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No Evidence of Mycoplasmas in Peripheral Blood Mononuclear Cell Fraction of HIV-Infected Patients

Summary: In this study, the prevalence of mycoplasmas in peripheral blood mononuclear cells (PBMC) from HIV-infected individuals was investigated using a mycoplasma genus-specific PCR assay. No mycoplasmas were detected in the PBMC samples from any of the 25 HIV-infected individuals (CDC 2, n = 8; CDC 3, n = 2; CDC 4, n = 15) or ten HIV-seronegative controls. As an internal control, HIV specific sequences were detected in the samples from all HIV-seropositives. These negative results do not support a suggested role of mycoplasmas as co-factor in the progression of HIV infection towards AIDS.

Introduction

In HIV infection, functional defects and deletions in antigen-reactive T cells have been reported to occur more frequently than can be explained by direct viral infection. Therefore, it was suggested that a yet unidentified co-factor could play a role in the development of AIDS. Mycoplasmas are considered as candidates for a role as co-factor. After the initial isolation of *Mycoplasma incognitus,* in fact a strain of *Mycoplasma fermentans* [1], from a patient with AIDS [2, 3], this organism has been found in blood, urine, organs and several other tissues of both AIDS patients and asymptomatic HIV-seropositive individuals [4–6]. Besides *M. fermentans,* also *Mycoplasma genitalium, Mycoplasma pirum* and *Mycoplasma penetrans* have been isolated from HIV-infected individuals [7, 8]. A possible role of mycoplasmas as co-factor was suggested after *in vitro* studies with HIV-producing cell lines. In these studies it was demonstrated that mycoplasmas were able to fuse with T lymphocytes [9, 10], that mycoplasmas enhanced the cytotoxic effects of HIV in cultures of T lymphocytes [11–13], and that elimination of mycoplasmas from HIV-producing cells prevented cell killing without affecting virus replication [14].

Whether mycoplasma infections simply represent opportunistic infections or whether they enhance the pathogenicity of HIV and play a role in the progression to AIDS is still unclear. To study the prevalance of mycoplasmas in PBMC of HIV-infected individuals, we tested PBMC from 25 HIV-seropositives with different stages of disease progression by a mycoplasma genus-specific PCR assay [15].

Patients and Methods

The group of HIV-infected individuals consisted of 25 persons attending the Department of Internal Medicine of the University Hospital Nijmegen. The CDC stages of the 25 HIV-infected individuals were CDC 2 (n = 8), CDC 3 (n = 2), and CDC 4 (n = 15). The $CD4^+$ T-cell numbers varied between 20 and 540 x 10^6/l (median value: 130 $\times$ 10^6 $CD4^+$ cells/l). Eighteen of these HIV-seropositives were homosexual men and two were intravenous drug users (one male and one female). Of the remaining five HIV-seropositives (four male and one female), risk factors were unknown. Ten healthy HIV-seronegative volunteers served as controls.

For PCR analysis, PBMC were isolated from 10 ml of citrated blood by standard Ficoll Hypaque density centrifugation [16]. Nucleic acid was isolated from 1 $\times$ 10^6 PBMC according to standard procedures [17]. One µg of nucleic acid was tested with the mycoplasma genus-specific primers (forward primer GPO-3, 5′-GGGAGCAAACAGGATTAGATACCCT-3′; reverse primer MGSO, 5′-TGCACCATCTGTCACTCTGTTAACCTC-3′), which amplify a 280 bp fragment [15]. These primers were selected from 16S rRNA sequences and have been demonstrated to react with mycoplasmal members from each of the different phylogenetic subgroups [15]. The internal oligonucleotide probe GPO-4, 5′-CTTAAAGGAATTGACGGGAACCCG-3′ [15], was used for Southern blot hybridization. As internal control, 10 µl of nucleic acid was tested with HIV-specific primers (primers SK 145 and SK 431; oligonucleotide probe SK 102; Perkin Elmer, Gouda, The Netherlands). Amplification by PCR and analysis of the amplified samples by agarose gel electrophoresis and Southern blot hybridization were performed as previously described [18]. The thermal profile involved 40 cycles of denaturation at 94° C for 1 min, primer annealing at 55° C for 1 min and primer extension at 72° C for 1 min.

