesus AA. *Primary immunodeficiencies in juvenile systemic lupus erythematosus patients* [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2011. 46 p.

Objectives. The objectives of this study were: to establish the frequency of primary immunoglobulin and Complement deficiency in Juvenile SLE (JSLE); to evaluate possible associations between the presence of primary immunodeficiency and demographic data, occurrence of infections, JSLE clinical manifestations, disease activity, cumulative damage and therapy; and to determine the frequency of anti-C1q antibody, establishing its sensitivity, specificity and predictive values for JSLE diagnosis. **Methods.** Seventy-two JSLE patients were analyzed for serum levels of immunoglobulin classes (IgG, IgA, IgM e IgE) and IgG subclasses and early components of the classical Complement pathway (C1q, C1r, C1s, C4, C2, C3). Sixty-seven patients and 26 healthy controls were evaluated for the presence of anti-C1q antibody. C4 gene copy number was determined by real time PCR (polymerase chain reaction) in C4 deficient patients. Seventy patients were analyzed by PCR for the presence of type I C2 deficiency. **Results.** Evidence of PID was identified in 16 patients (22%): 3 with C2 deficiency (D), 3 with C4D, 2 with C1qD, 4 with IgG2D (<20mg/dL), 3 with IgAD (<7mg/dL), and 3 with IgMD (<35mg/dL); one of these patients presented concomitant IgA, C2 and C4 deficiency. Four out of the 13 boys (30%) and 12 out of 59 girls (20%) had PID diagnosis. SLE features did not differ between patients with and without PID. The median SLICC/ACR-DI was higher among PID subjects

(p=0.0033), as was the frequency of SLICC/ACR-DI>1 (p=0.023). Both groups did not differ regarding the occurrence of infections and therapeutic for JSLE. The only 2 cases with age of onset below 2 years presented C1qD and IgMD, respectively. For JSLE diagnosis, the anti-C1q antibodies presented a specificity of 100% (CI 86.7-100%), sensitivity of 19.4% (CI 10.7-30.8%), positive predictive value of 100% (CI 75.3-100%) and negative predictive value of 32,5% (CI 22,4-43,9%). **Conclusions.** A high frequency of immunoglobulin and Complement deficiency was observed in this JSLE series, suggesting that these defects may contribute to lupus development. Our findings indicate that these two groups of PID should be investigated in early-onset and severe lupus.

Descriptors: 1.Juvenile systemic lupus erythematosus 2.Immunologic deficiency syndromes 3.Classical Complement pathway 4.Immunoglobulins 5.Complement C1q 6.Antinuclear antibodies

1 INTRODUÇÃO

O lúpus eritematoso sistêmico (LES) juvenil (LESJ) é uma doença autoimune sistêmica, caracterizada por alta complexidade, tanto quanto à diversidade de suas manifestações clínicas quanto em relação à sua etiopatogenia. O LESJ é causado pela interação de múltiplos fatores genéticos com diversos fatores ambientais, sendo considerado um protótipo de doença autoimune [1,2].

As imunodeficiências primárias (IDP) representam entidades monogênicas com defeito genético estabelecido em sua maioria [3]. A auto-imunidade é a segunda manifestação mais frequente das IDPs, ficando somente após a susceptibilidade a infecções. A frequência de associação com autoimunidade pode variar de 0 a 100%, como em algumas IDPs caracterizadas pela marcante presença de manifestações autoimunes [3,4]. As IDPs mais frequentemente associadas à autoimunidade são: *Autoimmune- Polyendocrinopathy- Candidiasis- Ectodermal- Dystrophy* (*APECED*) (causada por mutações em *AIRE*), *Immunedysregulation- Polyendocrinopathy- Enteropathy X-linked syndrome* (*IPEX*) (causada por mutações em *FOXP3*) (ainda sem denominações consagradas na língua portuguesa), síndrome de Omenn, síndrome linfoproliferativa autoimune (ALPS) e deficiência homozigótica de C1q [2,4,5].

Três grupos de IDPs podem ser considerados como frequentemente associados mais especificamente à LES ou a manifestações da doença, tais como: a) deficiência homozigótica das primeiras frações da via clássica do sistema Complemento [6,7]; b) deficiência parcial ou seletiva de imunoglobulinas, principalmente de IgA [8,9] e raramente de IgM [10,11], de subclasse IgG2 [12,13] e imunodeficiência comum variável [14, 15]; c) doença granulomatosa crônica (DGC) [16,17]. Em contraste, em algumas IDPs nas quais manifestações autoimunes órgão-específicas são frequentes (*APECED*, *IPEX*, *ALPS*, síndrome Wiskott-Aldrich e síndrome de hiper IgM), não há associação com LES ou suas manifestações [18-22].

A deficiência congênita completa de C1q, de herança autossômica recessiva, é o fator de risco genético que, isoladamente, está associado mais fortemente ao desenvolvimento de LES, com uma frequência desta doença ou de suas manifestações em 93% dos indivíduos com esta IDP [14,23]. A presença de deficiência de C1q em pacientes com LESJ determina doença de início precoce e forte associação com glomerulonefrite e vasculite de sistema nervoso central, bem como uma maior frequência de fotossensibilidade [23-25]. As peculiaridades clínicas do LES associado a essa deficiência genética têm sido um estímulo ao estudo das vias moleculares envolvidas na tolerância e no desenvolvimento de autoimunidade.

O C1q tem sido implicado na depuração de produtos celulares apoptóticos, o que determinaria um acúmulo de autoantígenos e à auto-imunidade. Foi demonstrado que o C1q se liga diretamente e de modo

específico à superfície de queratinócitos em apoptose, mesmo na ausência de anticorpos, determinando o seu clareamento e exercendo, desta forma, importante papel na autotolerância. A depuração dos corpúsculos apoptóticos mediada por C1q pode ocorrer por dois mecanismos distintos: o C1q ligado aos corpúsculos pode interagir diretamente com receptores de C1q na superfície de fagócitos ou pode mediar a ativação e deposição de C3 e C4 na superfície da célula apoptótica, levando à sua interação com receptores dessas frações do Complemento na superfície dos fagócitos [26-28]. Essa hipótese explica a acentuada fotossensibilidade como principal característica fenotípica de pacientes com LES relacionado à deficiência de C1q.

A importância do C1q na atividade do LES é evidenciada mesmo em indivíduos sem deficiência primária deste fator do Complemento. Anticorpos anti-C1q têm sido descritos em 20% a 40% dos pacientes com LES, especialmente durante períodos de atividade da doença. No contexto de uma depuração inadequada das células apoptóticas, o C1q ligado à superfície dos corpúsculos apoptóticos torna-se antigênico após exposição prolongada ao sistema imune. O anticorpo anti-C1q tem sido implicado na atividade da nefrite lúpica em pacientes adultos, visto que foi observada uma elevação dos seus níveis em períodos de atividade renal, bem como o seu decréscimo de acordo com a resposta ao tratamento [29-32]. Entretanto, a associação do anti-C1q e nefrite lúpica ainda não foi bem estabelecida no lúpus juvenil [33-35].

O estudo das IDPs, seja as fortemente associadas ao LES, como a deficiência de C1q, seja daquelas em que não há essa associação, pode ser muito útil para a compreensão da fisiopatologia desta complexa doença autoimune.

2 OBJETIVOS

1. Avaliar a frequência de imunodeficiências primárias do Complemento e imunoglobulinas em 72 pacientes com LESJ

2. Avaliar a possível associação entre dados demográficos, presença de infecções, manifestações da doença, atividade, dano cumulativo e terapia utilizada de acordo com a presença de IDP.

3. Avaliar a frequência do anticorpo anti-C1q e determinar sua sensibilidade, especificidade e valores preditivos para o diagnóstico de LESJ.

3 MÉTODOS

3.1 - Pacientes

Após a aprovação da Comissão de Pesquisa e Ética do Hospital das Clínicas (HC) da Faculdade de Medicina da Universidade de São Paulo (FMUSP) e assinatura pelos pais ou responsáveis do termo de consentimento informado dos pais ou responsáveis, foram avaliados 72 pacientes com LESJ em seguimento na Unidade de Reumatologia do Instituto da Criança do HC-FMUSP. Todos os pacientes preencheram critérios de classificação do Colégio Americano de Reumatologia (ACR) para o diagnóstico de LESJ [36] e eram submetidos mensalmente a avaliação clínica e laboratorial padronizada. A maioria dos pacientes estava recebendo tratamento imunossupressor para controle da doença (glicocorticóides e antimaláricos, em associação com azatioprina, ciclofosfamida endovenosa ou micofenolato mofetil). Todos os pacientes eram regularmente investigados quanto à presença de parasitose intestinal.

3.2 - Avaliação dos prontuários médicos

Os prontuários médicos dos 72 pacientes foram avaliados para o registro de dados demográficos (idade de início dos sintomas de LESJ,

idade de diagnóstico de LESJ, gênero e etnia) e história familiar de IDP e doença autoimune. Foram registradas as manifestações clínicas específicas de LESJ: envolvimento cutâneo, mucoso, renal, neuropsiquiátrico, cardiovascular, pulmonar, hematológico, articular e gastrintestinal; bem como a presença de síndrome antifosfolípide.

Doenças autoimunes órgão-específicas (tiroidite, hepatite autoimune, diabetes mellitus tipo 1 – DM1, doença celíaca, gastrite autoimune e cirrose biliar primária) também foram investigadas de acordo com características clínicas e determinação do específico auto-anticorpo: antitireoglobulina, antiperoxidase e antirreceptor de tireotropina; antimúsculo liso, antimicrossomal de fígado e rim tipo 1 e anticitosol hepático; anti-insulina e antidescarboxilase do ácido glutâmico; antiendomísio; anticélula parietal; antimitocondrial.

Os prontuários médicos foram também rastreados para a ocorrência de infecções: infecções piogênicas de repetição, micobacteriose, infecções fúngicas, herpes zoster e presença de sepse grave, definida de acordo com as definições do consenso internacional de sepse [37]. Para avaliação da atividade e dano cumulativo da doença foram determinados: *Systemic Lupus Erythematosus Disease Activity Index 2000* (SLEDAI-2K) [38] e *Systemic Lupus International Collaborating Clinics/ACR-Damage Index* (SLICC/ACR-DI), respectivamente, em cada paciente, no momento da coleta de sangue [39]. A atividade atual da doença foi arbitrariamente definida como SLEDAI-2K ≥4. Dados relativos aos tratamentos do LESJ foram: uso de prednisona, cloroquina, imunossupressores (azatioprina, metotrexate, ciclosporina,

ciclofosfamida endovenosa e micofenolato mofetil) e imunoglobulina intravenosa.

3.3 - Dosagem sérica de Complemento e imunoglobulinas

O nível sérico de C1q e C2 foi determinado por imunodifusão radial (The Binding Site, Birmingham, UK). Níveis baixos de C1q ou C2 foram confirmados em nova amostra por imunodifusão radial e imuno-hemólise, respectivamente [40]. Níveis séricos de C3 e C4 eram mensurados mensalmente como parte da rotina para pacientes lúpicos por nefelometria (Dade Behring/Siemens, Deerfield, USA). A presença de C1r e C1s foi avaliada no soro pela técnica de imunodifusão dupla (The Binding Site, Birmingham, UK). Uma vez que o consumo de proteínas do sistema Complemento é uma característica de atividade da doença no LES, deficiência de C2 ou C4 foi definida pela demonstração pelo menos duas vezes de níveis baixos de C2 ou C4 na presença de níveis normais de outras frações do Complemento em mais de uma amostra e SLEDAI-2K <4 no momento da coleta de sangue. O achado de baixo número de cópias do gene *C4* ou a presença de deficiência de C2 tipo I também foram considerados suficientes para o diagnóstico de deficiência de C4 e C2, respectivamente. Os métodos utilizados para a determinação do número de cópias de *C4* e de deficiência de C2 tipo 1 serão descritos posteriormente. Deficiência de C1q (C1qD) foi definida como a combinação de C1q

indetectável e níveis normais de outros componentes do Complemento em duas ou mais amostras.

Níveis séricos de IgG, IgA e IgM foram determinados por imunoturbidimetria (Roche Diagnostics, Indianapolis, USA), enquanto que os níveis de IgE e de subclasses de IgG1 e IgG2 foram avaliados por nefelometria (Dade Behring/Siemens, Deerfield, USA). Diagnóstico de deficiência seletiva de imunoglobulina foi estabelecido apenas em pacientes acima de 10 anos de idade e com níveis normais ou altos das outras classes de imunoglobulinas numa mesma amostra. O valor abaixo do qual foi definida deficiência de IgA (IgAD) foi 7 mg/dL [41]; para deficiência de IgM (IgMD) foi 35 mg/dL [42] e para deficiência de IgG2 foi 20 mg/dL.

Todos os resultados de imunoglobulinas e Complemento abaixo dos limites da normalidade para a faixa etária foram confirmados em uma ou mais amostras em laboratório clínico (Laboratório Fleury) certificado pelo Colégio Americano de Patologistas (CAP).

3.4 - Dosagem sérica do anticorpo anti-C1q

O nível sérico do anticorpo anti-C1q foi determinado em 67 pacientes com LESJ e 26 controles saudáveis em seguimento Unidade de Adolescentes do Instituto da Criança do HC-FMUSP. A determinação do anticorpo anti-C1q foi realizada pela técnica ELISA (enzyme-linked immunosorbent assay) (Inova Diagnostics - QUANTA Lite^{TM} Anti-C1q, San Diego, California, USA)

em amostras de soro em duplicata. O protocolo do fabricante foi estritamente seguido e foi utilizado o *cut-off* de 20 unidades.

3.5 - Determinação do número de cópias de *C4*

O DNA genômico foi obtido de células mononucleares do sangue periférico dos pacientes com LESJ e C4D. O número de cópias do gene que codifica o C4 foi determinado por *polymerase chain reaction* (PCR) em tempo real quantitativo utilizando sondas Taqman ligadas a fendas de ligação menor (non-fluorescent quencher e 6-carboxifluoresceína) em um Rotor Gene 3000 (Corbett, Sidney, Australia) como descrito previamente [43]. A amplificação do DNA foi realizada utilizando os seguintes *primers* (*forward* e *reverse*): 5’-GCAGGAGACATCTAACTGGCTTCT-3' e 5'-CCGCACCTGCATGCTCCT-3'. A detecção do número de cópias foi realizada com utilização de sondas específicas para *C4A* (5’ FAM-ACCCCTGTCCAGTGTTAG-MGB 3’) e *C4B* (5' FAM-ACCTCTCTCCAGTGATAC-MGB 3') em reações simultâneas independentes. Amostras de DNA com número de cópias de *C4A* e *C4B* conhecidas foram gentilmente cedidas pelo Dr. A. Szilagyi, da Hungria.

3.6 - Detecção de deficiência de C2 tipo I

Setenta pacientes foram avaliados para C2D tipo 1, que é a causa mais frequente de deficiência hereditária de C2. C2D tipo 1 é causada por uma deleção de 28 pares de base (pb) que remove 9 pb do final 3’ do exon 6 e 19

pb do final 5'do intron 6, levando à deleção completa do exon 6 e falência da síntese da proteína C2 [44]. DNA genômico foi amplificado por PCR para detecção da deleção de 28 pb do gene do C2. Os primers: C2-F:5´-GCCTGGGCCGTAAAATCCA-3´; C2-R:5´-GCACAGGAAGGCCTCTGCTGCAGGC-3´ foram desenhados como descrito previamente [45]. Um *primer* reverso foi sintetizado utilizando a sequência de nucleotídeos do gene do C2 (GenBank accession N° NG_011730, in www.ncbi.nlm.nih.gov/nuccore/225903390) para confirmação dos resultados: C2-R2: 5´-TTGGAGGAGGTGGGCTGTGAG -3´. O sequenciamento foi realizado utilizando Dyenamic ET Dye Terminators kit em MegaBace1000 (GE Healthcare, Amersham, UK). Os *primers* utilizados foram C2-F e C2-R2.

3.7 - Análise estatística

Pacientes portadores de LESJ com e sem IDP foram comparados em relação às manifestações clínicas, atividade (SLEDAI-2K) e gravidade e dano cumulativo (SLICC-ACR/DI) da doença, bem como para a ocorrência de infecções. Variáveis contínuas foram analisadas por teste de Mann Whitney e variáveis categóricas por teste exato de Fisher. Pacientes e controles saudáveis foram também comparados para a presença do anticorpo anti-C1q, assim como foi determinada associação estatística entre o anticorpo anti-C1q e atividade da doença (SLEDAI-2K) e presença de

nefrite lúpica. O coeficiente de Spearman foi utilizado para avaliar a correlação entre os níveis de anticorpo anti-C1q e anti-dsDNA e valores do SLEDAI-2k. Foram também determinados a sensibilidade, especificidade, valor preditivo positivo e negativo do anticorpo anti-C1q para o diagnóstico de LESJ. Valor de $p < 0,05$ foi considerado significante.

4 RESULTADOS

4.1 - Identificação de IDP em pacientes com LESJ (Tabela 1).

A idade dos 72 pacientes com LESJ avaliados (59 do sexo feminino e 13 do sexo masculino) variou de 1 a 16 anos ao diagnóstico da doença. Evidência de IDP foi encontrada em 16 pacientes (22%). Sete pacientes apresentaram deficiência de Complemento: 2 com C1qD completa (Figura 1a), 3 com C4D (Figura 1b), e 3 com C2D (Figura 1c). Dez pacientes apresentaram evidência de deficiência de imunoglobulinas: 3 com IgAD seletiva (Figura 2a), 3 com IgMD (Figura 2b), e 4 com IgG2D (Figura 2d). Uma paciente (P9) apresentou simultaneamente IgAD, C4D e C2D. Quatro dos 13 pacientes do sexo masculino (30%) apresentaram evidência de IDP: 1 C2D, 1 IgG2D, 1 C1qD, e 1 IgMD. Os pacientes com C1qD e IgMD foram os únicos casos que desenvolveram manifestações clínicas de LESJ antes dos 2 anos de idade. Doze dos 59 pacientes do sexo feminino (20%) apresentaram evidência de IDP: 3 C4D, 1 C1qD, 2 C2D, 3 IgAD, 3 IgG2D e 2 IgMD. Uma das pacientes apresentava concomitantemente C4D, C2D e IgAD. Todos os pacientes com C2D eram de etnia mista: afro-americana, indígena e hispânica. Nenhum paciente apresentou deficiência de C1r ou C1s ou níveis persistentemente

baixos de C3. Embora 6 pacientes tenham apresentado níveis de IgG abaixo do percentil 3 para a idade (Figura 2c), nenhum deles foi classificado como deficiência primária de IgG. Além disso, nenhum paciente apresentou níveis de IgG abaixo de 500 mg/dL, não sendo então considerado o diagnóstico de imunodeficiência comum variável (IDCV). Níveis simultaneamente baixos de IgA e IgG2 também não foram encontrados. Os 4 pacientes com IgG2D apresentaram níveis absolutos de IgG2 de 3, 4, 5 e 16 mg/dL, resultando em razões IgG2/IgG muito baixas: 0,3%, 0,7%, 0,3% e 2%.

Os 3 pacientes com IgMD apresentaram níveis absolutos de IgM de 23, 29 e 35 mg/dL, e razão IgM/IgG de 1,7%, 2,3% e 1%, respectivamente. A média das razões considerando todos os pacientes foi de 18,7±7,4% para IgG2/IgG, e 9,4±5,7% para IgM/IgG. Nenhum dos pacientes com evidência de deficiência de imunoglobulina estava recebendo medicações anti-convulsivantes ou utilizou rituximabe. Nenhum dos pacientes apresentou evidência clínica ou história familiar sugestiva de DGC.

Os níveis de IgE foram determinados em 68 pacientes, 30 (44%) dos quais apresentaram níveis acima de 100 UI/mL. Não foram encontradas parasitoses intestinais em nenhum deles. Quinze dos 72 pacientes apresentaram sintomas de alergia respiratória, 8 dos quais apresentaram níveis altos de IgE, 3 destes com dermatite atópica associada. Em relação aos pacientes sem manifestação de atopia, 2 apresentaram IgE acima de 1000 UI/mL e 5 apresentaram níveis de IgE entre 500 e 1000 UI/mL.

4.2 - Comparação entre pacientes lúpicos com e sem evidência de IDP (Tabela 2).

Trinta por cento (4/13) dos pacientes do sexo masculino e 20% (12/59) dos pacientes do sexo feminino apresentaram diagnóstico de IDP (p=0,74). Os grupos de pacientes com e sem IDP não diferiram quanto à idade de início dos sintomas de LESJ [mediana 9 (1 - 15) vs. mediana 9 (2 - 16) anos, p=0,43]. Entretanto, os dois pacientes com lúpus de início abaixo de 2 anos apresentaram IDP. As frequências de manifestações muco-cutâneas, renais, neuropsiquiátricas, cardiovasculares, pulmonares, hematológicas, articulares e gastrintestinais foram semelhantes entre os dois grupos (p>0,05).

Adicionalmente, os dois grupos não diferiram na frequência de síndrome antifosfolípide (0% vs. 7%, p=0,56), nem na evidência clínica ou laboratorial de autoimunidade órgão-específica (42% vs. 37%, p=0,78). Os níveis séricos de anticorpo anti-dsDNA [mediana 6 (0 – 33) vs mediana 5 (0 - 20), p=0,38] e o valor do SLEDAI-2K [mediana 6 (0-33) vs. 5 (0-20), p=0,14] também foram semelhantes nos dois grupos. O grupo de pacientes com IDP apresentou mediana do SLICC/ACR-DI significantemente maior que o grupo de pacientes sem IDP [mediana 1.5 (0 - 5) vs. 0 (0 – 3), p=0,0033]. A frequência de SLICC/ACR-DI ≥ 1 também foi significantemente maior no grupo com IDP (81% vs. 48%, p=0,023). Pacientes com IDP não apresentaram frequência significantemente maior de sepse grave quando comparados com pacientes sem IDP (31% vs. 7.5%, p=0,21). Além disso, a frequência de infecções piogênicas de repetição, micobacteriose, infecções

fúngicas ou herpes zoster não diferiu estatisticamente entre os dois grupos. Durante o período de seguimento, óbito ocorreu em 3 dos 16 pacientes com IDP (18%) e em apenas 1 dos 59 pacientes sem IDP (1,7%) (p=0,11). Pacientes com e sem IDP não diferiram quanto à terapia utilizada para o tratamento do lúpus (ciclofosfamida endovenosa, micofenolato mofetil, azatioprina e imunoglobulina endovenosa).

