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CONSEQÜÊNCIAS CLÍNICAS E IMONOLÓGICAS DA CO-INFECÇÃO HTLV-1 E HELMINTOS.

MARIA AURÉLIA DA FONSECA PORTO

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Conseqüências Clínicas e Imunológicas da Co-Infecção HTLV-1 e Helmintos

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LISTA DE ABREVIATURAS

ADCC	Citotoxidade celular dependente de anticorpo
LLcTA	Leucemia/linfoma de células T de adultos
CD	"Cluster of diferentation" (marcador de membrana de células)
CMSP	Células mononucleares de sangue periférico
DMID	Diabetis mellitus insulino dependente
HAM	Mielopatia associada ao HTLV-1
HLA	Antígeno de histocompatibilidade leucocitário
HTLV-1	Vírus linfotrópico de células T humanas tipo 1
IFN-α	Interferon -α
IFN-β	Interferon-β
IFN-γ	Interferon-γ
IL	Interleucina
IL-2R	Receptor de interleucina-2
LTR	Terminações longas repetidas
MHC	Complexo de histocompatibilidade principal
MMP	Matriz metaloproteinases
TIMP	Inibidor tecidual de metaloproteinases
NK	Células matadoras naturais
NOD	Diabético não obeso
SEA	Antígeno de ovo solúvel
SNC	Sistema nervoso central
SWAP	Aintígeno de verme adulto do *S. mansoni*
TGF-β	Fator de crecimento transformador l-β
Th1	Linfócitos T auxiliares do tipo 1
Th2	Linfócitos T auxiliares do tipo 2
TNF-α	Fator de necrose tumoral-α
TSP	Paraparesia espástica tropical
TT	Toxóide tetânico

RESUMO

CONSEQÜÊNCIAS CLÍNICAS E IMUNOLÓGICAS DA CO-INFECÇÃO HTLV-1 E HELMINTOS. **MARIA AURÉLIA DA FONSECA PORTO**. Estudos prévios têm mostrado que a infecção pelo HTLV-1 pode resultar em uma ativação e proliferação linfocitária, e uma exacerbada resposta imune Th1 com níveis altos de IFN-γ. A infecção por helmintos está relacionada com produção de IgE e citocinas com um perfil Th2. Neste trabalho foi caracterizada a resposta imune de portadores de HTLV-1 e de pacientes com mielopatia asssociada ao HTLV-1 e a influência da infecção pelo HTLV-1 no curso clínico e na resposta imune de pacientes com estrongiloidíase e esquistossomos. Adcionalmente, foi avaliada a influência da infecção por helmintos na resposta imune (determinação de citocinas em sobrenadante e análise por FACS) e na carga proviral de indivíduos infectados pelo HTLV-1. Foi observado que indivíduos com mielopatia apresentaram níveis de citocinas pro-inflamatórias, especificamente o IFN-γ, bem mais altos do que portadores assintomáticos, porém neste último grupo houve uma variação nestes níveis e 40% destes indivíduos tiveram níveis semelhantes aos pacientes com mielopatia. Além disso, os pacientes com mielopatia apresentaram maior proliferação linfocitária e maior freqüência de células T CD8+. Quando foi avaliada a influência do HTLV-1 na resposta imune ao *S. stercoralis*, foi documentado que pacientes com estrongiloidíase quando co-infectados pelo HTLV-1 apresentaram níveis mais baixos de IL-5, IL-13 e níveis mais altos de IFN-γ do que pacientes que apresentavam somente estrongiloidíase. Estes achados podem justificar o fato de que pacientes co-infectados pelo HTLV-1 e *S. stercoralis* desenvolvam formas disseminadas da doença e menor resposta terapêutica a drogas anti-helmínticas. Achados imunológicos semelhantes foram observados em relação à co-infecção com HTLV-1 e *S. mansoni*. Os pacientes dualmente infectados pelo HTLV-1 e *S. mansoni* produziram mais IFN-γ e menos IL-5, IL-10 e IgE específica para *S. mansoni* do que pacientes apenas com infecção pelo *S. mansoni*. A despeito de pacientes com HTLV-1 e *S. mansoni* apresentarem menor resposta ao tratamento a drogas anti esquistossomóticas, não foi encontrado fibrose hepática importante nesta população. Como helmintos levam a um ambiente rico em citocinas Th2, foi avaliado se a infecção por *S. stercoralis* e *S. mansoni* interfere na produção de citocinas e na carga proviral de indivíduos infectados pelo HTLV-1. Neste estudo foi documentado que quando os indivíduos infectados pelo HTLV-1 eram co-infectados por helmintos apresentaram níveis mais baixos de IFN-γ em sobrenadante de culturas de linfócitos, menor freqüência de células CD4+ e células CD8+ expressando IFN-γ, maior freqüência de células T expressando IL-5 e IL-10 e menor carga proviral. Adcionalmente, a infecção por helmintos foi menor no grupo de pacientes com mielopatia. Estes últimos dados sugerem que a infecção por helmintos pode modular a resposta inflamatória e se associa negativamente com doença neurológica associada a este vírus.

ABSTRACT

CLINICAL AND IMMUNOLOGICAL IMPACT OF THE ASSOCIATION OF HTLV-1 AND HELMINTHIC INFECTION. **MARIA AURÉLIA DA FONSECA PORTO**. Lymphocytes from patients infected with HTLV-1 proliferate without stimulus and have a predominant type 1 immune response. In contrast, helminthic infection are associated with a response Th2 and secretion of high levels of IgE. In the present study, the immune response in HTLV-1 carriers was compared with that observed in patients with myelopathy and the influence of HTLV-1 in the clinical and immunological response of patients with strongyloidiasis and schistosomiasis was studied. Moreover, the influence of helminthic infection in the immune response and in the proviral load of patients infected with HTLV-1 was studied. It was observed that patients with mielopathy associated to HTLV-1 had significant higher levels of IFN-γ than HTLV-1 carriers, although 40% of HTLV-1 carriers have similar immunological abnormalities than patients with mielopathy. Furtermore, patients with mielopathy had more CD8+ T cells and lymphocyte proliferation than HTLV-1 carriers. Regarding the immune response of patients dually infected with HTLV-1 and helminths, it was shown that patients co-infected with HTLV-1 and *S. stercoralis* had higher IFN-γ levels and lower IL-5, IL-13 levels than patients with strongyloidiasis without HTLV-1. These immunological abnormalities may be the basis for the occurrence of disseminated *S. stercoralis* infection and the decreasing in efficacy of anti-helminthic drugs in patients co-infected with HTLV-1 and *S. stercoralis*. Similar immunolgical abnormalities of those observed in patients dually infected with HTLV-1 and *S. stercoralis* were detected in patients co-infected with HTLV-1 and *S. mansoni*. Patient dually infected with HTLV-1 and *S. mansoni* produced more IFN-γ, less IL-5, IL-10 and *S. mansoni* specific IgE than patients with *S. mansoni* without HTLV-1 infection. As helminths induce a type 2 immune response that can down modulate the response Th1, it was evaluated if helminthic infection modify the immune response and the proviral load in patients with HTLV-1. This study shows that lymphocytes from HTLV-1 patients co-infected with helminths produces lower levels of IFN-γ and have lower frequency of CD4+ and CD8+ T cells expressing IFN-γ than HTLV-1 carriers without helminthic infection. Moreover, HTLV-1 carriers with helminthic infection had more T cells expressing IL-5 and IL-10 and had lower proviral load than HTLV-1 carriers without helminthic infection. We have previously shown that the prevalence of *S. mansoni* and *S. stercoralis* is higher in HTLV-1 infected individuals than in non infected controls. Here in, we show that the prevalence of helminthic infection is higher in HTLV-1 carriers than in patients with HAM/TSP. These data sugest that helminthic infection down modulate the immune response of HTLV-1 infected individuals and is inverselly associated with HAM/TSP.

SUMÁRIO

REVISÃO DE LITERATURA

Resposta imune na infecção pelo HTLV-1

O vírus linfotrópico de células T humanas tipo 1 (HTLV-1) pertence à família *Retroviridae,* gênero *Deltaretrovírus* e foi o primeiro retrovirus a ser isolado de um paciente com leucemia por Poiesz et al.nos fins da década de 70. O HTLV-1 possui duas moléculas iguais de RNA de fita simples, caracterizado pela presença no seu genoma do gene que codifica a transcriptase reversa. Esta enzima é importante na transcrição do seu RNA em DNA, o que permite a integração do seu DNA como um provírus no genoma da célula infectada (Porterfield et al., 1980).

O genoma deste vírus possue três genes estruturais básicos que são o gag (que origina proteínas do capsídeo, da matriz, e do nucleocapsídeo), o pol (onde situam-se os genes para produção da transcriptase reversa e integrase viral) e o env (que origina proteínas do envelope viral) e mais dois outros genes regulatórios, o tax e o rex. O gene tax é importante na replicação viral por ativar a transcrição das terminações longas repetidas (LTR) no genoma viral dando início à sua replicação (Lin et al., 1998). Além disso, ele codifica uma proteína que amplifica a transcrição de alguns genes celulares. Este mecanismo de ativação a distância de genes promotores do desenvolvimento celular é denominado de transativação viral. O vírus HTLV-1 tem tropismo especial por linfócitos T CD4+, podendo, também, infectar outras linhagens celulares como linfócitos T citotóxicos (CD8+) (Kaplan et al., 1993; Richardson et al., 1990; Richardson et al., 1997). Adicionalmente, já foi demonstrada infecção de células dendríticas *in vitro* por HTLV-1 (De Revel et al., 1993; Knight et al., 1993).

Este vírus está comprovadamente associado a malignidades hematológicas, desordens neurológicas, sendo estas duas patologias as mais documentadas em relação à este vírus como também as de maior morbidade e mortalidade. Tem-se relatado também outras patologias associadas ao HTLV-1, como uveíte, síndrome de Sjogren, artropatias, dermatite infectiva e pneumonite linfocitária. A infecção pelo HTLV-1 tem sido detectada em diferentes regiões do mundo, e estima-se que cerca de 10 a 20 milhões de pessoas sejam infectadas por este vírus (Edlich et al., 1999; Edlich et al., 2003). Um estudo epidemiológico realizado em Salvador revelou uma soroprevalência em doadores de sangue de 1,35% (Galvão-Castro et al., 1997) e na população geral de 1,76% (Dourado et al., 2003). Esta alta soroprevalência nos mostra uma necessidade de conhecer melhor este vírus e as doenças a ele relacionadas, como também nos permite estudar os mecanismos de atuação deste vírus.

A resposta imune contra os vírus, organismos intracelulares, caracteriza-se, inicialmente, pela produção de interferon-α (IFN-α) e interferon-β (IFN-β) por células infectadas pelo vírus. Estas citocinas agem inibindo a replicação viral, levam a uma resistência a infecção viral de células não infectadas, aumentam o potencial citotóxico de células matadoras naturais (NK) e aumentam a expressão de moléculas do complexo de histocompatibilidade principal (MHC) classe I. A expressão destas moléculas juntamente com antígenos virais ativam a fase efetora da resposta imune adaptativa, neste caso mediada por células T citotóxicas (CD8+), que são as principais células efetoras na imunidade contra os vírus. Os anticorpos servem para neutralizar os agentes virais presentes em locais extracelulares e na circulação e lise dos vírus mediada por anticorpo e complemento pode ocorrer. O vírus HTLV-1 por penetrar nos linfócitos T, escapa do

primeiro ataque do sistema imune, ou seja, da virólise mediada pelo complemento. Apesar de uma variedade de proteínas levar a uma resposta imune humoral, incluindo proteínas gag, env e tax, a soroconversão com o aparecimento de anticorpos anti HTLV-1 não leva à eliminação do vírus. O HTLV-1 tem predileção por infectar células T CD4+, e estas células após serem infectadas, expressam a proteína tax, dando origem a uma reposta imune contra este vírus. Trabalhos prévios têm demonstrado a importância de células T CD8+ no mecanismo de defesa contra o HTLV-1. Nesse caso, células T CD8+ destroem células T CD4+ infectadas que expressam a proteína tax, levando a uma menor porcentagem de células T CD4+ infectadas (Hanon et al., 2000).

São múltiplas as conseqüências da interação do HTLV-1 com o sistema imune. Sabe-se que o HTLV-1 se beneficia-se da ativação de células T, favorecendo uma infecção permanente. Estas células, quando infectadas, sofrem alterações importantes na expressão gênica e no controle do crescimento celular. Nesse caso, a proteína tax não somente altera o ciclo celular como também interfere na transcrição de proteínas e fatores celulares. Alguns trabalhos têm demonstrado que estas células T encontram-se sempre ativadas (Copeland & Heeney, 1996; Hollsberg & Hafler, 1993). Constituem-se evidências desta constante ativação a capacidade destas células de proliferar espontaneamente e de responder indiscriminadamente a estímulos sem restrição do antígeno de histocompatibilidade leucocitário (HLA) (Popovic et al., 1984). Além dessa constante ativação, estudos têm demonstrado que células T infectadas pelo HTLV-1 são insensíveis ao efeito inibitório do fator de crescimento transformador-β (TGF-β) (Hollsberg et al., 1994; Hollsberg et al.,1999). A ativação celular em indivíduos infectados pelo HTLV-1 parece ser induzida pelo contato entre células infectada/não infectada, com participação da ativação via CD2-

CD58 (LFA-3), que é uma via alternativa de ativação de linfócito T (Holsberg et al., 1999; Kimata et al., 1993; Wucherpfennig et al., 1992) e normalmente estas moléculas têm pouca expressão em indivíduos sadios. Na infecção pelo HTLV-1 existe uma alta expressão de CD58, facilitando o contato célulaT-célulaT. O contato célulaT-célulaT acaba beneficiando este vírus e levando à sua propagação (Banghan et al., 2003; Holsberg et al., 1999). Células infectadas pelo HTLV-1, quando estimuladas apresentam maior expressão de tax que pode contribuir para a patogênese das doenças relacionadas com este vírus (Lin et al., 1998), visto que a proteína tax interfere na transcrição de proteínas e fatores celulares. Foi documentado que o aumento da expressão de tax está relacionado a um rápido aumento da produção de interferon-γ (IFN-γ) porém, neste mesmo estudo, não houve aumento da expressão de interleucina (IL)-2 relacionada ao aumento de tax (Hanon et al., 2001). Um outro estudo realizado com clones de células Th1 e Th2 infectadas pelo HTLV-1 demonstrou que a infecção pelo HTLV-1 altera a produção de citocinas por estes clones infectados. Enquanto os clones Th1 mantiveram a capacidade de produzir IFN-γ, os clones Th2 perderam a expressão de IL-4, mantiveram uma produção pequena de IL-5 e adquiriram expressão de IFN-γ (Macchi, et al., 1998). Um estudo prévio realizado em nosso Serviço mostrou que a infecção pelo vírus HTLV-1 leva a uma forte resposta imune Th1 com produção espontânea bastante aumentada de IFN-γ em sobrenadante de culturas de células mononucleares de sangue periférico (CMSP) em indivíduos assintomáticos. Neste estudo foi também observada uma produção aumentada de fator de necrose tumoral-α (TNF-α), IL–5 e IL-10, porém o que mais chamou a atenção foi a exacerbada produção de IFN-γ (Carvalho et al., 2000). Também tem sido documentada alta expressão de receptor de interleucina-2 (IL-2R), de IL-2 e de IL-15 nos indivíduos infectados (Azimi et al., 1999;

combater a infecção, porém estas células tornam-se danosas, visto que a infecção não é debelada e essa grande quantidade de citocinas pro-inflamatórias produzidas constantemente pode levar a um dano para o sistema nervoso central. Estes achados questionam se esta resposta citotóxica é boa ou ruim para o indivíduo infectado. A razão pela qual apenas uma minoria de indivíduos infectados desenvolvem a doença, ainda não é bem compreendida, porém estudos prévios têm mostrado a importância da carga viral e de fatores genéticos no desenvolvimento de doença. Tem sido demonstrada a participação do HLA no controle da carga viral e consequentemente no desenvolvimento de HAM/TSP, onde o subtipo HLA-A 02 previne em 28% o desenvolvimento desta patologia, reduzindo a carga viral nos portadores do vírus (Jeffery et al., 1999). Posteriormente, também foi demonstrado que outro subtipo de HLA, o HLA-C*w**8 está associado a proteção para o desenvolvimento de doença (Jeffery et al., 2000). A carga proviral também tem sido relacionada ao desenvolvimento de mielopatia. Alguns trabalhos têm demonstrado que a carga proviral de HTLV-1 e a expressão de tax são mais altas em pacientes com HAM/TSP do que em portadores assintomáticos, associando esta alta carga pró-viral ao desenvolvimento da mielopatia e à progressão da doença (Matsuzaki et al., 2001). ***A primeira hipótese deste trabalho é que pacientes com mielopatia apresentam uma maior produção de citocinas pro-inflamatórias, maior proliferação e ativação linfocitária que indivíduos assintomáticos infectados pelo HTLV-1.***

Associação entre HTLV-1 e *Strongyloides stercoralis*

A estrongiloidíase é uma helmintíase com uma distribuição mundial e uma das mais importantes infecções por helmintos em países tropicais. O *S. stercoralis* é o menor dos nematódeos que infecta o homem. A única forma parasitária adulta é a fêmea

partenogenética. A deposição de ovos ocorre cerca de 28 dias após a infecção inicial. Após eclodirem, os ovos liberam as larvas rabditóides no solo. Pode haver um ciclo de vida livre, mas quando as condições climáticas não são apropriadas as larvas rabditóides transformam-se em larvas filarióides infectantes. Estas penetram na pele, alcançam os vasos sanguíneos, chegam aos pulmões onde rompem os capilares e caem nos alvéolos. Posteriormente, ascendem até a faringe e são deglutidas, sendo o intestino delgado o habitat dos vermes adultos. Completando o ciclo, existe a eclosão dos ovos e liberação das larvas rabditóides que são eliminadas nas fezes. Um processo de auto-endo-infecção pode ocorrer quando estas larvas rabditóides se transformam em larvas infectantes (filarióides) ainda no intestino. Clinicamente esta helmintíase se apresenta de duas formas: aguda e crônica. A forma aguda raramente é vista e sua manifestação é decorrente da penetração das larvas de *S.stercoralis* na pele do indivíduo. Esta forma tem como manifestação uma erupção eritemato-papulosa, pruriginosa e é denominada de larva currens. A forma crônica pode ser leve, moderada ou grave. Geralmente as formas leve e moderada manifestam-se através de sintomas intestinais como diarréia, dor abdominal e vômitos. Na forma grave estas manifestações são mais intensas e pode ocorrer desidratação, distúrbios hidroeletrolíticos, hipoalbuminemia e íleo paralítico, como também outros órgãos podem ser acometidos: pulmão, fígado, coração e sistema nervoso central (SNC). Nesta forma pode também ocorrer infecções bacterianas e septicemia podendo levar ao óbito. Inicialmente a apresentação de formas graves foi associada ao uso de drogas imunodepressoras e quimioterápicos, porém recentemente esta helmintíase vem sendo relacionada à infecção pelo vírus HTLV-1. Estudos epidemiológicos têm demonstrado uma forte associação entre este vírus e a infecção pelo *S. stercoralis* em regiões onde ambos os agentes são endêmicos. Inicialmente, Nakada et al. demonstraram que 60% de portadores de *S. stercoralis* em

Okinawa, Japão, apresentavam sorologia positiva para HTLV-1. Outros estudos, nesta mesma cidade, também demonstraram esta associação (Hayashi et al., 1997; Sato et al., 1989). Embora um estudo realizado na Jamaica não tenha mostrado maior freqüência de anticorpos contra o *S. stercoralis* em indivíduos infectados pelo HTLV-1 comparados a indivíduos não infectados (Neva et al., 1989), houve associação significativa entre estas duas condições, em um outro estudo nesta mesma região (Robinson et al., 1994). Um estudo realizado em doadores de sangue da cidade de São Paulo, Brasil, documentou que enquanto portadores do HTLV-1 apresentaram freqüência de 12,1% desta helmintíase nos exames de fezes, os indivíduos não infectados pelo vírus apresentaram apenas uma freqüência de 1,6% (Chieffi et al., 2000). Nós também documentamos uma maior freqüência de estrongiloidíase em 153 portadores assintomáticos do vírus HTLV-1 quando comparados com 394 indivíduos negativos para o HTLV-1. Neste estudo a freqüência de *S. stercoralis* detectada nas fezes pela técnica de Baermann foi de 16% no grupo infectado pelo HTLV-1 e de 3,5% no grupo controle negativo (dados não publicados). Desta forma, a maioria dos estudos mostram uma forte associação destes dois agentes infecciosos. Além de estudos epidemiológicos, implicações clínicas também têm sido atribuídas a esta co-infecção. Estrongiloidíase disseminada e recorrente tem sido associada à co-infecção pelo HTLV-1(Gottuzo et al., 1999; Newton et al., 1992; O'Doherty et al., 1984; Patey et al., 1992; Phelps et al., 1993) podendo levar ao óbito (Adedayo et al,2001). Na estrongiloidíase disseminada o aumento da carga parasitária leva à migração do *S. stercoralis* para outros órgãos. O fígado, pulmão e cérebro são os órgãos mais comumente envolvidos, porém relatos de casos têm demonstrado formas atípicas da estrongiloidíase associadas à infecção pelo HTLV-1 (Lambertucci et al, 2003). Recentemente documentamos um paciente com apresentação atípica da estrongiloidíase, no qual foi encontrado larvas de *S. stercoralis* no

espermograma, urina e fezes durante uma investigação para infertilidade. Este paciente queixava-se de dor em região escrotal e abdominal e teve sorologia positiva para HTLV-1. Após tratamento houve regressão da sintomatologia (Porto et al. in press).

A associação de formas graves da estrongiloidíase ao uso de corticosteróides, drogas imunossupressoras e neoplasias já é bem documentada. A realização do exame de Baermann antes e durante o uso de imunossupressores tem reduzido consideravelmente o aparecimento de formas disseminadas da estrongiloidíase. Nos últimos 10 anos, 08 indivíduos com forma grave da estrongiloidíase e 57 com forma assintomática ou leve acompanhados no Hospital Universitário Professor Edgard Santos e no Hospital Santo Antônio em Salvador-Bahia foram classificados de acordo com a forma clínica. Dos 08 pacientes com forma grave da estrongiloidíase, 06 eram infectados pelo HTLV-1 enquanto que no grupo assintomático ou com forma leve, apenas 01 tinha sorologia positiva para este vírus (Porto et al., 2002). Estes dados mostram a forte associação entre HTLV-1 e estrongiloidíase grave fazendo com que a infecção pelo HTLV-1 seja, no nosso meio, o principal fator predisponente para o desenvolvimento de estrongiloidíase grave.

Outro aspecto importante em relação à associação entre estas infecções é uma aparente falha terapêutica no tratamento da estrongiloidíase, em pacientes portadores de HTLV-1. Enquanto a cura da parasitose após tratamento com thiabendazol foi observada em 31 (94%) de 33 pacientes com estrongiloidíase sem HTLV-1, em pacientes co-infectados, a cura foi documentada em somente 39 (70%) dos 55 pacientes tratados (Sato et al., 1994). Neste mesmo estudo, a cura da estrongiloidíase foi também significativamente maior em pacientes não co-infectados pelo HTLV-1, comparado aos pacientes co-infectados, nos quais o tratamento da parasitose foi feito por Albendazol ou Pamoato de pirvínio. Outro estudo documentou que 80% dos pacientes com estrongiloidíase resistente

ao tratamento com ivermectina eram infectados pelo HTLV-1 (Shikiya et al., 1994)

Embora os mecanismos imunológicos de defesa contra helmintos não estejam bem esclarecidos e a maioria dos estudos tenham sido realizados em modelos experimentais, existem evidências de que a resposta imune Th2, através da síntese de IL-4, IL-5, IL-13 (Finkelman et al., 1997; King et al., 1992; Urban et al., 1991) e conseqüentemente produção de Imunoglobulina (Ig) E, eosinofilia e mastocitose, participam da destruição do parasita. IL-12 e IFN-γ, que estão relacionados a resposta imune com o perfil Th1, inibem a imunidade protetora contra helmintos (Finkelman et al., 1994; Rotman et al., 1997). Níveis elevados de IgE total e específica têm sido documentados em pacientes com estrongiloidiase (Neva et al., 1998; Porto et al., 2001). Estes anticorpos podem atuar através do mecanismo de citotoxidade celular dependente de anticorpos (ADCC). Reforça esta hipótese o fato de ser observada uma infiltração de eosinófilos em torno das larvas de *S. stercoralis* (German et al., 1992; Poltera & Katsimubara, 1974) e também a demonstração de uma atividade helmintotóxica dos grânulos liberados pelos eosinófilos (David et al., 1980). Além disso, tem sido demonstrado uma ação citotóxica dependente de anticorpos no mecanismo de defesa contra esquistossômulos *in vitro* (Butterworth et al., 1974; Capron et al., 1978; Ottesen et al., 1977). Foi documentado que a infecção pelo HTLV-1 reduz os níveis de IgE total e específica para o antígeno de *S. stercoralis* em pacientes com estrongiloidíase (Neva et al., 1998; Porto et al., 2001) e quando os indivíduos apresentam a forma grave e foram avaliados com relação à produção de anticorpos IgG e IgE anti *S. stercoralis*, teste cutâneo e sorologia para HTLV-1, nenhum dos pacientes com forma grave apresentou positividade para o teste cutâneo e os níveis de IgE específico foram indetectáveis (Porto et al., 2002). ***A segunda hipótese desse trabalho é que a infecção pelo***

HTLV-1 reduz a produção de citocinas Th2 em pacientes infectados pelo HTLV-1 e S. stercoralis.