Tabela 2 – Comparação entre paciente com LESJ com e sem IDP

Variáveis	Pacientes com IDP (n=16)	Pacientes sem IDP (n=56)	p
Sexo masculino n (%)	4 (31)	9 (69)	0,74
Idade de início do LESJ (anos) mediana (variação)	9 (1-15)	9 (2-16)	0,43
Idade atual (anos) mediana (variação)	15 (8-23)	16 (8-22)	0,32
SLICC/ACR-DI mediana (variação)	1,5 (0-5)	0 (0-3)	**0,0033**
SLICC/ACR-DI ≥ 1 n (%)	13 (81)	27 (48)	**0,023**
SLEDAI-2K mediana (variação)	6 (0-33)	5 (0-20)	0,38
SLEDAI-2K ≥ 4 n (%)	10 (62)	39 (69)	0,76
Manifestações clínicas e autoanticorpos no LESJ n (%)			
Cutâneas (eritema malar)	10 (63)	43 (77)	0,33
Nefrite	12 (75)	47 (84)	0,46
Artrite	14 (87)	44 (79)	0,72
Neuropsiquiátricas	5 (31)	31 (55)	0,15
Hematológicas	13 (81)	54 (96)	0,069
Serosite	7 (44)	19 (34)	0,55
Síndrome anti-fosfolípide	0 (0)	4 (7)	0,56
Anti-dsDNA (UI/mL)	39 (0-558)	33 (0-1000)	0,54
Anti-C1q (Unidades)	10 (6,2-127)	9,6 (5,6-103)	0,52
Ocorrência de infecções n (%)			
Bacterianas	10 (63)	31 (64)	0,77
Herpes zoster	5 (26)	12 (25)	0,50
Sepse grave	4 (31)	6 (7,5)	0,21
Óbito	3 (18)	3 (1,8)	0,11
Tratamento n (%)			
Ciclofosfamida	7 (52)	27 (47)	0,78
Micofenolato mofetil	4 (26)	10 (17)	0,49
Azatioprina	11 (68)	41 (69)	0,75
Imunoglobulina intravenosa	3 (26)	9 (13)	0,72

4.3 - Níveis de anticorpos anti-C1q em pacientes com LESJ e em controles saudáveis.

A média de idade no momento da coleta de sangue foi semelhante entre os pacientes e controles (14,6 ± 3,79 vs. 13,8 ± 2,56 anos, p=0,26), bem como a frequência de sexo feminino (83% vs. 73%, p=0,23). Uma maior frequência da presença do anticorpo anti-C1q foi observada nos pacientes com LESJ quando comparados aos controles (20% vs. 0%, p=0,016). Para o diagnóstico de LESJ, o anticorpo anti-C1q demonstrou especificidade de 100% (IC 86,7-100%), sensibilidade de 19,4% (IC 10,7-30,8%), valor preditivo positivo de 100% (IC 75,3-100%) e valor preditivo negativo de 32,5% (IC 22,4-43,9%). A mediana dos níveis do anticorpo anti-C1q também foi superior nos pacientes em relação aos controles [9,4 (5,5-127) vs. 7,3 (5-20) Unidades, p=0,004]. Por sua vez, a frequência (20% vs. 19%, p=1,0), e os níveis [9,9 (5-127) vs. 9,0 (5,6-30), p=0,62] do anticorpo anti-C1q foram semelhantes entre pacientes com e sem nefrite lúpica. Adicionalmente, pacientes com e sem atividade da doença (SLEDAI-2K<4) apresentaram frequências semelhantes do anticorpo anti-C1q (20% vs. 19%, p=1,0). Não houve diferença também nos níveis de anticorpo anti-C1q entre os pacientes com e sem doença ativa [10,85 (5,6-127) vs. 8,9 (5,5-34) Unidades, p=0,41]. Adicionalmente, o SLEDAI-2K foi semelhante em pacientes com e sem anticorpo anti-C1q [6,0 (0-18) vs. 4,0 (0-15), p=0,55]. Houve concordância entre anticorpo anti-C1q e anti-dsDNA em 61% dos pacientes e foi

observada uma correlação de Spearman positiva entre os níveis dos dois anticorpos (r=0,42, p=0,0004, IC=0,19-0,60).

4.4 - Características clínicas dos pacientes com deficiência de Complemento (Tabela 1 e Figura 1).

Os 2 pacientes com deficiência completa de C1qD (P1 e P2) apresentaram idade precoce de início do LESJ (1 e 5 anos de idade, respectivamente), fotossensibilidade acentuada, eritema malar intenso e lúpus subagudo. Enquanto a paciente P2 apresentou glomerulonefrite membranosa grave, o paciente P1 apresentou sintomas neuropsiquiátricos (mielite transversa, convulsões e psicose lúpica) e evoluiu para óbito aos 15 anos de idade. Não foram detectados anticorpos anti-C1q nos dois pacientes.

Uma das duas pacientes com deficiência isolada de C4 (P3) apresentou manifestações leves de LESJ, principalmente artrite e fotossensibilidade. A segunda paciente (P4) apresentou início precoce das manifestações de LESJ, com convulsões, serosite, anemia hemolítica e linfopenia acentuadas.

Dentre os 2 pacientes com deficiência isolada de C2, um paciente do sexo masculino (P5) apresentou C2 indetectável na presença de níveis normais de C1q, C3 e C4, e idade de início do LESJ de 7 anos. Ele apresentou quadro agudo de artrite, proteinúria, serosite e acidente vascular cerebral, e foi a óbito por meningite pneumocócica um mês após início dos sintomas. Neste paciente, que apresentou episódio prévio de meningite

pneumocócica aos 5 anos de idade, não foi possível realizar segunda dosagem de C2, e seu DNA não estava disponível para pesquisa genética de deficiência de C2 tipo I. A segunda paciente (P6) teve início da doença aos 6 anos, com alopécia, fotossensibilidade, úlceras orais, pericardite, artrite e linfopenia. Esta paciente apresentou tuberculose ganglionar previamente ao início dos sintomas do LESJ.

4.5 - Características clínicas dos pacientes com deficiência de imunoglobulinas (Tabela 1 e Figura 2).

Duas das 3 pacientes com IgAD (P10 e P11) apresentavam tireoidite autoimune e diabetes mellitus tipo 1, e faziam uso de bomba de insulina. Uma delas apresentava pneumonias de repetição. A terceira paciente com IgAD (P12) apresentava anticorpo anti-insulina positivo, sem diabetes, e também apresentava C4D e C2D. Sua história familiar revelou um irmão com LESJ, um irmão materno com IgAD e mãe com síndrome de Sjögren.

Todos os pacientes com IgG2D (P13, P14, P15 e P16) eram adolescentes ao diagnóstico de LESJ e 3 deles apresentavam infecções bacterianas recorrentes (celulite, pneumonia e sepse).

O único paciente do sexo masculino com IgMD (P17) apresentava lúpus discóide e síndrome de Sjögren, enquanto as duas pacientes do sexo feminino (P18 e P19) apresentavam fotossensibilidade acentuada e infecções bacterianas cutâneas de repetição. Todos os pacientes com IgMD apresentavam níveis normais de linfócitos B (CD19+).

4.6 - Número de cópias do gene de C4.

O paciente P3 apresentou 1 cópia do gene C4A e 1 cópia do gene C4B, enquanto o paciente P4 apresentou número de cópias adequado para C4A e C4B (2 e 3 cópias respectivamente). O paciente P12, que recebeu também diagnóstico de C2D, apresentou 2 cópias para C4A e 1 cópia para C4B. Dois pacientes com LESJ foram utilizados como controles e apresentaram pelo menos 2 cópias para C4A e C4B. O paciente P7, com C2D, também foi avaliado como controle e apresentou 2 cópias para C4A e C4B.

4.7 - Deficiência de C2 tipo I.

A mutação mais frequentemente encontrada em indivíduos caucasianos com C2D (deleção de 28 pares de base em 5'-splice site do intron 6 do gene para C2) foi pesquisada em 70 pacientes com C2D e foi evidenciada em dois (2,8%) deles.

Figura 1a

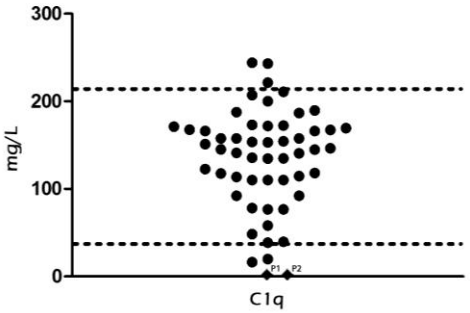

Figura 1b

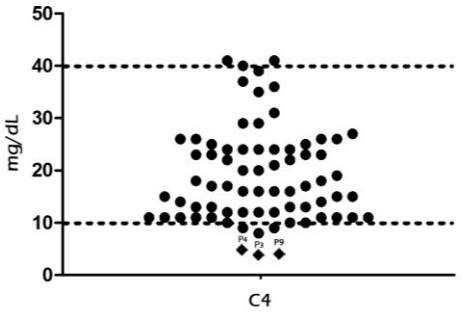

Figura 1c

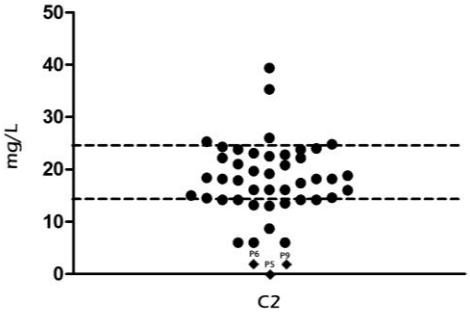

Figura 1d

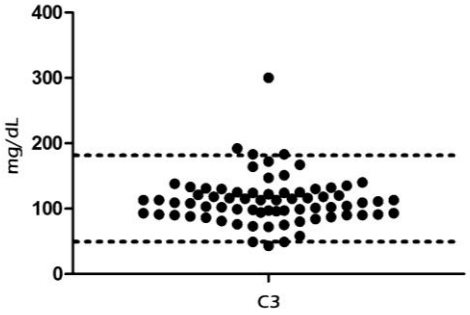

Figura 1 – Dados representativos da determinação de componentes do sistema Complemento em um único ensaio para C1q (n=54)(1a), C4 (n=72)(1b), C2 (n=46)(1c) e C3 (n=72)(1d) em pacientes com LESJ. Os valores individuais foram dispostos em gráficos com variação normal para cada componente estabelecida previamente pelo ensaio comercial: 1a) 33 – 209 mg/L (The Binding Site Birmingham, UK); 1b) 10 – 40 mg/dL (Dade Behringer/Siemens, Deerfied, USA); 1c) 14 – 25 mg/L (The Binding Site Birmingham, UK); 1d) 50 – 180 mg/dL (Dade Behringer/Siemens, Deerfied, USA). P1 - P6 e P9 representam pacientes com IDP do Complemento.

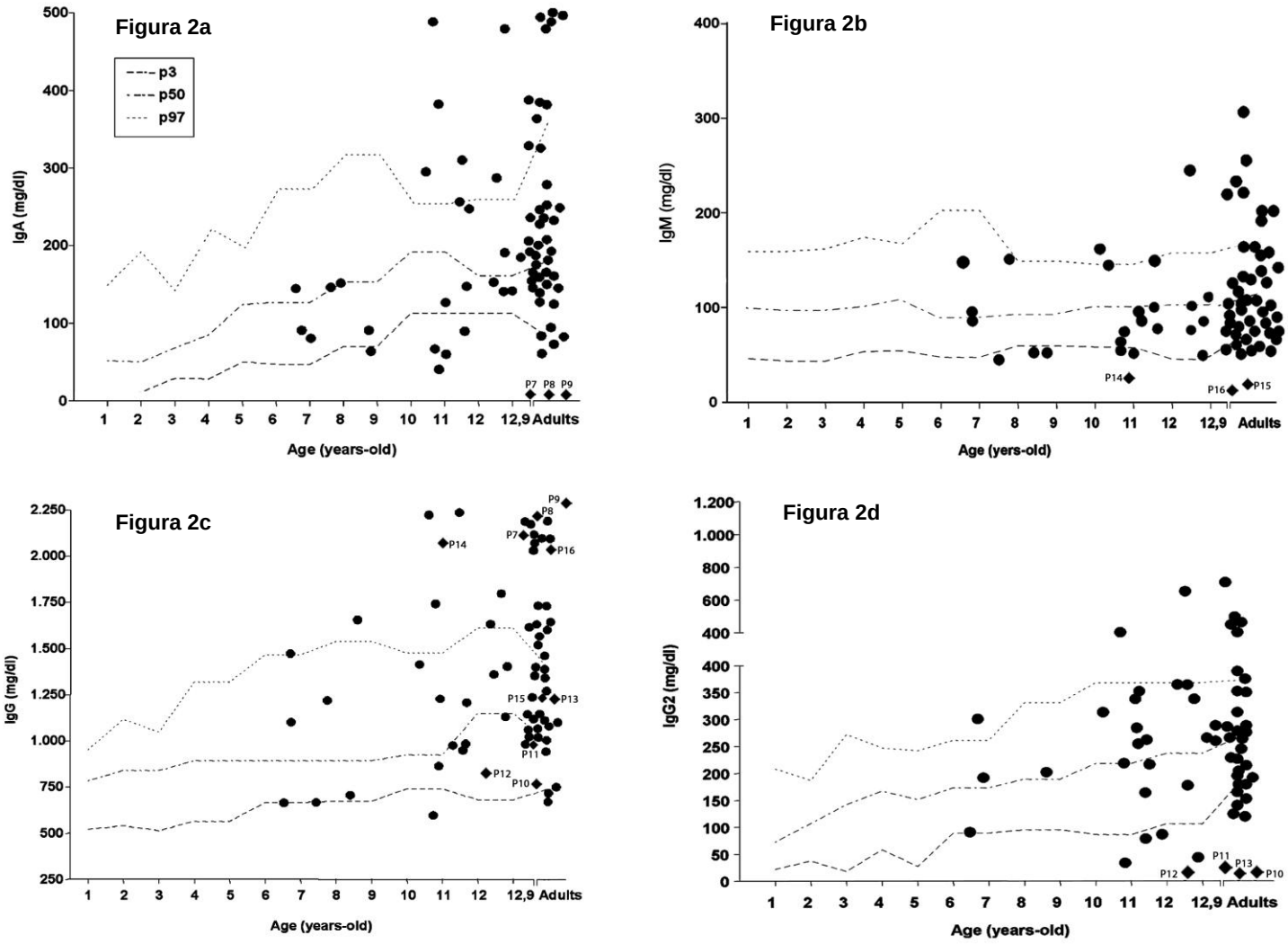

Figura 2 – Concentrações séricas de IgA (2a), IgM (2b), IgG (2c) e IgG2 (2d) de pacientes com LESJ. Valores individuais foram dispostos em gráficos, com valores normais (percentis 3 e 97) para cada imunoglobulina, obtidos de indivíduos brasileiros saudáveis. O grupo controle consistiu de 300 crianças de diferentes idades e 30 adultos jovens (Valores séricos de classes e subclasses de imunoglobulinas em uma população brasileira saudável. Tese de Doutorado de Maria Danisi Fujimura. Faculdade de Medicina da Universidade de São Paulo, 1990). P7 – P16 representam pacientes com IDP de imunoglobulinas.

5 DISCUSSÃO

O presente estudo demonstrou uma elevada frequência de IDP em pacientes com LESJ: seis pacientes (8%) apresentaram deficiência de um dos componentes da via clássica do sistema Complemento, e em dez pacientes (14%) foi evidenciada deficiência de imunoglobulinas.

A deficiência completa de um dos componentes iniciais da via clássica do sistema Complemento representa o fator de risco genético que, isoladamente, está mais fortemente relacionado ao desenvolvimento de LES. A frequência de associação entre deficiência homozigótica da fração do Complemento e LES ou suas manifestações é de 93% para C1q, 75% para C4, 66% para C1r/C1s e 25% para C2 [6, 7, 14]. É importante ressaltar que deficiências homozigóticas do Complemento conferem susceptibilidade ao lúpus e não estão associadas com doenças autoimunes em geral [7, 24]. Uma forte associação entre alelos nulos de C4A e LES também tem sido demonstrada em diferentes grupos étnicos [6, 48], enquanto que deficiência parcial de C4 ou C2 tem sido relacionada com o desenvolvimento de lúpus em menores prevalências [6, 7, 48, 49].

O consumo de proteínas do sistema Complemento, levando à redução dos seus níveis séricos, ocorre frequentemente em episódios de atividade lúpica, sendo utilizada a detecção de C3, C4 e CH50 para cálculo do SLEDAI-2K [38]. Portanto, para o diagnóstico de deficiência primária de

Complemento, foram consideradas necessárias pelo menos duas dosagens séricas com níveis muito baixos ou indetectáveis de apenas um dos componentes (C1q, C4 ou C2) na presença de concentrações normais de C3 e das outras frações, assim como SLEDAI-2K abaixo de 4 no momento da coleta de sangue.

Para corroborar o diagnóstico de deficiência de C4 e C2, também foram considerados a presença de um baixo número de cópias do gene *C4* e o achado de C2D tipo I, respectivamente.

Um dos pacientes com evidência de C4D isolada apresentou baixo número de cópias para *C4A* e *C4B*, enquanto o outro paciente apresentou pelo menos duas cópias para ambos os genes. Adicionalmente, o paciente com deficiências de C4, C2 e IgA apresentou duas cópias para *C4A* e apenas uma cópia para *C4B*.

A ocorrência de deficiência heterozigótica de C2 encontrada (2,7%) foi similar à descrita em estudos prévios em pacientes com LES (2,4 a 5,8%) [14, 46]. A proteína C2 é codificada por um gene de 20-kb, composto de 18 *exons* e localizado no braço curto do cromossomo 6. Dois tipos de C2D já foram descritos: tipo I e tipo II. Na C2D tipo I, não ocorre a tradução da proteína C2 e o defeito é causado por uma deleção de 28pb do gene C2, que resulta na deleção do *exon* 6 [44]. A C2D tipo II é caracterizada pelo bloqueio seletivo na produção da proteína C2 e ocorre geralmente em decorrência de mutações pontuais [50]. No presente estudo foi investigada a presença de C2D tipo I em 70 pacientes e dois deles foram heterozigotos para a deleção.

Em relação às imunodeficiências de imunoglobulinas, o estabelecimento do seu diagnóstico deve levar em consideração o efeito da terapia imunossupressora. O uso de ciclofosfamida e azatioprina, por exemplo, tem sido descrito como indutor de hipogamaglobulinemia transitória em pacientes com LES. Entretanto, esta deficiência é caracterizada por uma pan-hipogamaglobulinemia, envolvendo a queda das concentrações de IgG e dos outros isotipos de imunoglobulinas [11, 15, 47]. Desta forma, todos os 10 casos de deficiência de anticorpos relatados neste estudo apresentaram redução de apenas uma classe ou subclasse de imunoglobulina (Figura 2). Adicionalmente, seis pacientes desta casuística apresentaram níveis de IgG total abaixo do percentil 3 para a idade, mas não foram considerados como imunodeficientes, uma vez que eles também apresentaram níveis baixos das outras classes de imunoglobulinas (Figura 2c). Nestes casos, a pan-hipoglobulinemia é possivelmente secundária à terapia imunossupressora.

A frequência de IgAD desta casuística (4,2%) foi semelhante à encontrada por Cassidy *et al* em 77 pacientes com LESJ (5,2%) [9], enquanto que a frequência de IgG2D foi inferior à descrita por Oxelius *et al* [12] (4/14; 28%). Vale ressaltar que um caso adicional de IgAD não foi mencionado neste estudo porque o paciente, atualmente no grupo dos não imunodeficientes, apresentou concentrações de IgA persistentemente baixas (abaixo de 7 mg/dL) até os 10 anos de idade, quando desenvolveu sintomas de LESJ. Como os níveis de IgA sérica normalizaram após surgimento do

lúpus, é possível que a presença de IgAD tenha favorecido o aparecimento da doença autoimune neste caso.

Em contraste com a baixa frequência de manifestações autoimunes órgão-específicas na presente casuística (6/72), duas das três pacientes com IgAD apresentaram endocrinopatia autoimune (DM1 e/ou tireoidite), enquanto que a terceira paciente apresentou anticorpos anti-insulina. Esta associação sugere que um defeito comum possa estar envolvido no desenvolvimento de IgAD e DM1 tipo 1. Esta hipótese é corroborada pelo fato de que estas duas pacientes com associação entre DM1 e IgAD são as únicas pacientes com diabetes desta casuística. A terceira paciente com IgAD apresentou também C4D e C2D, além de uma história familiar positiva para doença autoimune sistêmica e IDP (Tabela 1). Sua avaliação genética demonstrou baixo número de cópias de *C4B* e C2D tipo I. Estudo prévio evidenciou a presença de alelos de HLA DRB1*15 and DRB1*13[51], os quais são transmitidos em conjunto com o alelo BF*S, que por sua vez está associado às C2D tipo I e II [44, 45, 50]. Estas observações sugerem que a IgAD provavelmente é determinada por defeitos genéticos na região do HLA classe III, onde os genes *C2*, *C4A* e *C4B* estão localizados [49, 52].