Resposta imune na esquistossomose

A esquistossomose tem distribuição mundial e estima-se que 200 milhões de pessoas em todo o mundo apresentam esta helmintíase (Butterworth et al., 1994). O *Schistosoma mansoni* é responsável por uma infecção parasitária crônica presente em grandes áreas de continentes sub-tropicais como o Brasil e outros países da América do Sul, como Suriname e Venezuela. Destes, o Brasil é o país de maior endemicidade. Apesar de existir outras espécies de *Schistosoma,* o *S. mansoni* é o único encontrado na América do Sul. No Brasil, a esquistossomose mansônica apresenta maior números de casos registrados na região Nordeste e no estado de Minas Gerais. O ciclo do *S. mansoni* inicia-se com a liberação de ovos pelas fezes de indivíduos infectados. Os ovos alcançam lagos, lagoas e rios, eclodem e liberam o miracídio. O miracídio infecta o caramujo e transforma-se em cercáreas. As cercáreas, liberadas na água podem penetrar na pele do indivíduo, quando perdem a cauda sendo denominadas de esquistossômulos. Estes ganham a circulação, fazem um ciclo pulmonar e migram para o sistema porta, onde acasalam. As fêmeas fazem a postura e os ovos passam para a luz intestinal e são excretados pelas fezes. Alguns ovos ficam presos na mucosa intestinal ou retornam ao fígado levando a formação de granuloma, podendo posteriormente levar à fibrose hepática.

A apresentação clínica da esquistossomose pode ser aguda ou crônica. A forma crônica pode ter comprometimento apenas intestinal, hepatointestinal e hepatoesplênico. Nas formas mais graves da doença ocorre um comprometimento geral do paciente, hepaesplenomegalia com hipertensão portal. O óbito pode ocorrer geralmente devido a

sangramentos digestivos. O principal achado patológico da esquistossomose é a fibrose hepática. A forma mais grave ocorre em cerca de 6% dos pacientes cronicamente infectados (De Jesus et al., 2000).

Os mecanismos de defesa contra o *S. mansoni* e a razão pela qual apenas alguns indivíduos desenvolvem fibrose hepática ainda não são bem esclarecidos. Porém, sabe-se que o "background"genético, o grau de infecção e a resposta imune do hospedeiro estão relacionados ao desenvolvimento desta fibrose (Secor et al., 1996; Sleigh et al., 1985). Tanto a resposta imune humoral quanto a resposta imune celular participam do mecanismo de defesa contra o *S. mansoni*. A presença de IgE específica para antígenos do parasito é correlacionada à uma resistência a re-infecção (Demeure et al., 1993; Dunne et al., 1992; Rihet et al. 1991), através do mecanismo de ADCC, que tem sido demonstrado *in vitro* contra esquistossômulos. (Butterworth et al., 1974; Capron et al., 1978; David et al., 1980; Ottesen et al., 1977). Além disso, um estudo realizado por Caldas e col. documentou que os níveis IgE/IgG4 estão associados à resistência a re-infecção. Quanto à resposta imune celular, tem sido demonstrado um predomínio de citocinas do tipo Th2 em culturas estimuladas com antígeno de verme adulto do *S. mansoni* (SWAP), com maior secreção de citocinas IL-4, IL-5 e IL-10, ausência de produção de IFN-γ e pouca ou nenhuma proliferação linfocitária (Araújo et al., 1996). Esta resposta, ocorre mais na fase crônica após a ovoposição. Na fase inicial desta infecção (fase aguda) uma resposta Th1 é induzida por antígenos do esquistossômulo (Grzych et al., 1991; Pearce et al., 1991). Esta resposta é caracterizada por alta produção de IFN-γ e TNF-α (De Jesus et al., 2002) e sintomatologia sistêmica como febre, astenia, caquexia, diarréia, dor abdominal e dispnéia pode ocorrer. Na fase crônica da doença, o principal achado patológico é a fibrose hepática, decorrente da

formação de granulomas ao redor do ovo do *S. mansoni*. A evolução da patologia pode levar a um quadro de fibrose, com danos para o hospedeiro. A formação do granuloma é dependente de células T, caracterizada como uma resposta de hipersensibilidade tardia com participação de citocinas Th1 e Th2 e contém uma alta percentagem de células como eosinófilos, macrófagos, fibroblastos e linfócitos. Esta resposta tem sido estudada principalmente em modelos experimentais. O papel da resposta Th2 na formação do granuloma tem sido demonstrado em alguns trabalhos, com participação de citocinas IL-4 e IL-13. Um trabalho experimental realizado em animais, no qual a resposta Th2 foi inibida através de tratamento (injeção intraperitoneal) de IL-13R α 2-Fc, observou-se uma redução significante na formação de granulomas nos pulmões desses animais (Chiaramonte et al., 1999a, Chiaramonte et al., 1999b). Este resultado sugere uma participação da citocina IL-13 nesta patologia. Um outro estudo em animais "knockout" para IL-4, a formação do granuloma foi similar aos animais tipos selvagens. Quando o "knockout" foi para o receptor de IL-4, houve uma redução no granuloma, sugerindo que a IL-13 está envolvida no desenvolvimento do granuloma (Jankovik et al., 1999). Isso se deve ao fato de que a IL-4 e a IL-13 compartilham o mesmo receptor. Adcionalmente, um outro trabalho experimental documentou que animais sensibilizados com ovos de *S. mansoni* e IL-12 desenvolveram granulomas mínimos. Este achado foi associado a um aumento dos níveis de IFN-γ (Wynn et al., 1994). Apesar destes estudos mostrarem a importância de citocinas Th2 no desenvolvimento do granuloma, existem dados que são contrários, sugerindo uma participação importante de citocinas inflamatórias, como TNF-α e IFN-γ na formação do mesmo (Leptak et., 1997; Mwatha et al., 1998) porém, poucos estudo têm sido realizados em humanos. Um outro estudo realizado observou uma maior participação de citocinas

inflamatórias na formação do granuloma, onde em animais com ausência do receptor para IFN-γ, observou-se uma redução do tamanho e da arquitetura do granuloma (Rezende et al., 1997). Um trabalho recente realizado neste serviço sugere uma participação das citocinas IL-5 e IL-13 na patogenia da fibrose hepática na esquistossomose. Avaliando indivíduos através de ultrassonografia, foi documentada uma associação entre níveis elevados de IL-5 e IL-13 e graus mais graves de fibrose. Além disso, avaliação imunológica realizada em diferentes períodos de tempo mostrou que o aumento da fibrose hepática (grau I para grau II) foi associada a um aumento da produção de IL-5 e IL-13. Não foi documentado associação entre fibrose hepática e produção de TNF-α e IFN-γ (De Jesus et al., 2004). Considerando que tanto a resistência a reinfecção como o desenvolvimento de fibrose hepática estão relacionados a uma resposta Th2, ***a terceira hipótese desse trabalho é que a freqüência de infecção pelo S. mansoni é maior em indivíduos infectados pelo HTLV-1 e que a infecção pelo HTLV-1 não leva a formas graves da doença.***

Modulação da resposta imune na esquistossomose mansônica

A fase aguda da esquistossomose é caracterizada por uma elevada produção de citocinas pro-inflamatórias e manifestações graves que podem levar ao óbito. Recentemente, um estudo realizado em 31 indivíduos na fase aguda da esquistossomose, no estado de Sergipe, foi documentada uma produção espontânea de IFN-γ, TNF-α, IL-1 e IL-6 em culturas de CMSP, além de altos níveis séricos de TNF-α. Quando comparado aos níveis de pacientes com esquistossomose crônica, os níveis destas citocinas secretados na fase aguda foram significantemente maior (De Jesus et al, 2002). Após a ovoposição ocorre uma redução nos níveis de IFN-γ que se tornam indetectáveis a partir da 8° a 12 ° semanas

da infecção em camundongos (Grzych et al., 1991; Pearce et al., 1991). Nesta fase crônica existe uma maior produção de citocinas Th2 como IL-4, IL-5 e IL-10. A mudança de um perfil com produção de citocinas pro-inflamatórias para um perfil Th2 está relacionado `a uma resposta moduladora no controle da inflamação associada a passagem de ovos através da parede intestinal. Em um estudo realizado com modelos experimentais, no qual os animais eram deficientes em produção de citocinas Th2, observou-se uma morbidade severa associada a caquexia e conseqüentemente morte destes animais (Brunet et al., 1997). O predomínio da resposta Th2 na forma crônica da doença coincide com uma baixa produção de IFN-γ em culturas estimuladas com antígeno de *S. mansoni*. O papel da IL-10 nesta modulação tem sido enfatizado desde que a neutralização com anticorpo monoclonal restaura a produção de IFN-γ (Araújo et al., 1996). O fato de na fase crônica da esquistossomose haver um predomínio de citocinas Th2 como uma tentativa de modulação de um processo inflamatório, levou alguns pesquisadores a estudar a interferência de um ambiente rico nestas citocinas na resposta imune a antígenos, como no desenvolvimento de doenças que têm na sua patogênese uma resposta imune celular com carácter inflamatório. Em um estudo realizado por Sabin e col. no qual pacientes infectados com *S. mansoni* foram vacinados com toxóide tetânico (TT) e foi estudada a produção de IFN-γ e IL-4 em sobrenadante de culturas de células mononucleares estimuladas com o antígeno de TT, observou-se que quando os indivíduos eram infectados pelo *S. mansoni* havia produção de IL-4 enquanto que nos indivíduos não infectados pelo *S. mansoni* houve produção tanto de IL-4 quanto de IFN-γ, sugerindo que a infecção pelo *S. mansoni* interfere na resposta imune a este antígeno. Posteriormente, Cooke e col. observaram que camundongos diabético não obeso (NOD) que têm tendência a desenvolver diabetes mellitus insulino dependente

(DMID) através de uma resposta Th1 contra células beta, quando infectados pelo *S. mansoni* apresentavam uma menor incidência desta doença. Reforçando que a infecção pelo *S. mansoni* pode interferir no desenvolvimento de doenças autoimunes, um estudo recente com modelo experimental para a esclerose múltipla (encefalite experimental autoimune) demonstrou que a infecção por este helminto reduziu a inflamação do SNC e alterou a progressão da encefalomielite. Neste estudo observou-se uma menor produção de IFN-γ, TNF-α por esplenócitos em animais infectados pelo *S. mansoni*. Além disso, a expressão de mRNA para IL-12p40 em cordão espinhal foi drasticamente reduzida nestes animais (La Flamme et al., 2003). Estes estudos sugerem uma ação moduladora da infecção pelo *S. mansoni* em situações onde se observa um perfil predominante Th1. Porém, também tem sido demonstrado que a infecção por este helminto pode inibir a reatividade ao teste cutâneo a aeroalérgenos como também foi observada uma menor gravidade da asma em indivíduos residentes em área endêmica para esquistossomose (Medeiros et al., 2004; Medeiros et al., 2003). Neste mesmo estudo, também foi observada pióra da sintomatologia da asma após o tratamento da esquistossomose. Com relação a resposta imune, foi observado que pacientes com *S. mansoni* e asma produzem menos IL-5 e mais IL-10 quando estimulado com antígeno de *Dermatophagoides pteronyssinus* do que pacientes que têm asma sem esquistossomose (Araújo et al., in press). Ainda não está claro como a infecção pelo *S. mansoni* pode interferir na progressão da asma e na resposta a testes cutâneos. A asma está relacionada a um processo inflamatório e é possível que a infecção por helmintos, através de mecanismos regulatórios, como a produção de IL-10, possa modular esta inflamação. ***A quarta hipótese desse trabalho é que a infecção por helmintos pode interferir na resposta imune e no curso clínico da infecção pelo HTLV-1.***

OBJETIVOS

GERAIS

- Avaliar a resposta imune em portadores assintomáticos do HTLV-1 e em pacientes com mielopatia associada a este vírus.
- Avaliar as implicações clínicas e imunológicas da associação HTLV-1 e helmintos.

ESPECÍFICOS

- Comparar a resposta imunológica através da determinação de citocinas em sobrenadante, da proliferação celular e da análise por citometria de fluxo de portadores assintomáticos do HTLV-1 com pacientes com mielopatia associada a este vírus.
- Determinar o perfil de citocinas em indivíduos com *S. stercoralis* co-infectados ou não pelo HTLV-1.
- Comparar a prevalência da esquistossomose em pacientes HTLV-1 positivos e negativos e descrever as formas clínicas desta parasitose apresentadas na presença ou ausência de co-infecção.
- Determinar o perfil de citocinas e de IgE especifica em indivíduos com *S. mansoni* co-infectados ou não pelo HTLV-1.
- Avaliar a resposta imune (determinação de citocinas em sobrenadante e análise por FACS) e a carga proviral em indivíduos infectados pelo HTLV-1 co-infectados ou não por helmintos (*S. stercoralis e S. mansoni*).

- Determinar a freqüência de infecção por helmintos (*S. stercoralis e S. mansoni*) em indivíduos portadores assintomáticos do HTLV-1 e em pacientes com mielopatia.

JUSTIFICATIVA

O HTLV-1 é um retrovírus que infecta predominantemente células T e é o agente causal da mielopatia associada ao HTLV-1 e da leucemia/linfoma de célula T de adultos (LLcTA). Apesar destas patologias serem as mais graves e de maior morbidade, este vírus está relacionado a outras doenças como uveíte, síndrome de Sjogren, artropatias, dermatite infectiva e pneumonite linfocitária. Embora o HTLV-1 tenha uma distribuição mundial, só é altamente prevalente em algumas áreas. A alta prevalência da infecção em Salvador, Bahia, oferece a oportunidade para esclarecer os mecanismos patogênicos e as características clínicas desta infecção. Apesar da literatura mostrar uma baixa prevalência de doenças relacionadas a este vírus, nós e outros autores temos observado que a prevalência da mielopatia é maior do que a documentada na literatura. As patologias relacionadas a este vírus estão associadas a desrregulação do sitema imune. Nesse caso, torna-se importante conhecer as alterações imunológicas causadas por este vírus, como também o mecanismo que pode levar ao desenvolvimento de doença. Nós tivemos a oportunidade de receber no ambulatório multidisciplinar de HTLV-1 pacientes com mielopatia e foi possível avaliar as alterações imunológicas relacionadas com esta patologia. O conhecimento sobre a patogênese desta doença pode oferecer suporte para alternativas de tratamento e também estabelecer marcadores que sejam importantes para um diagnóstico precoce, visto que o tratamento na forma inicial da doença é mais eficaz.

A infecção pelo HTLV-1 tem sido associada a uma maior freqüência de estrongiloidíase, ao desenvolvimento de formas graves e a um quadro de hiperinfecção, podendo levar ao óbito. No nosso meio, a estrongiloidíase disseminada tem sido atribuída à

co-infecção pelo HTLV-1. Inicialmente foi documentada uma maior prevalência de infecção pelo *S. stercoralis* em doadores de sangue infectados pelo HTLV-1comparados ao grupo de doadores não infectados. Além disso, documentamos uma menor resposta ao teste cutâneo de hipersensibilidade imediata para estrongiloidíase e menor níveis de IgE total e especifica para antígeno de *S. stercoralis*. A IgE está envolvida no mecanismo de defesa contra helmintos, porém a imunidade celular também participa desta defesa. Juntamente com a documentação de formas graves e uma menor resposta terapêutica a drogas anti-helmintícas, tornou-se importante conhecer melhor quais alterações imunológicas podem levar a estes achados, avaliando a imunidade celular em pacientes com estrongiloidíase e co-infectados pelo HTLV-1.

Salvador tem uma alta prevalência de HTLV-1 e a esquistossomose é altamente prevalente no nordeste do Brasil. A resposta imune participa não somente dos mecanismos de defesa contra o *S. mansoni* como também da patogênese da doença. Sendo o HTLV-1 uma infecção que se caracteriza por uma forte resposta Th1, a co-infecção entre HTLV-1 e *S. mansoni* é um importante modelo para avaliar a repercussão clínica e imunológica desta associação. A esquistossomose na sua forma crônica caracteriza-se por uma diminuição ou ausência de produção de IFN-γ, fenômeno que pode ser revertido com a neutralização de IL-10. Em modelos experimentais de doenças imunes mediadas por uma resposta Th1, a infecção com o *S. mansoni* diminui a freqüência e a severidade destas doenças. A mielopatia associada ao HTLV-1 está relacionada com uma resposta Th1 exacerbada e produção elevada de TNF-α e de IFN-γ. Como nós temos uma alta prevalência de HTLV-1 no nosso meio e estes pacientes apresentam co-infecção por helmintos, foi avaliada a

influência da infecção por helmintos na resposta imune e no desenvolvimento de mielopatia associada a este vírus.

ARTIGO 1

SANTOS SB, PORTO AF, MUNIZ AL, DE JESUS AR, MAGALHAES E, MELO A, DUTRA WO, GOLLOB KJ, CARVALHO EM. **Exacerbated inflammatory cellular immune response characteristics of HAM/TSP is observed in a large proportion of HTLV-I asymptomatic carriers**. *BMC Infect Dis*. 2004 Mar 2;4(1):7.

Neste artigo foi estudada a resposta imune através de produção espontânea de citocinas em sobrenadante, freqüência de células expressando citocinas, proliferação linfocitária e marcadores de ativação linfocitária em pacientes com HAM/TSP e portadores assintomáticos do vírus. Os pacientes com mielopatia apresentaram níveis mais elevados de citocinas pro-inflamatórias (IFN-γ e TNF-α) em sobrenadante de culturas de CMSP que portadores assintomáticos. Não houve diferença em relação aos níveis de IL-5 e IL-10. Foi observado uma grande variabilidade nos níveis de IFN-γ nos portadores assintomáticos, dentre os quais 40% apresentaram níveis semelhantes aos dos pacientes com mielopatia. Estes níveis se mostraram semelhantes em uma segunda avaliação. Adcionalmente, houve uma maior proliferação linfocitária no grupo com mielopatia. A análise pela citometria de fluxo mostrou maior freqüência de linfócitos T produzindo IFN-γ e TNF-α no grupo com mielopatia, como também foi observado neste grupo que a produção de IFN-γ foi predominantemente por linfócitos T CD8+. Nos pacientes assintomáticos as células T CD4+ e T CD8+ contribuíram de forma similar na produção de IFN-γ. Uma maior freqüência de células T CD8/CD28- foi observada em pacientes com mielopatia. Nós concluímos que pacientes com mielopatia têm uma maior ativação da resposta imune que indivíduos portadores assintomáticos. Entretanto, existiu uma variabilidade em relação aos níveis de IFN-γ nos portadores assintomáticos e em 40% destes, a produção foi semelhante aos pacientes com mielopatia.

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Exacerbated inflammatory cellular immune response characteristics of HAM/TSP is observed in a large proportion of HTLV-I asymptomatic carriers

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Abstract

Background: A small fraction of Human T cell Leukemia Virus type-I (HTLV-I) infected subjects develop a severe form of myelopathy. It has been established that patients with HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) show an exaggerated immune response when compared with the immunological response observed in HTLV-I asymptomatic carriers. In this study the immunological responses in HAM/TSP patients and in HTLV-I asymptomatic carriers were compared using several immunological assays to identify immunological markers associated with progression from infection to disease.

Methods: Immunoproliferation assays, cytokine levels of unstimulated cultures, and flow cytometry analysis were used to evaluate the studied groups. Nonparametric tests (Mann-Whitney U test and Wilcoxon matched-pairs signed ranks) were used to compare the difference between the groups.

Results: Although both groups showed great variability, HAM/TSP patients had higher spontaneous lymphoproliferation as well as higher IFN-γ levels in unstimulated supernatants when compared with asymptomatic carriers. Flow cytometry studies demonstrated a high frequency of inflammatory cytokine (IFN-γ and TNF-α) producing lymphocytes in HAM/TSP as compared to the asymptomatic group. This difference was accounted for mainly by an increase in CD8 cell production of these cytokines. Moreover, the HAM/TSP patients also expressed an increased frequency of CD28-/CD8+ T cells. Since forty percent of the asymptomatic carriers had spontaneous lymphoproliferation and IFN-γ production similar to HAM/TSP patients, IFN-γ levels were measured eight months after the first evaluation in some of these patients to observe if this was a transient or a persistent situation. No significant difference was observed between the means of IFN-γ levels in the first and second evaluation.

Conclusions: The finding that a large proportion of HTLV-I carriers present similar immunological responses to those observed in HAM/TSP, strongly argues for further studies to evaluate these parameters as markers of HAM/TSP progression.

Background

Human T cell leukemia virus-type 1 (HTLV-I) infects an estimated 10 to 20 million people worldwide, making it a serious public healthy problem. The HTLV-1 infection has a high prevalence in Brazil, and Salvador, the capital of the state of Bahia, has the highest prevalence of HTLV-1 in the country in blood donors (1,35%) [1,2]. It is estimated that 95% of HTLV-I infected individuals are asymptomatic carriers. A small percentage of infected individuals (2 to 5%) develop adult T cell leukemia/lymphoma (ATL) [3,4] or a chronic inflammatory disease, involving the central nervous system, termed HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) [5,6]. One of the most important immunological observations of HTLV-I infection is the demonstration that lymphocytes spontaneously proliferate *in vitro* in the absence of stimulus [7]. It has been shown that both infected CD4+ and CD8+ lymphocytes infiltrate spinal cord and peripheral blood and produce cytokines such IFN-γ, TNF-α and IL-6, which are considered important inflammatory mediators of the tissue damage in HAM/TSP [8-10]. Extensive previous studies have compared the immunological response of asymptomatic HTLV-I carriers to that of HAM/TSP patients [11,12]. Additionally, a recent study [13] has emphasized that the percentage of HTLV-I carriers that develop other immunological abnormalities, including HAM/TSP, is much higher than that previously cited in the literature. The aim of the present study is to compare in HTLV-I asymptomatic carriers, and in HAM/TSP patients, the spontaneous lymphoproliferative response and cytokine production, the overall *ex vivo* T cell activation states, and the production of immunoregulatory cytokines by CD4+ and CD8+ T cells. The documentation that some HTLV-I carriers have immunological alteration similar to that observed in HAM/TSP suggests that potential markers of disease progression may be determined in HTLV-I infection.

Methods

Patients selection and neurological exam

Patients were selected from the HTLV-I clinic of the Hospital Universitário Professor Edgard Santos, Federal University of Bahia, Brazil. The diagnosis was confirmed by Western blot (HTLV blot 2.4, Genelabs, Singapore). Seventeen patients with HAM/TSP were selected based on WHO criteria and thirty-six HTLV-I asymptomatic carriers were referred from two blood banks. Exclusion criteria included the use of antiviral drugs or immunomodulators in the previous 90 days, helminth infection, co-infection with HIV, HCV or hepatitis B and presence of other neurologic diseases. Motor dysfunction was determined by Osame's Motor Disability Score (OMDS) [14] and Expanded Disability Status Scale (EDSS) [15]. Patients with HAM/TSP had a marked neurological impairment with EDSS ≥ 3 and OMDS ≥ 1 and all asymptomatic subjects had OMDS and EDSS of zero. Seronegative normal donors were also referred from the same blood banks and used as negative controls. The Ethical Committee of the Hospital Universitário Professor Edgard Santos approved this study and informed consent was obtained from all prospectively enrolled patients.

Cell preparation and proliferation assay

Peripheral blood mononuclear cells (PBMC) were isolated and cultivated in RPMI 1640 (Gibco, Grand Island, NY, USA) plus 10 % heat inactivated human AB Rh+ serum (Sigma Chemical Co., St. Louis, MO), antibiotics and glutamine. A total of 2×10^5 cells/mL were incubated at 37°C in 5 % CO_2 atmosphere in a 96 well flat-bottom microtiter plates. The cells were kept unstimulated (media alone) and after 5 days, the cultures were pulsed with ^{3}H-Thymidine (1 μCi/well) for a final 6–16 hours period, and then harvested. The ^{3}H-Thymidine uptake was measured using a LKB beta scintillation counter. The average of counts per minute (cpm) was plotted and PHA (1.0 μg/mL, Sigma) was used as a positive control in the proliferation assay.

Cytokine determination

PBMC were adjusted to 3×10^6 cells/mL in RPMI 1640 plus 10 % serum AB Rh+. The cells were cultured unstimulated or stimulated with PHA (5 μg/mL) and all cultures were incubated at 37°C in 5 % CO_2 atmosphere for 72 hours until supernatants were collected. IFN-γ, TNF-α, IL-5 and IL-10 levels were measured by sandwich ELISA technique (R & D system, Minneapolis, MN).

Ex vivo staining of lymphocyte profiles

2×10^5 PBMC from HTLV-I patients were incubated with FITC, PE, or Cychrome-labeled antibody solutions for 20 minutes at 4°C. After staining, preparations were washed with 0.1% sodium-azide PBS, fixed with 2% formaldehyde in PBS and kept at 4°C until data acquisition using a FACScalibor (Becton Dickinson, San Jose, CA). The antibodies used were all directly conjugated either for FITC, PE, or Cychrome and consisted of: Ig controls, anti-CD4, CD8, CD28, CD69 (Pharmingen, San Diego, CA) and anti-CD62L (Caltag, Burlingame, CA).

Single cell cytoplasmic cytokine staining

Briefly, 2×10^5 PBMC were cultured in RPMI 1640 plus 5% AB Rh+ serum in 96 well plates. Based on preliminary results all the cytokine staining was performed after 20 hours of incubation. During the last 4 hours of culture, Brefeldin-A (1 μg/mL) was added to the culture. The cells were then washed and centrifuged using ice-cold PBS plus sodium-azide, stained for surface markers and fixed using 2% formaldehyde. The fixed cells were then permeablized with a solution of Saponin and stained, for 30 minutes at 4°C, using anti-cytokine mAbs directly conjugated with

PE (IFN-γ, TNF-α, IL-4 and IL-10) (Pharmingen). Preparations were then washed, fixed and analyzed using a FACScalibor. In all cases the cells were double stained for cytokine and for cell surface markers. In all cases, 30,000 gated events were acquired for later analysis due to the low frequency of positive events being analyzed.

Statistical analysis

A nonparametric Mann-Whitney U test and Wilcoxon matched-pairs signed rank tests were used to evaluate differences between the groups. An alpha (α) of 5% ($p < 0.05$) was considered for statistical significance. Lymphocytes were analyzed for their intracellular cytokine expression patterns and frequencies and for surface markers using the program Cell Quest. Statistical analysis was performed using the ANOVA "comparison of all pairs" contained in the statistical program from SAS, JMP.

Results

The mean age of the seventeen myelopathy patients was 53 ± 16 (mean ± SD) years and of the thirty-six HTLV-I healthy carriers was 39 ± 11 years. To determine if HAM/TSP patients and asymptomatic subjects produce different levels of secreted cytokines, IFN-γ, TNF-α, IL-10 and IL-5 were measured in supernatants of unstimulated cultures of HTLV-I infected groups and compared with negative controls. There was a high variability in IFN-γ levels in asymptomatic carriers (Figure 1A). The mean and SD of IFN-γ levels in myelopathy patients (4,246 ± 2,924 pg/mL, range: 375 to 10,750), was higher than that observed in asymptomatic carriers (1,362 ± 1,408 pg/mL range: 15 to 6,995) or in negative controls (1 ± 4 pg/mL), $p = 0.0001$, Mann-Whitney U test. There was also a tendency for higher TNF-α levels in HAM/TSP patients (378 ± 316 pg/mL) when compared with levels observed in asymptomatic carriers (259 ± 366 pg/mL) or in negative controls (60 ± 63 pg/mL), $p = 0.06$. No differences between IL-5 levels (151 ± 141 versus 166 ± 231 pg/mL), $p = 0.17$ and IL-10 levels (70 ± 66 versus 94 ± 110 pg/mL), $p = 0.9$, were observed between HTLV-I infected groups or negative controls (2 ± 2 versus 2.6 ± 10), Figure 1B.

The lymphoproliferative assays performed using PBMC from ten HAM/TSP patients and eleven asymptomatic individuals showed that lymphoproliferation was higher in HAM/TSP than HTLV-I asymptomatic carriers. The five day spontaneous proliferation of the HAM/TSP group gave a mean and SD of 21,404 ± 30,859 cpm (range: 919 to 102,242), while the asymptomatic HTLV-I group had a mean and SD of 3,365 ± 5,188 cpm (range: 139 to 18,169). The magnitude of the responses was higher in HAM/TSP than in HTLV-I carriers ($p = 0.006$) although a great variability of the spontaneous lymphoproliferation had been observed in both groups (date not shown).

Based on IFN-γ production, the immunological responses in 40% of the HTLV-I carriers were similar to that observed in patients with HAM/TSP ($p > 0.05$). These individuals had IFN-γ levels higher than 1,322 pg/mL, representing the mean minus one standard deviation of the IFN-γ levels obtained in HAM/TSP patients. To determine if the cytokine levels in HTLV-I carriers was reflecting a transient or persistent situation, IFN-γ levels were measured between 6 months to one year (with a mean of eight months) after the first evaluation. No significant differences were observed between the means of IFN-γ levels from fourteen asymptomatic carriers in the first and second evaluation (1,723 ± 450 and 1,670 ± 1,396, respectively), $p = 0.67$. HAM/TSP patients ($n = 11$) also had similar levels in the first and second evaluation (5,771 ± 3,365 versus 4,718 ± 2,427, respectively), $p = 0.17$, Wilcoxon matched-pairs signed ranks test (Figure 2).

To determine the activation states and relative lymphocyte proportions, as well as the cellular sources of immunoregulatory cytokines, four HAM/TSP patients and eight asymptomatic carriers were randomly selected and analyzed using flow cytometry. To measure lymphocyte activation and regulation, the markers CD69 and CD28 respectively, where used in conjunction with CD4 and CD8 in separate staining protocols. The relative proportions of CD4 and CD8 cells expressing the adhesion molecule, CD62L was also determined for both groups. Figure 3A demonstrates that the HAM/TSP group expressed a significantly higher frequency of CD8+ T cells (21.4 ± 3.3) than the asymptomatic group (9.8 ± 1.7). A higher frequency (51.9 ± 3.5) of the hyper-activated, CD28-/CD8+ T cells within the CD8+ T cell population in HAM/TSP than in asymptomatic group (35.2 ± 7.4) was also observed. There was no difference in CD69 and CD62L lymphocyte populations between both HTLV-I infected groups (data not shown).

To further investigate the differences in cytokine profile between the HAM/TSP and asymptomatic groups, flow cytometry was performed to determine the frequency of T cells producing IFN-γ, TNF-α, IL-4 and IL-10. A significant increase in the frequency of lymphocytes expressing TNF-α and IFN-γ was seen in the HAM/TSP group (12 ± 6 and 5 ± 0.3, respectively) as compared to the asymptomatic group (4 ± 2 and 1 ± 0.5, respectively) (Figure 3B). Moreover, CD8+ T cells where the major cellular source responsible for the difference in IFN-γ producing cells between the two groups. The frequency of CD8+ T cells producing TNF-α was higher in HAM/TSP as compared to asymptomatic carriers, with both CD4+ and CD8+ T cells contributing equally to the difference seen in TNF-α production (Figure 3C). In contrast, no difference was seen for the frequency of cells producing IL-4 or IL-10 (data not shown). While there was no difference regarding the frequency of

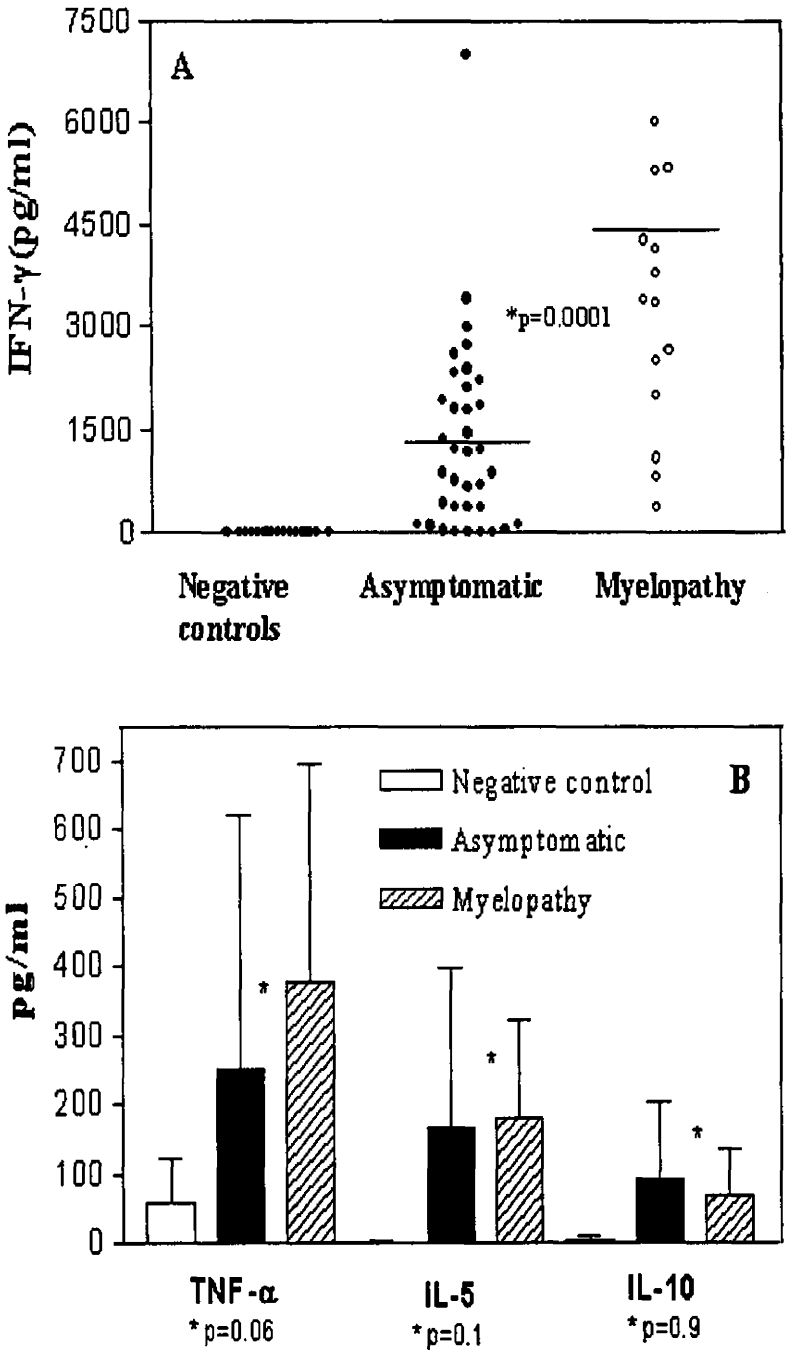

Figure 1

HAM/TSP patients display higher production of IFN-γ as compared with others cytokines synthesis. Unstimulated cultures supernatants of PBMC from 17 HAM/TSP patients and 36 asymptomatic carriers were compared with negative controls (n = 15) and assayed by ELISA to observe IFN-γ, TNF-α, IL-5 and IL-10 synthesis. Figure 1A shows IFN-γ levels (pg/ml) In HAM/TSP patients as compared with asymptomatic carries or negative controls. Figure 1B shows TNF-α, IL-5 and IL-10 levels in both groups. The bars represent the median of IFN-γ concentrations and the difference were considered significant when $p < 0.05$ (Mann-Whitney U Test).

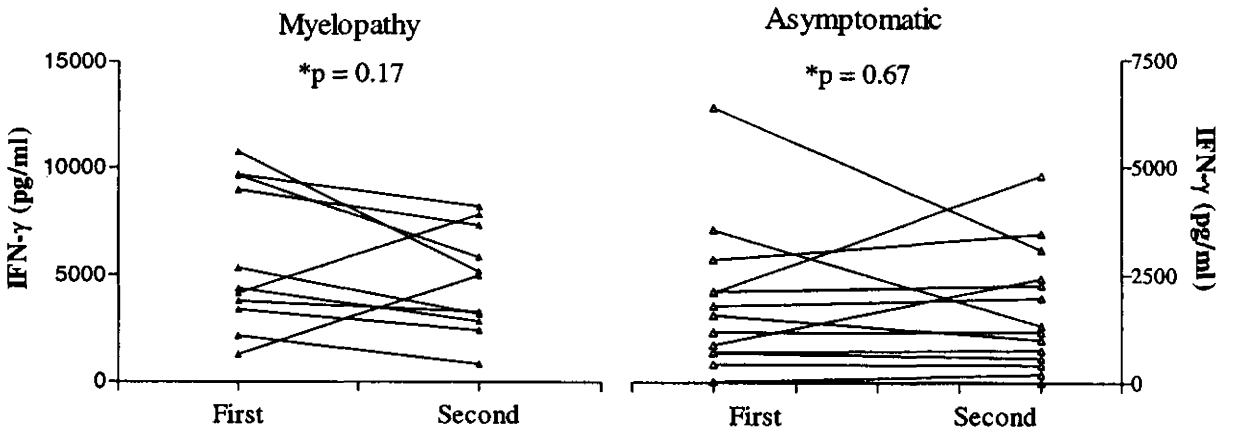

Figure 2

IFN-γ levels are relatively constant when evaluated in two different periods. Unstimulated cultures supernatants of PBMC from 11 HAM/TSP patients and 14 asymptomatic carriers were assayed by ELISA and analyzed at the Initial evaluation and eight months following the first evaluation. The data represent IFN-γ levels (pg/ml) of each patient in first and second evaluation. The differences were not statistically significant ($p > 0.05$, Wilcoxon matched-pairs signed ranks test).

CD4+ and CD8+ T cells producing IFN-γ within asymptomatic carriers (0.5 ± 0.4 vs. 0.5 ± 0.3), there was a significant difference ($p < 0.05$) in the frequency of CD4 cells producing IFN-γ in HAM/TSP (1.4 ± 0.5) compared to the CD8+ T cells (3.6 ± 0.5).

Discussion

The present study shows that lymphocytes from patients with HAM/TSP displayed higher spontaneous proliferation and IFN-γ synthesis, a higher frequency of TNF-α and IFN-γ producing lymphocytes and a significant increase in the deregulated T cell population, CD28-/CD8+, as compared to those from HTLV-I asymptomatic carriers.

The pathogenesis of HAM/TSP is not completely understood. Although initially considered a rare and late complication of infection, the disease has been identified in children, and in some cases, a rapidly progressive disease has been observed [16]. Increased proviral load, proinflammatory cytokines and the expansion of HTLV-I tax-specific CD8+ cytotoxic T lymphocytes, both in cerebrospinal fluid and in peripheral blood, have been associated with the central nervous system involvement in patients with HAM/TSP [17-21]. A recent report [13] established a cohort of HTLV-I asymptomatic carries to study clinical events and documented an increased frequency of abnormalities, including a case of HAM/TSP.

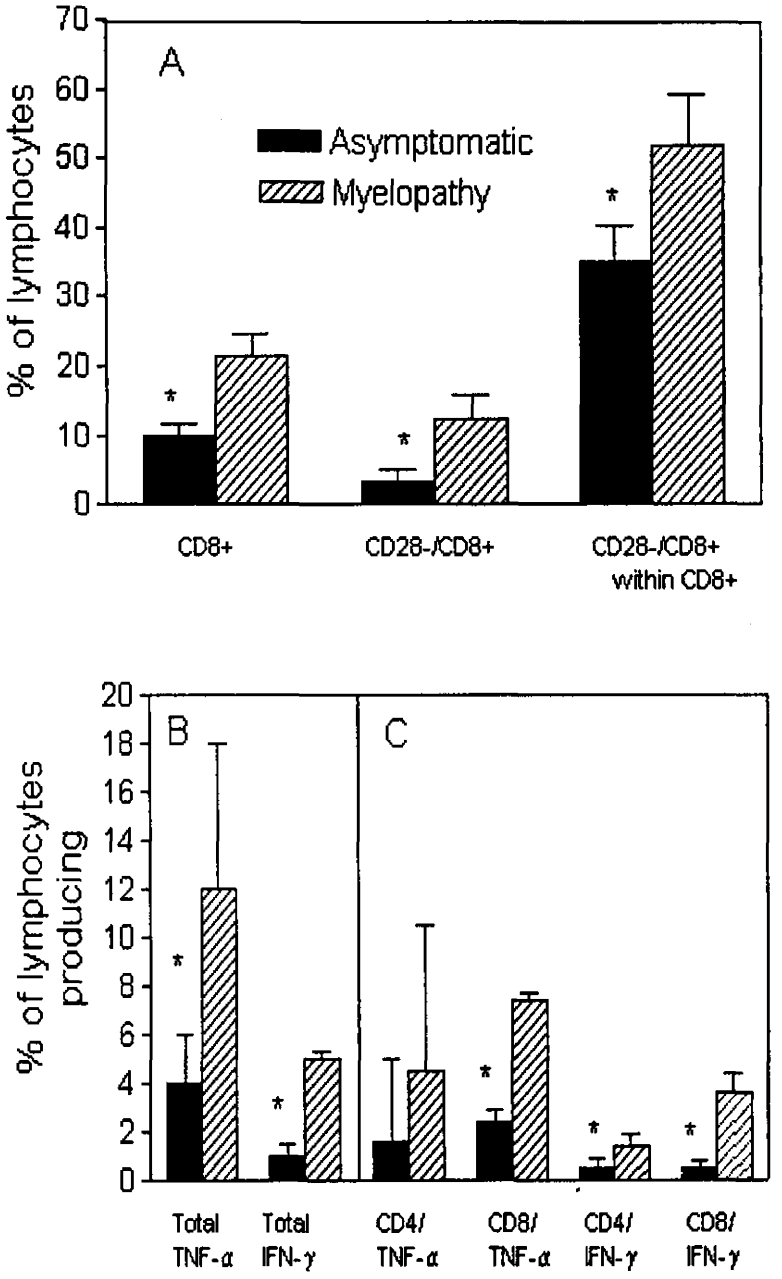

Figure 3

HAM/TSP patients display a higher frequency of CD28-/CD8+ T cells and inflammatory cytokine producing T cells than do asymptomatic carriers. PBMC from 4 HAM/TSP and 8 asymptomatic carriers were analyzed ex vivo or following a 20 hour media alone culture for the expression of CD4+ and CD8+ T cell subpopulations using flow cytometry. Figure 3A shows the frequeney of CD8 cells and the subpopulations defined by CD28 expression. Figure 3B shows the frequency of total lymphocytes producing TNF-α and IFN-γ. Figure 3C shows the relative contribution of CD4+ or CD8+ T cells to the overall cytokine producing population shown in Figure 3B. The data represent the mean ± S.D and the asterisk represents differences with a $p < 0.05$ (Mann-Whitney U Test).

Evaluation of immunological responses in patients with HAM/TSP and asymptomatic carries is important for the understanding of the pathogenesis of the HAM/TSP, and to identify early immunological markers associated with progression from infection to disease. This study indicates a higher and significant spontaneous lymphoproliferation and IFN-γ production in HAM/TSP patients as compared to HTLV-I asymptomatic carriers. Additionally, lymphocyte responses were quite variable, and in some asymptomatic carriers the lymphocyte proliferation and IFN-γ production were similar to those found in patients with myelopathy. These data induce us to observe if the parameters could be considered good markers of disease progression. This is in agreement with our previous observations that asymptomatic carriers can be divided in high and low producers according to IFN-γ levels [22]. A great variability of immune response observed in about 40% of HTLV-I infected subjects was not reflecting a temporary situation, since the IFN-γ levels were relatively constant within an individual over time (eight months). During this period of time no neurological manifestation was documented and no other disease that could alter the immune response was observed.

Many studies have demonstrated that IFN-γ and TNF-α produced in large quantities contribute to tissue damage of the central nervous system [23,24], and are likely involved in the pathogenesis of several infections and non-infections disease. The flow cytometric determination of cytokine producing lymphocytes extend the observations made in supernatants of lymphocytes cultures showing a significant increase in the frequency of IFN-γ and TNF-α producing cells in patients with HAM/TSP. This difference was accounted for both CD4+ and CD8+ T cells for the TNF-α producing cells, and mainly by CD8+ T cells for IFN-γ producing lymphocytes. Since CD4+ T cells are the main source of IFN-γ in HTLV-I carriers [22,25,26] this data may indicate that during the evolution from asymptomatic to myelopathy there is a switch from CD4+ to CD8+ in relation to the main cell producing IFN-γ. These findings support studies suggesting that CD8+ T cells may play an important role in the pathogenesis of HAM/TSP [27].

Previous studies have shown that CD28-/CD8+ cells display high cytotoxic activity, playing an important role in the pathology associated with viral diseases [28,29]. Additionally, recent studies have shown that HIV-1 can incorporate CD28 and the acquisition of this specific host surface glycoprotein modulates the virus life cycle [30]. Moreover, the role of CD28-/CD8+ T cells in HAM/TSP needs to be further investigated since these cells may induce cell damage and / or death in infections disease [31].

In conclusion, these results show an exacerbated type 1 immune response in HAM/TSP patients, characterized by elevated IFN-γ production, an increased frequency of TNF-α and IFN-γ producing lymphocytes, and by an increase in the frequency of CD28-/CD8+ T cells. Given that cellular activation and pro-inflammatory cytokine production are likely directly involved in the pathogenesis of HAM/TSP disease, longitudinal studies of HTLV-I infected asymptomatic carriers who present with high lymphoproliferative response, high production of IFN-γ and TNF-α and high expression of CD28-/CD8+ T cells should be conducted to determine the frequency of disease progression in this group. The identification of markers of HAM/TSP progression will allow for earlier initiation of current therapeutic interventions, and hopefully delay the fast and progressive development of the motor disability observed in HTLV-I infected individuals.

Competing interests

None declared.

Authors' contributions

SBS carried out the immunological studies, performed statistical analysis and drafted the manuscript. ALM, EM and AM participated in the coordination of neurological evaluations. AFP was involved in clinical evaluation of the patients. ARJ participated in the design and statistical analysis. WOD and KJG carried out FACS analysis and EMC conceived the study and participated in its design and coordination.

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ARTIGO 2

PORTO AF, NEVA FA, BITTENCOURT H, LISBOA W, THOMPSON R, ALCANTARA L, CARVALHO EM. **HTLV-1 decreases Th2 type of immune response in patients with strongyloidiasis.** *Parasite Immunol.* 2001 Sep;23(9):503-7.

A infecção pelo HTLV-1 tem sido associada a estrongiliodíase disseminada, à formas clinicas atípicas e à maior falha terapêutica com drogas anti-helmintícas. Já foi documentado que pacientes com estrongiloidíase, quando co-infectados pelo HTLV-1, apresentam níveis de IL-4 em culturas de CMSP, de IgE total e específica para antígeno de *S. stercoralis* inferiores aos níveis de pacientes apenas com estrongiloidíase. Neste artigo foi avaliada a resposta imune em pacientes com estrongiloidíase co-infectados ou não pelo HTLV-1. Enquanto os pacientes infectados unicamente pelo HTLV-1 apresentaram níveis mais elevados de IFN-γ, em culturas estimuladas com antígeno de *S. stercoralis*, os níveis de IL-5 e IL-13 foram mais baixos, quando comparados a indivíduos infectados apenas pelo *S. stercoralis*. Além disso, houve uma correlação inversa entre os níveis de IFN-γ e de IL-5 e de IFN-γ e de IgE específica para antígeno de *S. stercoralis*. Nossos dados mostram uma redução na resposta imune Th2 mediada por altos níveis de IFN-γ em pacientes co-infectados pelo HTLV-1, alteração essa que pode estar relacionada ao desenvolvimento de formas graves desta helmintíase em pacientes infectados por este vírus.

Parasite Immunology, 2001: **23**: 503–507

HTLV-1 decreases Th2 type of immune response in patients with strongyloidiasis

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SUMMARY

Eosinophils, immunoglobilin (Ig)E and cytokines have important roles in defence mechanisms against helminths. In this study, the influence of HTLV-1 infection, characterized by a Th1 type of immune response, was evaluated on the cytokine pattern and parasitic specific IgE response in patients with strongyloidiasis. Patients were divided into four groups: strongyloidiasis without HTLV-1 infection, strongyloidiasis with HTLV-1, HTLV-1 without strongyloidiasis and controls without either helminth infection or HTLV-1. The cytokine profile was determined in supernatants of mononuclear cells stimulated with Strongyloides stercoralis *crude antigen and the parasite specific IgE was measured by ELISA. Patients coinfected with HTLV-1 had higher levels of interfron (IFN)-γ and interleukin (IL)-10 ($P < 0.05$) and lower levels of IL-5 and IgE ($P < 0.05$) than patients with strongyloidiasis without HTLV-1. There was an inverse relationship between IFN-γ and IL-5 ($P = 0.01$; $r_s = -0.37$) and between IFN-γ and parasite specific IgE ($P = 0.01$; $r_s = -0.39$), and a direct relationship between IFN-γ and IL-10 ($P = 0.04$; $r_s = 0.35$). These data show that coinfection with HTLV-1 decreases IL-5 and IgE responses in patients with strongyloidiasis consistent with a relative switch from Th2 to Th1 response. Immunological responses such as these are important in the control of this helminthic infection.*

Keywords *HTLV-1, strongyloidiasis, IgE,* S. stercoralis

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INTRODUCTION

An association between strongyloidiasis and HTLV-1 infection has been documented in areas where these infections are endemic (1–4). While there is no agreement that HTLV-1 increases the prevalence of strongyloidiasis, there are strong data indicating that HTLV-1 has important clinical and immunological implications in this helminthic infection. For example, a high rate of therapeutic failure with thiabendazol and, consequently, chronic *Strongyloides stercoralis* infection has been documented in patients infected by HTLV-1 (5), and a severe form of disease with larval dissemination has been reported in patients coinfected with these two agents (6–9). Additionally, there is an inverse correlation between interferon (IFN)-γ levels and total immunoglobulin (Ig)E in patients with HTLV-1 and strongyloidiasis (10).

Individuals infected with HTLV-1 have spontaneous T cell proliferation (11–13) and high levels of IFN-γ (10), which are immunological functions asssociated with a Th1 type of immune response. The immune response in strongyloidiasis is not completely understood. Considering that helminthiases usually have a Th2 type immune response (14–18) and that levels of IgE in serum and interleukin (IL)-4 in cell supernatant fluids of patients with strongyloidiasis are elevated (10,19,20), it appears that these patients have a predominantly Th2 type of immune response. This type of immune response may be important in controlling hyperinfection due to *S. stercoralis*, since both IgE and IL-4 participate in killing or expulsion of helminths from the host (21–24). Moreover, since IL-4 and IL-13 share receptor components (25), it is posible that there also is participation of IL-13 in the defence mechanisms against helminths. The aim of the present study was to determine the cytokine profile in patients with

strongyloidiasis either coinfected or uninfected with HTLV-1 and to evaluate whether the increased IFN-γ production observed in patients coinfected with HTLV-1 and *S. stercoralis* may modulate the production of IL-5, IL-10, IL-13 and antigen specific IgE responses.

MATERIALS AND METHODS

Patients

Participants of the present study included HTLV-1 positive seroreactors from blood banks, and patients who lived in a rural endemic area for *S. stercoralis* near Salvador, state of Bahia, Brazil, with positive faecal examinations for *S. stercoralis* infection. A clinical history was taken and physical examination performed. The labouratory analysis included serology (IgE) for *S. stercoralis*, confirmation of HTLV-1 by Western blot and determination of cytokines (IFN-γ, IL-5, IL-10 and IL-13) in supernatant fluids of *S. stercoralis* antigen stimulated peripheral blood mononuclear cells (PBMC). Subjects were divided into four groups based on serology for HTLV-1 and *S. stercoralis* infection: group I comprised 20 individuals with negative serology for HTLV-1 and infected with *S. stercoralis*, group II comprised 20 patients coinfected with *S. stercoralis* and HTLV-1, group III comprised 20 individuals with positive serology for HTLV-1 and three negative stool examinations by the method of Baermann and group IV comprised 15 healthy subjects with negative serology and absence of helminths in the stool examination. The mean ages of patients in group I, group II group III and in group IV were 39 ± 9 years, 26 ± 16 years, 21 ± 3 and 20 ± 4, respectively, and the male/female ratio was 2·3 : 1, 4 : 1, 3 : 1 and 1·4 : 1, respectively. All the patients were asymptomatic in relation to strongyloidiasis. The criterion for a diagnosis of strongyloidiasis was a positive faecal examination (Baermann technique). After blood collection, all patients were treated with cambendazol (5 mg/kg weight). Informed consent was obtained and the human experimentation guidelines of the Hospital Universitário Prof. Edgard Santos were followed in the conduct of this clinical research.

Immunological studies

Antigen

Antigen for serology was prepared from infective larval stage 3 (L3) of the parasite recovered from faecal specimens of infected monkeys, after being allowed to develop at 25°C in charcoal cultures. Larvae were separated from the charcoal by the Baermann procedure and washed repeatedly by centrifugation. They were then exposed to 0·25% chlorox (sodium hypoclhorite) for 3–5 min for surface sterilization followed by multiple cycles of centrifugation in RPMI medium (Gibco, Grand Island, NY, USA) containing 100 μg per ml gentamicin. A soluble supernatant of sonicated larvae provided the somatic antigen used in the ELISA test.