A ocorrência de LES em indivíduos com deficiências dos primeiros componentes da via clássica do sistema Complemento tem sido relacionada à ineficácia na depuração de imunocomplexos e de debris apoptóticos [27, 53]. Os debris não depurados podem corresponder a autoantígenos, que ficam então expostos ao sistema imune e estimulam a ativação de células T *helper* e a produção de autoanticorpos [27, 53]. Uma vez que os anticorpos

IgM se ligam diretamente aos corpúsculos apoptóticos e ao C1q, a mesma hipótese poderia explicar a ocorrência de lúpus em indivíduos com IgMD [54]. Adicionalmente, autoanticorpos IgM dependentes de células T foram descritos como neutralizadores de autoanticorpos IgG [55], e a deficiência de IgM sérica ou secretória resultou em níveis elevados de anticorpos IgG lúpus-símile em estudos experimentais [56, 57].

A explicação fisiopatológica da depuração inadequada de debris apoptóticos não parece se aplicar, entretanto, a IgAD e IgG2D, já que estas imunoglobulinas não se ligam ao C1. Apesar de ser considerada frequente [58], a associação entre IgAD e IgG2D não foi encontrada no presente estudo. Em camundongos, a produção de IgA é dependente de IL-5 e TGF-beta, sendo que esta citocina está relacionada com a geração e propriedades funcionais das células T regulatórias (Treg) [59]. Por sua vez, defeitos das células Treg têm sido envolvidos na ocorrência de LES em pacientes adultos [60] e a participação da proteína C1q na geração dos Treg tem sido considerada [61].

Vale ressaltar que os casos de imunodeficiências de anticorpos relacionados ao desenvolvimento de LES correspondem a deficiências parciais ou seletivas, enquanto que deficiências generalizadas ou graves de imunoglobulinas parecem prevenir a evolução para lúpus [14]. Não há relatos, por exemplo, de casos de lúpus em grandes amostras de agamaglobulinemia ligada ao X, embora 15% destes pacientes apresentem outras manifestações autoimunes como artrite crônica, esclerodermia e dermatomiosite [62, 63, 64].

Adicionalmente, casos raros de LES têm sido descritos em pacientes com IDCV [14, 15]. A presente casuística não evidenciou nenhum caso de IDCV. Entretanto, durante a execução deste estudo, uma paciente com IDCV, em seguimento na Unidade de Alergia e Imunologia do Instituto da Criança do HC-FMUSP por 9 anos consecutivos, desenvolveu manifestações clínicas autoimunes e preencheu os critérios para o diagnóstico de LESJ (ulceração oral, artrite, nefrite, pericardite importante, citopenias e presenças de vários autoanticorpos). Neste momento, foram excluídas neoplasias e a paciente foi tratada com glicocorticóide e imunossupressores, com melhora progressiva do quadro clínico. Dois meses após, apresentou choque séptico e evoluiu para óbito. Marcadamente, a necrópsia evidenciou um linfoma hepatoesplênico de células T, sem evidências de doenças autoimunes associadas. Este foi o primeiro relato da literatura deste raro linfoma nesta IDP, que mimetizou LESJ [65].

A determinação dos anticorpos anti-C1q neste estudo foi realizada não só para afastar presença de deficiência secundária de C1q nos dois pacientes com C1qD, mas para avaliar a utilidade da nova ferramenta diagnóstica no LESJ. Foi evidenciado que estes anticorpos apresentam uma elevada especificidade e valor preditivo positivo para o diagnóstico de LESJ [66], o que também é observado em estudos em adultos [29-32]. Entretanto, não houve uma associação direta da presença destes anticorpos com atividade da doença ou nefrite, o que contrasta com evidências em lúpus de adulto [29-32] e de um estudo em lúpus pediátrico [35] em que o anticorpo se associa com atividade e nefrite lúpica. Um estudo subsequente incluindo

62 pacientes comparou os anticorpos anti-C1q com anticorpos anti-nucleossomo e anti-dsDNA e corroborou o seu valor para o diagnóstico de LES [67]. Para avaliação de atividade da doença e presença de nefrite lúpica o anticorpo anti-C1q não se mostrou superior aos anticorpos previamente descritos como marcadores de LESJ [35, 66, 68].

O presente estudo revelou que doença de maior gravidade deve ser considerada um fator de risco para a ocorrência de IDP em pacientes com LESJ. O instrumento de avaliação de dano cumulativo relacionado à doença, seu tratamento e co-morbidades (SLICC/ACR-DI) pode ser considerado um marcador de atividade e gravidade da doença ao longo do tempo [39]. A forte associação entre o SLICC/ACR-DI e a presença de IDP sugere que indivíduos com LESJ e IDP associados têm uma doença mais agressiva e de pior prognóstico. Além disso, a presença de deficiência de Complemento também deve chamar a atenção para um pior prognóstico uma vez que 3 dos 6 pacientes com esta imunodeficiência evoluíram a óbito.

6 CONCLUSÕES

1. O presente estudo evidenciou uma elevada frequência de imunodeficiências primárias de Complemento e de imunoglobulinas em pacientes com LESJ.

2. Maior dano cumulativo foi considerado fator de risco para IDP nos pacientes com LESJ.

3. O anticorpo anti-C1q apresentou alta especificidade e valor preditivo positivo para o diagnóstico de LESJ.

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8 ANEXOS

Anexo I – **"Complement and antibody primary immunodeficiency in juvenile systemic lupus erythematosus patients"**
Submetido à revista *Lupus*

Anexo II – **"Anti-C1q antibodies in juvenile-onset systemic lupus erythematosus"**
Publicado na revista *New York Academy of Sciencies*

Anexo III– **"Anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies in juvenile systemic lupus erythematosus patients"**
Submetido à revista *Clinical and Experimental Rheumatology*

Anexo IV– **"Common variable immunodeficiency associated with hepatosplenic T-cell lymphoma mimicking juvenile systemic lupus erythematosus"**
Submetido à revista *Lupus*

Anexo V– **"Organ-specific autoantibodies and autoimmune diseases in juvenile systemic lupus erythematosus and juvenile dermatomyositis patients"**
Submetido à revista *Lupus*

Anexo VI– **"Understanding systemic lupus erythematosus physiopathology in the light of primary immunodeficiency"**
Publicado na revista *Journal of Clinical immunology*

Anexo VII – **"Autoimmunity in hyper-IgM syndrome"**
Publicado na revista *Journal of Clinical immunology*

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COMPLEMENT AND ANTIBODY PRIMARY IMMUNODEFICIENCY IN JUVENILE SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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ABSTRACT

Objective To evaluate the frequency of primary immunodeficiencies (PID) in juvenile systemic lupus erythematosus (JSLE) patients.

Methods Seventy-two JSLE patients were analyzed for levels of immunoglobulin classes and IgG subclasses and early components of the classical Complement pathway. Determination of C4 gene copy number (GCN) and detection of type I C2 deficiency (D) was also performed.

Results PID was identified in 16 patients (22%): C2D in 3, C4D in 3, C1qD in 2, IgG2D(<20mg/dL) in 4, IgAD(<7mg/dL) in 3, and IgMD(<35mg/dL) in 3; one of these patients presented IgA, C2 and C4D. Two patients had low C4 GCN and two had type I C2D. Demographic data, family history of autoimmune disease and PID, JSLE clinical findings, occurrence of infections, disease activity and therapies were similar in patients with and without PID ($p>0.05$). Remarkably, the median of systemic lupus international collaborating clinics/ACR-damage index was significantly higher in JSLE patients with PID compared with patients without these abnormalities ($p=0.0033$), likewise the high frequency of SLICC/ACR-DI>1 ($p=0.023$).

Conclusions A high frequency of PID was observed in JSLE patients, suggesting that these defects may contribute to lupus development. Our findings indicate that these two groups of PID should be investigated in pediatric severe lupus.

keywords – juvenile systemic lupus erythematosus, primary immunodeficiency, antibody deficiency, complement deficiency, C1q deficiency, IgA deficiency

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex disease as to its underlying genetics and clinical presentation, having often been considered the prototype of autoimmune diseases[1]. Moreover, primary immunodeficiencies (PID) are associated with autoimmune diseases, as recently revised by our group[2,3].

Three groups of PIDs have been described as associated with SLE or lupus-like manifestations: homozygous deficiency of early components of the classical Complement pathway[4,5,6]; partial or selective immunoglobulin deficiency (mainly IgA[7,8], seldom IgG2[9,10] and IgM deficiency[11,12,13,14]); and chronic granulomatous disease (CGD), affecting both patients and female carriers of the X-linked CGD allele[15,16,17]. In contrast, several PIDs that are strongly associated with tissue or organ-specific autoimmune manifestations are selectively spared of SLE or lupus-like clinical and laboratory features. These include: autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) (due to *AIRE* mutations)[18,19], immunedysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) (due to *FOXP3* mutations)[20,21] – both resulting in defects of regulatory T cells (Treg); autoimmune lymphoproliferative syndrome (ALPS) (caused by genetic defects in apoptosis)[22]; and Wiskott-Aldrich syndrome[23,24].

To our knowledge, there is no study evaluating simultaneously Complement and immunoglobulin immunodeficiencies in a large population of

pediatric lupus. Therefore, the aims of the present study were to evaluate the frequency of these PIDs in juvenile SLE (JSLE) patients and to assess the possible association of the presence of PID in these patients with demographic data, clinical findings, disease activity and damage, and therapies.

MATERIAL AND METHODS

Patients. After Institutional Ethics Committee approval and informed consent from parents, Complement and immunoglobulin serum levels were measured in a cohort of 72 consecutive JSLE patients (59 girls and 13 boys; age varying from 1 to 16-years-old at diagnosis). All patients met the American College of Rheumatology (ACR) SLE classification criteria[25]. This cohort of patients has gone through a standardized monthly routine clinical and laboratory investigation at the Children's Hospital of Faculdade de Medicina da Universidade de São Paulo.

Medical records evaluation. Medical records were retrospectively evaluated regarding demographic data (gender, ethnic background, age at disease onset and age at disease diagnosis), family history of PID and autoimmune diseases. Screening for SLE-related clinical findings included: cutaneous, mucosal, renal, neuropsychiatric, cardiovascular, pulmonary, hematological, articular and gastrointestinal manifestations as well as evidence for anti-phospholipid syndrome. Organ-specific autoimmune diseases (thyroiditis, auto-immune hepatitis, diabetes mellitus, celiac disease, auto-immune gastritis and primary biliary cirrhosis) were investigated based on characteristic clinical features and determination of the respective

autoantibody profile: anti-thyroglobulin (TGA), anti-thyroid peroxidase (TPO) and anti-thyrotropin receptor (TRAb); anti-smooth muscle (SMA), anti-liver kidney microsome type 1 (LKM1) and anti-liver cytosol (LCA); anti-insulin (IAA) and anti-glutamic acid decarboxilase (GAD); anti-endomisium (EMA); anti-parietal cell (APC); anti-mitochondria (AMT). Medical records were also screened for the presence of infections: recurrent pyogenic infections, mycobacteriosis, fungal infections, herpes zoster and severe sepsis. Severe sepsis was determined according to international pediatric sepsis consensus conference definitions[26]. The SLE disease activity index 2K (SLEDAI-2K)[27] and the systemic lupus international collaborating clinics/ACR-damage index (SLICC/ACR-DI)[28] were determined for each patient at the time of blood withdrawal. Active lupus was arbitrarily defined as SLEDAI-2K ≥ 4.

Complement components and immunoglobulin serum levels. Serum levels of C1q and C2 were determined by radial immunodiffusion (The Binding Site, Birmingham, UK). Low C1q and C2 levels were confirmed in a new sample by radial immunodiffusion and immunohemolysis[29], respectively. Serum C3 and C4 levels were monthly measured by nephelometry (Dade Behring/Siemens, Deerfield, USA) as part of the routine follow-up. Serum C1r and C1s were determined by double immunodiffusion (The Binding Site, Birmingham, UK).

C4 deficiency (C4D) and C2 deficiency (C2D) were defined as the demonstration of low levels of C4 or C2 in the presence of normal levels of other Complement fractions in at least two samples and SLEDAI-2K score below 4 at the moment of sample withdrawal, and/or the finding of low C4

gene copy number or presence of type I C2D, respectively. Methods for C4 gene copy number determination and for detection of type I C2D are described later.

C1q deficiency (C1qD) diagnosis was defined as the combination of undetectable C1q and normal levels of other Complement components in at least two serum samples.

IgG, IgA and IgM immunoglobulin serum levels were measured by immunoturbidimetry (Roche Diagnostics, Indianapolis, USA), while IgG1 and IgG2 subclasses serum levels were determined by nephelometry (Dade Behring/Siemens, Deerfield, USA). Diagnosis of selective immunoglobulin deficiency was established only in patients who were older than 10 years of age and that presented normal or high levels of the other immunoglobulin classes and subclasses in the same sample. The threshold for IgA deficiency (IgAD) was below 7mg/dL[30]; for IgM deficiency (IgMD) below 35mg/dL[31], and for IgG2 deficiency (IgG2D) below 20m/dL.

Determination of C4 gene copy number (GCN). Genomic DNA was obtained from peripheral blood mononuclear cells from JSLE patients with persistently low C4 serum levels. The number of C4 gene copies was determined by quantitative real time polymerase chain reaction (PCR) using Taqman probes labeled with minor groove binding (MGB) non-fluorescent quencher and 6-carboxifluorescein (FAM) in a Rotor Gene 3000 (Corbett, Sidney, Australia), as previously reported[32]. Amplification was performed using forward primer (5'-GCAGGAGACATCTAACTGGCTTCT-3') and reverse primer (5'-CCGCACCTGCATGCTCCT-3') and detection was obtained with

specific probes for C4A (5' FAM-ACCCCTGTCCAGTGTTAG-MGB 3') and C4B (5' FAM-ACCTCTCTCCAGTGATAC-MGB 3') in independent simultaneous reactions. Normalization and estimation of the delta-delta CT ($\Delta\Delta$CT) was obtained by amplification of the RNAse P gene simultaneously in a third separate reaction, using a commercial kit with RNase P-specific primer and FAM-labeled probe (ABI Cat. No. 4316844). PCR conditions included incubation at 50ºC for 2 minutes followed by activation of the DNA polymerase (AmpliTaq Gold DNA polimerase, Applied Biosystems, ORIGEM) at 95°C for 10 minutes and 45 cycles (denaturing at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute). All reactions were run in triplicate at a final 25uL volume and using 3ng DNA. DNA samples with known C4A and C4B GCN were kindly provided by Dr. A. Szilagyi, from Hungary, and used as control. GCN was estimated by the $\Delta\Delta$CT method, where the GCN in the test sample is equal to the GCN in the control sample x $2^{-(\Delta\Delta CT)}$, the $\Delta\Delta$CT = control ΔCT – test sample ΔCT, and the ΔCT = RNase P CT – C4A/C4B CT.

Determination of C4 gene copy number

Detection of type I C2 deficiency. Seventy JSLE patients were evaluated for type I C2D, which is the most common cause of hereditary C2D. Type I C2D is caused by a 28 bp deletion that removes 9bp of the 3'-end of exon 6 and 19bp of the 5'-end of intron 6, leading to a premature stop codon, that results in deletion of exon 6 and a failure to synthesize detectable C2 protein[33]. The genomic DNA from individuals was amplified by PCR for detection of 28bp deletion at the 5'-splice site of exon 6 in C2 gene. The primers: C2-F:5´-GCCTGGGCCGTAAAATCCA-3´; C2-R:5´-GCACAGGAAGGCCTCTGCTGCAGGC-3´ were designed as previously

described[34]. A new reverse primer was synthesized using the nucleotide sequence of the complement component C2 gene (GenBank accession N° NG_011730 in www.ncbi.nlm.nih.gov/nuccore/225903390) to confirm the results as follows: C2-R2: 5´-TTGGAGGAGGTGGGCTGTGAG -3´. Each reaction was performed in a final volume of 25 µl containing 100 ng of DNA, 20 mM Tris-HCl (pH 8,4), 50 mM KCl, 1.5 mM $MgCL_2$, 50 µM of each dATP, dCTP, dGTP and dTTP, 0.25 µM of each primer, and 1.0 U of Taq DNA polymerase. The amplification conditions for 35 cycles were: 95°C for 30 sec, 66°C for 30 sec, and 72°C for 30 sec, with an initial denaturing step of 95°C for 5 min and a final extension step of 72°C for 7 min. The amplification products were detected by agarose gel electrophoresis. Subsequently, each amplicon was purified using GFX PCR DNA and Gel Band Purification Kit (GE Healthcare). The concentration was determined by NanoVue. The amplicons were nucleotide sequenced using the Dyenamic ET Dye Terminartors kit performed on a MegaBace1000 (GE Healthcare, Amersham, UK). The primers used were C2-F and C2-R2.

Anti-double stranded DNA (anti-dsDNA) and anti-C1q antibodies serum levels determination. Anti-dsDNA (The Binding Site, Birmingham, UK) and anti-C1q (Inova Diagnostics, San Diego, USA) antibodies serum levels were assessed by enzyme-linked immunosorbent assay (ELISA) commercial kits at the moment of clinical evaluation.

Statistical analysis. JSLE patients with and without PID were compared regarding demographic data, clinical findings, SLEDAI-2K and SLICC-

ACR/DI) indexes, as well as for occurrence of infections. Continuous variables were analyzed by Mann Whitney's test and categorical variables by Fisher's exact test.

RESULTS

Identification of PID in JSLE patients (Table 1)

Evidence of underlying PID was found in 16 (22%) of the 72 JSLE patients. Seven patients presented evidence of Complement deficiency: 2 with complete C1qD (Figure 1a), 3 with C4D (Figure 1b), and 3 with C2D (Figure 1c). Ten patients presented evidence for immunoglobulin deficiency: 3 with selective IgAD (Figure 2a), 3 with IgMD (Figure 2b), and 4 with IgG2D (Figure 2d) (Table 1). One female patient (P9) had simultaneous IgAD, C4D and C2D. Four out of the 13 males (30%) presented evidence of PID: 1 C2D, 1 IgG2D, 1 C1qD, and 1 IgMD. The later two boys were the only cases in the series that presented clinical manifestations before 2 years of age. Twelve out of the 59 females (20%) presented evidence of some type of PID: 3 C4D, 1 C1qD, 3 C2D, 3 IgAD, 3 IgG2D and 2 IgMD. One of them had concomitant C4D, C2D and IgAD (P9). There was no patient with C1r or C1s alterations, neither with persistent low C3 levels. Although 6 patients presented IgG levels below the 3rd percentile for the age group (Figure 2c), no one was classified as primary IgG deficiency. No patient presented IgG below 500 mg/dL, and none could be diagnosed as common variable immunodeficiency (CVID). No patient presented simultaneous IgA and IgG2 deficiency. The four IgG2 deficient patients presented values of 3, 4, 5 and 16mg/dL, resulting in IgG2/IgG ratios of 0.3%, 0.7%, 0.3% and 2%, respectively. The three patients with IgMD had

values of 23, 29 and 35mg/dL, and IgM/IgG ratios of 1.7%, 2.3% and 1%, respectively. The mean ratio considering all patients was 18.7±7.4% for IgG2/IgG, and 9.4±5.7% for IgM/IgG. None of the patients with evidence of immunoglobulin deficiency was receiving anti-epileptic drugs. No patient presented clinical evidence or had familial history suggestive of CGD.

Comparison between JSLE patients with and without evidence of underlying PID (Table 2)

30% of the boys (4/13) and 20% (12/59) of the girls presented an underlying PID (p=0.74). The JSLE groups with and without PID did not differ with respect to median age at disease onset [9 (1 - 15) vs. 9 (2 - 16) years-old, p=0.43]. However, the two patients with infantile lupus (age of disease onset below 2-years-old) presented evidence of PID.

The frequencies of muco-cutaneous, renal, neuropsychiatric, cardiovascular, pulmonary, hematological, articular and gastrointestinal manifestations were similar in both groups (p>0.05). In addition, the two groups did not differ with respect to the frequency of anti-phospholipid syndrome (0% vs. 7%, p=0.56) as well as clinical and/or laboratory evidence of organ-specific autoimmunity (42% vs. 37%, p=0.78). The median of anti-dsDNA antibody levels [39 (0-558) vs 33 (0 – 1000), p=0.54] and SLEDAI-2K [6 (0 – 33) vs. 5 (0 -20), p=0.38] were equivalent in both groups. Remarkably, the PID group presented a significantly higher median of SLICC/ACR-DI score than the patients with no evidence of PID [1.5 (0 - 5) vs. 0 (0 – 3), p=0.0033]. The frequency of SLICC/ACR-DI ≥ 1 was also significantly higher in PID group (81% vs. 48%, p=0.023). JSLE patients with evidence of PID did not present an increased

frequency of severe sepsis than JSLE with no evidence for PIDs (31% vs. 7.5%, p=0.21). Additionally, the frequencies of recurrent pyogenic infections, mycobacteriosis, fungal and herpes zoster infections were similar in the two groups. Death occurred in 3 out of the 16 PID patients (18%) and in 1 out of the 59 patients without PID (1.7%) (p=0.11), during the period of follow-up. Patients with and without evidence for PID did not differ regarding the therapeutic approach for JSLE (cyclophosphamide, mycophenolate mofetil, azathioprine and intravenous immunoglobulin use).

Clinical data from Complement and antibody deficiencies in JSLE patients are shown in Table 1.

C4 gene copy number (GCN)

P3 patient presented 1 copy for C4A and 1 copy for C4B. P4 patient, that presented persistent low C4 levels despite normal C2/C3 levels at the moment of blood withdrawal, presented 2 copies for C4A and 3 copies for C4B. P9, who additionally presented C2D and IgAD, had 2 copies for C4A and 1 copy for C4B. Three other lupus patients without PID (used as controls) presented at least 2 copies for C4A and C4B.

C2 type I deficiency

P6 (C2D) and P9 (IgAD, C4D and C2D) were heterozygous for the described 28bp deletion. DNA sequencing confirmed the deletion in both patients. Genomic DNA from P5 (phenotypically C2 deficient) was not available for investigation.

DISCUSSION

The present data demonstrated a high frequency of PID associated with JSLE: 8% with deficiency of one of the early components of the classical Complement pathway and 14% had evidence of selective immunoglobulin deficiency.