Serum specific-IgE assays

S. stercoralis specific serum IgE was measured by ELISA on microtitre plates (Immulon 2; Dynatech Laboratories, Chantilly, VA, USA) as previously described (10). Sera were first depleted of IgG by treatment with Gamma Bind G Sepharose (Pharmacia Biotechnology, Uppsala, Sweden) before reaction with antigen overnight at 4°C. Detection of antibody was performed with goat antihuman IgE conjugated to alkaline phosphatase (Sigma, St Louis, MO, USA); the substrate was *p*-nitrophenylphosphate (Sigma) and the results are expressed as international units (IU).

Cytokine determination

Cytokine levels (IFN-γ, IL-5, IL-10 and IL-13) in supernatants of mononuclear cells were measured by ELISA. Briefly, peripheral blood mononuclear cells were obtained by density gradient centrifugation using lymphocyte separation media (LSM; Organon Teknika Coorporation, Durham, NC, USA). After washing in saline, the cells were adjusted to 3×10^6/ml in RPMI 1640 (Gibco) supplemented with 10% AB + sera containing 100 U penicillin/G and 10 μg/ml of streptomycin. The cells were either unstimulated or stimulated with *S. stercoralis* antigen (1 μg/ml). All cultures were incubated at 37°C in 5% CO_2 for 72 h. Supernatant fluids were collected and stored at − 20°C. IFN-γ (Genzyme Corp., Cambridge, MA, USA), IL-5, IL-10 and IL-13 (PharMingen, San Diego, USA) levels were measured by ELISA sandwich technique (26) and the results were expressed in pg/ml based on a standard curve generated using recombinant cytokines. Values represent the difference between the value of stimulated cultures minus the values of unstimulated cultures. Because we found that IFN-γ levels in subjects infected with HTLV-1 were similar in unstimulated or antigen stimulated cultures, for these groups of patients, the IFN-γ data presented correspond to the values observed in unstimulated cultures.

Serology for HTLV-1

HTLV-1 serology was performed by ELISA test (Cambridge Biotech, Cambridge, MA, USA). Positive ELISA tests were confirmed by Western blot (HTLV Blot 2·4, Genelabs, Singapore).

Statistical analysis

The correlations were analysed by Spearman correlation test. The Rank Sum Test was used to compare the means.

RESULTS

With the aim of determining the cytokine profile in patients with strongyloidiasis, coinfected or not with HTLV-1, the levels of IFN-γ, IL-5, IL-10 and IL-13 were determined in supernatants from *S. stercoralis* antigen stimulated lymphocyte cultures (Figure 1). The mean ± SD of IFN-γ levels in patients only infected with *S. stercoralis* was 20 ± 46 pg/ml (0–192 pg/ml) and the mean in supernatants of patients coinfected with *S. stercoralis* and HTLV-1 was 919 ± 944 pg/ml (0–3470 pg/ml) ($P = 0.01$). Although there was a tendency for the IFN-γ levels be higher in subjects only infected with HTLV-1 without strongyloidiasis (2063 ± 2499 pg/ml with variation of 15–9675 pg/ml) than in patients with HTLV-1 and strongyloidiasis 919 ± 944 pg/ml. (0–3470 pg/ml), this diference was not statisticaly significant. The mean ± SD of IL-5 levels in patients only infected with *S. stercoralis* was 727 ± 554 pg/ml (0–1683 pg/ml). This value was higher than that observed in patients coinfected with *S. stercoralis* and HTLV-1 (173 ± 168 pg/ml with variation of 0–488 pg/ml) ($P < 0.0001$) and in subjects without HTLV-1 and without *S. stercoralis* infection (2 ± 2 pg/ml). There was also a tendency for higher IL-13 levels in patients with strongyloidiasis without HTLV-1 (220 ± 361 pg/ml) than in patients coinfected with HTLV-1 (43 ± 45 pg/ml) ($P = 0.41$). Although the levels of IL-5 and IL-13 in subjects only infected with *S. stercoralis* were higher than that observed in subjects coinfected with *S. stercoralis* and HTLV-1, there was a higher production of IL-10 in this last group. The mean ± SD of IL-10 levels in first group was 5 ± 11 pg/ml (0–37 pg/ml) and in the group II was 35 ± 53 pg/ml (0–532 pg/ml) ($P = 0.02$). The level of IL-10 in patients only infected with HTLV-1 was 141 ± 115 pg/ml (0–390 pg/ml).

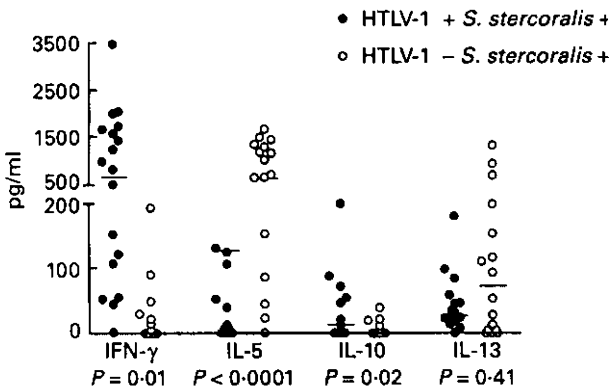

Figure 1 Cytokine profile in patients with strongyloidiasis coinfected or not with HTLV-1. Data for IFN-γ and IL-5 were obtained from all 40 patients. IL-10 levels were documented in 17 blood donors only infected with *S. stercoralis* and in 15 blood donnors coinfected with *S. stercoralis* and HTLV-1. IL-13 levels were documented in 17 blood donnors only infected with *S. stercoralis* and in 20 blood donnors coinfected with *S. stercoralis* and HTLV-1. Values of IL-5, IL-10 and IL-13 represent the differences between the values of stimulated cultures minus the values of unstimulated cultures. The IFN-γ data presented correspond to the value observed in unstimulated cultures.

Parasite specific IgE levels in patients only infected with *S. stercoralis* were higher than in patients coinfected with HTLV-1 and *S. stercoralis* ($P = 0.01$). The mean ± SD in the group I was 251 ± 437 IU and in the group II was 74 ± 94 IU.

The relationship between IFN-γ production in supernatants of lymphocyte cultures and IL-5, IL-13 and serum specific IgE levels in 40 patients with strongyloidiasis, with or without HTLV-1 coinfection, is shown in Table 1. An inverse relationship between IFN-γ and serum specific IgE ($P = 0.01$; $r_s = -0.39$) and between IFN-γ and IL-5 ($P = 0.01$; $r_s = -0.37$) was observed by Spearman analysis. When IL-5 production in supernatants of lymphocyte cultures was related to parasite specific IgE levels and to IL-13 in the same subjects, there was a direct relationship ($P = 0.0001$; $r_s = 0.57$ and $P < 0.0001$; $r_s = 0.75$, respectively). A direct relationship was also found between IFN-γ and IL-10 levels in supernatants of lymphocyte cultures ($P = 0.04$; $r_s = 0.35$). However, IL-10 levels were so low that it was difficult to interpret the results.

DISCUSSION

The present study shows that the cytokine profile in patients with strongyloidiasis is characterized by a predominance of IL-5 in relation to IFN-γ and that high levels of antigen specific IgE antibodies against *S. stercoralis* are observed. Coinfection with HTLV-1 changes this immunological

Table 1 Correlations between IFN-γ, IL-5, IL-13 and specific IgE levels in patients infected with strongyloidiasis with or without HTLV-1 coinfection

Variables	*r*	*P*
IFN-γ and IgE	− 0·39	0·01
IFN-γ and IL-5	− 0·37	0·01
IFN-γ and IL-13	− 0·04	0·92
IL-5 and IL-13	0·75	< 0·0001
IL-5 and IgE	0·57	0·0001

response leading to a decrease of IL-5 and specific IgE antibodies against *S. stercoralis*.

The documentation that $CD4^+$ T cells are a heterogeneous population formed by Th1 and Th2 cells has contributed to our understanding of the modulation of the immune response and the pathogenesis of several diseases. CD4 Th1 cells secrete predominantly IL-2, IFN-γ and TNF-α, while Th2 cells produce mainly IL-4, IL-5 and IL-10 (27). A predominant Th1 type response suppresses Th2 cell differentiation (28) and Th2 cytokines such as IL-4 and IL-10 downregulate IFN-γ Th1 functions (29). We have previously documented that HTLV-1 infection decreases IL-4 synthesis and total IgE levels in patients with strongyloidiasis (10). In this study, we extend these observations showing that coinfection with HTLV-1 leads to a decrease in levels of IL-5 and specific IgE antibodies against *S. stercoralis*.

IL-10 is a cytokine produced predominantly by macrophages, B cells and CD4 Th2 cells (30,31). IL-10 has an important modulatory effect in the immune response, mainly suppressing macrophage function (32,33), lymphocyte proliferation (34) and IFN-γ synthesis (29). In comparison to IL-5 that was reduced in patients coinfected with HTLV-1 and *S. stercoralis*, there was a direct relationship between IFN-γ and IL-10. Because high IFN-γ levels decrease Th2 cell function, it is likely that, in patients coinfected with HTLV-1 and *S. stercoralis*, the source of IL-10 was not CD4 Th2 cells. In these cases, it is possible that the increased levels of IL-10 observed in coinfected patients may be a host attempt to modulate the high levels of IFN-γ production.

The majority of subjects infected with *S. stercoralis* have an asymptomatic or mild infection. When autoinfection occurs on a large scale, severe disease with parasitic dissemination is observed. Although the defence mechanism against *S. stercoralis* is not completely understood, based on histopathological findings in strongyloidiasis and on observations in other helminthic infections, cytokines, IgE, eosinophils and mast cells participate in helminthic expulsion and killing. IL-4 is the major cytokine that differentiates B cells to produce IgE, and both IL-4 and IL-13 increase the intestinal fluid content, a phenomenon that may contribute to parasite rejection (25,35). IL-5 is an important cytokine for differentiation, activation and proliferation of eosinophils (36,37), which are cells that are involved in the killing of helminths (37). Mast cell degranulation mediated by IgE and parasite antigens is also involved in the expulsion of parasites (38). A reduction in numbers of eosinophils has been observed in patients with disseminated strongyloidiasis (39) and decreased total IgE antibody levels have been observed in patients with severe strongyloidiasis associated with HTLV-1 infection (3,7,40). Additional recent evidence for HTLV-1 as an important factor for disseminated strongyloidiasis has been reported from Peru (9). Our data showing decreases of IL-5, IL-13 and specific IgE in patients coinfected with HTLV-1 and *S. stercoralis*, suggest that a decrease in the Th2 type immune response mediated by high levels of IFN-γ may be the immunological basis for the increased susceptibility of the coinfected patients to develop disseminated strongyloidiasis.

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ARTIGO 3

PORTO AF, SANTOS SB, ALCANTARA L, GUERREIRO JB, PASSOS J, GONZALEZ T, NEVA F, GONZALEZ D, HO JL, CARVALHO EM. **HTLV-1 modifies the clinical and immunological response to schistosomiasis.** *Clin.Exp.Immunol.*2004Aug;137(2):424-9.

Neste artigo foi avaliada a associação entre HTLV-1 e *S. mansoni* em relação à resposta imune, avaliação clínica e ultrassonográfica da esquistossomose e resposta terapêutica ao praziquantel. Foi observado que indivíduos infectados pelo HTLV-1 apresentaram uma maior prevalência de infecção pelo *S. mansoni* (8,4%) quando comparado com indivíduos sem infecção por este vírus (1,8%). Estes pacientes co-infectados apresentaram níveis de IgE especifica para antígeno de *S. mansoni* e níveis de IL-5 e IL-10 em sobrenadante de culturas de CMSP inferiores aos pacientes apenas com esquistossomose. Em contraste, os níveis de IFN-γ foram mais altos em pacientes co-infectados pelo HTLV-1 e *S. mansoni* que pacientes apenas com esquistossomose. A fibrose hepática foi leve em todos os pacientes co-infectados. Além disso, a eficácia do praziquantel foi inferior em pacientes com esquistossomose co-infectados pelo HTLV-1, que pacientes somente infectados pelo *S. mansoni.* Estes dados mostram que a infecção pelo HTLV-1 interfere na resposta imune ao *S. mansoni*, que a fibrose hepática foi leve em indivíduos com altos níveis de IFN-γ, sugerindo que outras citocinas estão relacionadas com o desenvolvimento de fibrose hepática e que, apesar dos pacientes apresentarem forma leve da esquistossomose, eles tiveram uma resposta terapêutica reduzida a drogas anti-esquistossomóticas quando co-infectados pelo HTLV-1 sugerindo que uma resposta imune adequada é importante na resposta terapêutica a estas drogas.

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HTLV-1 modifies the clinical and immunological response to schistosomiasis

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SUMMARY

The immunological response in HTLV-1 infected individuals is characterized by a prominent Type-1 cytokine response with high production of IFN-γ and TNF-α. In contrast, helminthic infections and in particular chronic schistosomiasis are associated with a predominant production of IL-4, IL-5, IL-10 and IL-13. Liver fibrosis is the main pathological finding in schistosomiasis that occurs after many years of infection. This pathology is T cell dependent but the immune response mechanisms are not completely understood. The North-east region of Brazil is endemic for both HTLV-1 and schistosomiasis. In the present study the immune response, clinical severity, and therapeutic response to praziquantel of patients with schistosomiasis coinfected with HTLV-1 were compared with patients infected only with *S. mansoni*. Patients with HTLV-1 and *S. mansoni* had lower levels of IL-5 ($P < 0{\cdot}05$) and higher levels of IFN-γ ($P < 0{\cdot}05$) in cultures stimulated with *S. mansoni* antigen and decreased *S. mansoni* antigen specific IgE levels when compared with patients with schistosomiasis without HTLV-1 coinfection. Liver fibrosis was mild in all HTLV-1 coinfected patients and efficacy of praziquantel was lower in patients dually infected than in patients infected only with *S. mansoni*.

Keywords HTLV-1 schistosomiasis liver fibrosis

INTRODUCTION

Human T cell leukaemia virus type-1 (HTLV-1) infects an estimated 20 million people worldwide [1]. Adult T cell leukaemia and lymphoma (ATLL) and HTLV-1 associated myelopathy (HAM/TSP) are the main diseases caused by HTLV-1, but other diseases such us Sjögren syndrome, polyarthritis, uveitis, infective dermatitis and lymphocytic alveolitis are documented clinical manifestation of HTLV-1 infection. The immunologic response in HTLV-1 infection is characterized by an exaggerated T cell response with high production of IFN-γ and TNF-α [2,3]. The predominant IFN-γ production and lymphocyte proliferation occur even in unstimulated cultures [2,4]. In contrast, helminthic infections and in particular chronic schistosomiasis are associated with a predominant type-2 immune response [5,6]. The main pathological finding in schistosomiasis is liver fibrosis, and late severe fibrosis occurs in about 6% of chronically *S. mansoni* infected patients [7]. The hepatic pathology of schistosomiasis is T cell dependent but the immunologic mechanisms mediating liver damage are not completely understood.

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The impact of HTLV-1 on helminthic infection has been reported in patients coinfected with *Strongyloides stercoralis* [8–10]. In such cases, coinfection with HTLV-1 decreases the predominant type-2 immune response observed in strongyloidiasis [11] as well as *S. stercoralis* specific and total IgE antibodies [12,13]. In addition, disseminated and recurrent strongyloidiasis are associated with HTLV-1 coinfection [14–16].

Salvador, the capital of the state of Bahia, located in the North-east of Brazil has the highest (1·35%) prevalence of HTLV-1 infection as reported in blood donors throughout Brazil [17]. The North-east region of Brazil is also endemic for schistosomiasis making it possible to evaluate the association of these two diseases. In the present study the immune response in patients with schistosomiasis coinfected with HTLV-1 was compared with that patients only infected with *S. mansoni*. Additionally, clinical and ultrassonography evaluation of liver fibrosis and therapeutic response to praziquantel were determined.

MATERIALS AND METHODS

Patients

Patients infected with HTLV-1 were recruited in the HTLV-1 multidisciplinary clinic, located in Hospital Universitário Prof Edgard Santos (HUPES) in Salvador, Bahia, Brazil. The clinic started in

2000 and follows more than 500 HTLV-1 infected individuals, most of them referrals from two blood banks in Salvador. All patients admitted in the clinic are asked to undergo 3 stool examinations. Of the 309 HTLV-1 individuals who had stool examination, *S. mansoni* eggs were found in 26 and 22 of them agreed to participate of this study (Group 1, schistosomiasis and HTLV-1 coinfected). These 22 patients had no clinical manifestations associated with HTLV-1 being considered HTLV-1 infected individuals. To determine the frequency of schistosomiasis, 331 HTLV-1-seronegative blood donors were screened by three stool examinations for schistosomiasis. To obtain patients with schistosomiasis who were HTLV-1-seronegative ($n = 44$, Group II) an existing cohort of patient ($N = 164$) in the *S. mansoni* endemic area of Caatinga do Moura, was used to select at a ratio of 2 to one by matching age and sex with Group I individuals (schistosomiasis and HTLV-1 coinfection). HTLV-1 positive and *S. mansoni* negative individuals (Group III) were selected from the HTLV-1 clinic matched by age and sex with Group II individuals (HTLV-1 and *S. mansoni* infection). Healthy University Hospital employees ($n = 19$) who were seronegative for HTLV-1 and absent helminthes by three stool examinations served as a control (group IV). The diagnosis of schistosomiasis was made by a positive fecal examination (Hoffman technique) and the Kato-Katz method was used to quantify the number of eggs per gram of stool [18]. After informed consent, a clinical history by standardized questionnaire was obtained and a complete physical examination and abdominal ultrasound were performed. The laboratory analysis included determination of cytokines (IFN-γ, IL-5, IL-10 and TNF-α) in supernatants of unstimulated cultures and in supernatant of soluble adult worm antigen preparation (SWAP) stimulated peripheral blood mononuclear cells (PBMC). After blood collection all patients with schistosomiasis were treated with praziquantel (50 mg/kg weight ($n = 18$) or oxamniquine 20 mg/kg weight ($n = 2$). The informed consent and experimental protocol were approved by the committee on human subjects of the Hospital Universitário Prof Edgard Santos, Salvador Bahia, Brazil that conforms to national guidelines.

Ultrassonography

Ultrassonography examination was performed with the Quantum 2000 Siemens ultrasound with a convex transductor of 3·5 Mhz, according to a previously published technique [19]. Grading of hepatic fibrosis was determined according with WHO criteria established in 1993 and previously revalidated [7]. Patients were classified in four different degrees according to the mean thickness of four portal tracts after the first division from the right and left branches of portal vein: degree 0: <3 mm thickness, degree I: 3–5 mm, degree II: 5–7 mm, degree III thickness: >7 mm.

Immunological studies

HTLV-1 serology. A commercial HTLV-1 ELISA test (Cambridge Biotech, Cambridge, MA, USA) was used and positive tests were confirmed by commercial Western blot (HTLV Blot 2·4, Genelabs, Singapore).

Cytokine determination. IFN-γ, IL-5, IL-10 and TNF-α in supernatants of mononuclear cells were measured by commercial ELISA. Briefly, peripheral blood monoclonal cells were obtained by density gradient centrifugation using lymphocyte separation media (LSM; Organon Teknika Coorporation, Durham, NC, USA) immediately the blood have been drawn. After washing in saline, the cells were adjusted to 3×10^6/ml in RPMI 1640 (Gibco, Grand Island, NY, USA) supplemented with 10% AB sera containing 100 U Penicillin G and 10 μg/ml of streptomycin. The cells were either unstimulated or stimulated with 2 μg/ml soluble adult Schistosoma mansoni worm antigen (SWAP). All cultures were incubated at 37°C in 5% CO_2 for 72 h. Supernatant fluids were collected and stored at −20°C. IFN-γ (Genzyme Corp., Cambridge, MA, USA), IL-5, IL-10 and TNF-α (PharMingen, San Diego, CA, USA) levels were measured by sandwich ELISA and the results were expressed as pg/ml using a standard curve generated using recombinant cytokines. Although the expression of cytokines *in vitro* may not necessarily reflects what occurs in vivo, this is not the case in the infection caused by HTLV-1 or *S. mansoni*. TNF-α, IL-1 and IFN-γ are increased in perivascular infiltrating cells of HAM/TSP patients [20] and high levels of IFN-γ in serum and in cerebrospinal fluid are observed in HAM/TSP patients [21]. Similarly, in schistosomiasis high expression of type-2 cytokines are found in the liver [22] and in peripheral blood cells [5]. Reported values represent the difference between the stimulated cultures minus unstimulated cultures. Since we found that IFN-γ levels in subjects infected with HTLV-1 were similar in unstimulated or stimulated cultures, the IFN-γ data presented were the values observed in unstimulated cultures.

IgE specific to S. mansoni *antigen.* Analysis of IgE specific to SWAP was performed by ELISA. Briefly, plates were coated with SWAP antigen at 40 μg/mi and left overnight at 4°C. In order to eliminate competition from IgG antibodies, immunoassay were performed with 100 μl of serum diluted 1 : 10 in phosphate-buffered saline with 0·05% Tween (PBS-T) containing 25% Sepharose–Protein G (Pharmacia, Uppsala, Sweden) following manufacturer's instructions. After incubation at room temperature for 15 min samples (tests and controls) were centrifuged at 650× g for 5 min. The IgG preabsorbed serum samples were used at final dilution of 1 : 4. The coated plates were blocked with PBS-5% bovine serum albumin and 100 μl of serum samples were incubated overnight to develop primary reactions. Secondary reactions were carried out with 100 μl of affinity-purified antibody phosphatase labelled goat anti-human IgE (Kirkegaard & Perry Laboratories, MD, USA). The ELISA was developed with 100 μl of p-nitrophenyl phosphate, and the absorbance changes (optical density [OD]) were measured by a spectrophotometer at 405 nm. The cut-offs of the immunoassay were determined using the mean plus 3 SD of the absorbance obtained with serum from 15 healthy unexposed individuals.

Treatment of S. mansoni *infection*

The treatment of schistosomiasis was performed with praziquantel (50 mg/kg weight) divided in two doses and given in the same day or oxamniquine (20 mg/kg weight) in a single dose. The use of one or another drug was dependent on their availability, since the medications were provided by the Healthy Secretary's office. Cured was defined as three negative stool examinations by the Hoffman technique 60 days after therapy [22,23], because patients continue to release viable *S. mansoni* eggs shortly after treatment. The possibility of re-infection was ruled out based on epidemiological and parasitological data. The patients were not living in endemic areas of *S. mansoni* at the time of the study and all of them denied exposure to contaminated water after therapy.

Statistical analysis

The Rank Sum Test was used to compare the means. Fisher's exact test was used to compare the proportions. The Chi-

Table 1. Clinical and ultrasound evaluation in *S. mansoni* and HTLV-1 Co-infected patients and patients only infected by *S. mansoni*

	Schistosomiasis groups		
Patient characteristics	HTLV-1 coinfected ($n = 22$)	HTLV-1-noninfected ($n = 44$)	*P*-value
Age (mean ± SD)	40 ± 10	32 ± 12	>0·05
Gender (male/female)	13/9	26/18	>0·05
Hepatomegaly	1/22 (4·5%)	11/44 (25%)	= 0·05
Splenomegaly	0/22 (0%)	2/44 (4·5%)	>0·05
Ultrasound degree I	21/22 (95·5%)	29/44 (66%)	<0·05
Ultrasound degree II	1/22 (4·5%)	15/44 (34%)	<0·05
Kato Katz (X)	24 eggs/g stool gram	399 eggs/g stool	<0·05

Grade III fibrosis was not observed. In this study only 8 of 66 patients evaluated had other parasites (*S. stercoralis*, *A. lumbricoides* and *T. trichiura*) that was distributed in each group. These patients were treated for other helminthes prior to the immunological evaluation.

square test was used to compare the prevalence of *S. mansoni* infection.

RESULTS

The frequency of *S. mansoni* infection was 4·7 fold higher (26/309, 8·4%) in individuals infected with HTLV-1 than a comparable group of HTLV-1-seronegative individuals (6/331, 1·8%) ($P < 0·05$). The clinical characteristics and the ultrasound findings of 22 schistosomiasis patients coinfected with HTLV-1 and schistosomiasis HTLV-1-seronegative controls are shown on Table 1. The control group had a slightly lower mean age than the HTLV-1 coinfected patients because the majority of individuals over 40 years of age living in the endemic area are free of schistosomiasis. With the exception of three schistosomiasis and HTLV-1 coinfected patients, the remaining patients were symptom-free. One coinfected patient complained of abdominal pain and diarrhea. A second coinfected patient had only abdominal pain and the third had only diarrhoea. None of the 22 patients had splenomegaly, an index of severe hepatic fibrosis. Only one case (4·5%) had mild hepatomegaly. In contrast, age- and sex-matched control schistosomiasis and HTLV-1-seronegatvie cases showed a significantly higher frequency of clinical parameters for hepatic fibrosis. Splenomegaly was observed in 4·5% and hepatomegaly in 25% of the HTLV-1-seronegative schistosomiasis controls from Caatinga do Moura. Ultrassonography studies were used to further quantify the observed clinical findings. An absence of or a mild degree of fibrosis was noted in 21 schistosomiasis patients coinfected with HTLV-1 studied by ultrasound (Table 1). In contrast, 34% of the HTLV-1-seronegative schistosomiasis control group had degree II evidence of liver fibrosis that was significantly different ($P < 0·05$) between the two groups. We also quantified current *Schistosoma* infection burden by measuring *Schistosoma* eggs in the stool. The number of eggs/gram of stool was significantly lower in HTLV-1 coinfected schistosomiasis patients than in HTLV-1 seronegative *Schistosoma* infected controls (Table 1).