The frequency of IgAD (4.2%) in this series was comparable to the 5.2% frequency in 77 JSLE patients reported by Cassidy et al.[8], and the frequency of IgG2D (5.5%) was below that reported by Oxelius et al.[9] in JSLE (4/14; 28%).

The frequency of heterozygous C2D in the present series was 2.7%, what is in harmony with previous reports of heterozygous C2D prevalence between 2.4% and 5.8% in SLE patients[35].

Importantly, one relevant aspect of our study was the definition of Complement deficiency, that required at least two determinations with very low or absent levels of only one component (C1q, C4 or C2) in the presence of normal concentration of C3 and other components, as well as SLEDAI-2K scores below 4. Moreover, in order to corroborate Complement deficiency diagnosis, the finding of low C4 GCN or type I C2D diagnosis were also considered for C4D and C2D, respectively.

As suggested by Boeckler et al.[34] for a further investigation of Complement deficiency, and considering that it has been previously described that a low copy number of C4 is a risk factor for SLE development, we additionally determined the C4 GCN in patients with evidence of C4D and the 28bp deletion for C2 gene in patients with C2D[36,37]. Although one patient presented

low GCN for *C4A* and *C4B* genes, the other had at least 2 copies for both C4A and C4B genes. Interestingly, the patient with also C2 and IgA deficiencies presented 2 copies for C4A and only 1 copy for C4B. C2 protein is encoded by a 20-kb gene comprised of 18 exons on the short arm of chromosome 6. Two genetic types of C2D have been previously described. In type I deficiency there is no translation of C2 protein caused by a 28pb deletion in the C2 gene that results in the deletion of exon 6[37]. The type II deficiency is associated with a selective block in C2 production and is generally due to punctual mutations[38]. We investigated the presence of type I deficiency in 70 patients and two of them were heterozygous for the deletion. In addition to the above requirements, the two patients with C1qD did not present anti-C1q antibodies as observed in our previous study[39].

The definition of selective immunoglobulin deficiency requires consideration related to the effect of immunosuppressive therapy. Both cyclophosphamide and azathioprine have been reported to cause reversible hypogammaglobulinemia in SLE patients[14,40,41]. Yet, such secondary hypogammaglobulinemic states present as panhypogammaglobulinemia involving decreased levels of IgG and other immunoglobulin isotypes. Remarkably, all 10 cases presently reported as immunoglobulin PIDs had consistently very low levels of one immunoglobulin class or subclass with normal or high levels of other immunoglobulin classes, including total IgG and subclasses (Figure 2). In fact, our JSLE series included six other patients with total IgG levels below the 3rd percentile for the age group (Figure 2c) who were not considered as PID cases, but as probably secondary to

immunosuppressive therapy, because they also showed low levels of at least one other immunoglobulin class.

In contrast with the rarity of organ-specific autoimmune clinical manifestations in the overall cohort of JSLE patients (6/72), two of the three IgA-deficient girls presented autoimmune endocrinopathy (diabetes mellitus and/or thyroiditis), and the third had anti-insulin antibodies, suggesting that a common defect may underlie this particular form of PID and diabetes mellitus. This former patient had C4 and C2 deficiency and presented only one copy of *C4B* and two copies of *C4A* gene. Genotyping showed type I C2 deficiency. This patient also presented HLA alleles DRB1*15 and DRB1*13 determined in a previous study[42]. These alleles are transmitted together with the BF*S allele that is associated with both type I and type II C2 deficiencies[34,37,38]. These observations are also consistent with the finding of genetic linkage of IgAD to the HLA class III region where C2 and *C4A* and *C4B* genes map to[43,44].

Complete deficiency of any of the early components of the classical Complement pathway represents the strongest single genetic risk factor for the development of SLE identified to date. SLE or lupus-like manifestations have been detected in 93% of homozygous C1q-deficient, 75% of homozygous C4-deficient, 66% of homozygous C1r/C1s deficient, and 25% of homozygous C2-deficient individuals[4,5,6]. A strong association between null alleles of C4A and SLE has also been demonstrated in different ethnic groups[5,45].

The well-known association of SLE with deficits in the early components of the classical Complement pathway has been interpreted to suggest poor clearance of immune complexes and apoptotic debris[46,47]. In

turn, such non-cleared debris would operate as antigens to stimulate helper T cells and autoantibody production. IgM deficiency might operate in a similar way, since these IgM antibodies bind both directly to apoptotic debris and to C1q[48]. However, this pathophysiologic explanation is not promptly valid for the association of SLE with IgA and IgG2 deficiency, since these immunoglobulin classes do not efficiently bind to C1. Both IgA and IgG2 deficiency are often associated and, therefore, are likely to be regulated together, by similar mechanisms[49]. In mice, IgA production is known to depend on IL-5 and TGF-beta. The latter cytokine is intimately related to the generation and functional properties of regulatory T cells (Treg cells)[50]. Treg cell deficits have been invoked to play a role in adult-onset SLE[51] and the participation of C1q in the generation of Treg cells has been claimed[52]. This may suggest a novel interpretation of some of the above associations.

Moreover, T cell-dependent IgM autoantibodies were described in the past as neutralizers of IgG autoantibodies[53], and deficiency of serum and/or secretory IgM in the mouse was shown to result in elevated levels of lupus-type IgG antibodies in the serum[54,55].

Remarkably, immunoglobulin PID cases reported as associated to SLE correspond to partial or selective deficiencies. Otherwise, severe generalized immunoglobulin deficiency seems to prevent lupus development[24]. For instance, there is no report of SLE manifestations among the large series of X-linked agammaglobulinemia patients, although 15% of them present chronic arthritis, and some have scleroderma or dermatomyositis[56,57,58]. Moreover, only rare SLE cases have been described among CVID patients[24,41].

Disease severity was identified as a major factor associated with PID in the present study. The association between damage index and evidence of PID is striking. The SLICC/ACR-DI describes accrual damage and may indicate a measure of disease activity and severity over time. The observation herein reported suggests that an associated PID might contribute to a more aggressive and to a worse prognosis in JSLE patients. In particular, Complement deficiency represented a significant indicator of bad prognosis: three out six Complement deficient patients in this series died during the short period of follow-up.

In conclusion, all the PID forms identified in this JSLE cohort had already been reported as associated to lupus. The novel insight brought by the present study was the demonstration of a high frequency of PID overall in pediatric SLE. This observation appears to suggest that a PID background may eventually turn into an imbalance represented by an SLE syndrome. Therefore, early onset SLE patients, particularly those with severe disease should be investigated for primary Complement and immunoglobulin deficiency. Further studies shall investigate if PID-associated JSLE patients may present distinctive pathophysiologic and prognostic features, and shall define more effective forms of therapy for this specific group of patients.

ACKNOWLEDMENTS

The authors are thankful to Leuridan Torres, Mariana Kmiliauskis, Patricia Palmeira, Kaline Medeiros Costa Pereira and Natalia Umetsu Sobrinho, for their valuable help with laboratorial analyses. This work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo - grants

02/05880-4 and 08/58238), and CNPq (Conselho Nacional de Ciência e Tecnologia, grants 302469-05-2 to CAS, and 34802-0 to MC-S).

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Anti-C1q Antibodies in Juvenile-Onset Systemic Lupus Erythematosus

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The objective of this study was to evaluate the presence of anti-C1q antibodies Hospital Israelita Albert Einstein Research Institute, São Paulo, Brazil in 67 juvenile systemic lupus erythematosus (JSLE) patients and 26 healthy controls and to assess the association of these antibodies with disease activity, nephritis, and presence of anti-double-stranded (ds)DNA. Anti-C1q antibodies were detected by ELISA. A higher frequency of anti-C1q antibodies was observed in JSLE patients compared to controls (20% vs. 0%, $P = 0.016$). Specificity of these antibodies was 100% [95% confidence interval (CI) 86.7–100%] and sensitivity was 19.4% (95% CI 10.7–30.8%) for a lupus diagnosis. The median anti-C1q antibodies was higher in JSLE patients compared to controls [median (range) 9.4 (5.5–127) vs. 7.3 (5–20) units, $P = 0.004$]. Remarkably, a positive Spearman's coefficient was found between anti-dsDNA and anti-C1q units ($r = 0.42$, $P = 0.0004$, 95% CI 0.19–0.60). Our results confirm a low frequency of anti-C1q antibody in our lupus populations, but the presence of anti-Clq antibodies appears to be a good marker for JSLE diagnosis.

Key words: **juvenile systemic lupus erythematosus; anti-C1q; nephritis; anti-dsDNA; ELISA**

Introduction

The complement system plays an important role in systemic lupus erythematosus (SLE) pathogenesis because tissue damage is strongly mediated by immune complex deposition.[1] Paradoxically, hereditary classical complement component deficiencies are frequently associated with SLE development as a result of an impairment in the clearance of apoptotic debris. Homozygous C1q deficiency is the strongest genetic risk factor related to SLE, and 93% of C1q-deficient patients develop SLE or lupus-like manifestations.[2–7] However, the majority of SLE patients frequently have secondary complement deficiency caused by the presence of anti-C1q antibodies.[8,9]

Of note, anti-C1q antibodies have been detected in up to 65% of adult SLE patients and associated with disease activity, especially with lupus nephritis.[10,11] However, there are only two studies that evaluated anti-C1q antibodies in juvenile SLE (JSLE), and these studies had discordant results and did not have a comparative healthy control group. In 1997, Ravelli *et al.*[12] found serum anti-C1q antibody in 17/29 (59%) JSLE patients and this was not associated with active nephritis. More recently, Kozyro

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Contemporary Challenges in Autoimmunity: Ann. N.Y. Acad. Sci. 1173: 235–238 (2009).
doi: 10.1111/j.1749-6632.2009.04675.x

et al.[13] reported the presence of anti-C1q antibody in 7/12 (58%) lupus nephritis patients. Six of these seven patients were in renal flare.

We have therefore evaluated the presence of anti-C1q antibody in JSLE and controls. The possible association of these antibodies with disease activity, nephritis, and presence of anti-double-stranded DNA (anti-dsDNA) was also assessed.

Patients and Methods

Sixty-seven consecutive JSLE patients followed at the Pediatric Rheumatology Unit, University of São Paulo, Brazil, were studied for the presence of anti-C1q antibodies from 2006 to 2007. All patients fulfilled the American College of Rheumatology (ACR) SLE classification criteria.[14] The control group included 26 healthy age-matched subjects from the adolescents unit at the same university hospital. The local ethical committee approved this study, and an informed consent was obtained from all participants.

Anti-C1q antibodies were detected by ELISA (QUANTA Lite™ anti-C1q, Inova Diagnostics, San Diego, CA) with duplicated samples. Tests were performed strictly according to manufacturer protocols, including the suggested cutoff values of 20 U. Anti-dsDNA antibodies were also detected by ELISA.

SLE disease activity was measured in all patients, using the SLE disease activity index (SLEDAI).[15] Lupus activity was defined arbitrarily as SLEDAI >4. The diagnosis of nephritis was established according to the presence of proteinuria $\geq$ 0.5g/24 h, presence of cellular casts, persistent hematuria $\geq$ 10 red blood cells under high-power field, and renal failure. The renal histopathology was described according to the World Health Organization (WHO) classification.[16]

Results were presented as the mean for continuous variables and as the number (%) for categorical variables. Data were compared by Mann–Whitney *U* test for continuous variables to evaluate differences between JSLE patients and controls and with or without disease activity and nephritis. For categorical variables, differences were assessed by Fisher's exact test. Spearman's coefficient was used to evaluate correlations between serum anti-C1q and anti-dsDNA. The sensitivity, specificity, and positive- and negative-predictive values of anti-C1q antibodies for JSLE diagnosis were also evaluated. $P < 0.05$ was considered significant.

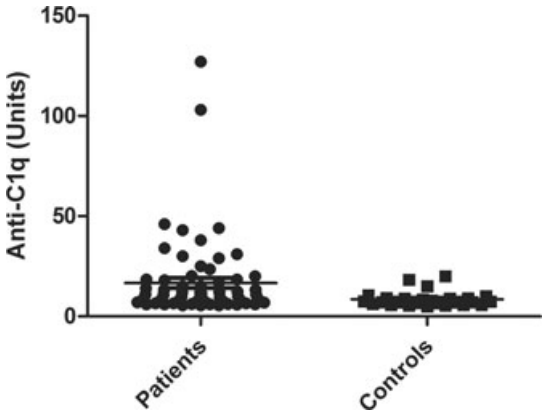

Figure 1. Levels of anti-C1q antibodies in juvenile systemic lupus erythematosus patients compared to controls ($P = 0.016$).

Results

The mean current age was similar in JSLE patients and controls (mean $\pm$ SD 14.6 $\pm$ 3.79 vs. 13.8 $\pm$ 2.56 years, $P = 0.26$). The female gender was similar in both groups (83% vs. 73%, $P = 0.23$). The age at JSLE onset ranged from 3–16 years.

A higher frequency of anti-C1q antibodies was observed in JSLE compared to controls (20% vs. 0%, $P = 0.016$) (Fig. 1). Interestingly, specificity of these antibodies was 100% [95% confidence interval (CI) 86.7–100%], sensitivity was 19.4% (95% CI 10.7–30.8%), positive-predictive value was 100% (95% CI 75.3–100%), and negative-predictive value was 32.5% (95% CI 22.4–43.9%) for the lupus diagnosis. Remarkably, the median anti-C1q antibody levels were significantly higher in JSLE

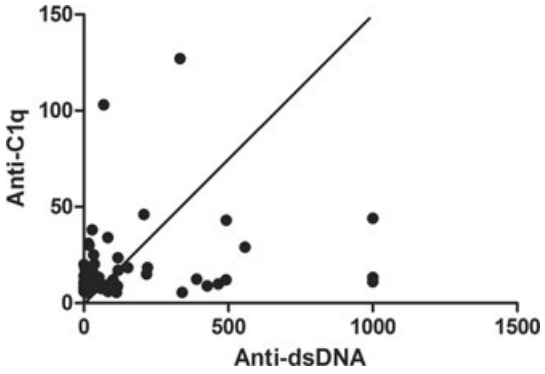

Figure 2. Positive correlation between anti-C1q and anti-double-stranded (ds)DNA antibodies in juvenile systemic lupus erythematosus patients compared to controls ($r = 0.42$, $P = 0.0004$, 95% confidence interval 0.19–0.60).

patients compared to controls [median (range) 9.4 (5.5–127) vs. 7.3 (5–20) units, $P = 0.004$].

The frequency of anti-C1q antibody in JSLE patients with and without nephritis was alike (20% vs. 19%, $P = 1.0$). Likewise, the median anti-C1q level was similar in both groups [9.9 (5–127) vs. 9.0 (5.6–30), $P = 0.62$]. According to WHO classification, active focal or diffuse proliferative lupus nephritis was observed in 6/13 JSLE patients with anti-C1q antibodies.

The frequency of anti-C1q antibody in JSLE patients with and without disease activity (SLEDAI > 4) was alike (20% vs. 19%, $P = 1.0$). No difference in the median of anti-C1q antibodies was observed in active and inactive disease [10.85 (5.6–127) vs. 8.9 (5.5–34) units, $P = 0.41$]. Additionally, no difference was found in the median of the SLEDAI score in lupus patients with present or absent anti-C1q [6.0 (0–18) vs. 4.0 (0–15), $P = 0.55$].

Anti-C1q and anti-dsDNA antibodies agreed in 61% of JSLE patients. Remarkably, a positive Spearman's coefficient was found between anti-dsDNA and anti-C1q levels ($r = 0.42$, $P = 0.0004$, 95% CI 0.19–0.60) (Fig. 2).

Discussion

To our knowledge, this is the first study that evaluated the anti-C1q antibodies in a large JSLE population and clearly showed a higher frequency of this antibody compared to controls. Moreover, this laboratorial test had elevated specificity and positive-predictive value for lupus, in spite of a low sensitivity in our population.

Anti-C1q antibodies were reported in only two studies with a smaller pediatric lupus population and without a control group. The prevalence of these antibodies was 59% and 58%, respectively [12,13] and was higher than observed in this study (20%). In studies of adult patients, the prevalence ranged from 20–100%.[8–10] This disparity of frequency between the various studies could be attributed to technical issues, such as the assay methodology and adopted cutoff value.

Interestingly, anti-C1q antibodies may interfere with the clearance of apoptotic cells and induce autoimmunity. Moreover, these antibodies may be pathogenic and participate in immune complex-mediated renal disease.[17] The primary mechanism leading to active nephritis might be different in juvenile and adult lupus and could explain the low frequency of this antibody in our population.

Of note, the anti-C1q is associated with lupus activity, particularly with active nephritis, and could be a useful tool for monitoring the disease.[10] In our study the association of these autoantibodies with anti-dsDNA corroborates this hypothesis, despite a lower frequency of renal involvement in anti-C1q-positive JSLE patients.

Furthermore, lupus is characterized by the presence of broad types of autoantibodies, including antinuclear, antiphospholipid, anti-dsDNA, and anti-Sm antibodies.[18,19] These tests are included in ACR classification criteria and could be specific and useful for disease diagnosis, particularly anti-dsDNA and anti-Sm.[14] Recently, we showed that antinucleosome antibodies have a high specificity for pediatric lupus,[20] as observed in the current study for the presence of anti-C1q. Therefore, the detection of antinucleosome[20] and anti-C1q antibodies in a suspected JSLE patient

could help in its earlier diagnosis, especially in patients that were negative for anti-dsDNA and anti-Sm.

In conclusion, a low frequency of anti-C1q antibodies with high specificity was found in our patients with JSLE, indicating its usefulness as a new biomarker for the diagnosis and management of this disease.

Conflicts of Interest

The authors declare no conflicts of interest.

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Brief paper

Anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies in juvenile systemic lupus erythematosus patients

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Abstract

Objectives: To evaluate the presence of anti-C1q, anti-chromatin/nucleosome and anti-double stranded DNA (dsDNA) antibodies in juvenile systemic lupus erythematosus (JSLE) and controls. **Methods:** Sixty-two JSLE and 34 healthy controls were analyzed for the presence of anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies by ELISA. C1q levels were evaluated by radial immunodiffusion. **Results:** The mean current age was similar in JSLE patients and controls (14.6±3.86 vs. 13.6±2.93 years, p=0.14). Higher frequencies of anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies were observed in JSLE compared to controls (20% vs. 0%, p=0.0037; 48% vs. 0%, p<0.0001 and 69% vs. 3%, p<0.0001, respectively). The median of anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies were also significantly higher in JSLE patients than in controls [9.6(5.5-127) vs. 7.5(5-20)Units, p=0.0006; 18(1.9-212) vs. 3.2(1.7-17)Units, p<0.0001 and 111 IU/ml (6-741) vs. 14 (6-33)IU/mL, p<0.0001, respectively]. The sensitivities for anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies were 21% (CI 11-33), 49% (CI 36-62) and 70% (CI 57-81). The specificities were 100% (CI 88-100), 100% (88-100) and 97% (CI 83-99), respectively. A positive correlation was found between anti-dsDNA levels and both anti-C1q (r=0.51, CI: 0.29-0.68, p<0.0001) and anti-chromatin/nucleosome antibodies (r=0.87, CI: 0.79-0.92, p<0.0001) levels. A negative correlation was observed between anti-C1q and C1q serum levels (r= **-** 0.33, CI: **-** 0.56 - **-** 0.05, p=0.018). No differences were observed in the frequencies of these three autoantibodies and nephritis or SLEDAI≥4 (p>0.05). **Conclusion:** Our study demonstrated an elevated specificity for lupus diagnosis involving the three autoantibodies, especially anti-C1q and anti-chromatin/nucleosome antibodies.

Keywords: juvenile systemic lupus erythematosus, anti-C1q antibody, anti-chromatin/nucleosome antibody, anti-dsDNA antibody, nephritis.

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease characterized by multiple autoantibodies. Specific and pathogenic autoantibodies were previously studied in juvenile SLE (JSLE) patients, particularly anti-C1q (1-4), anti-double stranded DNA (anti-dsDNA) (3-5) and anti-chromatin/nucleosome antibodies (4,5). We have previously shown that in JSLE patients that the presence of these autoantibodies were associated with lupus activity (5) and may be a valuable tool for monitoring the disease course (3). However, to our knowledge, the concomitant evaluation of these three antibodies with assessments of sensitivity, specificity, positive and negative predictive values for JSLE diagnosis were not carried out in a pediatric lupus population.

Therefore, we evaluated the prevalence of anti-C1q, anti-dsDNA, anti-chromatin/nucleosome antibodies in JSLE and controls, and the possible association of these antibodies with lupus nephritis and disease activity. Additionally, we assessed the sensitivity, specificity, positive and negative predictive values of the three autoantibodies for JSLE diagnosis.

Materials and Methods

Sixty-two consecutive JSLE patients followed at the Pediatric Rheumatology Unit were evaluated. All patients fulfilled the American College of Rheumatology (ACR) SLE classification criteria (6). The control group included 34 healthy subjects followed at the Adolescent Unit at the same University Hospital. The Local Ethical Committee approved this study and an informed consent was obtained from all participants.

Auto-antibodies and C1q assessments

Anti-C1q antibodies were detected by enzyme-linked immunosorbent assay (ELISA) (Inova Diagnostics - QUANTA Lite^{TM} Anti-C1q, San Diego, California, USA). The cut-off for a positive test result was 20 Units, as

determined by the manufacturer. Anti-chromatin/nucleossome antibodies were determined by ELISA (Inova Diagnostics - QUANTA Lite^{TM} Anti-nucleossome, San Diego, California, USA). The cut-off for a positive test result was 20 Units, as also determined by the manufacturer. Anti-dsDNA antibodies were detected by Farrzyme assay (The Binding Site, Birmingham, UK) with cut-off of 30 Units, as determined by the manufacturer. All antibodies were assessed with duplicated samples in JSLE patients and controls. C1q levels were evaluated by radial immunodiffusion (The Binding Site, Birmingham, UK) in JSLE patients and the normal values were 33-209 mg/L.