We next evaluated whether HTLV-1 infection modified the immune response in patients with *S. mansoni* by measuring the cytokine profile. Participated as controls patients with schistosomiasis without HTLV-1, individuals only infected with HTLV-1 and healthy subjects without HTLV-1 and *S. mansoni*. The levels

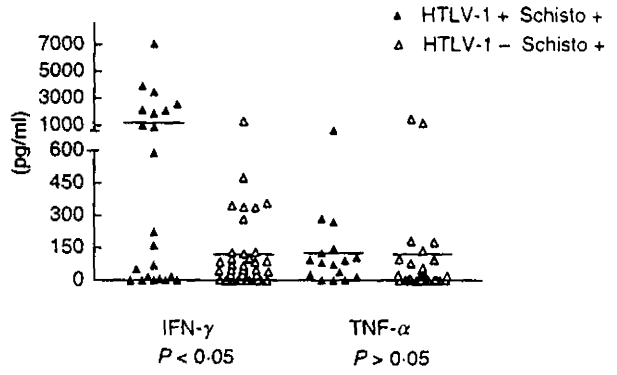

Fig. 1. IFN-γ and TNF-α production in PBMC stimulated with SWAP in *S. mansoni* and HTLV-1 coinfected patients (▲) and in chronic schistosomiasis patients (Δ) from an endemic area.

of IFN-γ, TNF-α determined in supernatants from adult SWAP stimulated lymphocytes cultures are shown in Fig. 1. The mean ± SD of IFN-γ levels in 22 patients coinfected with *S. mansoni* and HTLV-1 was 1182 ± 1785 pg/ml ranging from 0 to 7040 pg/ml. This was higher ($P < 0·05$) than the mean of IFN-γ of 40 patients only infected with *S. mansoni* (120 ± 229 pg/ml ranging from 0 to 1317 pg/ml). In supernatant of unstimulated cultures of subjects only infected with HTLV-1 without schistosomiasis, IFN-γ levels were 1540 ± 1628 pg/ml with variation of 0–6995 pg/ml (data not shown). The mean levels of TNF-α in supernatants of lymphocyte cultures of 16 patients with *S. mansoni* and HTLV-1 (127 ± 168 pg/ml) ranging from 0 to 667 were similar than that observed in 30 patients who were only infected with *S. mansoni* (122 ± 339 pg/ml).

Cytokines, IL-5, IL-10 and IL-4, are thought to reflect the Th2 immune response of schistosomiasis. We therefore, directly measured IL-5 and IL-10 production and indirectly evaluated IL-4 by assessing *S. mansoni* specific IgE production. The mean ± SD of IL-5 levels in 18 patients coinfected with *S. mansoni* and HTLV-1 was 260 ± 659 pg/ml with variation of 0–2716 pg/ml. This value was lower than ($P < 0·05$) that observed in 37 patients only infected with *S. mansoni* (907 ± 1289 pg/ml with ranging of 0–

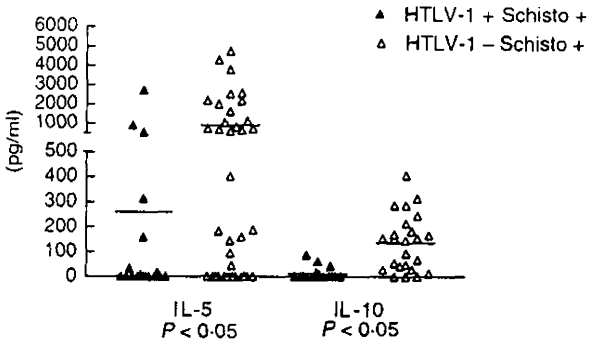

Fig. 2. IL-5 and IL-10 production in PBMC stimulated with SWAP in *S. mansoni* and HTLV-1 coinfected (▲) and in chronic schistosomiasis patients (Δ) from an endemic area.

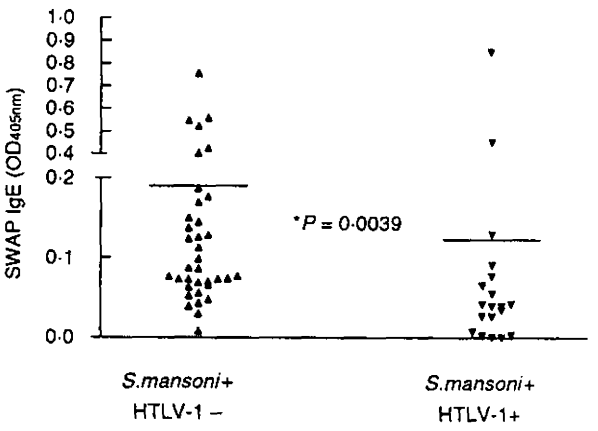

Fig. 3. Soluble (adult) worm antigen (SWAP)-specific IgE from patients with *S. mansoni* infection and *S. mansoni* coinfected with HTLV-1.

4747 pg/ml (Fig. 2). IL-5 levels in subjects without HTLV-1 and without *S. mansoni* infection were 2 ± 2 pg/ml (data not shown). Moreover the IL-10 levels in 18 patients coinfected with *S. mansoni* and HTLV-1 (12 ± 25 pg/ml with variation of 0–88 pg/ml) was lower ($P < 0·05$) than that observed in 24 patients only infected with *S. mansoni* (136 ± 114 pg/ml with variation of 0–407 pg/ml).

Considering that the degree of *S. mansoni* infection is associated with liver fibrosis and that egg load may alter the immunological response, the frequency of liver fibrosis determined by ultrasound and the cytokine levels in the HTLV-1 *S mansoni* coinfected patients were compared with that observed in a subgroup of patients who had only schistosomiasis and had low degree of *S. mansoni* egg excretion. In 17 of the 44 patients with only schistosomiasis the degree of egg was lower than 200 eggs/g/stool with mean of 57 ± 59 eggs/g/stool. The frequency of grade II of liver fibrosis in these patients was 50% compared to 4·5% in patients coinfected with HTLV-1 and *S. mansoni*. Furthermore levels of IFN-γ (1182 ± 1785 pg/ml) was significantly higher and of IL-5 (260 ± 659 pg/ml) significantly lower in the patients coinfected with HTLV-1 and *S. mansoni* than in the 17 patients with only schistosomiasis and similar egg load.

The distribution of IgE, expressed in OD in patients with schistosomiasis without HTLV-1 infection, and in those coinfected with HTLV-1 is shown on Fig. 3. The mean IgE in patients without HTLV-1 infection was 0·190 ± 0·170 compared to 0·123 ± 0·191 in patients with schistosomiasis associated with HTLV-1 infection ($P < 0·05$).

Twenty-two HTLV-1 and *S. mansoni* coinfected patients were treated (20 with praziquantel and 2 with oxamniquine) but repeated stool results were available in only 20 cases. Therapeutic failure, defined as the presence of *S. mansoni* eggs two months after therapy was documented in 4 of 20 (20%) HTLV-1 and *S. mansoni* coinfected patients; all were treated with praziquantel. The 20% of therapeutic failure in HTLV-1 and *S. mansoni* coinfected patients was significantly higher than the 2·3% rate (1 of 44) observed in HTLV-1-seronegative *S. mansoni*-infected controls ($P < 0·05$) (Table 2). Two patients with HTLV-1 and *S. mansoni* coinfection required three courses of treatment before becoming egg-free (two with praziquantel and once with oxamniquine). A third patient remained *S. mansoni*-infected, despite two courses of therapy with praziquantel and oxamniquine. The fourth patient has persistence of eggs of *S. mansoni* in the stool despite 8 courses of treatment with both drugs. Neither patient had re-exposure to contaminated water.

Table 2. Reduced therapeutic response to anti-schistosomal drugs in *S. mansoni* and HTLV-1 co-infected patients

S. mansoni infected groups	Numbers cured (%)
HTLV-1-noninfected	43/44 (98%)*
HTLV-1 coinfected	16/20 (80%)*

*$P < 0·05$, Fisher's exact test.

DISCUSSION

Although HTLV-1 and schistosomiasis occurs in areas where both diseases are endemic, no previous study has evaluated the impact of HTLV-1 on schistosomiasis. Here, we showed that the prevalence of *S. mansoni* is higher in HTLV-1 infected subjects than in HTLV-1-seronegative controls. Importantly, HTLV-1 modified the clinical outcome of schistosomiasis and altered the immune response to parasitic antigens. Additionally, HTLV-1 coinfection reduced the efficacy of antischistosomal drugs and at least one patient failed to eradicate *S. mansoni* infection after 8 courses of treatment.

Schistosomiasis is one of most important helminthic diseases, affecting two hundred millions of individuals mainly in Africa, Asia and South America. The majority of patients with schistosomiasis have an intestinal or hepatointestinal form of the disease characterized by diarrhea and/or constipation and abdominal pain. Liver fibrosis, the main pathology is observed in 6% of patients with long-standing chronic schistosomiasis [7]. In this severe form of the disease, patients develop hepatosplenomegaly, portal hypertension and died as a result of uncontrollable gastrointestinal bleeding. Several factors have been associated to liver fibrosis including the genetic background, the degree of infestation and host immunological response. Initial studies in experimental model of schistosomiasis suggested that type-1 cytokines were associated with granuloma formation [24].

However, more recent accumulated data point to the importance of IL-4 and IL-13 in inducing fibrosis [25–27] and the ability of IL-12 and IFN-γ to decrease the fibrosis in experimental model of schistosomiasis [28,29]. The changes in the immune response to *S. mansoni* antigen observed in patients coinfected with HTLV-1 may have modified the clinical manifestation of schistosomiasis in these patients. In such case it is possible that the high IFN-γ production by itself or by decreasing the Th2 type of immune response is preventing the development of fibrosis, and would be the clinical correlate to the experimental findings [28,29].

Patients coinfected with HTLV-1 and schistosomiasis did show a lower *S. mansoni* eggs burden in the stool. We cannot exclude a low infection/exposure rate as a contribution to lessen clinical disease. However, in such clinical study where the exposure/infection burden could not be accurately determined, it can also be argued that the lower egg burden may also be directly due to HTLV-1 infection. In such case the high Th1 response could facilitate a Th1-associated antischistosome response and contribute for parasite killing and reduced egg excretion. The low levels of *S. mansoni* egg excretion observed in our patients may also be due the fact that they live in urban areas were re-exposure of *S. mansoni* infection is uncommon. Alternatively, excretion of fewer eggs may be due to a decrease in *S. mansoni* type-2 cytokine production, since egg excretion is directly correlated with type-2 immune response [30]. This supposition can be tested in future studies by evaluating whether immunological perturbation by HTLV-1 interferes with the passage of eggs from the portal system to the intestinal lumen. Anyway the low egg excretion observed in the HTLV-1 coinfected patients does not seem to be the reason for the differences in the immunological and ultrasound findings observed between the two groups. When the ultrasound and immunological findings of the HTLV-1 *S mansoni* coinfected patients were compared with a subgroup of patients having only schistosomiasis but with similar degree of infection than those dually infected with *S. mansoni* and HTLV-1, this last group had significantly lower liver fibrosis, increasing levels of IFN-γ and decreasing in IL-5 than patients with only schistosomiasis.

Immunological response in patients with chronic schistosomiasis is characterized by decreased IFN-γ production and enhancement in IL-4, IL-5 and IL-10 levels. This predominant type-2 immune response is independent of the degree of infection measured by eggs/stool gram and occurs in all clinical forms of schistosomiasis [5,31]. It is induced by parasite antigen and has been related to host defense mechanism against *S. mansoni* [23,32,33]. HTLV-1 infection is associated with an exaggerated T cell response with spontaneous lymphocyte proliferation and production of high levels of IFN-γ and TNF-α [2,4]. We have previously shown that HTLV-1 decreases antigen specific type-2 immune response in patients with strongyloidiasis [12,13] another important helminthic infection in tropical countries. In such case HTLV-1 down regulate defense mechanisms against *S. stercoralis* leading to the development of disseminated and recurrent *S. stercoralis* infection [14–16,34,35]. In this study we demonstrated that IFN-γ secretion was up-regulated in patients with schistosomiasis and HTLV-1. In contrast IL-5, a typical Th2 cytokine highly secreted in schistosomiasis was down regulated in patients coinfected with HTLV-1 and *S. mansoni*. Since there was a tendency for an inverse correlation between IFN-γ and IL-5 levels, it is possible that the down regulation of IL-5 was related to the enhancement of IFN-γ observed during HTLV-1 infection. IgE is elevated in parasitic infection and *S. mansoni* parasite specific IgE [36] and high IgE:IgG4 ratio have been associated with resistance to reinfection with *S. mansoni* [37]. Herein, we showed that infection with HTLV-1 significantly reduces the levels of parasite-specific IgE for schistosomiasis in patients infected with this parasite.

Oxamniquine and praziquantel are the standard treatment for schistosomiasis and there is no report of resistance to these drugs in Brazil. In this study, we observed a high rate of therapeutic failure to these drugs. A high rate of therapeutic failure against another helminthes has also been observed in patients coinfected with HTLV-1 and *S. stercoralis* [38,39]. Although the mechanism of therapeutic failure observed in strongyloidiasis and herein in schistosomiasis is not clear, it is possible that the cure of this helminthic infection is also dependent of the presence of an appropriate immune response present.

HTLV-1 infection induces activation of T cells with a predominant and exaggerated production of IFN-γ. This study showed that HTLV-1 and *S. mansoni* coinfection changes the immunological response to *S. mansoni*, reduced clinical disease and fibrosis resulting from schistosomiasis and lowered the therapeutic response to antischistosomal drugs.

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ARTIGO 4

A.F. PORTO, S.B. SANTOS, A.L. MUNIZ, V.BASÍLIO, W. RODRIGUES JR, F.A. NEVA, W.O. DUTRA, K. GOLLOB, S. JACOBSON, E.M. CARVALHO. **Helminthic Infections down Modulate Type 1 Immune Responses in HTLV-1 Patients and are more Prevalent Among HTLV-1 Carriers than Patients with HTLV-1-Associated Myelopathy / Tropical Spastic Paraparesis,** submetido e aceito pelo *J. Infect. Dis.*

A infecção pelo HTLV-1 é associada com proliferação espontânea e ativação de células T e uma exacerbada resposta Th1, enquanto que a infecção por helmintos está associada com uma resposta Th2 e altos níveis de IL-4, IL-5 e IL-10. Neste artigo a produção de citocinas, marcadores de linfócitos e carga proviral foram avaliadas em pacientes co-infectados pelo HTLV-1 e helmintos (*S. stercoralis* e/ou *S. mansoni)* e em pacientes somente infectados pelo HTLV-1. Além disso, foi determinada a freqüência de infecção por helmintos em portadores assintomáticos do HTLV-1 e em pacientes com mielopatia. A produção de IFN-γ em sobrenadante de CMSP foi mais elevada nos indivíduos apenas infectados pelo HTLV-1 quando comparada com indivíduos co-infectados por helmintos. Adcionalmente, a freqüência de células T CD8+ e CD4+ expressando IFN-γ e a carga proviral foram inferiores em pacientes dualmente infectados quando comparadas com pacientes unicamente infectados pelo HTLV-1. Em contraste, a percentagem de células T expressando IL-5 e IL-10 em pacientes co-infectados por helmintos foi mais alta. Além disso, nós observamos que portadores do HTLV-1 tiveram uma freqüência de infecção por helmintos 7 vezes mais do que pacientes com HAM/TSP. Estes dados sugerem que a infecção por helmintos pode modular a ativação de células T tipo Th1 em indivíduos infectados pelo HTLV-1 e está inversamente associada com o desenvolvimento de mielopatia.

Running title: Helminthes regulate immune response in HTLV-1.

Helminthic Infections down Modulate Type 1 Immune Responses in HTLV-1 Patients and are more Prevalent Among HTLV-1 Carriers than Patients with HTLV-1-Associated Myelopathy / Tropical Spastic Paraparesis

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Key words: Human T cell lymphotropic virus type 1, (HTLV-1) and strongyloidiasis, HTLV-1 and schistosomiasis, HTLV-1 and helminthic infection, HTLV-1-associated myelopathy / tropical spastic paraparesis (HAM/TSP).

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Footnote page

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The Ethical Committee of the Hospital Universitário Prof. Edgard Santos approved this study and informed consent was obtained from all prospectively enrolled patients.

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Abstract

HTLV-1 infection is associated with an exacerbated type 1 immune response and secretion of high levels of pro-inflammatory cytokines. In contrast, helminthic infections induce a type 2 immune response. In this study, a comparison of the cytokine profile in HTLV-1 patients that were co-infected with parasitic helminths (*S. stercoralis* and/or *S. mansoni*) was made with persons who were infected with HTLV-1 alone. IFN-γ levels were higher ($p<.05$) in HTLV-1 carriers without helminthic infection than in co-infected patients. The overall frequency of IFN-γ expressing CD8+ and IFN-γ expressing CD4+ cells was decreased in patients dually infected ($p<.05$). The percentage of IL-5 and IL-10 expressing T cells in subjects co-infected was higher than in individuals only infected with HTLV-1 ($p<.05$). Moreover we found that the prevalence of helminthic infection was 7-fold higher ($p<.05$) among HTLV-1 carriers than in patients with HTLV-1-associated myelopathy / tropical spastic paraparesis. These data show that helminths decrease Th1 cell activation, which may influence clinical outcome of HTLV-1 infection.

Introduction

Human T cell lymphotropic virus type 1 (HTLV-1) infection is associated with spontaneous T cell activation, uncontrolled lymphocyte proliferation, and an exacerbated type 1 immune response with secretion of high levels of pro-inflammatory cytokines [1-3] The great majority of individuals infected with HTLV-1 display an asymptomatic form of the infection and are referred to as HTLV-1 carriers. The HTLV-1-associated myelopathy / tropical spastic paraparesis (HAM/TSP) and adult T cell leukemia / lymphoma (ATLL) are the main clinical manifestations associated with HTLV-1 infection. HAM/TSP is characterized by hyperreflexia, muscle weakness and spasticity in the lower extremities. Evidence that the immunological response participates in the pathogenesis of HAM/TSP includes: 1. cytotoxic activity against viral Tax protein in HAM/TSP patients [4,5]; 2. an increase in pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 is observed in the cerebral spinal fluid (CSF) of patients with HAM/TSP [6-8]; and 3. spinal cord lesions are associated with CD4+ and CD8+ T cell infiltration, presence of macrophages, proliferation of astrocytes, and fibrillary gliosis [9]. Although more prominent in HAM/TSP, HTLV-I carriers also have a high production of pro-inflammatory cytokines such as IFN-γ and TNF-α [2].

In contrast to HTLV-1, helminthic infections are associated with a type 2 immune response and high levels of IL-4, IL-5, and IL-10 and low IFN-γ levels [10,11]. It has been shown that as a regulatory mechanism of the immune response, cytokines secreted by Th2 cells may down regulate the Th1 type of immune response and vice-versa. For instance IL-4 and IL-10 may down regulate IFN-γ response [12] and IFN-γ decreases the secretion of Th2 cytokines [13]. We and others have previously shown a high frequency of

strongyloidiasis [14-17] and increased susceptibility to develop disseminated *S. stercoralis* infection in HTLV-I carriers [18-20]. It is also well known that helminthic infection and, in particular, schistosomiasis down regulate type 1 immune responses and decrease the severity of autoimmune disease in experimental animals [21,22]. To evaluate if helminthic infection may influence the immunologic response in individuals infected with HTLV-I, the cytokine profile, and proviral load were determined in HTLV-1 carriers co-infected with helminths (*S. stercoralis* and/or *S. mansoni)* and in patients only infected with HTLV-1. Additionally, the prevalence of helminthic infection in patients with HAM/TSP and in HTLV-1 carriers was compared.

Materials and Methods

1. Patients

Participants of the present study included 310 HTLV-1 carriers and 32 patients with HAM/TSP from the HTLV-1 multidisciplinary clinic located at Hospital Universitário Prof. Edgard Santos (HUPES) in Salvador, Bahia, Brazil. A clinical history was taken and physical examination performed on all patients. All patients had confirmation of HTLV-1 infection by Western blot and had 3 stool examinations (Hoffman and Baermann techniques). Twenty-five per cent of the patients infected with *S. stercoralis* had complained of diarrhea. Patients with schistosomiasis were asymptomatic and had less than 25 eggs per gr of stool. Immunological evaluation was performed in 35 HTLV-1 carriers co-infected with helminths (*S. stercoralis* and for *S. mansoni*) and in a control group of 35 HTLV-1 carriers matched by age and sex but without evidence of helminthic infection. Immunological studies were also performed in 18 patients with HAM/TSP including the

one with HAM/TSP and helminthic infection. Immunological evaluation consisted of determination of cytokines (IFN-γ, IL-5) in supernatant of unstimulated PBMC cultures by enzyme-linked immunoabsorbent assay (ELISA) and measurement of intracellular cytokines (IFN-γ, IL-10, IL-5), and phenotypic immunological markers by flow cytometry. Moreover, proviral load was determined. The mean age of HTLV-1 carriers co-infected with helminthes and individuals only infected with HTLV-1 were 45 ± 17 years and 46 ± 12 years respectively, and the male/female ratio was 6:1 and 5:1, respectively. This was the naturally occurring bias found in the sample population. The criterion for a diagnosis of strongyloidiasis and schistosomiasis was a positive stool examination by identification of *S. stercoralis* larvae (Baermann technique) or *S. mansoni* eggs (Hoffman technique). Thirteen patients had only *S. stercoralis*, 15 subjects had *S. mansoni*, and 7 individuals had *S. stercoralis* and *S. mansoni* in the stool examination. After blood collection all patients infected with *S. stercoralis* were treated with cambendazol (5 mg/kg weight) and those infected with *S. mansoni* were treated with praziquantel (50mg/kg weight divided in two doses) or oxaminiquine (20mg/kg weight in a single dose).

For the evaluation of the prevalence of helminthic infection, participants in the study consisted of all 342 individuals of the HTLV-I clinic. These patients had been evaluated by 2 neurologists and were divided into two groups according to the Osames' Motor Disability Score (OMDS) [23] and Expanded Disability Status Scale (EDSS) [24]: HAM/TSP patients and HTLV-I carriers who did not fulfill the WHO criteria for HAM/TSP.

Informed consent was obtained for all participants and human experimentation guidelines of the Hospital Universitário Prof. Edgard Santos were followed in the conduct of this clinical research.

2. Immunological studies

Cytokine determination

Cytokine levels (IFN-γ, IL-5) in supernatants of unstimulated mononuclear cells were measured by ELISA. Briefly, peripheral blood mononuclear cells were obtained by density gradient centrifugation using lymphocyte separation media (LSM; Organon Teknika Coorporation, Durham, NC, USA). After washing in saline, the cells were adjusted to 3 x 10^6/ml in RPMI 1640 (Gibco, Grand Island, NY, USA) supplemented with 10% AB+ sera containing 100 U Penicillin/G and 10μg/ml of streptomycin. All cultures were incubated without stimulus at 37°C in 5% CO_2 for 72 hours. Supernatant fluids were collected and stored at -20°C. IFN-γ (Genzyme Corp., Cambridge, MA, USA) and IL-5 (PharMingen, San Diego, CA, USA) levels were measured by ELISA sandwich technique and the results were expressed in pg/ml based on a standard curve generated using recombinant cytokines.

Single cell cytoplasmic cytokine staining

Briefly, 2 x 10^5 PBMC were cultured in RPMI 1640 plus 5% AB Rh+ serum in 96 well plates. Based on preliminary results all the cytokine staining was performed after 20 hours of incubation with and without αCD3/CD28 stimulus. During the last 4 hours of culture, Brefeldin-A (1 μg/mL) was added to the culture. The cells were then washed and centrifuged using ice-cold PBS plus sodium-azide, stained for surface markers and fixed using 2%

formaldehyde. The fixed cells were then permeablized with a solution of Saponin and stained, for 30 minutes at 4°C, using anti-cytokine mAbs directly conjugated with PE (IFN-γ, IL-5 and IL-10) (Pharmingen). Preparations were then washed, fixed and analyzed using a FACScalibor. In all cases the cells were double stained for cytokine and for cell surface markers. In all cases, 30,000 gated events were acquired for later analysis due to the low frequency of positive events being analyzed.

3. Proviral load

Subjects and cells

The sample consisted of 12 HTLV-1 carriers without helminthic co-infection and 17 HTLV-1 carriers with helminthic co-infection who had available frozen PBMC cells, pro-viral load was performed only in a sub-group of patients because other immunological tests were performed with the same patients not leaving cells left for this kind of study.

Real-time PCR of DNA

HTLV-I proviral DNA load in the PBMC was measured using an ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA) as previously described [25] DNA was extracted from $1x10^6$ cells using Puregene DNA Isolation Kit (Gentra, Minneapolis, MN) according to manufacturer's instruction and 100 ng of sample DNA solution per well was analyzed. The HTLV-I proviral DNA load was calculated by the following formula: copy number of HTLV-I (*pX*) per 100 cells = (copy number of *pX*) / (copy number of β-actin / 2) x 100.

4. Serology for HTLV-1

All sera were screened for HTLV-1/2 antibodies by ELISA (Cambridge Biotech Corp., Worcester, MA, U.S.A.). Repeatedly reactive samples were subjected to Western blot analysis to distinguish between HTLV-1 and HTLV-2 using HTLV blot 2.4, Genelabs, Singapore according to the manufacturers instructions.

5. Statistical Analysis

The Wilcoxon Rank Sum Test was used to compare the means. Fisher's exact test was used to compare the proportions. The Chi-square test was used to compare the prevalence of helminthic infection.

Results

The levels of IFN-γ in supernatants of lymphocyte cultures from HTLV-1 carriers with helminthic infection and without helminthic infection are shown on figure 1. The IFN-γ levels (1,465 ± 1,648 pg/ml) were higher (P<.05) in 35 HTLV-1 patients without helminthic infection than that observed in 35 patients with helminthic infection (913 ± 1163 pg/ml). Both *S. mansoni* and *S. stercoralis* contribute to the decreasing in IFN-γ levels but the down modulation of IFN-γ was mainly observed in patients who had HTLV-1 and *S. mansoni* (474 ± 838 pg/ml). Although the IL-5 levels did not differ in the two groups there was a tendency for higher IL-5 levels in patients with helminthic infection (199 ± 476 pg/ml) compared to patients with HTLV-1 without helminthic infection (132 ± 258 pg/ml, P>.05, data not shown). As we have previously observed [2], IFN-γ levels in HTLV-1 carriers were quite variable and individuals could be divided into low IFN-γ producers (levels < 400 pg/ml and ranging from 0 to 370 pg/ml) and high IFN-γ producers (levels > 400 pg/ml and ranging from 430 to 6,995 pg/ml). While the frequency of low IFN-γ

producers was 52% in the group only infected with HTLV-1, it was 80% ($P<.05$) in individuals co-infected with HTLV-1 and helminths (Table 1). The mean of IFN-γ production in patients with HAM/TSP was 4246 ± 2924 pg/ml.