Demographic data, nephritis and disease activity JSLE evaluations

Demographic data included current age, age at JSLE onset and gender. Renal involvement was defined according to proteinuria ≥ 0.5g/24h, presence of cellular casts or persistent hematuria ≥ 10 red blood cells per high power field. SLE disease activity at the time of study entry was measured in all patients, using the SLE Disease Activity Index 2000 (SLEDAI-2K) (7). Disease activity was arbitrarily defined as SLEDAI-2K ≥ 4.

Statistical analysis

Results were presented as the mean ± standard deviation (SD) or median for continuous and number (%) for categorical variables. Data were compared by *t* test in continuous variables to evaluate differences between JSLE and controls, and in JSLE subgroups. Categorical variables differences were assessed by Fisher's exact test. Spearman´s coefficient was used to evaluate correlations between serum auto-antibodies, and between anti-C1q antibodies and C1q levels. The sensitivity, specificity, positive and negative predictive values of these antibodies for JSLE diagnosis were also evaluated. In all the statistical tests the level of significance was set at 5% ($p < 0.05$).

Results

The mean current age was comparable in JSLE patients and controls (14.6 ± 3.86 vs. 13.6 ± 2.93 years, p=0.14). The percentage of female gender was similar in both groups (83% vs. 79%, p=0.58) (Table 1) and the age at JSLE onset ranged from 3 to 16 years.

Higher frequencies of elevated anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies were observed in JSLE compared to controls (20% vs. 0%, p=0.0037; 48% vs. 0%, p<0.0001 and 69% vs. 3%, p<0.0001, respectively). The median of anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies were also significantly higher in JSLE patients than in the controls [9.6 (5.5-127) vs. 7.5 (5-20) Units, p=0.0006; 18 (1.9-212) vs. 3.2 (1.7-17) Units, p<0.0001 and 111 (6-741) vs. 14 (6-33) IU/mL, p<0.0001, respectively] (Table 1).

Nephritis was evidenced in 50 (81%) of JSLE patients. No differences were observed in JSLE patients with and without nephritis in the frequencies of anti-C1q (26% vs. 8%, p=0.26), anti-chromatin/nucleosome (48% vs. 50%, p=1.00) and anti-dsDNA antibodies (72% vs. 58%, p=0.3).

Disease activity was observed in 43 (69%) of JSLE patients. No differences were observed in JSLE patients with SLEDAI ≥ 4 *versus* SLEDAI < 4 in the frequencies of anti-C1q (20% vs. 21%, p=1.0), anti-chromatin/nucleosome (56% vs. 31%, p=0.1) and anti-dsDNA antibodies (72% vs. 63%, p=0.55).

The sensitivities for anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies were 21% (confidence interval - CI: 11-33%), 49% (CI: 36-62%) and 70% (CI: 57-81%); and the specificities were 100% (CI: 88-100%), 100% (88-100%) and 97% (CI: 83-99%), respectively. Positive predictive value was 100% (CI: 75-100%), 100% (CI: 88-100%) and 97% (CI: 87-99%) and negative predictive value was 39% (CI: 28-50%), 50% (CI: 37-62%) and 62% (CI: 47-76%), for anti-C1q, anti-chromatin/nucleosome and anti-dsDNA antibodies, respectively.

Regarding the discordance between the three auto-antibodies, remarkably, one patient was positive only for anti-C1q antibody (negative for anti-

chromatin/nucleosome and anti-dsDNA antibodies) and another patient was positive only for anti-chromatin/nucleosome antibody (negative for anti-C1q and anti-dsDNA autoantibodies).

Figure 1 shows a positive correlation between anti-C1q and anti-dsDNA antibodies in JSLE patients (r=0.51, IC: 0.29-0.68, p<0.0001). Figure 2 demonstrates a positive correlation between anti-chromatin/nucleosome and anti-dsDNA antibodies in JSLE patients (r=0.87, CI: 0.79-0.92, p<0.0001).

Additionally, a negative correlation was observed between anti-C1q and C1q serum levels (r= - 0.33, CI: - 0.56 - - 0.05, p=0.018). None of them had undetectable C1q levels compatible with a primary C1q immunodeficiency.

Discussion

Our study demonstrated an elevated specificity and positive predictive value for lupus diagnosis of these three autoantibodies, especially anti-chromatin/nucleosome anti-C1q antibodies. The anti-chromatin/nucleosome antibody showed a fairly strong positive correlation with anti-dsDNA.

Anti-C1q antibodies have been associated to lupus nephritis and disease activity in adult (8,9) and juvenile lupus patients (4). However, we recently demonstrated that this antibody in our cohort was not associated with nephritis in 67 JSLE patients (3), as observed herein. Likewise, Ravelli et al did not observe any association between anti-C1q levels and renal involvement (1). Nonetheless, the present study showed a positive correlation between anti-C1q and anti-dsDNA antibodies. The latter is a well-known biomarker of renal disease activity in active SLE. Another important consideration is that, a standardized set of reagents for the determination of anti C1q antibodies has not been established yet, this may partially explain some discrepancies in the previous results.

Additionally, in our lupus patients, a negative correlation was observed between anti-C1q and C1q serum levels, as also evidenced in a Chinese study with pediatric lupus patients (4). Therefore, the presence of these antibodies could lead to

a secondary decrease in C1q levels and to an impairment of auto-antigens clearance, contributing to lupus pathogenesis (10).

Anti-chromatin/nucleosome antibodies have also been described as a marker of disease activity in adult patients (11-14). Regarding pediatric lupus, we previously showed that the presence of this antibody was associated with lupus activity but not with renal manifestations (2), as also observed in the current study.

Importantly, these autoantibodies had a markedly high specificity and positive predictive value, greater than 97%, for lupus diagnosis, and can be considered as a reliable tool in a clinical practice, especially anti-chromatin/nucleosome and anti-C1q antibodies. Indeed, in previous studies, the specificity and positive predictive value for anti-chromatin/nucleosome antibodies for JSLE diagnosis were reported from 96 to 98% (4,5) and 97% (5), respectively. Regarding the anti-C1q antibodies, the specificity for JSLE diagnosis was 92-100% (3,4).

The determinations of anti-chromatin/nucleosome and anti-C1q antibodies should be carried out for evaluation of both lupus diagnosis, especially in JSLE patients who are negative for anti-dsDNA autoantibodies. These exams may be considered as lupus biomarkers, particularly in patients with incomplete lupus (up to three ACR classification lupus criteria) and prospective studies are necessary.

In conclusion, although the anti-C1q and anti-nucleosome auto-antibodies presented a lower sensitivity compared to anti-dsDNA, the exceedingly high specificity and positive predictive value of both antibodies could help in JSLE diagnosis, especially in patients with negative anti-dsDNA.

Acknowledgments

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (grant 2008/58238-4), Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPQ (grants 303165/2008-1 to MCS, and 300248/2008-3 to CAS) and Federico Foundation Grant to CAS. We thank Mariana Acenjo for C1q analysis and Rufus Burlingame from INOVA for providing the anti-C1q kit.

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Table 1 – Demographic data and anti-C1q, anti-chromatin/nucleosome and anti-dsDNA autoantibodies in juvenile systemic lupus erythematosus (JSLE) patients and controls.

Variables	JSLE (n=62)	Controls (n=34)	p
Demographic data			
Current age, yrs	14.6 ± 3.86	13.6 ± 2.93	0.14
Female gender	52 (83)	27 (79)	0.58
Autoantibodies			
Anti-C1q, Units	9.6 (5.5-127)	7.5 (5-20)	**0.0006**
Elevated levels (> 20 U)	13 (20)	0 (0)	**0.0037**
Anti-chromatin/nucleosome, Units	18 (1.9-212)	3.2 (1.7-17)	**<0.0001**
Elevated levels (> 20 U)	30 (48)	0 (0)	**<0.0001**
Anti-dsDNA, IU/mL	111 (6-741)	14 (6-33)	**<0.0001**
Elevated levels (>30 IU)	43 (69)	1 (3)	**<0.0001**

Values were expressed in mean ± standard deviation, median (range) or n (%).

Figure 1 - Positive correlation between anti-C1q and anti-dsDNA antibodies in 62 juvenile systemic lupus erythematosus patients (r=0.51, IC: 0.29-0.68, p<0.0001)

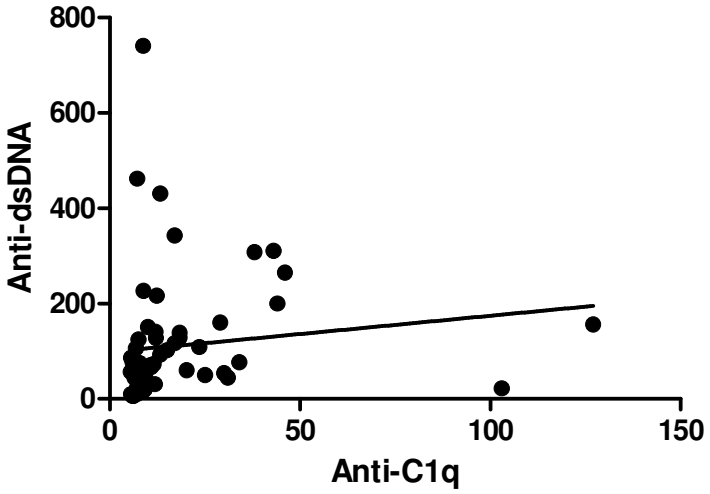
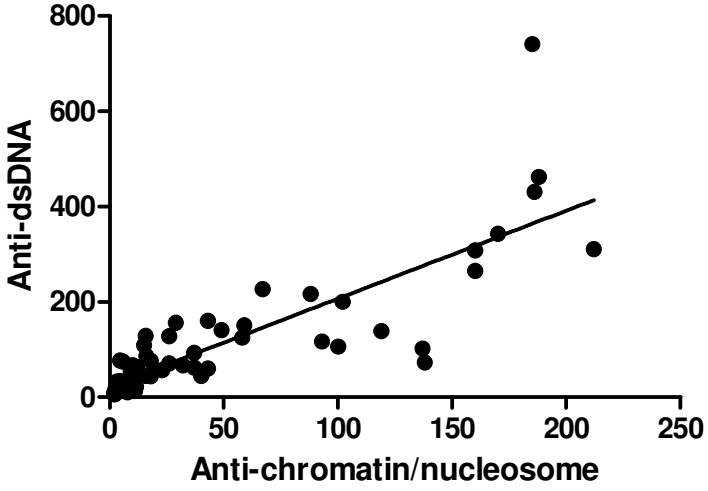


Figure 2 – Positive correlation between anti-chromatin/nucleosome and anti-dsDNA antibodies in 62 juvenile systemic lupus erythematosus patients (r=0.87, CI: 0.79-0.92, p<0.0001)



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Case Report

COMMON VARIABLE IMMUNODEFICIENCY ASSOCIATED WITH HEPATOSPLENIC T-CELL LYMPHOMA MIMICKING JUVENILE SYSTEMIC LUPUS ERITHEMATOSUS

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SUMMARY

Common variable immunodeficiency (CVID) is a heterogeneous disorder with susceptibility to infections, autoimmune manifestations and cancer. To our knowledge, CIVD with T-cell lymphoma mimicking juvenile systemic lupus erythematosus (JSLE) was not described in the literature and one case was reported herein. An 8-year-old female was admitted in our Pediatric Immunology Unit with a clinical history of hypogammaglobulinemia, recurrent upper respiratory infections and pneumonias. She had a marked decrease of three serum immunoglobulin isotypes and the diagnosis of CVID was established. At the age of 17-years, she presented oral ulceration, nonerosive arthritis, nephritis, serositis, cytopenia, positive antiphospholipid antibodies and positive antinuclear antibody fulfilling the American College of Rheumatology (ACR) criteria for SLE. She was treated with intravenous methylprednisolone for three consecutive days and intravenous immunoglobulin, and after chloroquine, azathioprine and prednisone 40 mg/day. Two months later, she died of septic shock secondary to acute pneumonia. The necropsy showed hepatosplenic T-cell lymphoma with diffuse involvement of bone marrow, spleen, liver and lungs. The lymphoma cells were positive for CD3 immunostaining and negative for CD20 and lysozyme. In conclusion, the association of CVID and hepatosplenic T-cell lymphoma may simulate JSLE diagnosis.

Key words: common variable immunodeficiency, juvenile systemic lupus erythematosus, hepatosplenic T-cell lymphoma, sepsis.

INTRODUCTION

The juvenile form of systemic lupus erythematosus (SLE) is a rare autoimmune disorder that may affect multiple organs and systems[1]. Of note, some primary immunodeficiencies (PIDs) are frequently associated to early-onset SLE or lupus manifestations, such as the deficiencies of the first components of the classical complement pathway and selective IgA deficiency[2]. On the other hand, PIDs with severe antibody synthesis deficiency, such as agammaglobulinemia and common variable immunodeficiency (CVID), have been rarely associated to SLE development[1]

CVID is a heterogeneous disorder with susceptibility to infections, autoimmune manifestations and cancer[3], and has been classified as a predominantly antibody deficiency according to the International Union of Immunological Societies (IUIS) updated classification[4]. This PID is characterized by a marked decrease of two serum immunoglobulin isotypes, usually IgG and IgM and/or IgA, over two standard deviations below mean values for age, in addition to impaired ability to specific antibody production after vaccination or exposure to a known infectious agent[3].

Autoimmune manifestations have been described in up to 20% of CVID patients[3]. The most common autoimmune complications reported are the cytopenias, especially immune thrombocytopenic purpura, and autoimmune hepatitis[3]. Additionally, systemic lupus erythematosus (SLE) was rarely reported in CVID patients[5], generally diagnosed during the disease follow-up.

Furthermore, CVID patients have 2-8% of non-Hodgkin´s lymphoma, especially B-cell origin[3]. However, to our knowledge, CVID with T-cell lymphoma mimicking

juvenile SLE (JSLE) was not described in the literature and one case was reported herein.

CASE REPORT

An 8-year-old female was admitted to the Pediatric Immunology Unit with a clinical history of recurrent upper respiratory infections, pneumonias and hypogammaglobulinemia. She presented the first severe infection when she was 6 months old, needing hospitalization in intensive care unit (ICU). At 5 and 7 years-old, she had two pneumonias with pleural effusion. At her admission with 8-year-old, physical examination detected weight and height at 25 percentile. Laboratory exams demonstrated: hemoglobin 12.5 g/L, hematocrit 40.1%, white blood cell count 6500cels/mm^3, platelets 211,000/mm^3, reduced serum levels of IgG 497mg/dL (normal 952-1538 mg/dL), IgA <6mg/dL (normal 111-335), IgM 55mg/dL (normal 59-151), CD3 2085 cells/mm^3 (normal 605-2460), CD4 936 cells/mm^3 (normal 493-1666), CD8 937 cells/mm^3 (normal 224-1112), CD56 233 cells/mm^3 (normal 73-654) and CD19 69 cells/mm^3 (normal 72-520). Therefore, CVID was diagnosed according to IUIS criteria[5], and prophylactic antibiotics and intravenous immunoglobulin (IVIG) were started. Antinuclear antibody (ANA) and rheumatoid factor (RF) were negative at that moment. The treatment resulted in the maintenance of IgG $\geq$ 600mg/dL and in a reduced frequency of infectious episodes. However, during the follow up, she was hospitalized eight times due to: septic shock (n=3), pneumonia with pleural effusion (n=2), otomastoiditis (n=1), acute cytomegalovirus infection (n=1) and urinary tract infection (n=1). At 12 years-old, she developed pancytopenia [hemoglobin 10.2 g/L, hematocrit

34.2%, white blood cell count 3,790/mm³ (39% neutrophils, 54% lymphocytes, 2% eosinophils and 5% monocytes), platelets 108,000/mm³] associated to hepatosplenomegaly. Bone marrow aspiration was performed twice and showed hyperplasia of erythrocyte and hypoplasia of granulocyte series. At that moment, autoantibodies were not detected, such as: ANA, RF, anti-double stranded-DNA (anti-dsDNA), anti-Sm, anti-RNP, anti-Ro, anti-La, anti-P ribosomal, anticardiolipin IgG and IgM, lupus anticoagulant, anti-Scl70, anti-Jo1, anti-insulin, anti-neutrophil cytoplasmic (ANCA), anti-glutamic acid decarboxylase (anti-GAD), anti-insulin, anti-thyroglobulin, anti-peroxidase, anti-parietal cell, anti-endomysium, anti-smooth muscle and anti-liver-kidney microsome antibodies. At the age of 17-years, the patient presented fever, oral ulcers, alopecia, arthritis of wrists and elbows, headache and cough, being hospitalized. She evolved to septic shock associated to pleural and large pericardial effusion and was admitted to ICU. Laboratory exams revealed: hemoglobin 7.9 g/L, hematocrit 22%, white blood cell count 1,000/mm³, platelets 17,000/mm³, reticulocyte count 0.32% and proteinuria 3.0 g/day. Polymerase chain reaction (PCR) for Epstein-Barr virus was negative. The following autoantibodies were observed: ANA (1:320, dense fine speckled pattern), anticardiolipin IgM (100 MPL), RF and lupus anticoagulant. At that moment, she fulfilled the American College of Rheumatology (ACR)[6] criteria for SLE. Additionally, she also had hepatosplenomegaly, and aspartate aminotransferase (AST) 58 IU/l (normal 0-20 IU/l), alanine aminotransferase (ALT) 111 IU/l (normal 6-20 IU/L), triglycerides 456 mg/dL (normal<130 mg/dL) and ferritin 1668 ng/ml (36-92 ng/ml). She was treated with intravenous methylprednisolone for three consecutive days and IVIG (2 g/kg/dose), and after chloroquine (250mg/day), azathioprine (100mg/day) and prednisone 40 mg/day. At that moment, bone marrow

aspiration evidenced hypoplasia in all cell lineages, renal biopsy showed isolated tubular necrosis without glomerular injury (direct immunofluorescence study was negative) and liver biopsy showed drug-induced hepatitis. Despite treatment, two months later, she died of septic shock secondary to acute pneumonia. Remarkably, the necropsy showed hepatosplenic T-cell lymphoma (HSTL) with diffuse involvement of bone marrow, spleen, liver and lungs. The lymphoma cells were positive for CD3 immunostaining and negative for CD20 and lysozyme.

DISCUSSION

Lymphomas, especially from B-cell origin, have been described as associated to 2-8% of CIVD patients[3]. However, to our knowledge, this was the first case reported in the literature that described CVID with HSTL. Moreover, the clinical and laboratorial features mimicked JSLE.

Peripheral T-cell lymphomas are rare neoplasms. Two different subtypes are known: HSTL and primary cutaneous gammadelta T-cell lymphoma. Of note, HSTL is an extranodal lymphoma characterized by intrasinusoidal infiltration of the bone marrow, liver and spleen[7], as observed in our patient. This entity has a rapidly and acute progressive course, with pancytopenia and lung involvement, without significant lymphadenopathy, as also evidenced herein[8]. This cancer commonly affects male adolescents and young adults[8], and may occur in patients under immune system suppression[7], particularly long-term immunosuppressive therapy for solid organ-transplantation[8]. Lymphocyte immunophenotyping is important for definitive diagnosis, especially expression of CD3[8].

Importantly, CVID patients may present non-Hodgkin´s lymphoma generally in 6th and 7th decades and rarely in pediatric population. The most frequent subtype of this neoplasm is B-cell without EBV infection[3]. Our female patient had a T-cell lymphoma in the second decade of life.

In addition, the patient described herein had JSLE clinical manifestations eleven years after CVID diagnosis. These findings contrast with the few case reports on the association between CVID and SLE that showed this primary immunodeficiency occurred during SLE follow-up. Fernandez-Castro et al[5] described two cases and reviewed the literature regarding SLE and CVID association. In all the 18 cases reported in the literature, CVID diagnosis was established one to 22 years after SLE diagnosis[5].

Our patient had 7 of the 11 ACR classification lupus criteria (oral ulceration, nonerosive arthritis, nephritis, serositis, hematologic abnormalities, positive antiphospholipid antibodies and positive ANA)[6]. Interestingly, the sensitivity and specificity of ACR 1982 criteria for JSLE were 96% and 100%, respectively[9].

Lupus mimicking lymphoma in adult population was rarely described. Subcutaneous panniculitis-like T-cell lymphoma simulating a lupus erythematosus panniculitis with systemic manifestations was reported in one 29-year-old woman[10]. In contrast, JSLE with nephrotic syndrome and mediastinal lymphadenopathy mimicking lymphoma was described in one female adolescent[11].

In conclusion, the association of CVID and hepatosplenic T-cell lymphoma with diffuse involvement of bone marrow, spleen, liver and lungs may simulate JSLE diagnosis.

ACKNOWLEDGMENTS

This study was sponsored by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (grant 08/58238-4) by Conselho Nacional de Desenvolvimento Científico - CNPQ (300248/2008-3 to CAS and to 34802/2008-0 to MC-S) and Federico Foundation to CAS.

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Concise Report

ORGAN-SPECIFIC AUTOANTIBODIES AND AUTOIMMUNE DISEASES IN JUVENILE SYSTEMIC LUPUS ERYTHEMATOSUS AND JUVENILE DERMATOMYOSITIS PATIENTS

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Conflicts of interest: none

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Summary

To our knowledge, no study has assessed simultaneously a large number of organ-specific autoantibodies, as well as the prevalence of organ-specific autoimmune diseases in juvenile systemic lupus erythematosus (JSLE) and juvenile dermatomyositis (JDM) populations. Therefore, the purpose of this study was to evaluate organ-specific autoantibodies and autoimmune diseases in JSLE and JDM patients. Forty-one JSLE and 41 JDM patients were investigated for serum autoantibodies associated with autoimmune hepatitis, primary biliary cirrhosis, type 1 diabetes mellitus (T1DM), autoimmune thyroiditis, autoimmune gastritis and celiac disease. Patients with positive organ-specific antibodies were assessed for the presence of the respective organ-specific autoimmune diseases. Mean age at diagnosis was significantly higher in JSLE compared to JDM patients (10.3±3.4 vs. 7.3±3.1years, p=0.0001), whereas the mean disease duration was similar in both groups (p=0.92). The frequencies of organ-specific autoantibodies were similar in JSLE and JDM patients (p>0.05). Of note, the high prevalence of autoantibodies related to T1DM and autoimmune thyroiditis were observed in both groups (20% vs. 15%, p=0.77 and 24% vs. 15%, p=0.41; respectively). Higher frequencies of antinuclear antibody - ANA (93% vs. 59%, p=0.0006), anti-dsDNA (61% vs. 2%, p<0.0001), anti-Ro (35% vs. 0%, p<0.0001), anti-Sm (p=0.01), anti-RNP (p=0.02), anti-La (p=0.03) and IgG aCL (p=0.001) were observed in JSLE compared to JDM patients. Organ-specific autoimmune diseases were evidenced only in JSLE patients (24% vs. 0%, p=0.13). Two JSLE patients had T1DM associated with Hashimoto thyroiditis and another had subclinical thyroiditis. Another JSLE patient had celiac disease diagnosis based on iron deficiency anaemia, presence of anti-endomysial antibody, duodenal biopsy compatible to celiac disease and response to a gluten-free diet. In conclusion, organ-specific diseases were observed solely in JSLE patients and required specific therapy. The presence of these antibodies recommends the evaluation of organ-specific diseases and a rigorous follow-up of these patients.

Keywords: organ-specific, autoantibodies, juvenile systemic lupus erythematosus, juvenile dermatomyositis.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune multisystemic disease characterized by the presence of autoantibodies[1]. Juvenile dermatomyositis (JDM) is a connective tissue disease characterized by muscle and cutaneous vasculitis, which can also compromise other organs and systems[2]. JDM etiology is unknown. However, the presence of chronic muscle inflammation, positivity for serum autoantibodies and association with other autoimmune diseases suggest that autoimmune mechanism is involved in its pathogenesis[2].

Of note, studies on organ-specific autoimmunity in juvenile SLE (JSLE) patients have shown a high prevalence of anti-thyroid antibodies and subclinical hypothyroidism[1]. Additionally, autoimmune hepatitis was rarely reported in our JSLE patients[3]. On the other hand, few studies have described organ-specific antibodies in JDM[4,5], including rare cases of Hashimoto thyroiditis (HT)[6] and type 1 diabetes mellitus (T1DM)[7]. To our knowledge, other organ-specific autoimmune diseases, such as celiac disease (CD), autoimmune gastritis and primary biliary cirrhosis, were not evaluated in both diseases.

Moreover, no study assessed simultaneously a large number of organ-specific autoantibodies, as well as the prevalence of subclinical organ-specific autoimmune diseases in JSLE and JDM patients.

Therefore, the objectives of the present study were to investigate the organ-specific and other serum autoantibodies in JSLE and JDM populations, and to evaluate the possible association between demographic data, disease activity and treatment in JSLE and JDM patients according to the presence of organ-specific antibodies. In addition, the organ-specific diseases were also described.

Patients and methods

Forty-one JSLE and 41 JDM patients regularly followed at the Pediatric Rheumatology Unit of our University Hospital were enrolled from January 2008 to January 2009. All patients fulfilled the American College of Rheumatology (ACR) criteria for JSLE[8] and the Bohan and Peter criteria for JDM[9]. The Local Ethical Committee approved this study and an informed consent was obtained from all participants.

Organ-specific and other autoantibodies

Serum autoantibodies associated with the following organ-specific autoimmune diseases were assessed: **autoimmune thyroiditis** - anti-thyroid peroxidase (anti-TPO) antibody by fluoroimmunoassay, anti-thyroglobulin (anti-TG) antibody and anti-thyroid stimulating hormone (TSH) receptor antibody (TRAb) by chemiluminescence; **T1DM** - insulin autoantibody (IAA), anti-glutamic acid decarboxylase (anti-GAD) antibody and anti-tyrosine phosphatase (anti-IA2) antibody by radioimmunoassay; **autoimmune hepatitis** - anti-type I liver-kidney microsomal (anti-LKM-1) antibody and anti-smooth muscle antibody (SMA) by indirect immunofluorescence on rat liver and kidney tissue sections; **primary biliary cirrhosis** - antimitochondrial antibody (AMA) by indirect immunofluorescence on rat liver, kidney and stomach parietal cells, and confirmation of the positive cases by enzyme-linked immunosorbent assay (ELISA); **autoimmune gastritis** - parietal cell autoantibody (PCA) by indirect immunofluorescence; **celiac disease** – immunoglobulin A (IgA) class anti-endomysial (EMA) antibody by indirect immunofluorescence. Patients who were positive for organ-specific autoantibodies had the test repeated for confirmation. After that, they were investigated for the presence of the organ-specific autoimmune disease.

The following other serum autoantibodies were also measured: antinuclear antibody (ANA) by indirect immunofluorescence using human cell epithelioma (HEp-2), rheumatoid factor (RF) by immunonephelometry, anti-double-stranded DNA (anti-dsDNA), anti-Sm, anti-RNP, anti-SSA/Ro, anti-

SSB/La , anti-topoisomerase 1 (anti-Scl70), anticardiolipin (aCL) isotypes IgG and IgM by ELISA and lupus anticoagulant (LAC) by the dilute Russell's viper venom time with confirmatory testing, anti-neutrophil cytoplasm antibody (ANCA) by ELISA and direct immunofluorescence in human neutrophils fixed with ethanol and anti-Jo1 by ELISA.

All autoantibodies were assessed at Central Laboratory Division of our Hospital.

Organ-specific autoimmune diseases

HT was defined according to the reduced levels of free thyroxine (T4) and elevated TSH levels, and subclinical hypothyroidism as elevated TSH levels associated with normal levels of T4[10]. The presence of antithyroid antibodies was required to characterize autoimmune thyroiditis. T1DM was diagnosed by the presence of polyuria, polydipsia and unexplained weight loss, and increased plasma glucose ≥ 200 mg/dL at any time of day or fasting glucose ≥ 126 mg/dL[11]. Autoimmune hepatitis was defined as a progressive chronic hepatitis of unknown origins, characterized by elevated transaminase levels, hypergammaglobulinemia, serum autoantibodies and histological characteristics[12]. Primary biliary cirrhosis was defined as the presence of at least two of the following: elevated alkaline phosphatase (≥ 2 times the upper limit of normal) or gamma-glutamiltrasferase (≥ 5 times the upper limit of normal), positivity for AMA, and liver biopsy with nonsuppurative cholangitis and destruction of bile ducts[13]. Autoimmune gastritis was defined by the presence of gastric fundus and body atrophy in histology, positivity for PCA and anti-intrinsic factor, hypo/achlorhydria, low concentrations of serum pepsinogen and anemia secondary to vitamin B12 and iron deficiency[14]. Celiac disease diagnosis was defined by the presence of at least four of the following criteria: clinical manifestations (chronic diarrhea, stunting and/or iron deficiency anemia), positivity for celiac disease IgA class antibodies, HLA-DQ2 or DQ8 genotype, small intestine biopsy compatible with celiac enteropathy, and response to gluten-free diet[15].

Disease activity, disease damage and treatment in JSLE and JDM patients

SLE disease activity and cumulative damage were measured at the moment of organ-specific antibodies and disease evaluations in JSLE patients using the SLE Disease Activity Index 2000 (SLEDAI-2K)[16] and the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SLICC/ACR-DI)[17].

JDM activity was assessed by disease activity score (DAS)[18], and muscle strength was evaluated by childhood myositis assessment scale (CMAS)[19] and manual muscle testing (MMT)[19]. The serum muscle enzymes performed concomitantly to organ-specific antibodies and disease assessments were creatine phosphokinase (CPK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and aldolase.

Data concerning the current JSLE and JDM treatments included: prednisone, methotrexate, azathioprine, chloroquine, cyclosporine, cyclophosphamide, mycophenolate mofetil and intravenous immunoglobulin.

Statistical analysis

Results were presented as mean ± standard deviation or median (range) for continuous and number (%) for categorical variables. Data were compared by t-Student or Mann-Whitney tests for continuous variables to evaluate differences between JSLE and JDM patients. For categorical variables differences were assessed by Fisher's exact test. In all the statistical tests the level of significance was set at 5% ($p < 0.05$).

Results

Demographic features: The mean age at JSLE diagnosis was significantly higher compared to JDM patients (10.3 ± 3.4 vs. 7.3 ± 3.1 years, p=0.0001). However, the mean duration of disease was similar in both groups (4.4 ± 3.7 vs. 4.4 ± 3.3, p=0.92), as well as the frequency of female gender (85% vs. 71%, p=0.18).

Organ-specific and other autoantibodies

The frequencies of at least one serum organ-specific antibody were similar in JSLE and JDM patients [17 (41%) vs. 11 (27%), p=0.24]. High frequencies of autoantibodies related to autoimmune thyroiditis (anti-TG, anti-TPO antibodies and/or TRAb) and T1DM (IAA, anti-GAD and/or anti-IA2 antibodies) were observed in both diseases (24% vs. 15%, p=0.41; 20% vs.15%, p=0.77; respectively). The frequencies of EMA and PCA were comparable in both groups (2% vs. 2%, p=1.0; 2% vs. 0%, p=1.0; respectively). Likewise, the frequencies of autoimmune hepatitis antibodies were similar: anti-LKM-1 antibody and/or SMA (2% vs. 5%, p=1.0). None of JSLE and JDM patient had AMA (Table 1).

Higher frequencies of ANA (93% vs. 59%, p=0.0006), anti-dsDNA (61% vs. 2%, p<0.0001), anti-Ro (35% vs. 0%, p<0.0001), anti-Sm (p=0.01), anti-RNP (p=0.02), anti-La (p=0.03) and IgG aCL (p=0.001) were observed in JSLE compared to JDM patients (Table 1).

No differences were observed in the demographic data, disease activity, treatment and other autoantibodies frequencies in 17 JSLE patients with at least one organ-specific autoantibody compared to 24 without organ-specific autoantibodies (p>0.05) (Table 2). Additionally, no differences were evidenced in the demographic data, disease activity, treatment and other autoantibodies in 11 JDM patients with at least one organ-specific autoantibody *versus* 30 without organ-specific autoantibodies (p>0.05) (Table 2).

Organ-specific autoimmune diseases

Organ-specific autoimmune diseases were evidenced only in JSLE patients (24% vs. 0%, p=0.13) (Table 1). Two of them fulfilled both T1DM and HT diagnosis criteria and were treated with insulin and levothyroxine. Another patient had subclinical hypothyroidism with presence of anti-TG antibody. The fourth patient had diagnosis of celiac disease based on the following features: chronic iron deficiency anaemia, presence of AEM antibody, duodenal biopsy compatible to celiac disease and response to a gluten-free diet (Table 3).

None of our 41 JDM patients had evidence of organ-specific autoimmune diseases.

Discussion

As far as we know, this was the first study to evaluate simultaneously a variety of organ-specific antibodies in JSLE and JDM populations, and demonstrated a high prevalence of these antibodies in both diseases. Other antibodies were demonstrated in lupus patients, and organ-specific autoimmune diseases were evidenced exclusively in JSLE patients, particularly autoimmune endocrine and gastrointestinal illnesses that required specific treatment.

Of note, JSLE is a chronic multisystem autoimmune disease, characterized by the presence of autoreactive cells and a marked risk for the development of multiple organ and non-organ-specific autoantibodies[1]. The profile of other specific and non-specific antibodies was clearly evidenced in our JSLE patients compared to JDM.

Clinical autoimmune thyroiditis, specially HT, is the most important organ-specific autoimmune disease in female pediatric lupus, and the frequency of anti-thyroid antibodies were previously reported in 14% to 26% of these patients[1,20], as observed in the current study. Autoimmune subclinical hypothyroidism was evidenced in 0-7% of JSLE population, as also observed herein[1,20]. On the other hand, autoimmune hyperthyroidism was not diagnosed in our study and has been already described in 2-3% of these patients[1,20].

Interestingly, no study evaluated the frequency of T1DM-associated antibodies in JSLE population, and, to our knowledge, few reports have described pancreas autoantibodies in adult and pediatric lupus[7]. In our population, we found 5% of JSLE patients with these antibodies and controlled insulin-dependent T1DM. This autoimmune disease is under diagnosed in JSLE patients, probably due to a high frequency of diabetes mellitus induced by glucocorticoids use.

Other relevant aspect of our study was the assessment of gastric and intestinal autoimmunity. Remarkably, one of our JSLE patients with chronic iron deficient anemia and without gastrointestinal manifestations had CD. Indeed, the most common manifestations of CD are weight loss and diarrhea, and

chronic anemia is one of the extra-intestinal manifestations found in the subclinical forms of the disease[15].

The PCA antibody is highly correlated with chronic autoimmune gastritis[14]. The absence of gastrointestinal manifestations may indicate that the autoimmune process was at an initial stage in one of our JSLE patients with PCA. Importantly, atrophic alteration of the gastric mucosa can progress to chronic autoimmune gastritis within 20 to 30 years period[14], and this patient requires a rigorous follow-up. Likewise, three of our lupus and JDM patients had liver autoantibodies, and also should be constantly monitored[12].

Furthermore, previous studies were reported with small JDM populations and incomplete evaluations reported assessment of organ-specific and other antibodies. We observed only endocrine, liver and intestinal autoantibodies in JDM patients without autoimmune diseases, as previously described[5]. Montecucco et al[4] did not evidence autoimmune liver and gastric autoantibodies in 14 JDM patients, and Martinez-Cordero et al[5] described one JDM patient with SMA. ANA and aCL-IgM were observed in almost 50% of our patients, and the later had a higher frequency compared to previous studies[4].

Disease activity and treatment was not associated with organ-specific autoantibodies in our two autoimmune disease populations. In contrast, a high frequency of anti-thyroid antibodies and subclinical thyroiditis were previously evidenced in mild JSLE patients[1]. Moreover, fluctuation of these antibodies may occur during the course of the disease, as described in JSLE with autoimmune thyroid disease[1,10].

In conclusion, organ-specific diseases were observed solely in JSLE patients and required specific treatment. The presence of these antibodies recommends the evaluation of organ-specific diseases and a rigorous follow-up of these patients.

ACKNOWLEDGMENTS

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (grant #08/58238-4 to MCS), by Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (grant #300248/2008-3 to CAS) and Federico Foundation Grant to CAS. We thank Dr. Leila Antonângelo of Central Laboratory Division of our Hospital.

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Table 1 – Organ-specific autoantibodies and diseases, and other autoantibodies in juvenile systemic lupus erythematosus (JSLE) *versus* juvenile dermatomyositis (JDM) patients

Variables	JSLE n=41	JDM n=41	p
Organ-specific antibodies	17 (41)	11 (27)	0.24
Autoimmune thyroiditis (anti-TG and/or anti-TPO and/or TRAb)	10 (24)	6 (15)	0.41
Type 1 diabetes mellitus (IAA and/or anti-GAD and/or anti-IA2)	8 (20)	6 (15)	0.77
Celiac disease (EMA)	1 (2)	1 (2)	1.0
Autoimmune hepatitis (SMA and/or anti-LKM-1)	1 (2)	2 (5)	1.0
Primary biliar cirrhosis (AMA)	0 (0)	0 (0)	1.0
Autoimune gastritis (PCA)	1 (2)	0 (0)	1.0
Organ-specific diseases	4/17 (24)	0/11 (0)	0.13
Other autoantibodies			
ANA	38 (93)	24 (59)	**0.0006**
RF	4 (10)	0 (0)	0.12
Anti-dsDNA	25 (61)	1 (2)	**< 0.0001**
Anti-Sm	11 (27)	2 (5)	**0.01**
Anti-RNP	9 (22)	1 (2)	**0.02**
Anti-Ro	14 (35)	0 (0)	**< 0.0001**
Anti-La	6 (15)	0 (0)	0.03
Anti-Scl-70	0 (0)	0 (0)	1.0
Anti-Jo1	0 (0)	2 (5)	0.49
aCL-IgM	20 (49)	17 (41)	0.66
aCL-IgG	19 (46)	5 (12)	**0.001**
LAC	5 (12)	1 (2)	0.2
p-ANCA	4 (10)	0 (0)	0.12
c-ANCA	1 (2)	1 (2)	1.0

Data are expressed in n (%); anti-TG - anti-thyroglobulin antibody, anti-TPO - anti-thyroid peroxidase antibody, TRAb - anti-thyroid stimulating hormone (TSH) receptor antibody, IAA - insulin autoantibody, anti-GAD - anti-glutamic acid decarboxylase antibody, anti-IA2 - anti-tyrosine phosphatase antibody, EMA - anti-endomysial antibody, SMA - anti-smooth muscle antibody, anti-LKM-1 - anti- type I liver-kidney microsomal antibody, AMA - antimitochondrial antibody, PCA - parietal cell autoantibody, ANA - antinuclear antibody, RF -rheumatoid factor, anti-dsDNA - anti-double-stranded DNA, anti-Scl70 - anti-topoisomerase 1, aCL – anticardiolipin, LAC - lupus anticoagulant, p-ANCA – perinuclear anti-neutrophil cytoplasm antibody, c-ANCA – cytoplasmic anti-neutrophil cytoplasm antibody.

Table 2 – Demographic data, disease activity, treatment and other autoantibodies in juvenile lupus erythematosus (JSLE) and juvenile dermatomyositis (JDM) patients with and without organ-specific autoantibodies

JSLE Variables	JSLE with organ-specific antibody (n=17)	JSLE without organ-specific antibody (n=24)	p
Demographic data			
Female gender	13 (76)	22 (92)	0.21
Current age, years	11 (4-13)	11 (6-17)	0.18
Disease duration, years	3.1 (0.3-7.6)	3.8 (0-12.3)	0.78
SLEDAI-2K	5 (0-12)	3 (0-14)	0.36
SLICC/ACR-DI	1 (0-2)	1 (0-2)	0.93
Current treatment			
Prednisone	14 (83)	23 (96)	0.29
Immunosuppressive use	7 (41)	17 (71)	0.11
Other autoantibodies			
ANA	15 (88)	23 (96)	0.56
anti-dsDNA	11 (65)	14 (58)	0.75
anti-Sm	6 (35)	5 (21)	0.48
anti-RNP	5 (29)	4 (17)	0.45
anti-Ro	8 (47)	6 (25)	0.19
anti-La	3 (18)	3 (13)	0.68
aCL-IgM	10 (59)	11 (46)	1.69
aCL-IgG	7 (41)	12 (50)	0.75
JDM Variables	**JDM with organ-specific antibody (n=11)**	**JDM without organ-specific antibody (n=30)**	**p**
Demographic data			
Female gender	10 (91)	19 (63)	0.13
Current age, years	12 (5-17)	11 (6-18)	0.92
Disease duration, years	3.7 (1.1-8.1)	3.7 (0-13.5)	0.86
JDM scores and muscle enzymes			
CMAS (0 - 52)	50 (44-52)	48.5 (4-52)	0.27
MMT (0 - 80)	80 (75-80)	80 (38-80)	0.47
DAS (0 - 20)	2 (0-7)	3 (0-17)	0.71
AST (10 – 36 UI/L)	23.5 (12-43)	25.5 (14-90)	0.57
ALT (24 – 49 UI/L)	34 (29-103)	33.5 (10-123)	0.76
CPK (39 – 170 UI/L)	93 (26-165)	92.5 (40-27381)	0.72
Aldolase (<7.6 UI/L)	7.05 (3.5-9.4)	7.2 (2.7-49.9)	0.95
LDH (240 – 480 UI/L)	159.5 (135-238)	200 (87-522)	0.19
Current treatment			
Prednisone	6 (55)	15 (50)	1.0
Immunosuppressive use	7 (64)	17 (57)	0.74
Other autoantibodies			
ANA	5 (45)	19 (63)	0.48
aCL-IgM	5 (45)	12 (40)	1.0

Data are expressed in n (%) or median (range), SLEDAI-2K - Systemic Lupus Erythematosus Disease Activity Index 2000, SLICC/ACR-DI- Systemic Lupus International Collaborating Clinics/ACR Damage Index, ANA – antinuclear antibody, anti-dsDNA - anti-double-stranded DNA, aCL – anticardiolipin, CMAS – Childhood Muscle Assessment Score, MMT – Manual Muscle Testing, DAS – Disease Activity Score, AST – aspartate aminotranspherase, ALT – alanine aminotranspherase, CPK – creatine phosphokinase, LDH – lactate dehydrogenase.