Due to the fact that only one patient with HAM/TSP had helminthic infection, no comparison could be performed between HAM/TSP patients with and without helminthic infection.

The frequency of cytokine producing cells in HTLV-1 carriers or helminth co-infected HTLV-1 carriers after stimulation with αCD3/CD28 was determined by FACS analysis. In Figure 2 it is shown that while the frequency of the total number of cells secreting IFN-γ was 2.33% in three HTLV-1 carriers without helminths, in three HTLV-1 carriers co-infected with helminths only 0.70% of cells were secreting IFN-γ ($p<.05$). In contrast, the frequency of cells secreting IL-5 was two fold higher ($p<.05$) in HTLV-1 carriers with helminthic infection (0.58%) compared to HTLV-1 carriers not infected with helminths (0.24%). Most of the IFN-γ was secreted by CD4+ T cells, and the frequency of CD4+ IFN-γ+ T cells (1.18%) was higher ($p<.05$) in HTLV-1 carriers without helminthic infection than in individuals co-infected with helminths (0.22%) (Figure 2).

Previously we have shown that most of the IFN-γ producing cells in HTLV-1 carriers were CD4+ T cells, although both CD4+ and CD8+ T cells are responsible for the high levels of IFN-γ observed in the HTLV-1 infected individuals [26]. Figure 3 shows the frequency of CD8+ T cells secreting IFN-γ or IL-10 and the total frequency of cells secreting IL-10 in unstimulated cultures. Co-infection of HTLV-1 with helminths significantly decreases the frequency of CD8 T cells secreting IFN-γ ($p<.05$). In contrast

the total frequency of cells secreting IL-10 and the frequency of CD8 T cells secreting IL-10 was higher ($p<.05$) in four HTLV-1 individuals co-infected with helminths (0.58%) in comparison with those (n=7) only infected with HTLV-1 (0.21%). Moreover, the frequency of total CD8 T cells was higher in individuals co-infected with HTLV-1 and helminths (data not shown).

Figure 4 shows the pro-viral load in a subset of individuals from the two groups of patients from whom mononuclear cells were frozen. Although the number of copies were quite variable in both groups, the proviral load was significantly lower in 17 HTLV-1 co-infected with helminths (2.2 ± 1.5 copies/100 cells) than in 12 individuals only infected with HTLV-1 (3.7± 1.2 copies/100 cells) ($p<.05$).

The frequency of infection with intestinal helminths *S. stercoralis* and *S. mansoni* in patients infected with HTLV-1 is higher than that observed in seronegative individuals [27]. Comparing the prevalence of these helminths in HTLV-1 carriers versus patients with HAM/TSP we found that HTLV-1 carriers had 7 fold higher prevalence of infection with intestinal helminths than patients with HAM/TSP (Table 2).

Discussion

This study shows that helminthic infections decrease IFN-γ production in HTLV-I infected individuals and the overall frequency of IFN-γ expressing CD8+ and IFN-γ expressing CD4+ cells. In contrast, the percentage of IL-10 expressing cells in subjects co-infected with helminthes was higher than that observed in individuals only infected with

HTLV-1. Moreover, the prevalence of helminthic infection was significantly lower in patients with HAM/TSP than in HTLV-1 carriers.

Co-infection of HTLV-1 with helminths has clinical and immunological implications. It is known that the prevalence of strongyloidiasis and schistosomiasis is higher in HTLV-1 infected individuals than in seronegative controls [14-17, 27] and co-infection of HTLV-1 with *S. stercoralis* is associated with parasite dissemination and development of severe forms of strongyloidiasis [18-20]. We have previously shown that HTLV-1 decreases the Th2 type of immune response in patients with strongyloidiasis and schistosomiasis [27-29]. Herein we show that helminthic infection can down modulate the exaggerated inflammatory response observed in HTLV-1 infected individuals. Additionally, co-infection of HTLV-1 with helminths was associated with a decreased proviral load and with a decreased frequency of myelopathy.

HTLV-1 infects predominantly T cells leading to spontaneous lymphocyte proliferation and increased cytokine secretion. Although both type 1 and type 2 cytokines are increased in unstimulated lymphocyte cultures of HTLV-1 infected individuals compared to controls, the most striking finding in this regard is the high IFN-γ levels secreted by both CD4 and CD8 T cells [26]. Considering that helminthic infections are associated with increasing levels of IL-4, IL-5 and IL-10 [10-11] the immunological consequences of the association of HTLV-1 with helminthic infection was evaluated. The documentation that IFN-γ levels and the number of CD4+ and CD8+ positive T cells was decreased in HTLV-1 carriers co-infected with helminths indicate that helminthic infection may down regulate IFN-γ production in HTLV-1 carriers.

We have previously shown that exogenous IL-10 can decrease IFN-γ production in lymphocyte cultures of HTLV-1 carriers [26]. The documentation that patients co-infected with HTLV-1 and helminths have an increased frequency of cells secreting IL-10 than individuals only infected with HTLV-1 indicates that helminths may down regulate IFN-γ production in HTLV-1 through the induction of IL-10.

Although little is known about defense mechanisms against HTLV-1, killing of infected T cells by CD8 T cells participates in this phenomenon [5]. Considering that helminthic infections down regulate the type 1 immune response, it would be plausible that helminthic infection increases the HTLV-1 proviral load. In fact a previous study [30] showed that co-infection with *S. stercoralis* increases HTLV-1 proviral load. Herein we show that proviral load in HTLV-1 carriers co-infected with helminths is lower than that observed in patients only infected with HTLV-1, suggesting that helminths may inhibit HTLV-1 transcription. As the spread of the virus is accelerated by activation of T cells [31], it is possible that low proviral load in patients co-infected with HTLV-1 and helminths may be due to the down regulation of the immune system observed in these patients. Interestingly, a study [32] of patients with T cell Non-Hodgkin's lymphoma and ATLL patients showed that when the patients were infected with *S. stercoralis* there was a better response to treatment and longer survival rate than uninfected patients

HAM/TSP is one of the most important consequences of HTLV-1 and is characterized by weakness, hyperreflexia, urinary manifestations, and spastic paraparesis Although the pathogenesis of HAM/TSP is not completely understood the participation of the abnormal immune response observed in HTLV-1 in inducing tissue damage have been suggested by several studies: 1) Infiltration of the spinal cord by T lymphocytes with an

increasing number of CD8+ T cells expressing *tax* [9]; 2) Increasing levels of pro-inflammatory cytokines in lymphocyte cultures and CSF [6-8]; 3) Occurrence of fibrosis of the neurological tissue associated with inflammation [9]. Previously, we have shown that the prevalence of *S. stercoralis* and *S. mansoni* was higher in HTLV-1 carriers than in HTLV-1 seronegative blood donors [17,27]. In this study we found that the frequencies of *S. mansoni* and *S. stercoralis* infections were much lower in patients with HAM/TSP than in HTLV-1 carriers. Although it can be argued that HAM/TSP patients are potentially less exposed to *S. stercoralis* and *S. mansoni* due to their physical limitations, the group of co-infected individuals reported here had no recent exposure to these helminths. In fact, all of the HTLV-1 infected individuals in this study now live in urban areas, where *S. mansoni* transmission is not documented and contamination of the adult population with *S. stercoralis* is less likely. These observations, together with the data that HTLV-1 increases the failure rate of anti-helminthic drugs [33,34], suggest that most of the HTLV-1 individuals infected with *S. stercoralis* and *S. mansoni* acquired the helminthic infection during childhood. In such case the low frequency of helminthic infection in HAM/TSP may suggest that helminthes, by decreasing the IFN-γ production and proviral load, may protect HTLV-1 carriers from developing myelopathy. Interestingly, the majority of the studies of co-infection of HTLV-1 and *S.stercoralis* in Japan is performed in Okinawa and there is no data in the literature about prevalence of HAM/TSP in this area of Japan [34].

This study clearly demonstrates that HTLV-1 infected individuals co-infected with helminthes display an immune phenotype consistent with a suppression of the type 1 response, resulting in a decreased viral load. These findings, together with the findings of a lower prevalence of helminthic infections in the more severe HAM/TSP cases, aid in the

understanding of the events that lead to the development of this more severe clinical outcome of HTLV-1 infection. Lastly, they highlight an important interaction within the infected host between viral and parasitic pathogens.

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Legends

Figure 1. IFN-γ levels of HTLV-1 infected patients co-infected or not by helminths (*S. stercoralis* and *S. mansoni).* The levels of IFN-γ were determined in unstimulated 72 hours culture supernatants of PBMC. The horizontal lines represent the means of the populations.

Figure 2. HTLV-1 carriers co-infected with helminths have a lower frequency of CD4 + IFN-γ + cells and higher frequency of IL-5 secreting cells than individuals only infected with HTLV-1. The columns and error bars represent the mean +/- standard deviation, respectively. n=3 in each group.

Figure 3. HTLV-1 carriers co-infected with helminths display a lower frequency of CD8+ T cells secreting IFN-γ and higher number of cells secreting IL-10 than individuals only infected with HTLV-1. The columns and error bars represent the mean +/- standard deviation, respectively. n=4 co-infected and n=7 HTLV-1 alone.

Figure 4. Proviral load of HTLV-1infected patients co-infected or not with helminths (S. *stercoralis* and S. *mansoni).* The horizontal lines represent the means of the populations.

Figure 1

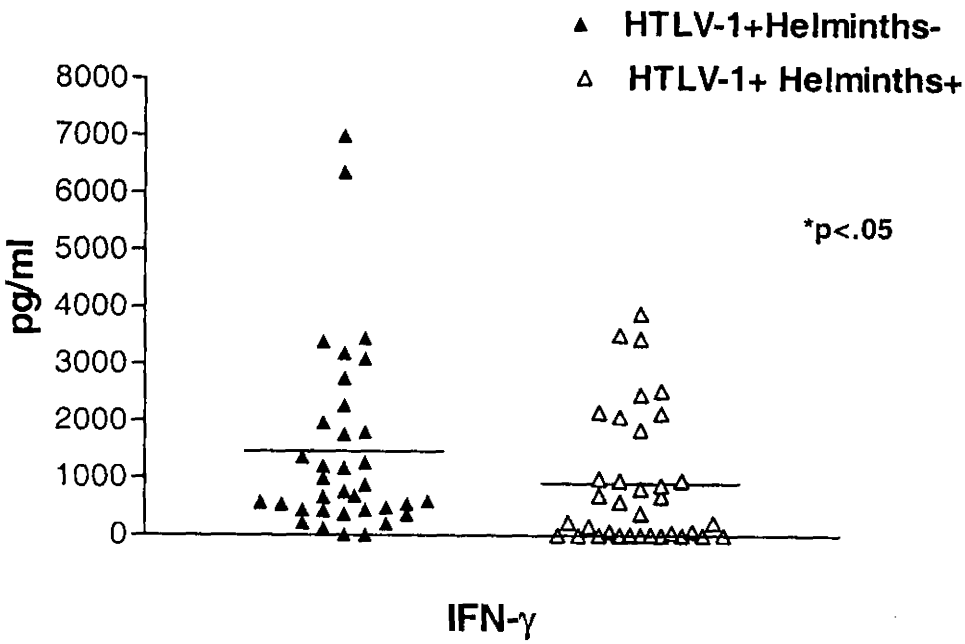
HTLV-1+Helminths-
HTLV-1+ Helminths+
*p<.05
8000
7000
6000
5000
4000
3000
2000
1000
0
pg/ml
IFN-γ

Figure 2

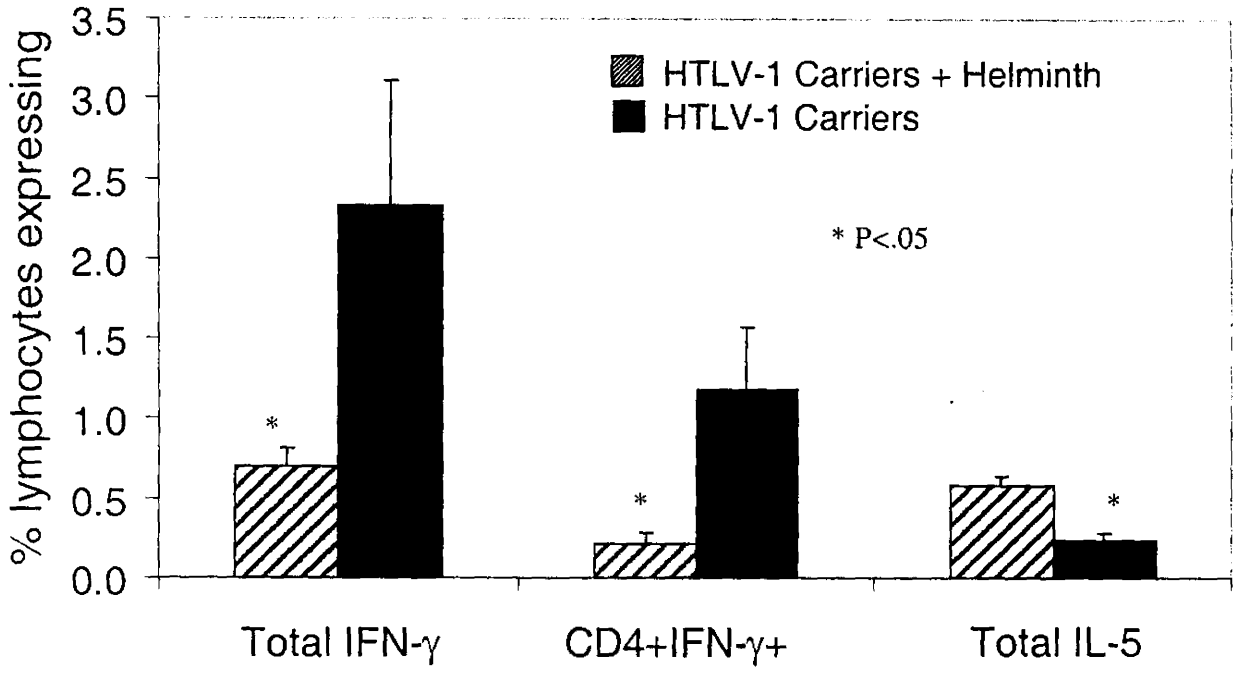
% lymphocytes expressing
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
HTLV-1 Carriers + Helminth
HTLV-1 Carriers
* P<.05
*
*
*
Total IFN-γ
CD4+IFN-γ+
Total IL-5

Figure 3

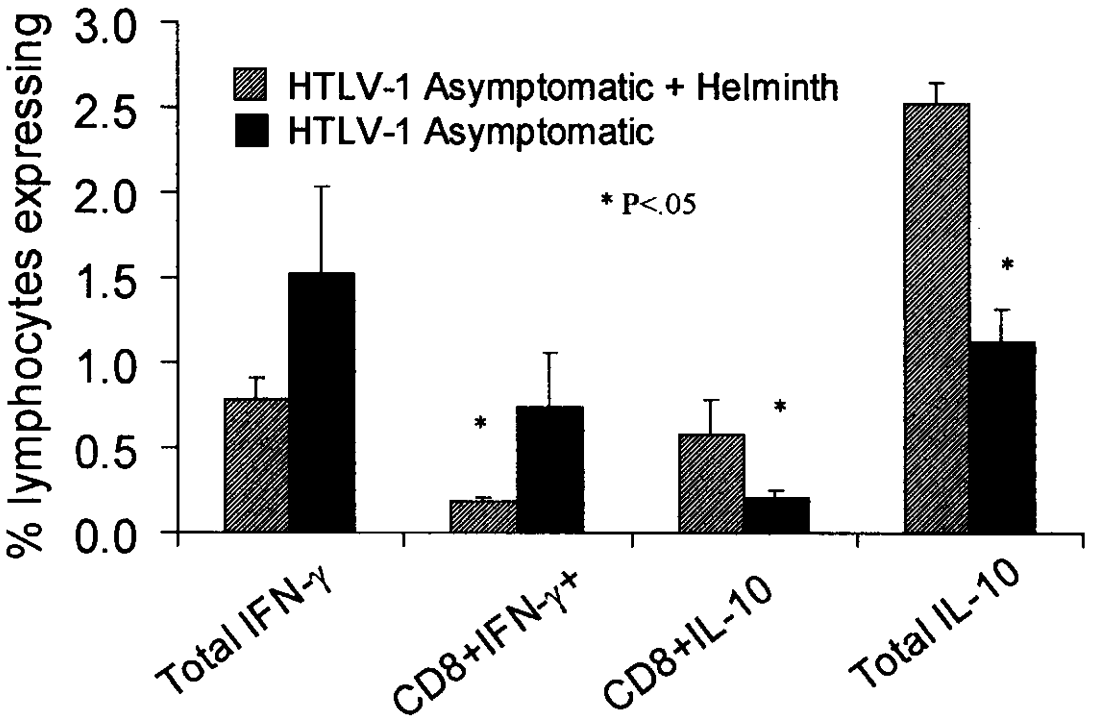
% lymphocytes expressing
3.0
2.5
2.0
1.5
1.0
0.5
0.0
HTLV-1 Asymptomatic + Helminth
HTLV-1 Asymptomatic
* P<.05
*
*
*
Total IFN-γ
CD8+IFN-γ+
CD8+IL-10
Total IL-10

Figure 4

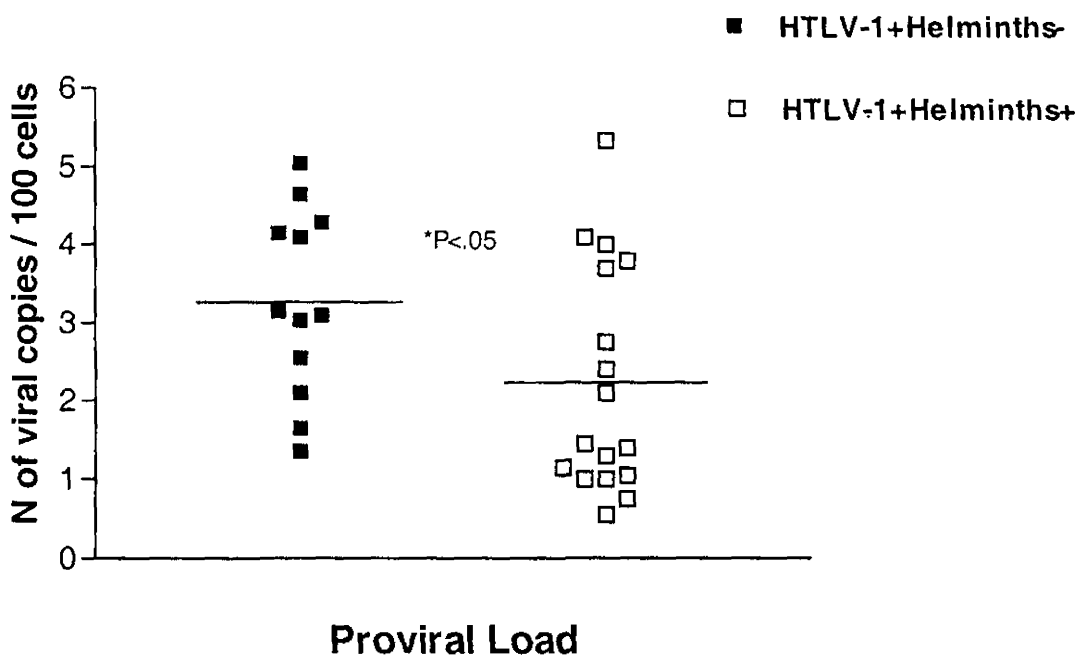
HTLV-1+Helminths-
HTLV-1+Helminths+
N of viral copies / 100 cells
6
5
4
3
2
1
0
*P<.05
Proviral Load

Table 1. Frequency of high levels of IFN-γ (>400pg/ml) in patients with HTLV-1 co-infected or not with helminths (*S. stercoralis* and *S. mansoni*)

	HTLV-1	**HTLV-1 + helminths**
High producers (> 400 pg/ml)	17/35 (48%)	7/35 (20%)
Low producers (<400 pg/ml)	18/35 (52%)	28/35 (80%)*

* P<.05 – Fisher's exact test

Table 2. Frequency of *S. stercoralis* and *S. mansoni* in HTLV-1 Carriers and in Patients with HAM/TSP

Clinical forms of HTLV-1 infection	
	S. stercoralis and/or *S. mansoni*
HTLV-1 Carriers	71/310 (23%)*
Myelopathy	1/32 (3%)

*P<.05 - Chi-square test

DISCUSSÃO

Resposta imune inflamatória exacerbada característica de pacientes com mielopatia é observada em uma grande proporção de portadores assintomáticos do HTLV-1.

A infecção pelo HTLV-1 e sua interação com o sistema imune tem sido relacionada a uma alta produção de citocinas pro-inflamatórias, uma alta resposta linfoproliferativa e uma ativação celular constante. Do ponto de vista clínico a mielopatia e LLcTA são as doenças com maior morbidade relacionadas a este vírus. Apesar de a maioria dos trabalhos documentarem que poucos indivíduos desenvolvem mielopatia (5%), esta doença tem sido muito estudada, visto que é uma doença debilitante, levando à incapacidade funcional do indivíduo. Além disso, tem sido descritas mielopatia em crianças (Kramer et al., 1995) e mielopatia juvenil (Muniz et al., 2002). Recentemente, um estudo de coorte documentou uma maior freqüência no desenvolvimento desta patologia (Taylor et al., 1999). A interação entre o HTLV-1 e o sistema imune leva a alterações imunológicas, mesmo em indivíduos infectados assintomáticos. Estes indivíduos apresentam ativação linfocitária, proliferação espontânea e um predomínio na produção de citocinas com um perfil Th1 (IL-2, IL-15, TNF-α e IFN-γ) (Azimi et al., 2001; Carvalho et al., 2001; Copeland & Heeney, 1996; Tendler et al., 1990; Uchiyama et al., 1985). No artigo número 1 foi avaliado o perfil de citocinas de sobrenadante de culturas de CMSP sem estímulo, a proliferação espontânea de linfócitos e análise por citometria de fluxo de marcadores linfocitários e expressão de citocinas intracelulares em indivíduos portadores assintomáticos e com mielopatia. Foi observado que pacientes com mielopatia apresentam, além de uma maior proliferação

linfocitária, níveis bem mais elevados de IFN-γ em sobrenadante de cultura de CMSP, comparados aos portadores assintomáticos. Houve igualmente um aumento significativo de linfócitos T expressando IFN-γ e TNF-α em indivíduos com mielopatia, sugerindo que estas citocinas pro-inflamatórias estão envolvidas na patogênese da mielopatia. Além disso, os indivíduos assintomáticos foram arbitrariamente dividido em dois grupos: altos produtores de IFN-γ e baixos produtores de IFN-γ. Esta divisão foi realizada devído à alta variabilidade na produção de IFN-γ no grupo de portadores assintomáticos. Quarenta por cento dos indivíduos assintomáticos apresentaram níveis elevados de IFN-γ semelhantes aos níveis de pacientes com mielopatia. Quando estes níveis foram reavaliados 06 meses após, os mesmos mantiveram-se elevados. Pode ser que estes indivíduos tenham uma tendência maior para desenvolver doença, visto que alguns estudos têm demonstrado que IFN-γ e TNF-α são encontrados em níveis elevados no líquor e em lesões inflamatórias da medula espinhal de pacientes com mielopatia. (Biddison et al., 1997; Umehara et al., 1994). Além disso, existe um aumento de células T expressando citocinas pro-inflamatórias e uma gliose reativa nos locais onde há lesão do SNC (Umehara et al., 1994). Estes astrócitos com o estímulo de citocinas produzidas pelos linfócitos tornam-se também uma fonte de TNF-α, podendo causar maior dano para o SNC (Méndez et al., 1997). Como os linfócitos T ativados infiltram o sistema nervoso central não é bem compreendido. Tem sido demonstrado que o vírus atravessa a barreira hemato-encefálica através de linfócitos T infectados (Cavrois et al., 2000). Estes linfócitos ativados podem alterar a expressão de matriz metaloproteinases (MMP) e seus inibidores (TIMP) resultando em um desequilíbrio MMP/TIMP, facilitando a penetração destas células no SNC (Giraudon et al., 2000). Diante destes dados, toma-se importante observar se portadores assintomáticos, com alterações

imunológicas semelhantes aos pacientes com mielopatia, irão desenvolver esta doença e observar parâmetros que possam ser considerados marcadores de progressão de doença. Um estudo comparando pacientes assintomáticos aos pacientes com mielopatia observou que ambos tinham expansão de células TCD8+. Esta expansão foi significantemente maior em pacientes com mielopatia, comparados aos indivíduos assintomáticos (Ureta-vidal et al., 2001). Um estudo inicial realizado em nosso serviço documentou que indivíduos assintomáticos apresentam altos níveis de IFN-γ. A produção desta citocina foi principalmente por células CD4+ , visto que a depleção de células CD8+ e células NK não afetaram a produção de IFN-γ, enquanto que a depleção de células CD4+ reduziu significantemente a produção desta citocina. Estes resultados sugerem que, em portadores assintomáticos, a principal fonte de IFN-γ são células T CD4+ (Carvalho et al., 2001). No presente trabalho nós observamos que em pacientes com mielopatia as células CD8+ são as principais responsáveis pela produção de IFN-γ. Pode ser que isso seja devido a um desvio da produção desta citocina de células CD4+ para células CD8+ e que realmente estas células CD8+ estejam mais relacionadas à patogênese dessa doença. Já tem sido demonstrado a presença de células CD8+ anti tax no líquor e no sangue de indivíduos com mielopatia. Como as células CD4+ são as que mais expressam a proteína tax, é possível que contribuam para uma constante ativação de células citotóxicas (Hanon et al., 2000), células estas, envolvidas no mecanismo de defesa contra este vírus (Kubota et al., 1998; Nagai et al., 2001). Porém, a infecção não é debelada e esta resposta do sistema imune acaba causando um dano tecidual, levando a alterações do sistema nervoso central. Tem sido demonstrado que indivíduos infectados pelo HTLV-1 apresentam ativação linfocitária constante, com maior expressão de marcadores HLA-DR em linfócitos T CD4+ e CD8+

(Al-Fahim et al., 1999). Estudos prévios têm demonstrado que células CD28-CD8+ têm um poder de alta citotoxidade, com um papel importante no controle de doenças virais (Azuma et al., 1993; Weekes et al, 1999). Neste trabalho foi avaliado, através da citometria de fluxo, marcadores linfocitários e foi documentado um aumento na freqüência de células T CD28-CD8+ em pacientes com mielopatia, porém, o papel destas células precisa ser melhor esclarecido, visto que elas têm sido relacionadas ao dano tecidual em doenças infecciosas (Dutra et al., 1996). Um aumento da carga proviral também tem sido relacionado ao desenvolvimento de mielopatia. Neste estudo a carga proviral não foi avaliada, porém um estudo em pacientes com HAM/TSP e portadores assintomáticos com a mesma carga pró-viral de HTLV-1 e mesmo nível de expressão espontânea de tax, determinada por citometria de fluxo, foi observado que em pacientes com HAM/TSP, os níveis de citocinas pró-inflamatórias (IFN-γ e TNF-α) em células que expressam Tax foram significativamente maiores comparados aos níveis de portadores assintomáticos (Furukawa et al., 2003). Este encontro, sugere, uma participação importante destas citocinas no desenvolvimento de mielopatia. Desta forma, são fatores considerados importantes para o desenvolvimento de HAM/TSP, a carga proviral, a ativação de células T CD8+ e a produção exagerada de citocinas pro-inflamatórias.