Table 3 – Demographic data, disease activity, other autoantibodies and treatment in four juvenile systemic lupus erythematosus (JSLE) patients with organ-specific autoimmune diseases

Case	1	2	3	4
Demographic data				
Age at JSLE diagnosis, years	11.6	15.6	11.1	9.3
Age at organ-specific autoimmune disease, years	11.4	12	11.6	13.2
Current age, years	15	18.9	16.3	12.6
Gender	female	female	female	female
Organ-specific autoimmune disease	T1DM and HT	T1DM and HT	Subclinical hypothyroidism	Celiac disease
Organ-specific antibodies	Anti-TPO and IAA	TRAb, IAA and Anti-GAD	Anti-TG	EMA
Clinical features at JSLE diagnosis				
Mucocutaneous involvement	-	+	-	+
Arthritis	+	-	+	-
Serositis	+	-	+	+
Hematological abnormalities	-	+	+	-
Neuropsychiatric involvement	-	-	-	-
Nephritis	-	+	+	-
Disease activity and damage at organ-specific antibodies assessment				
SLEDAI-2K	8	8	12	8
SLICC/ACR-DI	2	1	1	0
Current treatment				
JSLE	Prednisone	Prednisone, chloroquine and azathioprine	Prednisone, chloroquine and azathioprine	Prednisone and chloroquine
Organ-specific autoimmune disease	Insulin and levothyroxine supplementation	Insulin and levothyroxine supplementation	-	Gluten-free diet

T1DM - type 1 diabetes mellitus, HT - Hashimoto thyroiditis, Anti-TPO - anti-thyroid peroxidase antibody, IAA - insulin autoantibody, TRAb - anti-thyroid stimulating hormone (TSH) receptor antibody, Anti-GAD - anti-glutamic acid decarboxylase antibody, Anti-TG - anti-thyroglobulin antibody, EMA - anti-endomysial antibody, SLEDAI-2K - Systemic Lupus Erythematosus Disease Activity Index 2000, SLICC/ACR-DI - Systemic Lupus International Collaborating Clinics/ACR (SLICC/ACR) Damage Index, + positive, - negative

J Clin Immunol (2008) 28 (Suppl 1):S34–S41
DOI 10.1007/s10875-008-9187-2

Understanding Systemic Lupus Erythematosus Physiopathology in the Light of Primary Immunodeficiencies

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Received: 28 January 2008 / Accepted: 29 January 2008 / Published online: 11 April 2008

Abstract
Introduction Associations between systemic lupus erythematosus (SLE) and primary immunodeficiencies (PIDs) were analyzed to gain insight into the physiopathology of SLE. Some PIDs have been consistently associated with SLE or lupus-like manifestations: (a) homozygous deficiencies of the early components of the classical complement pathway in the following decreasing order: in C1q, 93% of affected patients developed SLE; in C4, 75%; in C1r/s, 57%; and in C2, up to 25%; (b) female carriers of X-linked chronic granulomatous disease allele; and (c) IgA deficiency, present in around 5% of juvenile SLE.
Discussion In the first two groups, disturbances of cellular waste-disposal have been proposed as the main mechanisms of pathogenesis. On the other hand and very interestingly, there are PIDs systematically associated with several autoimmune manifestations in which SLE has not been described, such as autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), immunedysregulation polyendocrinopathy enteropathy X-linked (IPEX), and autoimmune lymphoproliferative syndrome (ALPS), suggesting that mechanisms considered as critical players for induction and maintenance of tolerance to autoantigens, such as (1) AIRE-mediated thymic negative selection of lymphocytes, (2) Foxp3+ regulatory T cell-mediated peripheral tolerance, and (3) deletion of auto-reactive lymphocytes by Fas-mediated apoptosis, could not be relevant in SLE physiopathology. The non-description of SLE and neither the most characteristic SLE clinical features among patients with agammaglobulinemia are also interesting observations, which reinforce the essential role of B lymphocytes and antibodies for SLE pathogenesis.
Conclusion Therefore, monogenic PIDs represent unique and not fully explored human models for unraveling components of the conundrum represented by the physiopathology of SLE, a prototypical polygenic disease.

Keywords Systemic lupus erythematosus · primary immunodeficiencies · complement deficiencies · chronic granulomatous disease · agammaglobulinemia · APECED · IPEX · ALPS

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Introduction

Primary immunodeficiency diseases (PIDs) represent a large collection of monogenic entities whose genetic defects and whose defective underlying immune mechanisms have already been identified in most of them [1]. Interestingly, there are certain PIDs systematically associated with autoimmunity, while in others, autoimmune manifestations have rarely or never been described, even

with patients presenting recurrent and/or chronic infections [2]. Moreover, there are PIDs in which systemic lupus erythematosus (SLE) is practically the only autoimmune manifestation, such as in the complement deficiencies and in chronic granulomatous disease (CGD), while in others, even in those strongly associated with autoimmune manifestations, SLE has never been observed. Thus, monogenic PIDs may represent unique and privileged human models for unraveling the components of the conundrum represented by SLE physiopathology, a prototypical polygenic disease. Also, notably, some PIDs represent natural "knockout" human models for various lupus susceptibility genes, such as Fas, FasL, ICOS, TACI, Fc receptors, CD40L, IFN-γ, C1q, C1r/s, C4, C2, MBL, among others.

The aim of this review is to analyze the associations between PIDs and lupus to gain understanding of SLE pathophysiology. In Table 1, PIDs are classified according to the degree of association with SLE or lupus-like manifestations. The main PIDs consistently associated with SLE as well as some PIDs strongly associated with autoimmunity but not with SLE are discussed in the text.

PIDs Consistently Associated with SLE or Lupus-Like Manifestations

Complement Deficiencies

Although homozygous complement deficiencies represent rare conditions, and are estimated to be found in less than 1% of SLE patients, their study has substantially contributed to the development of new concepts concerning the pathogenetic mechanisms of SLE [3–5]. Complete deficiency of any of the early components of the classical complement pathway (C1q, C1r, C1s, C4, and C2) represents the strongest single genetic risk factor for the development of SLE identified up to now. A striking feature is that SLE or lupus-like syndromes have been the predominant autoimmune disorders observed in all complement deficiencies, while other autoimmune diseases were rarely described in association with them. SLE or lupus-like manifestations have been identified in respectively 93% of homozygous C1q-deficient, in 75% of C4-deficient, in two thirds of C1r/C1s-deficient, and in up to 25% of C2-deficient individuals (Table 2) [4–6]. Interestingly, lupus-like disease susceptibility drops to around or below 10% in patients with deficiency of C3, and only isolated cases have been reported as associated with deficiencies of the late components of the membrane attack complex (C5–9; see Table 2). Cases of SLE have also been reported among patients with hereditary C1 inhibitor deficiency, who present chronically low levels of C4 and C2 [5, 7]. In both human beings and murine models, the degree of association as well as the severity of the disease is hierarchical depending on the position of the deficient component in the classical pathway. SLE association is less clear for partial deficiencies, as well as for MBL (mannose-binding lectin) complete defects.

Taken collectively, these clinical data point out that the early components of the classical complement pathway, independently of C3 activation, have a critical protective activity against the development of SLE. The first hypothesis

Table 1 Association between PIDs and SLE or Lupus-like Manifestations

Consistently associated	Some isolated SLE or lupus-like case reports	Not associated
C1q deficiency [3–5]	C3 deficiency [4, 5]	APECED[a] [17–20]
C4 deficiency [4, 5]	Hereditary C1 inhibitor deficiency [4, 7]	IPEX[a] [2, 21, 22]
C1r/s deficiency [4, 5]	Deficiencies of MAC components [4, 5]	Wiskott-Aldrich syndrome[a] [43, 44]
C2 deficiency [4–6]	CVID[a] [31–35]	Omenn syndrome[a] [2]
Female carriers of X-linked CGD allele [9, 10]	IgG subclass deficiency [2]	X-linked agammaglobulinemia [28–30]
X-linked and AR CGD [9, 10]	Hyper-IgM syndrome probably due to AID or UNG deficiency [41]	X-linked Hyper-IgM syndrome (CD40L defects) [36, 37]
IgA deficiency[a] [13–15]	ALPS[a] [24–26]	Hyper-IgM due to CD40 deficiency [38]
	Hyper-IgE syndrome [2]	Defects of IL-12/IL-23-IFN-γ axis [45]
	Prolidase deficiency[b] [42]	DiGeorge anomaly [46]
		Ataxia-telangiectasia syndrome [2]
		Chédiak–Higashi syndrome [2]
		FcγRIIIb deficiency [47]
		Autoinflammatory disorders [1, 2]

CGD chronic granulomatous disease, *MAC* membrane attack complex, *ALPS* autoimmune lymphoproliferative syndrome, *APECED* autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, *IPEX* immune dysregulation, polyendocrinopathy, enteropathy X-linked, *CVID* common variable immunodeficiency, *AID* activation induced cytidine deaminase, *UNG* uracil-N-glycosilase

[a] PIDs frequently associated with other autoimmune manifestations

[b] Entity not classified as a PID, although affected patients are prone to infections

Table 2 Homozygous Complement Deficiencies and Susceptibility to SLE or Lupus-like Manifestations [3–7]

Complement deficiency	Frequency of SLE/lupus-like manifestations (%)	Main functional defects	Main lupus clinical features	Susceptibility to infections
C1q	93	Impaired clearance of apoptotic debris and dissolution of immune complexes (IC)	High frequency of glomerulonephritis and central nervous system disease; marked photosensitivity	Encapsulated bacteria
C4	75	Impaired clearance of IC; defective humoral immune response	Multi-organ involvement; glomerulonephritis in 30%	Encapsulated bacteria
C1r/C1s	57	Impaired clearance of IC	Multi-organ involvement; glomerulonephritis in 30%	Encapsulated bacteria
C2	10–25	Impaired dissolution of IC	Prominent cutaneous (photosensitivity) and articular involvement; mild or absent renal, neurological or pleuropericardial involvement; low frequency of ANA; high frequency of anti-Ro antibodies	Pyogenic infections; invasive infections due to encapsulated bacteria; *Streptococcus pneumoniae* sepsis and meningitis
Hereditary C1 inhibitor deficiency	23 case reports	Spontaneous activation of the complement pathway with consumption of C4 and C2	Systemic or discoid lupus erythematosus; high frequency of photosensitivity and ANA	Normal resistance to infections
C3 deficiency	3 case reports	Defective MAC, defective bactericidal activity, defective humoral immune response	SLE; malar rash, photosensitivity, arthralgia and Raynaud's phenomenon	Recurrent pyogenic infections
C7 deficiency	3 case reports	Defective MAC, defective bactericidal activity	Systemic lupus erythematosus	Neisserial infections
C6 deficiency	3 case reports	Defective MAC, defective bactericidal activity	One DLE and 2 SLE patients	Neisserial infections
C9 deficiency	2 case reports	Defective MAC, defective bactericidal activity	SLE with renal involvement	Neisserial infections
C5 deficiency	2 case reports	Defective MAC, defective bactericidal activity	One patient with membranous glomerulonephritis and arthritis and one patient with DLE	Neisserial infections
C8 deficiency	1 case report	Defective MAC, defective bactericidal activity	Photosensitivity, malar rash, alopecia, arthritis and nephritic syndrome	Neisserial infections

DLE discoid lupus erythematosus, *MAC* membrane attack complex, *IC* immune complexes, *ANA* antinuclear antibodies

to explain these observations invokes the role of complement in physiological waste disposal, in particular, the processing and clearance of dying cells and immunecomplexes [3, 5]. It has been demonstrated that C1q is essential for proper clearance of apoptotic cells, considered as the primary source of self antigens which break tolerance during SLE development. SLE associated with complete C1q deficiency is a severe condition, which tends to appear early in life, with no female predominance. There is no description of C1q deficiency among healthy individuals, reinforcing the crucial role of C1q for immune homeostasis [5]. The second hypothesis, which does not exclude the first one, proposes that complement also plays a role in activation of B and T lymphocytes and that complement deficiency causes autoantibody production by impairing the normal mechanisms of tolerance induction and maintenance [5, 8].

Chronic Granulomatous Disease

CGD is a genetically heterogeneous disease characterized by recurrent life-threatening infections with bacteria and fungi due to severely impaired phagocyte intracellular killing. CGD is caused by defects of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase system, which is responsible for the generation of superoxide and other reactive oxygen species in phagocytic cells. The X-linked form, caused by mutations of the *CYBB* gene, accounts for more than 75% of the cases [9]. In the large US series of

368 CGD patients, ten (2.7%) presented discoid lupus (DLE) and 2 (0.5%) SLE. [9]. In the same study, a number of first-degree female relatives were reported as having SLE or DLE (20 with DLE and two with SLE from 290 kindreds), lupus manifestations being significantly more common among the mothers, grandmothers, maternal counterparts, and/or maternal aunts of the X-linked CGD patients. Interestingly, female carriers do not present abnormal susceptibility to infections. More recently, Cale et al. [10] investigated 19 carrier mothers of the X-linked CGD allele for the presence of lupus manifestations. It is remarkable that 12 mothers presented photosensitivity, seven presented arthralgia, and eight had mouth ulcers. Anti-nuclear antibodies were positive in five patients, four of whom reported photosensitivity. One patient had anti-double-stranded DNA (dsDNA), and another presented lupus anticoagulant. An explanation for such a strong association between CGD and lupus was given by Brown et al. [11] and Sanford et al. [12] who observed impaired exposure of phosphatidyl serine on neutrophils from X-linked GCD patients and concluded that these patients may have an increased risk of developing lupus due to abnormal apoptosis coupled to abnormal clearance of apoptotic cells, a mechanism equivalent to that proposed in complement deficiencies. Therefore, it is possible that one of the pathogenetic factors underlying lupus-like features in CGD could be a defective neutrophil apoptosis. These data might suggest that *CYBB* and other CGD-related genes could be lupus-susceptibility genes.

Selective IgA Deficiency

Selective IgA deficiency (IgAD) is the most common PID (1:400–1:3,000 among healthy blood donors). The prevalence of this PID in various SLE series has been estimated as 1–4.6% and is, therefore, ten to 50 times higher than the described for the general population [13, 14]. More recently, Cassidy et al. [15] detected a frequency of 5.2% in 77 juvenile SLE (JSLE) patients, while the frequency of this PID in 152 adult onset SLE patients was of 2.6%. We detected three patients with selective IgA deficiency (<7 mg %) and two patients with partial IgA deficiency (14 and 21 mg%, respectively) in a series of 49 consecutive JSLE at our hospital (unpublished data). Additionally, anti-IgA antibodies have been detected in 58–100% of the patients with IgAD and SLE [14, 15]. No significant differences in clinical presentation and in disease severity have been found comparing IgA-deficient SLE patients with those with normal IgA levels [14, 15].

The strong association between IgAD and SLE, as well as with other systemic and organ-specific autoimmune disorders, has not been fully elucidated yet, and many hypotheses to explain this association have been proposed [15]. As secretory IgA plays a key role as an initial barrier to prevent infections and food antigen absorption, its lack in the mucosal tracts of IgAD patients may expose these individuals to several environmental antigens, favoring an enhanced immune response to such antigens and a chronic T and B cell activation. In its turn, serum monomeric IgA has been regarded as a discreet "housekeeper" of the immune system, with multiple anti-inflammatory functions. A novel hypothesis about the anti-inflammatory role of monomeric serum IgA is proposed in another review of this issue. According to that hypothesis, the interaction of serum IgA with the FcαRI on cell membrane would result in partial phosphorylation of FcRγ-associated FcαRI, notably in the ITAM motif, thus inducing recruitment of the SHP-1 tyrosine phosphatase [16]. This in turn leads to deactivation of several immune response pathways, and consequently, inflammatory reactions and autoimmunity would be prevented. In contrast, the lack of engagement of FcαRI by IgA in IgA-deficient individuals would result in the abolishment of FcαRI-inhibitory signaling.

PIDs Not Associated with SLE

In contrast to the previous group, there are some PIDs in which SLE or lupus-like manifestations have not been observed, and those may also be relevant for the comprehension of SLE physiopathology.

Diseases of Immune Dysregulation

It seems paradoxical that SLE or lupus-like manifestations have not been described in PIDs highly associated with autoimmunity, such as autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX), and autoimmune lymphoproliferative syndrome (ALPS) [1, 2]. Indeed, these disorders represent unique human models to analyze the involvement of pivotal mechanisms for tolerance induction and maintenance, such as (1) thymic negative selection of lymphocytes, that is, central tolerance; (2) regulatory T cell-mediated peripheral tolerance; and (3) Fas-mediated apoptosis of autoreactive lymphocytes in SLE pathogenesis, and their study strongly suggests that these mechanisms are not crucial players in SLE pathophysiology.

Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy

APECED, also called APS-1 (autoimmune polyglandular syndrome type 1), is associated with loss-of-function mutations of the autoimmune regulator (*AIRE*) gene [2]. AIRE was shown to exert its critical function in medullary epithelial

cells of the thymus by promoting ectopic expression of peripheral tissue antigens. It has been considered as a central molecule in the thymic selection of lymphocytes. Therefore, in defective AIRE expression, autoreactive T cells escape negative selection, migrate to the periphery, and thus cause disease. APECED has been considered a prototypical disease of central tolerance breakdown. Besides mucocutaneous candidiasis, APECED patients present a series of organ-specific autoimmune manifestations: hypoparathyroidism, hypoadrenalism, ovarian or testicular failure, hypothyroidism, insulin-dependent diabetes mellitus, chronic hepatitis, alopecia, and vitiligo have been the most frequently described [17–19]. Candidiasis usually manifests first, in the first years of life, while endocrinopathies appear later in childhood or adolescence or even in adulthood, as patients usually have a long survival if adequately treated. Strikingly, these patients rarely present systemic autoimmune manifestations and cytopenias. Although almost all APECED patients have multiple autoimmune manifestations, so far none have been described as fulfilling criteria for SLE diagnosis. In addition to this fact, although APECED patients present high levels of various organ-specific autoantibodies, ANA (antinuclear antibody) titers have been constantly observed as being negative or low (<1:80) [20]. In the series studied by Perniola et al., only one patient was described with positive anti-Sm antibodies, while none presented anti-dsDNA. The absence of SLE among the APECED series strongly suggests that AIRE-mediated thymic selection of lymphocytes is not a critical pathway in SLE pathogenesis, allowing us to speculate that this would be a pivotal mechanism for the induction of tolerance to tissue-restricted antigens but perhaps not to ubiquitously expressed autoantigens.

Immunodysregulation Polyendocrinopathy Enteropathy X-Linked

IPEX is associated with mutations of *FOXP3*, which is essential for development and functions of regulatory T cells (Treg). It is usually a fatal condition, with multiple organ-specific and systemic autoimmune manifestations, mediated by both T cells and antibodies, typically observed from the first weeks of life. Inflammatory enteropathy, insulin-dependent diabetes mellitus, thyroiditis, Coombs' positive hemolytic anemia, and thrombocytopenia are frequent. Neutropenia, autoimmune hepatitis, nephritis, and vasculitis have also been described, accompanied by a variety of autoantibodies to multiple targets [2, 21, 22]. Diagnosis of SLE has not been established neither in the "classical" IPEX babies nor in the few older children with less severe phenotypes. One can argue that most IPEX patients do not survive enough to develop SLE.

Studies concerning Treg cells in SLE patients have shown lower percentages with variable results in regards to their functional activity as well as to levels of FOXP3 mRNA and protein, and, thus, further observations are necessary to reach conclusions about the role of Treg cells in SLE pathogenesis [23].

Autoimmune Lymphoproliferative Syndrome

ALPS is characterized by defective lymphocyte apoptosis and homeostasis, due, in most cases, to mutations of the Fas gene, but ALPS phenotype has also been associated with mutations of the Fas ligand, caspases 10 and 8, and NRAS (neuroblastoma rat sarcoma oncogene) [24, 25]. Although cytopenias are the most characteristic autoimmune manifestations of ALPS patients, who also usually present high titers of autoantibodies, only one description of an ALPS patient (with Fas ligand mutation) fulfilling SLE diagnostic criteria has been found in more than 200 patients already described [24, 26]. Antinuclear antibodies have been observed in ALPS patients, but, except for the above-mentioned patient, there is no description of anti-dsDNA or other SLE-related autoantibodies.

On the other hand, homozygous Fas- (*lpr*) and FasL- (*gld*) deficient mice, a natural knockout model of ALPS, present features that resemble SLE. Regarding the study of apoptosis pathways in SLE patients, increased apoptosis rates as well as increased expression of Fas receptor on their lymphocytes have been observed, and the last finding correlated with disease activity [27]. There is a hypothesis that accelerated apoptosis of circulating lymphocytes or impaired clearance of apoptotic cells in patients with SLE may lead to the presence of increased amounts of intact nuclear antigens at the extracellular tissue and drive an autoimmune response with production of autoantibodies. However, the study of ALPS patients suggests that the Fas-mediated lymphocyte apoptosis pathway does not seem to be critical for the physiopathology of SLE.

Agammaglobulinemia and Common Variable Immunodeficiency

X-linked agammaglobulinemia (XLA) is a disorder of B-cell maturation characterized by the absence of mature B cells, very low serum levels of all immunoglobulin isotypes, and lack of specific antibody production, and which has been associated with *BTK* mutations. Remarkably, among the large series of XLA patients, there is no description of SLE, although these patients may develop chronic arthritis (around 15% of cases), dermatomyositis, and scleroderma [28–30]. It has not been established so far if the neutropenia frequently seen in agammaglobulinemic patients is of autoimmune nature. SLE diagnosis is obviously difficult to be established in the absence of autoantibodies, but it is noteworthy that XLA patients do not present the most characteristic SLE features,

such as cutaneous and oral manifestations, renal involvement and serositis. This "human model" highlights that B lymphocytes and antibodies are essential for SLE pathogenesis and has implications in the rationale for the indication of anti-CD20 therapy for severe manifestations of SLE.

The rarity of SLE descriptions among common variable immunodeficiency (CVID) patients (estimated as present in 1:25,000–1:50,000 in general population) reinforces the pivotal role of antibodies in SLE pathophysiology [31, 32]. To note, cytopenias are the most frequent autoimmune manifestations associated with CVID, described in up to 22% in large CVID series [32]. Interesting observations are those related to the CVID patients whose underlying molecular defects were already identified, i.e., defects of ICOS, TACI, BAFF-R, CD19, and MSH5, and no SLE was observed among the still restricted number of families so far described [33–35].

Hyper-IgM Syndrome due to Defects of CD40–CD40 Ligand Pathway

Immunodeficiency with hyper-IgM (HIGM) is characterized by normal to increased serum IgM levels and very low or undetectable IgG, IgA, and IgE, and part of the cases are due to defects in the CD40–CD40 ligand pathway. SLE or lupus-like manifestations have not been reported among X-linked HIGM series, associated with CD40L (CD154) defects [36, 37], nor in the few cases so far identified of HIGM due to CD40 deficiency [38]. These observations suggest that severely impaired CD40–CD40L pathway do not favor SLE development, perhaps as a consequence of defective B cell activation coupled to deficient immunoglobulin class switching. In their turn, SLE patients with active disease present increased percentages of CD4+, CD8+ T lymphocytes, and surprisingly, B lymphocytes expressing CD40L, favoring the idea that SLE patients present a chronic lymphocyte activation [39]. Thus, the study of HIGM may provide useful insights into the role of the CD40–CD40L pathway in SLE development.

Concluding Remarks

The "experiments of nature" represented by PIDs effectively teach us useful lessons for the comprehension of underlying mechanisms leading to the development of SLE. On one hand, the PIDs highly associated with SLE or lupus-like manifestations, i.e., deficiencies of the early components of classical complement pathway and CGD, point out to the relevance of defective mechanisms of cellular waste-disposal in SLE physiopathology. The most impressive lesson is given by complete C1q deficiency, which may be considered as practically a monogenic form of SLE. On the other hand, the group of "syndromes with autoimmunity" is also very informative, as APECED, IPEX, and ALPS, albeit highly associated with other autoimmune manifestations, and the human "knockout" models for *AIRE*, Foxp3+ Treg cells, and Fas-mediated apoptosis, respectively, are not associated to SLE.