O vírus HTLV-1 diminui a resposta imune Th2 em pacientes com estrongiloidiase

A infecção por parasitas intestinais como *Áscaris, Trichuris, Ancylostoma e S. stercoralis*, atinge aproximadamente 01 bilhão de pessoas em todo o mundo. Estima-se em 01 milhão, o número de mortes anual devido a estas infecções (Finkelman et al., 1997). Entre estes nematódeos destaca-se o *S. stercoralis*, pela sua capacidade de disseminação e

por causar doença grave e fatal. A auto-infecção é o fenômeno que justifica a manutenção de indivíduos infectados por períodos longos de tempo, mesmo afastado de áreas endêmicas, e também o desenvolvimento de formas disseminadas das doenças com alta carga parasitária (Carvalho et al., 1978). A associação entre HTLV-1 e estrongiloidíase tem sido demonstrada por estudos epidemiológicos em regiões onde estes dois agentes são endêmicos e até mesmo em região de baixa endemicidade para esta helmintíase (Dixon et al., 1989; Hayashi et al., 1997; Nakada et al., 1984; Sato et al., 1989). Além de uma maior frequência de estrongiloidíase em portadores do HTLV-1, estudos outros têm associado esta infecção viral com o desenvolvimento de formas graves e disseminadas desta helmintíase (Gottuzo et al., 1999; Newton et al., 1992; O'Doherty et al., 1984; Patey et al., 1992; Phelps et al., 1993). Enquanto no passado a principal causa de estrongiloidíase disseminada era o uso de corticosteróides e imunossupressores, hoje o HTLV-1 é considerado o principal fator predisponente para a apresentação de formas graves da parasitose. Sabe-se que o vírus HTLV-1 interfere na resposta imune com uma produção espontânea predominantemente de citocinas Th1. A descoberta de que a população de células T auxiliadoras (CD4+) é heterogênea e constituída por sub-populações de células T denominadas linfócitos T do tipo Th1 e linfócitos T do tipo Th2 contribuiu para um melhor entendimento da resposta imune nas doenças parasitárias. As células Th1 secretam IL-2, IFN-γ e TNF-α e TNF-β e são relacionadas com uma resposta imune celular enquanto que as céluals Th2 secretam IL-4, IL-5, IL-13 e IL-10 e estão relacionadas com uma resposta imune humoral (Mosmann et al., 1986; Romagnani, et al., 1991). Recentemente foram descritas as células Th3 que estão relacionadas com expressão de IL-10 e TGF-β, citocinas que modulam a resposta imune celular (Roncarolo et al., 2003; Mills et al., 2004).

Adicionalmente, existem evidências de mecanismos regulatórios da resposta imune onde citocinas secretadas por células Th1 podem modular células Th2 e vice-versa (Klimpel et al., 1990; Fiorentino et al., 1989). No segundo artigo foi documentado uma maior produção de IFN-γ e uma menor produção de IL-5 e IL-13 em culturas estimuladas com antígeno de *S. stercoralis* em indivíduos co-infectados pelo HTLV-1 e *S .stercoralis* comparado aos pacientes apenas infectados pelo *S. stercoralis*. Estes dados, associados a observações anteriores de que indivíduos co-infectados pelo HTLV-1 e *S. stercoralis* apresentam menor produção de IL-4, menor produção de IgE total e específica contra antígeno de *S. stercoralis* do que pacientes com estrongiloidíase isoladamente (Neva et al., 1998) sugerem que a co-infecção com HTLV-1 desvia a resposta imune para o tipo Th1 levando a uma redução da produção de IL-4, IL-5 e IL-13, citocinas diretamente envolvidas no mecanismo de defesa contra parasitas intestinais. IL-4 e IL-13 aumentam o fluido intestinal facilitando a eliminação de parasitas (Finkelman et al., 1997; Goldhill et al, 1997) e a IL-4 é diretamente envolvida na produção de IgE por linfócitos B. Desta forma, uma menor produção destas citocinas pode contribuir para uma maior transformação de larvas rabditóides em larvas filarióides facilitando o processo de auto-infecção. Adcionalmente, a redução dos níveis de IgE pode interferir na capacidade de mastócitos de destruir larvas que penetram através do intestino. A documentação de que animais infectados pelo *Trichinela spiralis* e *S. mansoni* destroem estes parasitos com a participação de mastócitos e de IgE implicam que desgranulação de mastócitos pode estar envolvida no mecanismo de defesa contra helmintos a nível de mucosa (Ahmad et al., 1991). Esta defesa a nível de mucosa neste caso estaria dependente de citocinas Th2 (IL-3 e IL-4) que estão relacionadas com a ativação dos mastócitos resultando na desgranulação destas células com liberação de

histamina, lesando diretamente o parasita. Reforça esta hipótese o fato de ratos hipotímicos apresentarem uma incapacidade de expulsar o *S. ratti* da mucosa intestinal e após administração de IL-3, haver uma expulsão associada à intensa mastocitose (Abe & Nawa, 1988; Abe et al., 1992). Como já foi citado anteriormente, existe uma participação da IL-4 e da IL-13 na fisiologia intestinal, aumentando o conteúdo de fluidos no trato digestivo e facilitando a expulsão de larvas pelas fezes (Finkelman et al., 1997; Goldhill et al, 1997). A IL-5 é uma citocina relacionada à produção, diferenciação e ativação de eosinófilos. Estas células têm capacidade *in vitro* de destruir helmintos através do mecanismo de ADCC, e eosinófilos são encontrados circundando larvas de *S. stercoralis* (German et al., 1992; Poltera & Katsimubara, 1974). Desta forma, a diminuição da produção de IL-5, em pacientes com HTLV-1 e estrongiloidíase, pode levar a uma redução da capacidade destas células em destruir parasitas e contribuir para ocorrência de disseminação da doença. Mais recentemente, foi demonstrado que a carga proviral do HTLV-1 tem valor preditivo para o desenvolvimento de formas graves da estrongiloidíase, e a falta de resposta ao tratamento desta helmintíase se associa a maior expressão de RNA mensageiro para IFN-γ e baixos níveis de IgE (Satoh et al., 2002; Satoh et al.,2003). Neste mesmo trabalho também foi observado aumento de IgG4 nos pacientes sem resposta ao tratamento. O aumento da expressão de tax, uma proteína viral relacionada a transcrição de proteínas e fatores celulares, está associada a um rápido aumento da produção de IFN-γ (Hanon et al., 2001). Neste nosso segundo artigo foi observada uma maior produção de IFN-γ em indivíduos co-infectados pelo HTLV-1. Esta alta produção foi inversamente proporcional aos níveis de IL-5 e de IgE. Estes resultados sugerem que níveis elevados de IFN-γ e redução dos níveis de IL-4, IL-5, e IgE, encontrados em indivíduos infectados pelo HTLV-1, esteja

relacionada a manutenção da infecção e a uma maior susceptibilidade para desenvolver estrongiloidíase disseminada.

A infecção pelo HTLV-1 modifica o curso clinico e a resposta imunológica a esquistossomose.

Nenhum estudo prévio tem avaliado a associação entre a infecção pelo *S. mansoni* e pelo HTLV-1. Como já foi dito anteriormente, a infecção pelo HTLV-1 tem sido associada a uma maior prevalência de *S. stercoralis,* a um maior desenvolvimento de formas graves e atípicas da estrongiloidíase, maior falha terapêutica e diminuição da resposta Th2 (Sato et al., 1994; Porto et al., 2002). Foi documentado neste terceiro trabalho uma maior prevalência de infecção pelo *S. mansoni* em indivíduos infectados pelo HTLV-1, comparada aos indivíduos não infectados pelo HTLV-1. A maioria destes pacientes eram assintomáticos, apenas três apresentavam sintomas como diarréia e dor abdominal. Todos os pacientes apresentaram a forma crônica da esquistossomose. Apenas um paciente apresentou hepatomegalia,, 95,5% apresentaram grau I de fibrose hepática e apenas um paciente (4,5%) foi classificado para grau II de fibrose hepática. Esplenomegalia não foi observada. A lesão hepática com fibrose grave ocorre em uma média de 6% dos pacientes cronicamente infectados e é decorrente da formação de granulomas ao redor do ovo do *S. mansoni* (de Jesus et al., 2000). "Background"genético, grau de infestação e resposta imune são fatores importantes associados ao desenvolvimento da fibrose hepática (Secor et al., 1996; Sleigh et al., 1985). Apesar de alguns estudos utilizando modelos experimentais sugerirem que citocinas tipo 1 estão associadas a formação de granuloma (Leptak et., 1997; Mwatha et al., 1998), estudos recentes têm associado uma maior participação de citocinas

IL-4 e IL-13 na formação do granuloma (Chiaramonte et al., 1999a, Chiaramonte et al., 1999b; Jankovik et al., 1999). Tem sido também demonstrado que pacientes com grau III de fibrose hepática apresentaram níveis elevados de IL-5, IL-10 e IL-13 em sobrenadantes de CMSP estimulados com antígeno de ovo solúvel (SEA) (de Jesus et al., 2004), sugerindo que a resposta Th2 tem um papel importante nas etapas iniciais da fibrose hepática. Como a infecção pelo HTLV-1 interfere na resposta imune ao *S. mansoni*, com maior produção de IFN-γ é possível que os altos níveis desta citocina encontrados nestes pacientes possam modular a formação do granuloma. Outra possibilidade é que estes pacientes apresentam uma menor resposta Th2 e não desenvolvem formas mais graves de fibrose hepática.

Neste trabalho foi observado que dentre os pacientes com esquistossomose, os co-infectados pelo HTLV-1 apresentaram excreção inferior de ovos, quando avaliada pelo método de Kato Katz. Estes pacientes vivem em região urbana e não apresentam re-exposição ao *S. mansoni*, o que poderia justificar uma baixa carga parasitária. A excreção de ovos tem sido associada a uma resposta Th2. Estudos realizados em pacientes com esquistossomose e baixa contagem de células CD4+, observou uma redução na excreção de ovos (Karanja et al., 1997). Em um outro estudo realizado em semelhantes grupos de pacientes, a baixa contagem de células CD4+ foi relacionada a um aumento na relação de citocinas Th1/Th2, representados por IFN-γ e IL-4 respectivamente (Mwinzi et al., 2001). Ou seja, menor produção de IL-4 foi relacionada a uma menor excreção de ovos. Como nesse terceiro trabalho a co-infecção pelo HTLV-1 reduziu a resposta Th2, é possível que tenha havido influência na excreção de ovos, porém seria esperado que esta baixa excreção de ovos estivesse relacionada com uma maior gravidade dos sintomas da esquistossomose, visto que em modelos experimentais uma elevada resposta Th1 e reduzida produção de

citocinas Th2, com conseqüente baixa excreção de ovos, está associada com hepatotoxidade, irritação intestinal e mortalidade (Fallon et al., 2000). A detecção de antígenos circulantes tem sido utilizada para avaliar a carga parasitária, porém este método somente detecta a excreção de ovos se for superior a 100 ovos/gr de fezes, e no caso dos pacientes co-infectados, estes antígenos circulantes provavelmente seriam indetectáveis. Estudos posteriores podem esclarecer se esta baixa excreção de ovos está associada a uma baixa carga parasitária ou se existe uma maior dificuldade na excreção de ovos. A resposta imune contra a esquistossomose crônica tem sido relacionada a diminuição dos níveis de IFN-γ e a aumento dos níveis de IL-4, IL-5 e IL-10 (Araújo et al., 1996). Neste terceiro estudo foi demonstrado que a co-infecção pelo HTLV-1 aumenta a produção de IFN-γ de culturas de CMSP estimuladas com antígeno de verme adulto do *S. mansoni* e reduz os níveis de IL-5 e de IL-10, comparado aos níveis em pacientes apenas com esquistossomose. Além disso, foi observada uma redução dos níveis de IgE específica contra antígeno de *S. manosni*. Estudos prévios têm demonstrado uma correlação entre altos níveis de IgE, alta relação IgE/Ig4 e resistência a reinfecção com *S. mansoni* (Caldas et al., 2000, Demeure et al., 1993; Dunne et al., 1992; Rihet et al. 1991). A documentação que a infecção pelo HTLV-1 reduz os níveis de IgE específico sugere que esta alteração da resposta imune pode estar associada a uma maior susceptibilidade de re-infecção em pacientes infectados pelo HTLV-1, porém, como já foi dito anteriormente, estes pacientes residem em região urbana e apresentam menor risco de contaminação. Neste trabalho houve uma menor resposta terapêutica a drogas anti-esquistossomóticas quando comparado ao grupo apenas com esquistossomose. Este último grupo apresentou uma taxa de resposta de 98%, o que é comparável à taxa relatada pela literatura (Veronesi, 1997; Ferrari et al., 2003). A

documentação neste terceiro trabalho que a infecção pelo HTLV-1 reduz os níveis de IL-5 e de IgE específico, sugere que esta maior falha terapêutica pode estar associada a estas alterações da resposta imune encontrada em pacientes com esquistossomose e co-infectados pelo HTLV-1 e conseqüentemente levar a uma maior prevalência de esquistossomose em indivíduos infectados pelo HTLV-1. Em resumo, a infecção pelo HTLV-1 interfere na resposta imune ao *S. mansoni*, reduz a resposta terapêutica a drogas anti-esquistossomóticas mas, apesar de permanecerem infectados, estes pacientes não apresentam grau importante de fibrose hepática.

Infecção por helmintos modula a resposta imune Th1 em pacientes com HTLV-1 e é mais prevalente em portadores assintomáticos do HTLV-1 que em pacientes com mielopatia.

Nos três primeiros trabalhos foi documentado que pacientes infectados pelo HTLV-1 apresentam uma resposta imune com produção de citocinas predominantemente com um perfil Th1 com altos níveis de IFN-γ e uma resposta linfoproliferativa aumentada. Estas alterações se apresentam de forma mais exacerbada quando estes indivíduos apresentam mielopatia. Também foi documentado que esta alteração da resposta imune nestes indivíduos interfere no mecanismo de defesa contra helmintos (*S. stercoralis e S. mansoni*) com redução de citocinas Th2 e de IgE específica. Como na infecção por estes helmintos, principalmente na fase crônica, há um ambiente rico em citocinas Th2, neste 4° trabalho foi avaliada a interferência destas helmintíases na produção de citocinas e na carga proviral do HTLV-1. Inicialmente foi documentado que pacientes infectados pelo HTLV-1 e co-infectados por helmintos apresentaram níveis inferiores de IFN-γ em sobrenadante de culturas de CMSP e menor freqüência de células espressando esta citocina, comparados aos

indivíduos apenas infectados pelo HTLV-1. IFN-γ é uma citocina produzida por linfócitos T CD4+, CD8+ e células NK. A porcentagem de células CD4+ e CD8+ expressando IFN-γ também foi reduzida com esta co-infecção. Em contraste aos níveis de IFN-γ, a freqüência de células T expressando IL-5 e IL-10 foi maior em indivíduos com co-infecção por helmintos. Como já foi descrito no primeiro trabalho, os níveis de IFN-γ em pacientes infectados pelo HTLV-1 são variáveis e existem pacientes com alta produção e baixa produção de IFN-γ. Neste trabalho a maioria dos pacientes com helmintos foram baixos produtores de IFN-γ.

Existem evidências de mecanismos regulatórios da resposta imune onde citocinas secretadas por células Th2 podem modular células Th1 e vice versa (Fiorentino *et al.* 1989). A IL-10 é uma das principais citocinas moduladoras da resposta imune. Tem capacidade de suprimir a síntese de citocinas por macrófagos e por células T, inibir a proliferação de células T e suprimir a ativação de macrófagos mediada por IFN-γ (Carvalho et al., 1995). A IL-10 suprime de modo importante a síntese de TNF-α (Fiorentino et al., 1991), e por esta razão, é considerada uma importante citocina na modulação do processo inflamatório. No homem, o papel de IL-10 na desregulação da resposta imune a agentes infecciosos tem sido documentada. Por exemplo, em pacientes com leishmaniose visceral existe uma supressão da proliferação linfocitária e da produção de IFN-γ, funções que podem ser restauradas *in vitro* por adição de anticorpos monoclonais anti-IL-10 e a conseqüente neutralização desta citocina (Carvalho et al., 1994). Recentemente, nós documetamos que adição de IL-10 em culturas de CMSP de pacientes com HTLV-1 pode reduzir a produção de IFN-γ (Carvalho et al., 2001) e um estudo realizado por Araújo e col. documentou que pacientes com esquistossomose mansônica apresentaram produção de citocinas Th2 (IL-4, IL-5 e IL-10) e

nenhuma produção de IFN-γ em culturas de CMSP. Quando a IL-10 foi neutralizada através de anti IL-10, a produção de IFN-γ foi restaurada. É possível que os pacientes co-infectados por helmintos possam modular a produção de IFN-γ, por apresentarem uma maior freqüência de células expressando citocinas Th2, como a IL-10. Além disso, pode existir uma participação de células T regulatórias, que inibem a ação de células T CD4+ e CD8+, células que atuam na patogênese da HAM/TSP (Kubota et al., 1998; Nagai et al., 2001b; Osame et al., 2002). A infecção pelo HTLV-1 é uma infecção crônica, e a maioria de células infectadas são células TCD4+ (Kaplan et al., 1993; Richardson et al., 1990; Richardson et al., 1997). Estas células apresentam expressão de tax (Hanon et al., 2000) e apesar do sistema imune tentar destruir células através de células citotóxicas, a infecção permanece ativa. A carga proviral tem sido relacionada ao desenvolvimento de doenças relacionadas a este vírus (Nagai et al., 2001; Yamano et al., 2002). A co-infecção por helmintos, por reduzir uma resposta Th1, poderia inibir o mecanismo imune de destruição de células infectadas e conseqüentemente aumentar a carga proviral. Todavia, foi documentado neste quarto trabalho que a infecção por helmintos (*S. mansoni e S. stercoralis*) reduziu a carga proviral de indivíduos infectados pelo HTLV-1. A propagação do vírus está relacionada à ativação de células T (Holsberg et al., 1999), então, é possível que esta menor carga proviral esteja relacionada à modulação da resposta imune, pelos helmintos. Reforçando esta hipótese, um estudo realizado por Agape e col. demonstra que pacientes com linfoma não Hodgkin de células T e pacientes com LLcTA infectados pelo *S. stercoralis* apresentam uma resposta significantemente melhor (100% e 28%, respectivamente) ao tratamento e maior sobrevida que pacientes não infectados por *S. stercoralis*. A sobrevida de pacientes infectados pelo *S. stercoralis* com LLcTA foi de 27

meses, enquanto nos pacientes com LLcTA sem infecção pelo *S. setrcoralis* foi de 5 meses. Não houve diferença na sobrevida nem na resposta ao tratamento quando os pacientes tinham linfoma de células B. Estes achados sugerem que a infecção por helmintos pode alterar o curso clínico de doenças relacionadas a uma maior proliferação de células T. A mielopatia é uma doença relacionada ao aumento de carga proviral, infiltração da medula por linfócitos T e aumento de citocinas pro-inflamatórias em culturas de linfócitos e no líquor. Neste quarto trabalho nós documentamos que a freqüência de helmintos (*S. mansoni e S. stercoralis*) foi significantemente maior em portadores assintomáticos que em indivíduos com mielopatia. Neste último grupo apenas um paciente estava infectado pelo *S. mansoni.* e o nível de IFN-γ foi bastante inferior à média dos níveis de IFN-γ encontrada nos pacientes com mielopatia. Pacientes com mielopatia têm menor exposição a helmintos, pela própria limitação física, porém este grupo co-infectado não apresenta exposição recente, reside em área urbana e apresenta falha na resposta terapêutica a drogas anti-helmínticas. Estudos prévios, em modelos experimentais, têm demonstrado que infecção com *S. mansoni* pode reduzir o desenvolvimento de DMID em animais que têm tendência a desenvolver esta doença (Cooke et al., 1999). Além disso, a infecção por *S. mansoni* reduziu significativamente a inflamação da encefalomielite e interferiu na progressão desta doença (La Flamme et al., 2003). Este 4° trabalho demonstra que a infecção por helmintos (*S. mansoni e S. stercoralis*) reduziu a resposta imune Th1 exacerbada encontrada em pacientes com infecção pelo HTLV-1 e também a carga proviral. Estes dados associados ao fato de que pacientes com mielopatia apresentam uma baixa freqüência de infecção por helmintos sugerem que a infecção por helmintos pode associar-se negativamente com desenvolvimento de doença neurológica associada a este vírus.

CONCLUSÕES

- Os pacientes com mielopatia mostraram níveis mais elevados de IFN-γ, maior proliferação linfocitária e maior freqüência de células T CD8+ que portadores assintomáticos do HTLV-1. Porém, 40% dos portadores assintomáticos do HTLV-1 têm alterações imunológicas semelhantes às descritas em pacientes com mielopatia.
- Os pacientes co-infectados pelo HTLV-1 e *S. stercoralis* tiveram níveis inferiores de IL-5 e IgE e níveis mais elevados de IFN- γ em culturas estimuladas com antígeno de *S. stercoralis*, comparado aos pacientes apenas com infecção pelo *S. stercoralis*. Houve uma correlação inversa entre os níveis de IFN-γ e os níveis de IL-5 e de IgE.
- A frequência de *S. mansoni* foi maior em pacientes com infecção pelo HTLV-1, comparada a pacientes soronegativos. Pacientes co-infectados pelo HTLV-1 e *S. mansoni* tiveram níveis inferiores de IL-5, IL-10 e IgE específica e níveis mais elevados de IFN-γ, comparado aos pacientes apenas com infecção pelo *S. mansoni*.
- A eficácia terapêutica do praziquantel foi reduzida em pacientes co-infectados pelo *S. mansoni* e HTLV-1.
- A fibrose hepática foi leve em todos os pacientes co-infectados pelo *S. mansoni* e pelo HTLV-1.
- As infecções por helmintos são mais freqüentes em portadores do HTLV-1 do que em pacientes com mielopatia associada ao HTLV-1.

- A infecção por helmintos modula os níveis elevados de produção de IFN-γ observada em indivíduos infectados pelo HTLV-1, aumenta a frequência de células T expressando IL-5 e IL-10 e reduz a carga proviral do HTLV-1.

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ANEXO

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Cytokine Profile and Immunomodulation in Asymptomatic Human T-Lymphotropic Virus Type 1–Infected Blood Donors

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Summary: The modulation of the immune response has been used as therapy for clinical disorders associated with human T-lymphotropic virus type 1 (HTLV-1) infection. In this study, the cytokine profile was evaluated in 26 asymptomatic HTLV-1 blood donors. Additionally, both the cell responsible for producing interferon-γ (IFN-γ) and the role of exogenous interleukin (IL)-10 in downregulating IFN-γ production were studied. Cytokine levels were determined in supematants of unstimulated lymphocyte cultures by enzyme-linked immunosorbent assay. The levels of IFN-γ, tumor necrosis factor-α, IL-5, and IL-10 were higher in supernatants of the lymphocyte cultures taken from HTLV-1–infected donors than in those taken from healthy subjects. Although depletion of $CD8^+$ T cells and natural killer cells did not affect IFN-γ production, depletion of $CD4^+$ T cells significantly decreased IFN-γ production. Furthermore, at a concentration of 2 ng/ml, IL-10 had only a minimum effect on IFN-γ production, although at high concentrations (100 ng/ml), IL-10 decreased IFN-γ production by 50% in HTLV-1–infected individuals. These data indicate that both T helper 1 and T helper 2 cytokines are elevated in HTLV-1 infection and that IL-10 in high concentrations modulates IFN-γ production in these patients. **Key Words:** Cellular immunity in HTLV-1—Cytokines in HTLV-1—HTLV-1—Immune response in HTLV-1—Immunomodulation in HTLV-1.

The immunologic response in human T-lymphotropic virus type 1 (HTLV-1) infection is characterized by spontaneous T-cell proliferation with increasing secretion of interleukin (IL)-2 and expression of the IL-2 receptor (1–3). Abnormalities in the response have been shown in patients with HTLV-1–associated myelopathy (tropical spastic paraparesis) compared with asymptomatic HTLV-1–positive individuals, including elevated levels of cytokines such as tumor necrosis factor-α (TNF-α), IL-6, and IL-2 in sera and cerebrospinal fluid (4,5). Both $CD4^+$ and $CD8^+$ T cells are infected by HTLV-1 (6,7). Although only a small percentage of infected individuals develop clinical manifestations associated with HTLV-1, the prevalence of this retrovirus is high in endemic areas such as Salvador, Bahia, Brazil, where 1.35% of blood donors are infected with HTLV-1 (8). Although high levels of IL-2 and interferon-γ (IFN-γ) have been documented in supernatants of lymphocyte cultures from asymptomatic HTLV-1 carriers (3), little is known about the secretion of T helper (Th) 2 cytokines or the ability of cytokines and cytokine antagonists to modulate the lymphocyte function in the course of this viral infection. The major aim of the current study was to evaluate the cytokine profile in asymptomatic subjects infected with HTLV-1, to determine which cells are secreting IFN-γ in such patients, and to evaluate the ability of IL-10 to downregulate IFN-γ production in patients infected with HTLV-1.

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INFLUENCE OF HUMAN T-CELL LYMPHOCYTOTROPIC VIRUS TYPE 1 INFECTION ON SEROLOGIC AND SKIN TESTS FOR STRONGYLOIDIASIS

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Abstract. The aim of this study was to determine whether human T-cell lymphocytotropic virus type 1 (HTLV-1) infection may affect the levels of parasite-specific immunoglobulin (Ig) G and IgE and the positivity of the skin test for strongyloidiasis. Participants included 67 patients with strongyloidiasis (40 without HTLV-1 infection and 27 coinfected with HTLV-1). We determined IgG and IgE levels by enzyme-linked immunosorbent assay, and the immediate hypersensitivity skin test was performed with the metabolic *Strongyloides stercoralis* antigen. Specific IgE levels and the size of skin reactions in patients without HTLV-1 were higher ($P < 0.01$) than those observed in patients coinfected with HTLV-1. Additionally, 89% of patients without HTLV-1 had specific IgE and 92.5% had positive skin tests; however, these values were significantly reduced ($P < 0.01$) in patients coinfected with HTLV-1 (44% and 59%, respectively). These data show that HTLV-1 infection decreases the sensitivity of detection of *S. stercoralis*-specific IgE, the size of the immediate hypersensitivity reaction, and the sensitivity of these tests in the diagnosis of strongyloidiasis.

INTRODUCTION

Although stool examination is the most direct and simplest test for diagnosis of strongyloidiasis, the diagnostic value is compromised by the following: irregular and scanty output of larvae by intestinal adult females[1]; failure to detect acute infection[2]; and occasional negative results in some patients with severe disease.[3] Immunodiagnostic tests such as an assay for specific immunoglobulin (Ig) G antibody and an immediate hypersensitivity skin test can also be used and are especially useful for epidemiologic studies.[4] Parasite-specific IgE antibodies have also been documented in patients with strongyloidiasis.[5] Because this isotype is involved in defense against helminths, interest in characterizing the IgE response has recently increased.

Considerable evidence has recently accumulated concerning certain complications of strongyloidiasis, such as development of severe disease, nonresponse to therapy, or both in patients concurrently infected with human lymphocytotropic virus type 1 (HTLV-1).[6–8] Some patients infected with HTLV-1 produce high levels of interferon gamma and reduced total serum IgE levels,[9] which may account for the more severe disease and impaired response to treatment of strongyloidiasis in patients infected with both agents. The aim of the present study was to determine whether coinfection with HTLV-1 decreases the humoral immune responses to *Strongyloides stercoralis* antigen, specifically parasite-specific IgG and IgE, and the immediate hypersensitivity skin test.

MATERIALS AND METHODS

Patients. Patients were recruited into the study from 3 sources: referrals of HTLV-1–positive seroreactors from blood banks, residents from a rural endemic area near Salvador with positive fecal examination for *S. stercoralis* infection, and 3 patients admitted at Hospital Santo Antonio due to severe strongyloidiasis. A clinical history was taken and physical examination performed. Laboratory analyses included serology (IgG and IgE) for *S. stercoralis*, serology for HTLV-1, and immediate hypersensitivity skin test for *S. stercoralis*. The criterion for a diagnosis of strongyloidiasis was a positive fecal examination for larvae by the Baermann concentration technique. Initial screening by direct fecal smears was not done because of low sensitivity of the method, especially in chronic infections.

Protocols, including consent forms written in Portuguese, were approved by ethical review committees of the University Hospital in Salvador, Brazil, as well as of the National Institutes of Health in the United States. All patients provided written informed consent.

Preparation of antigens. Antigens for the skin test and serology were prepared from infective larval stages (L3) of the parasites recovered originally as rhabditiform larvae from fecal specimens of infected monkeys and allowed to develop at 25°C in charcoal cultures for 7–10 days. Larvae were separated from the charcoal by the Baermann procedure and washed repeatedly by centrifugation. They were then exposed to 0.25% sodium hypochlorite for 3–5 min for surface sterilization, followed by multiple cycles of centrifugation in RPMI medium (Gibco, Grand Island, NY) containing 100 μg/mL gentamicin. The somatic antigen for the enzyme-linked immunosorbent assay (ELISA) was prepared from the soluble supernatant of sonicated larvae. Metabolic antigen for skin testing was prepared from 24- and 48-hr harvests of larval cultures incubated at 33°C. After separation of larvae by centrifugation and Millipore filtration, these fluids were pooled and lyophilized, reconstituted in distilled water, and dialyzed with phosphate-buffered saline (PBS) at pH 7.2. An equal volume of RPMI medium containing 100 μg/mL gentamicin was lyophilized and treated exactly as the antigen to provide a diluent control preparation. The antigen and control were then treated with 1:4,000 formalin at 37°C for 14 days and checked for sterility and endotoxin content before storage in multidose vials in the presence of 0.4% phenol at 4°C. Additional details concerning the skin test antigen are provided elsewhere.[10]

Skin test. The test was performed by intradermal injection into the forearm of 0.1 mL of metabolic antigen containing

Juvenile HAM/TSP of Subacute Evolution: Case Report and Literature Review

HAM/TSP Juvenil de Evolução Subaguda: Relato de Caso e Revisão de Literatura

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Abstract

Human T cell lymphotropic virus type I (HTLV-I) is an exogenous retrovirus that has shown to be the etiological agent in adult T cell leukemia (ATL) and a progressive neurological disease called HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The physiopathogenesis of this disease is not completely understood, although it has been suggested that virus-host interactions play a role in the pathogenesis of the disorder. The prevalence of this retrovirus is 1.35% among blood donors in Salvador, Bahia, Brazil. HAM/TSP may present a rapid evolution, with acute or subacute onset, even in young patients with a history of vertical transmission. The aim of the current study is to present a report of a probable juvenile HAM/TSP case and its clinical, epidemiological, and immunological features. A 15-year-old black male was admitted to the HTLV-I outpatient clinic because of large joint pain, paraparesis with signs of pyramidal liberation in the lower limbs, and a HTLV-I positive blood test. His mother, a 54-year-old woman, is also a HTLV-I carrier. An immunological analysis of the boy was performed attempting to evaluate type 1 (IFN-γ and TNF-α) and type 2 (IL-5 and IL-10) responses in supernatants of unstimulated peripheral blood mononuclear cells. In this report, we extend previous observations of high IFN-γ production in unstimulated cultures of HTLV-I-infected patients, showing that other cytokines such as TNF-α, IL-5, and IL-10 are also increased in cell supernatants of these patients.

Key words: human T-lymphotropic virus 1; paraparesis, tropical spastic; cytokines.

Resumo

O vírus linfotrópico humano de células T tipo I (HTLV-I) é um vírus exógeno que já foi demonstrado ser o agente etiológico na leucemia de células T do adulto (ATL) e em uma doença neurológica chamada de mielopatia associada ao HTLV-I/paraparesia espástica tropical (HAM/TSP). A fisiopatogenia desta doença não está completamente entendida, embora interações vírus-hospedeiro possam desempenhar um papel na patogênese da doença. A prevalência deste retrovirus é de 1,35% dentre os doadores de sangue em Salvador, Bahia, Brasil. HAM/TSP pode apresentar uma

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Implicações clínicas e imunológicas da associação entre o HTLV-1 e a estrongiloidíase

Clinical and immunological consequences of the association between HTLV-1 and strongyloidiasis

Maria Aurélia F. Porto[1], André Muniz[1], Jamary Oliveira Júnior[1] e Edgar Marcelino Carvalho[1]

Resumo *A estrongiloidíase é uma das mais importantes helmintíases em países tropicais e estudos epidemiológicos têm demonstrado associação desta parasitose com o vírus HTLV-1. Em regiões onde estes dois agentes são endêmicos a coinfecção pode resultar no desenvolvimento de formas disseminadas da estrongiloidíase assim como em estrongiioidíase recorrente. Enquanto que o vírus HTLV-1 está relacionado com uma alta produção de IFN-γ e desvio da resposta imune para o tipo Th1, a proteção contra helmintos está associada a uma resposta Th2. Devido a este viés da resposta imune, indivíduos infectados pelo HTLV-1 apresentam redução na produção de IL-4, IL-5, IL-13 e IgE, componentes participantes dos mecanismos de defesa contra* S. stercoralis. *Estas anormalidades constituem a base para a ocorrência de maior freqüência e de formas mais graves da estrongiloidíase em pacientes infectados pelo HTLV-1.*

Palavras-chaves: *Estrongiloidíase. HTLV-1.* Strongyloides stercoralis.

Abstract *Strongyloidiasis is one of most important forms of helminthiasis in tropical countries and epidemiologic studies have shown the association of this parasitic disease with HTLV. It has been observed in regions where both these agents are endemic and coinfection may result in an increase in the disseminated forms of strongyloidiasis as well as recurrent strongyloidiasis. While HTLV-1 is related to a high production of IFN-γ and deviation of the immune response towards a Th1 response, the protection against helminths is associated with Th2 like immune response. Individuals infected with HTLV and* S. stercoralis *have a reduction in the production of IL-4, IL-5, IL-13 and parasitic IgE response, all of which are factors participating in the defense mechanism against* S. stercoralis. *These abnormalities are the basis for the occurrence of an increase in the severe forms of strongyloidiasis among patients infected with HTLV-1.*

Key-words: *Strongyloidiasis. HTLV-1.* Strongyloides stercoralis.

A estrongiloidíase é uma das mais importantes helmintíases intestinais em países tropicais. Devido ao aumento de estrongiloidíase disseminada em conseqüência do grande uso de quimioterápicos e drogas imunossupressoras, tem ressurgido um interesse maior nesta helmintíase. Recentemente, tem sido demonstrada associação deste parasito com o vírus linfotrópico para células T humanas tipo 1 (HTLV-1), com apresentação de formas graves e recorrência após o tratamento. A transmissão da doença ocorre pela penetração das larvas filarióides infectantes (L3), através da pele humana intacta. As larvas migram para o pulmão pela corrente sangüínea. No pulmão, elas ultrapassam os capilares pulmonares e entram nos alvéolos, sofrendo uma muda para o estágio L4 neste órgão. Em seguida, elas ascendem até a faringe, são deglutidas e atingem a maturação final para verme adulto (fêmeas partenogenéticas) na mucosa do intestino delgado. Os ovos liberados atingem a maturidade e eclodem liberando as larvas rabditóides (L1) que migram para a luz do intestino. As larvas rabditóides nos estágios, L1 e L2, são eliminadas pelas fezes e podem transformar-se em vermes machos ou fêmeas de vida livre ou em larvas infectantes (L3). Um modo peculiar de infecção conhecida como auto-endo-infecção, ocorre pela transformação das larvas

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Bexiga neurogênica como primeira manifestação de infecção pelo HTLV-I*

Neurogenic bladder as the first manifestation of HTLV-I infection

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Resumo

Neste relato, uma mulher de 28 anos, soropositiva para HTLV-I, cursou com polaciúria, urgência miccional e urge-incontinência como primeira manifestação da infecção pelo vírus. Estava sendo acompahada no Ambulatório Multidisciplinar para HTLV-I da Universidade Federal da Bahia. Exames bioqúimicos da função renal, sumário de urina, urocultura e ultrassonografia das vias urinárias apresentaram resultados normais. O estudo urodinâmico evidenciou contrações hiper-reflexas do detrusor, aumento da sensibilidade e diminuição da capacidade vesical. A paciente foi tratada com brometo de propantelina por 3 meses, apresentando excelente resposta clínica com remissão dos sintomas.

Descritores: bexiga neurogênica; infecção; vírus 1 linfotrópico T humano; propantelina.

Abstract

In this report, a 28-year-old woman who was seropositive for HTLV-I presented urinary frequency, urgency and urge incontinence as the first manifestation of the viral infection. She was being treated at the HTLV-I Multidisciplinary Ambulatory Unit of the Federal University of Bahia. Biochemical testing of the renal function, urinalysis, urine culture, and ultrasound of the urinary tract presented normal results. Urodynamic study showed detrusor over activity, increased sensibility and decreased bladder capacity. The patient was treated with propantheline bromide for 3 months, presenting excellent clinical response with remission of symptoms.

Keywords: bladder, neurogenic; infection; humanT-lymphotropic virus 1; propantheline.

Introdução

O Vírus Linfotrópico para Células T Humano tipo 1 (HTLV-I) é um retrovírus exógeno humano que foi demonstrado ser o agente etiológico na leucemia de células T do adulto (ATL) e de uma doença neurológica progressiva chamada de mielopatia associada ao

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Paradoxical Coexistence Of Atopic Asthma And Human T-Lymphotropic Virus Type I (HTLV-I) Infection: A Case Report

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Abstract

Background: High levels of IL4 secretion and IgE synthesis characterize allergic respiratory diseases. Individuals infected with HTLV-I virus have spontaneous T-cell proliferation and high levels of IFN gamma production, which are immunological functions associated with a Th1 type of immune response.
Objective: To relate the occurrence of HTLV-I infection in an atopic patient with symptomatic asthma.
Methods: In this case report, the authors relate the presence of two supposedly antagonic immune diseases in

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Original article

Skin reactivity to aeroallergens is reduced in human T-lymphotropic virus type I-infected healthy blood-donors (asymptomatic carriers)

Mail

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Background: A type 2 immune response, characterized by high levels of interleukin-4 and immunoglobulin E synthesis is a hallmark of respiratory allergic diseases. Individuals infected with human T-lymphotropic virus type I (HTLV-I) virus have spontaneous T-cell proliferation and increased interferon γ productions, which are immunological functions, associated with a type 1 immune response.

Objective: To determine the frequency of asthma and rhinitis symptoms and immediate skin reactivity to aeroallergens in HTLV-I infected individuals, compared with noninfected subjects.

Methods: Cross sectional study of 101 HTLV-I infected and 101 control uninfected blood donors, assessed by enzyme-linked immunosorbent assay and Western blot assays. The subjects were age and sex-matched, identified as presenting allergy history by questionnaire, which was complemented by a complete clinical examination and skin prick tests for aeroallergens.

Results: The frequency of atopy was lower in infected than uninfected subjects, 14.9 and 29.7%, ($P = 0.017$), respectively. Skin reactivity to *Dermatophagoides pteronissynus*, *Dermatophagoides farinae* and *Blomia tropicalis* were the most frequently observed among all the tested antigens in both groups. Skin reactivity to histamine was also reduced in the infected individuals compared with uninfected subjects (medians 4.0 vs. 5.0, respectively; $P = 0.0001$). Infection by HTLV-I was found to be a factor of protection to atopy (RP 0.44; $P = 0.005$).

Conclusions: The HTLV-I infection reduces the frequency of respiratory allergy and skin reactivity to aeroallergens.

Key words: allergy; asthma; atopy; human T-lymphotropic virus type I; retrovirus; rhinitis.

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Human T-lymphotropic virus type I (HTLV-I) is a retrovirus highly prevalent in certain endemic areas such as Salvador, Bahia (Northeastern Brazil) where 1.35% of blood donors have circulating antibodies against this virus (1). The HTLV-I infects CD4+ and CD8+ T cells and establishes persistent infection (2). Individuals infected with HTLV-I have spontaneous T-cell proliferation and produce high levels of interleukin (IL)-2 and interferon (IFN) γ, which are immunological functions associated with a type 1 of immune response (2, 3).

Cytokines regulate both the initiation and the maintenance of immune responses against foreign antigens. The balance between Th1 and Th2 cells plays a major role in immunity and hypersensitivity reactions in several diseases (4, 5). Atopy is characterized by an immune system that is biased to type 2 activation. Th2 cells secrete IL-4, which stimulate B-cell proliferation and differentiation to produce immunoglobulin (Ig)E antibodies (6). Broadly stated, naturally occurring infections and microbial exposures might reduce the likelihood of the development of asthma and allergic diseases (4–5).

We have previously demonstrated that HTLV-I infections can suppress type 2 immune response, through suppression of IL-4 production and IgE, reducing immediate skin reactivity in patients co-infected with helminthes (7–9). However, there are evidences that Th2 cells might still be activated during HTLV-I infection despite strong polarization towards type 1 immune response, as we have observed the occurrence of asthma and high levels of IgE has been detected in patients with HTLV-I (10). The viral protein *tax* has been implicated in promoting IL-4 expression (12). In addition, IL-5 has been shown in PBMC supernatants from HTLV-I

Abbreviations. EDSS, Expanded Disability Status Scale; HTLV-I, human T-lymphotropic virus type I; HAM/TSP, HTLV-I associated myelopathy/tropical spastic paraparesis; OR, Odds ratio; PR, Prevalence ratio.

Clinical and Immunological Consequences of Human T Cell Leukemia Virus Type-I and *Schistosoma mansoni* Co-infection

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Human T cell leukemia virus type-I (HTLV-I) infection is associated with spontaneous T cell activation and uncontrolled lymphocyte proliferation. An exacerbated type-1 immune response with production of pro-inflammatory cytokines (interferon-γ and tumor necrosis factor-α) is significantly higher in patients with myelopathy associated to HTLV-I than in HTLV-I asymptomatic carriers. In contrast with HTLV-I, a chronic Schistosoma mansoni *infection is associated with a type-2 immune response with high levels of interleukin (IL-4, IL-5, and IL-10) and low levels of IFN-γ. In this study, clinical and immunological consequences of the HTLV-I and* S. mansoni *infection were evaluated. The immune response in patients with schistosomiasis co-infected with HTLV-I showed low levels of IL-5 ($p < 0.05$) in peripheral blood mononuclear cells cultures stimulated with* S. mansoni *antigen (SWAP) and decreased SWAP-specific IgE levels when compared with patients with only schistosomiasis ($p < 0.05$). Liver fibrosis was mild in all HTLV-I co-infected patients. Immunological response was also compared in individuals who had only HTLV-I infection with those who were co-infected with HTLV-I and helminths* (S. mansoni *and* Strongyloides stercoralis). *In patients HTLV-I positive co-infected with helminths the IFN-γ levels were lower than in individuals who had only HTLV-I. Moreover, there were fewer cells expressing IFN-γ and more cells expressing IL-10 in individuals co-infected with HTLV-I and helminths. These dates indicate that HTLV-I infection decrease type 2-response and IgE synthesis and are inversely associated with the development of liver fibrosis. Moreover, helminths may protect HTLV-I infected patients to produce large quantities of pro-inflammatory cytokines such as IFN-γ.*

Key words: human T cell leukemia virus type-I - *Schistoma mansoni* - co-infection

The human T cell leukemia virus type-I (HTLV-I) is an oncogenic exogenous retrovirus that infects between 10 and 20 million people worldwide (Edlich et al. 2000). HTLV-I is the recognized cause of adult T-cell leukemia (ATL) as well as HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Osame et al. 1986, Uchiyama 1997), but other disorders have been associated with HTLV-I infection. The immunological response in HTLV-I infection is characterized by a spontaneous lymphoproliferation and an exaggerated T cell response with high production of important inflammatory mediators of tissue damage as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and interleukin (IL-6) (Nishimoto et al. 1990, Kubota et al. 1998, Carvalho et al. 2001). Although the pathogenesis of neurological disease associated to HTLV-I is not completely understood, there are various evidences that immunological response participate and is responsible by inducing tissue damage (Hanon et al. 2000, Nagai & Jacobson 2001, Osame 2002). By the other hand, helminthes infections such as strongyloidiasis and in particular a chronic disease caused by infection with *Schistosoma mansoni* are associated with a predominant anti-inflammatory type-2 immune response with increased levels of IL-4, IL-5 and IL-10 and low levels of IFN-γ (Araujo et al. 1996, Finkelman et al. 1997). The high degree of infection and the host's immune reaction to parasite eggs contribute to granuloma formation. Liver fibrosis is the most important pathological finding in schistosomiasis, being registered in about 5% of chronically *S. mansoni* infected patients (Bina & Prata 2003). Although initial experimental studies suggested that type-1 cytokines were associated with granulomatous reaction to *S. mansoni* infection (Leptak & McKerrow 1997, Rezende et al. 1997), its clear from current data that type-2 cytokines play a primary role in inducing fibrosis, whereas the IFN-γ (type-1 cytokine) acts as an endogenous down regulator of the response (Wynn et al. 1994, Chiaramonte et al. 1999a, Jankovic et al. 1999). Simultaneous infection between HTLV-I and *Strongyloides stercoralis* decreases the predominant type-2 immune response in patients with strongyloidiasis (Neva et al. 1998, Porto et al. 2001a) as well as *S. stercoralis*-specific and total IgE antibodies (Neva et al. 1998, Porto et al. 2001b). Moreover, co-infection with HTLV-I is also associated with disseminated and recurrent strongyloidiais (Phelps et al. 1991, Newton et al. 1992). It is known that the prevalence of strongyloidiasis is higher in HTLV-I infected patients than in seronegative controls (Robinson et al. 1994, Hayashi et al. 1997). Based on these observations one of the aims of this study was to determine if HTLV-I infection decrease the type-2 im-

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ATYPICAL CLINICAL PRESENTATION OF STRONGYLOIDIASIS IN A PATIENT CO-INFECTED WITH HUMAN T CELL LYMPHOTROPHIC VIRUS TYPE I

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Abstract. Alterations in the immunologic response induced by human T cell lymphotrophic virus type I (HTLV-I) predispose the development of disseminated strongyloidiasis. We report a case of an atypical clinical presentation of strongyloidiasis in a patient co-infected with HTLV-I causing scrotal and perineal pain and infertility. *Strongyloides stercoralis* was found in the analysis of the sperm and specific therapy for strongyloidiasis was associated with disappearance of the symptoms.

INTRODUCTION

Strongyloidiasis has a worldwide distribution and is one of the most important enteric helminthic infections. Diarrhea, abdominal pain, and less frequently vomiting are the main clinical features of the disease. More recently, an association between human T cell lymphotrophic virus type I (HTLV-I) and disseminated strongyloidiasis has been reported.[1–4] This association is due to decreased immunity against *Strongyloides stercoralis* in patients co-infected with HTLV-I. The high production of interferon-γ (IFN-γ) resulting from HTLV-I infection leads to a decreased synthesis of interleukin-4 (IL-4), IL-5, parasite-specific IgE, and eosinophils, cytokines and cells that are involved in the expulsion and killing of *S. stercoralis*, respectively.[5–7] Herein, we report a patient co-infected with HTLV-I and *S. stercoralis* who sought medical evaluation for infertility. Upon evaluation, *S. stercoralis* was detected in the ejaculate mixed with spermatozoa and in the urine.

CASE HISTORY

A 27-year-old man born in Salvador, Bahia, Brazil had sperm and urine analyses performed to evaluate infertility. On clinical evaluation, the patient complained of mild pain in the scrotum and perineal region. He denied having diarrhea, abdominal pain, or vomiting. There was mild edema in the scrotum and a urologic examination found small varicoceles. An ultrasound examination showed no abnormalities in the abdomen, but a scrotal examination confirmed the clinical finding of varicoceles. A spermatogram showed a normal number of spermatozoae with normal motility and a large number of rhabditiform and filariform larvae and young adult female *S. stercoralis*. Contamination of the sperm by urine was ruled out by the aspect of the material and by the forms of parasite found. While rhabditiform larvae were detected only in the urine, all parasite stages were observed in the sperm. The total peripheral white blood cell count and number of eosinophils were normal. Rhabditiform larvae of *S. stercoralis* were also observed by urine analysis and in the stool. Because of the atypical presentation of *S. stercoralis* infection, HTLV-I serology was performed. A diagnosis of HTLV-I infection was made and confirmed by Western blot.[7] Cytokines were measured in supernatants of mononuclear cells stimulated and not stimulated with *S. stercoralis* antigen. The levels of IFN-γ, tumor necrosis factor-α, IL-5, and IL-13 (Table 1) were measured by an enzyme-linked immunosorbent assay sandwich technique (Genzyme Corp., Cambridge, MA) and the results were expressed in picograms per milliliter based on a standard curve generated using recombinant cytokines. [T1]

The patient was treated with a single dose of cambendazole (5 mg/kg of body weight). The pain in the scrotum and perineal region and edema resolved and the varicoceles were reduced in size. *Strongyloides stercoralis* was no longer seen on a spermatogram, but rhabditiform larvae were present in the stool examined two months after therapy. The patient was re-treated with a single dose of ivermectin (200 μg/kg of body weight) and the results of a repeated spermatogram and stool examinations every 2–3 months remained negative for *S. stercoralis* at one year of follow-up. After therapy, his wife became pregnant, indicating that the infertility had improved.

DISCUSSION

Disseminated *S. stercoralis* infection is a recognized consequence of immune suppression caused by corticosteroids or cytotoxic drugs.[8,9] More recently, severe and recurrent strongyloidiasis has been reported in association with HTLV-I co-infection.[1–4,10–12] In disseminated strongyloidiasis, increases in parasite load from autoinfection leads to systemic migration of *S. stercoralis* to other organs. The lungs, liver, and brain are the most common organs invaded by *S. stercoralis*, leading to severe clinical disease. Herein, we described a patient who complained of scrotal and perineal pain in whom *S. stercoralis* was found in a spermogram. The documentation of *S. stercoralis* larvae and adult worm in the sperm and the improvement of perineal symptoms and signs after specific anti-strongyloides treatment indicated not only the ability of this parasite to infect the genitourinary tract, but

TABLE 1

Levels of IFN-γ, TNF-α, IL-5, and IL-13 in supernatants of lymphocyte cultures stimulated with *Strongyloides stercoralis* antigen*

Cytokines	Levels (pg/ml)	
	Medium	*S. stercoralis* antigen
IFN-γ	70	246
TNF-α	0	0
IL-5	185	184
IL-13	207	191

* Peripheral blood mononuclear cells were stimulated with 5 μg/mL of *S. stercoralis* antigen as previously described.[5] IFN-γ = interferon-γ; TNF-α = tumor necrosis factor-α; IL-5 = interleukin-5.