Further unique lessons in the understanding of SLE pathogenesis are given by antibody deficiencies. XLA demonstrates in an unequivocal fashion the essential role of B lymphocytes and antibodies in SLE development. Moreover, the study of CVID patients reinforces the lessons learned from XLA. Observations from patients with various antibody deficiencies show that those with severely impaired IgG production (such as XLA, CVID, and HIGM syndrome) are not prone to SLE, even if they are able to synthesize IgM, as in HIGM due to AID (activation-induced cytidine deaminase) and UNG (uracil-*N*-glycosilase) deficiencies [40, 41]. Taken collectively, data from the different predominantly antibody deficiencies suggest that IgG is crucial for the development of SLE.

As human models of autoimmune disorders, PIDs represent unique and not fully explored opportunities for a better comprehension of SLE. While the PIDs strongly associated with SLE point out to mechanisms to be more extensively explored, the not-associated ones indicate mechanisms not relevant or pathways whose severe impairment may prevent SLE development, such as CD40-CD40L and IL-12/IFN-γ pathways (Table 1).

Addendum in proof After the acceptance of this manuscript, a study by Martire et al described one more X-linked CGD patient with SLE and another with discoid lupus erythematosus in a multicenter Italian series of 60 CGD patients (Clinical features, long-term follow-up and outcome of a large cohort of patients with Chronic Granulomatous Disease: an Italian multicenter study. Clin Immunol, 2008;126:144–156).

Acknowledgment This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo—FAPESP (grant 2002/05880-4 to MC-S) and Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq (grants 302469/2005-2 to CAAS and 34802/2005-0 to MC-S).

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J Clin Immunol (2008) 28 (Suppl 1):S62–S66
DOI 10.1007/s10875-008-9171-x

Autoimmunity in Hyper-IgM Syndrome

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Received: 17 December 2007 / Accepted: 7 January 2008 / Published online: 2 February 2008

Abstract
Introduction Immunodeficiency with hyper-IgM (HIGM) results from genetic defects in the CD40–CD40 ligand (CD40L) pathway or in the enzymes required for immunoglobulin class switch recombination and somatic hypermutation. HIGM can thus be associated with an impairment of both B-cell and T-cell activation.
Results and discussions There are seven main subtypes of HIGM and the most frequent is X-linked HIGM, resulting from *CD40L* mutations. In addition to the susceptibility to recurrent and opportunistic infections, these patients are prone to autoimmune manifestations, especially hematologic abnormalities, arthritis, and inflammatory bowel disease. Furthermore, organ-specific autoantibodies are commonly found in HIGM patients.
Conclusions The mechanisms by which HIGM associates to autoimmunity are not completely elucidated but a defective development of regulatory T cells, the presence of IgM autoantibodies and an impaired peripheral B-cell tolerance checkpoint have been implicated. This article reviews the main subtypes of HIGM syndrome, the clinical autoimmune manifestations found in these patients, and the possible mechanisms that would explain this association.

Keywords Autoimmunity · CD40 · CD40L · hyper IgM · inflammatory bowel disease

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Immunodeficiency with hyper-IgM (HIGM) was first described in 1960 and refers to a group of disorders characterized by normal to increased serum IgM and very low or undetectable IgG, IgA, and IgE [1]. This immunological phenotype is mainly due to the failure of B cells to complete their maturation through immunoglobulin-isotype class switch recombination (CSR) and somatic hypermutation (SHM). Among primary hyper-IgM syndromes, genetic heterogeneity is supported by the existence of X-linked, autosomal recessive, and autosomal dominant inheritance [2]. HIGM can be caused by defects in CD40L–CD40 pathway, which is essential for B-cell activation, or by defects involving the enzymes required for CSR and SHM [3] (Table 1). The binding of CD40L to CD40 induces CD40 cytoplasmic domain binding to members of TNF receptor-associated-factor (TRAF) proteins. Further signaling by TRAF proteins is mediated by nuclear factor (NF)-kB, and activates pathways leading to immunoglobulin gene switching. Figure 1 shows possible mechanisms involved in HIGM [1, 4].

The X-linked variant of hyper-IgM syndrome (XHIM or HIGM1) is the most frequent subtype of HIGM (65–70%) and results from defects of the *CD40L* gene, which encodes for the CD40 ligand (CD154) molecule expressed transiently on the surface of activated T cells [3, 5]. XHIM patients have a normal number of circulating B lymphocytes which expresses IgM and IgD but not other isotypes at cell surface. About 50% of the XHIM have normal IgM levels at diagnosis, although the majority develops increased IgM

Table I Characteristics of the Main Hyper-IgM Syndromes

Hyper-IgM syndrome	Defective gene	Inheritance	Frequency (%)	Autoimmune manifestations
Type 1	*CD40L*	XL	70	+
Type 2	AID	AR or AD	<1	++
Type 3	*CD40*	AR	10	+
Type 5	*UNG*	AR	5	−
Type 6/HIGM-ED	*NEMO*	XL	1–2	++

CD40L CD40 ligand, *HIGM-ED* hyper-IgM with ectodermal dysplasia, *AID* activation-induced cytidine deaminase gene, *UNG* uracil DNA glycosylase gene, *NEMO* NF-κB essential modulator, *XL* X-linked, *AR* autosomal recessive, *AD* autosomal dominant, *single positive symbol* (+) mild association with autoimmunity, *double positive symbol* (++) moderate association with autoimmunity, *negative symbol* (−) no association with autoimmunity

during follow-up [1, 6]. In addition to defective isotype switching, XHIM is also characterized by low affinity antibody production in response to T-dependent antigens and lack of memory B-cell generation. Lymphnodes of these patients contain primary follicles, but lack germinal centers [1, 7, 8].

CD40L is a member of the tumor necrosis factor (TNF) family of cytokines composed of 261 amino acids and three functional domains (intracellular, transmembrane, and extracellular) [9]. The human *CD40L* gene includes five exons and mutations are more common in the TNF-homology domain encoded by exon 5 [10]. Recently, the first mutation in the promoter for CD40L gene was identified [11]. Its natural receptor is CD40, which is expressed on antigen presenting cells, including B cells, dendritic cells, and macrophages. Nonsense, missense, insertion, deletion, and splice-site mutations have been reported in XHIM patients, and some of these mutations have been shown to prevent binding of CD40L to CD40, while others affect folding of the CD40L monomer or trimer formation [12].

All major CD4 T-cell subsets express substantial amounts of CD154 after activation, including the naïve and memory CD4 T cells, and Th0, Th1, and Th2 cells. Activated CD4 T cells expressing CD154 engage CD40 on B cells, directing B-cell growth and differentiation, formation of germinal centers, upregulation of immunoglobulin heavy-chain transcription, and isotype switching [12]. CD40–CD40L interactions also provide a costimulatory signal for T cells, leading to T-cell activation and inducing CD8 T-cells effector functions, cytokine production by CD4 T cells, and humoral immunity to T-cell-dependent antigens [3, 13].

XHIM patients have an increased susceptibility to infections caused by bacteria, viruses, fungi, and parasites, reflecting their defect in T-lymphocyte function, as well as their hypogammaglobulinemia. These patients are uniquely susceptible to interstitial pneumonia caused by *Pneumocystis carinii* (20–40%) and present protracted chronic diarrhea caused by *Cryptosporidium* sp (30%). Liver disease as a whole is common in XHIM patients, which

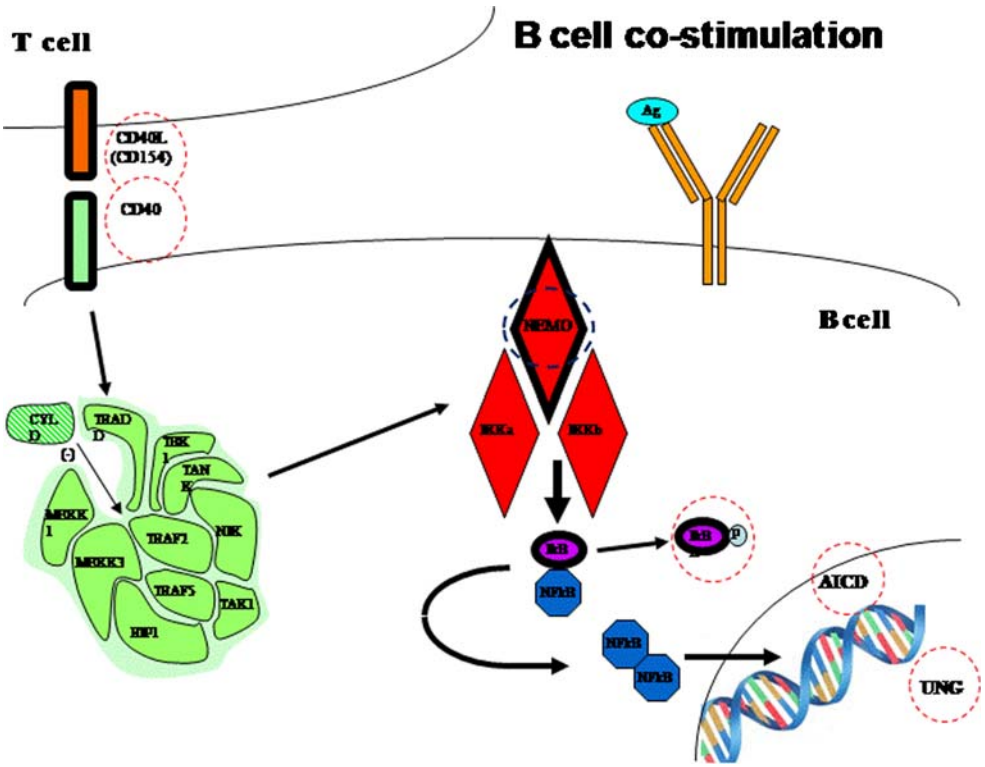

Fig. 1 Schematic illustration of the molecular defects leading to HIGM syndrome phenotype. When the interaction between activated T cell and B cells is impaired (CD40L and Cd40 deficiencies), B cells are unable to undergo class-switch recombination and affinity maturation. Mutations in downstream signaling molecules (NEMO and IkB) or intrinsic B cell enzymes (AICD and UNG) may also result in HIGM. *TRAF* TNF receptor-associated factor; *NF-κB* nuclear factor-κB; *IKKγ/NEMO* inhibitor of κB-kinase/NF-κB essential modulator; *AICD* activation-induced cytidine deaminase; *UNG* uracil DNA glycosylase; *IκBα* α inhibitor of NF-κB (adapted from Orange J, with permission)

frequently present sclerosing cholangitis (related to *Cryptosporidium parvum* infections), chronic hepatitis (due to hepatitis B virus, hepatitis C virus, and cytomegalovirus), and liver and biliary tract tumors. Other characteristic features are recurrent oral ulcers and proctitis, usually associated to neutropenia, observed in about 50% of the patients. Additionally, they may also develop autoimmune or inflammatory disorders and malignancies [1, 6, 14].

HIGM2 and HIGM3 are autosomal recessive variants of HIGM and are caused by mutations in the activation-induced cytidine deaminase gene, *AICDA* (AID, CDA2) and CD40 gene, *TNFRSF5*, respectively. Patients with HIGM3 are clinically undistinguishable from subjects carrying genetic defects of CD40L, while patients with HIGM2 present with a clinical phenotype characterized by enlargement of tonsils and lymphnodes and recurrent bacterial sinopulmonary infections, without increased susceptibility to opportunistic pathogens [2, 15].

HIGM4 is the least characterized subset, with patients clinically resembling a milder form of HIGM2 with residual IgG production. B cells from HIGM4 patients present defective CSR with normal SHM, and no genetic cause has been identified so far [14].

Mutations in *UNG* (uracil DNA glycosylase gene) have been found in a few patients clinically similar to HIGM2. This condition is termed HIGM5 and has an autosomal recessive inheritance. Mutations in *AICDA* and *UNG* are considered intrinsic B-cell defects, as they encode for enzymes involved in CSR and SHM [2].

Another type of HIGM affects males and is characterized by the association of hypogammaglobulinemia with hypohydrotic ectodermal dysplasia (HIGM-ED/HIGM6). This condition is caused by hypomorphic mutations in the NF-κB essential modulator (*NEMO*/*IKKG*) gene. More recently, a defect in another molecule of the same pathway, IκBα, was associated to Hyper IgM syndrome, and the disease named HIGM7. Both mutations result in a defective NF-κB translocation to the nucleus leading to an abnormal expression of multiple enzymes such as AID and UNG [2, 16].

Approximately 25% of HIGM patients have normal CD40L, CD40, AID, and UNG genes and some of these patients have been identified as having a defect in CSR [2, 13].

Autoimmune Manifestations in HIGM

In addition to the susceptibility to opportunistic and bacterial infections, HIGM patients are prone to develop autoimmune diseases such as immune thrombocytopenia, Coombs positive hemolytic anemia and nephritis, suggesting that tolerance is not correctly maintained in these patients [3]. It has been reported that sera from CD40L-deficient patients contain specific antibodies against self-antigens (antierythrocyte, antierythropoietin, antiplatelet, antismooth muscle, anticardiolipin, anti-Ro, anti-RNP, antinuclear, and antithyroid). Other autoimmune manifestations seen in these patients include inflammatory bowel disease, autoimmune hepatitis, seronegative arthritis, hypothyroidism, and discoid lupus erythematosus [2, 17–19].

Most data related to the frequency of autoimmune manifestations comes from XHIM patients. In the cohort of 56 patients with XHIM published by Levy et al. [6], inflammatory bowel disease was seen in 6% of the patients with protracted or recurrent diarrhea, seronegative arthritis in 11%, chronic neutropenia of unknown origin in 44.6%, and Coombs positive hemolytic anemia in 1 patient. Among 79 patients with XHIM registered by Winkelstein et al. [20], 60% presented neutropenia, while anemia and thrombocytopenia occurred in 15% and 4%, respectively. Parvovirus B19 infection was detected in only three patients (4%), raising the possibility of an autoimmune etiology for most of the hematologic manifestations. According to Webster et al. [21], from 62 XHIM patients registered at the European CD40L Defect Database until 1999, 11 presented arthritis as a manifestation, including a man with an aggressive form of polyarticular arthritis complicated by subcutaneous nodules and periarticular cysts. Taken together, these data suggest that autoimmune disease is a common and important feature of X-linked HIGM syndrome.

Regarding intrinsic B-cell defects, autoimmune manifestations occur in 25% of AID deficient patients, mainly hemolytic anemia, thrombocytopenia, and autoimmune hepatitis, and autoantibodies of the IgM isotype have also been described [14]. Also, Melegari et al. [22] described a woman with HIGM not related to CD40 or CD40L mutations, presenting systemic lupus erythematosus manifestations and autoantibodies against double strand-DNA and ribonucleoprotein (RNP).

Among 29 HIGM patients due to AID deficiency reported by Quartier et al., six presented autoimmune disorders, including a 21-year-old male with marked splenomegaly, oral ulcers, thrombocytopenia, and diabetes mellitus. Another patient presented a chronic, destructive, bilateral, and symmetrical polyarthritis. Other autoimmune manifestations observed in this study were autoimmune hepatitis (AIH), hemolytic anemia, and thrombocytopenia in a patient who presented several autoantibodies of IgM isotype (antihepatocyte membrane, antiliver–kidney microsome, antismooth muscle, anticardiolipin, antierythrocyte, and antiplatelets). Chronic hepatitis was observed in another patient and autoimmune etiology in this case was supported by liver histological findings, negative infection screening, and by the efficacy of corticosteroids and immunosuppressive therapy, since AIH autoantibodies were not detected. Other interesting manifestations were inflam-

matory bowel disease mimicking Crohn's disease in one patient and bilateral chronic uveitis in other [23].

Autoimmune manifestations were also reported in patients with HIGM6, related to NEMO defects. Orange et al. [24] described 13 NEMO patients presenting autoimmune manifestations, including inflammatory bowel disease in 10, arthritis in two and autoimmune hemolytic anemia in one.

Thus, it appears that autoimmunity occurs in all types of HIGM syndrome, with variable presentation according to the underlying genetic defect.

Mechanisms of Autoimmunity in HIGM

CD40–CD40L interactions may be involved in the selection of T-cell repertoire, since autoimmune diseases (thyroiditis, syaloadenitis, pancreatitis, oophoritis, and adrenalitis) can be elicited by transferring T cells from CD40-deficient mice into athymic nude mice that lack T cells but have B cells and other APCs expressing CD40 [24]. It was also observed a severe reduction of CD25+CD45RB^{lo} CD4+ T-cell numbers and impaired differentiation of Tr1 cells in CD40-deficienct mice, indicating that defective development of regulatory T cells may result in increased T-cell autoreactivity in the absence of CD40–CD40L interactions [3, 25].

The use of CD40L blocking antibodies in experimental animal models has also contributed to the comprehension of XHIM. These antibodies have demonstrated to be beneficial in murine collagen arthritis, thyroiditis, and experimental allergic encephalomyelitis [10]. Clonal deletion of thymocytes bearing T-cell receptors of low affinity is also altered by the functional blockage of CD40L by anti-CD40L antibodies [10].

Lacroix-Desmazes et al. [18] demonstrated that the IgM reactivity repertoire is skewed toward self-antigens in 19 HIGM patients as compared to normal subjects, while the repertoire of reactivity against foreign antigens did not differ between the two groups. Additionally, IgG antibodies of HIGM patients lacked reactivity with self antigens, in contrast with IgG of healthy controls.

Although the molecular mechanisms by which CD40L induces B-cell proliferation and differentiation are well described, its potential functions in counterselecting human autoreactive B cells are poorly understood. Transgenic mouse models have suggested that CD4 + T cells play an important role in the elimination of peripheral autoreactive B cells, and that CD40–CD40L and MHC class II T-cell receptor are required for the counterselection of transgenic autoreactive B cells [3].

In humans, auto-reactive B cells are removed during central and peripheral B-cell tolerance checkpoints. Whereas central B-cell tolerance involves B-cell receptor signaling pathways, the mechanisms involved in peripheral B-cell tolerance are not well characterized [3, 26]. Hervé et al. tried to determine the impact of CD40L and MHC class II expression on human B-cell tolerance through cloning antibodies from CD40L or MHC class II deficient patients. This study concluded that polyreactive B cells are properly counterselected in the bone marrow of CD40L-deficient patients, revealing a functional central B-cell tolerance checkpoint in the absence of CD40L expression [3]. In contrast, the peripheral B-cell tolerance mechanism seems to be defective in CD40L-deficient patients, resulting in an increase of circulating polyreactive B cells [26]. The same study also concluded that MHC class II is essential in the development of class-switched memory B cells in humans, since MHC class II deficient patients (bare lymphocyte syndrome-BLS) lacked an IgM^{-}CD27^{+} class-switched B-cell population [3].

A third conclusion was a significant decrease in the proportion of CD25^{+}Foxp3^{+}T reg cells in CD40L-deficient patients as compared to the normal controls. Finally, serum BAFF (a potent B-cell survival factor) levels were significantly increased in CD40L-deficient and BLS patients [3].

The CD40 molecule has also been implicated in the autoimmunity found in HIGM syndrome. Since CD40 is expressed not only on B cells, but also on all other APCs, the CD40–CD154 interaction may be important in T-cell-dependent macrophage-mediated immune response. Ligation of CD40 on the surface of monocytes induces the secretion of several cytokines, such as IL-1, IL-6, IL-8, IL-10, IL-12, and TNF-α. These cytokines promote the maturation of dendritic cells, enhancing their ability as APCs. Moreover, as DCs are the main producers of cytokines that regulate T-cell activation like IL-10 and IL-12, CD40-deficient patients may present an imbalance in the production of these cytokines [2].

CD40–CD40L System and Systemic Lupus Erythematosus

Although CD4 T cells do not play a direct role in systemic lupus erythematosus (SLE) tissue damage, they appear to be required for the production of pathogenic autoantibodies. An increased CD154 expression on T cells of lupus-prone mice has been reported, and treatment of these mice with a neutralizing anti-CD154 has been shown to delay and reduce the incidence of glomerulonephritis [12, 27]. In patients with SLE, it has been shown that CD40 expression is markedly increased in kidney and is associated with the presence of infiltrating CD154-expressing mononuclear cells [12]. Desai-Mehta et al. demonstrated increased frequencies of CD154 expressing CD4 T cells among patients with active SLE as compared to those in remission or normal controls. It was also shown that an antibody against CD154 significantly blocked the ability of lymphocytes from active SLE patients

to produce pathogenic antinuclear antibodies in vitro. Soluble CD154 (sCD154) has been also detected in SLE patients and levels of sCD154 correlated with disease activity and anti-dsDNA titers [28].

Dysregulated expression of CD154 has been correlated with other autoimmune diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis (RA), and systemic sclerosis (SS). CD154 has been shown to be expressed by T cells in the joints and peripheral blood of RA patients and in the sera of RA-associated vasculitis patients. Hyperexpression of CD154 by CD4 T cells in IBD intestinal lesions may contribute to pathogenic cytokine production in this disease. However, CD154 expression appears to be normal in patients with idiopathic thrombocytopenic purpura (ITP), suggesting that CD154 is not always overexpressed in autoimmune disorders [12].

Conclusions

HIGM syndrome can be caused by several defects involving the CD40L–CD40 pathway, NEMO, AID, UNG, and other unknown defects, all of them involved in a proper immunoglobulin production by B cells. In addition to the immunodeficiency state leading to recurrent or opportunistic infections, these patients are susceptible to autoimmune manifestations. The association between autoimmunity and X-linked HIGM could be explained by presence of IgM autoantibodies, decreased peripheral control of B lymphocytes, impaired development of T reg cells and increased levels of BAFF (also implicated in peripheral tolerance), as shown by recent studies.

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