Results

The PCR for mycoplasmas was negative for all PBMC samples of both the 25 HIV-infected individuals and the ten HIV-negative controls, while PBMC from an HIV-negative control spiked with mycoplasmas showed the 280 bp amplification product (data not shown). HIV sequences were detected in the PBMC from all HIV-seropositives, which indicates that the negative PCR results obtained with the mycoplasma genus-specific primers are not due to inhibiting substances or to the quality of the nucleic acid.

Discussion

No mycoplasmas were detected in the PBMC fraction of any of the HIV-infected patients or seronegative controls. The $CD4^+$ cell number as marker of disease progression in

Received: 18 March 1994/Accepted: 7 April 1994

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this study population of HIV-infected patients ranged between 20 and 540 $\times$ 10^6 cells/l, but no mycoplasmas were found. Thus, no evidence was found for a putative role of mycoplasmas in the progression of HIV infection towards AIDS. *Hawkins* et al. [19], *Katseni* et al. [20] and *Bebear* et al. [21], also found no correlation between the presence of *M. fermentans* and the clinical stage of HIV infection. In contrast to our study, however, these investigators found *M. fermentans* in 9–11% of the PBMC samples from both HIV-infected individuals and non-infected controls. It is not clear why *M. fermentans* was detected in approximately 10% of the individuals tested in these studies, while in our study all PBMC samples were negative for *M. fermentans* as well as for other mycoplasmas. Recently, IgG antibodies to *M. penetrans* were found at high frequencies in male homosexuals with AIDS and in HIV-infected asymptomatic homosexuals (37% and 26.5%, respectively), but not in HIV-infected intravenous drug users and HIV-infected hemophiliacs [22, 23]. In our study, the patient group of HIV-infected individuals consisted also mainly of male homosexuals. Nevertheless, no *M. penetrans* was detected. The reason for these conflicting results is unclear. In summary, in this study no mycoplasmas could be detected in PBMC of AIDS patients and asymptomatic HIV-infected individuals. These results do not support the suggested role of mycoplasmas as co-factor in the progression towards AIDS. The possibility that mycoplasmas can affect the progression towards AIDS, however, cannot be excluded on the basis of these results.

Zusammenfassung: Kein Nachweis von Mykoplasmen in der Fraktion peripherer mononukleärer Blutzellen bei HIV-infizierten Patienten. Bei HIV-infizierten Patienten wurde mittels eines Genus-spezifischen PCR-Assay versucht, die Prävalenz von Mykoplasmen in mononukleären Zellen des peripheren Blutes zu bestimmen. In keinem der 25 untersuchten Fälle (CDC-Stadium 2, n = 8; Stadium 3, n = 2; Stadium 4, n = 15) und zehn HIV-seronegativen Kontrollen waren Mykoplasmen in peripheren mononukleären Zellen nachzuweisen. In allen HIV-seropositiven Fällen waren HIV-spezifische Sequenzen mittels PCR nachzuweisen, was als interne Kontrolle dienen kann. Diese negativen Ergebnisse unterstützen die Hypothese nicht, daß Mykoplasmen eine Bedeutung als Kofaktoren der Progression der HIV-Infektion zu AIDS zukommt.

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Book-Review

R. D. Feigin, J. D. Cherry (eds.)

Textbook of Pediatric Infectious Diseases (3rd edition)
Volumes 1 and 2, 2,395 pages plus index (87 pages), numerous figures and tables
W. B. Saunders Company, Philadelphia 1992
Price: about $ 250,–

Eleven years after the publication of the first edition of the "Textbook of Pediatric Infectious Diseases" in 1981, the third edition of this well-known and internationally accepted reference book was published in late 1992.

Within the last decade, several new infectious diseases in children have been described and novel infectious agents have been discovered so that a further extensive revision and expansion of the text was necessary and is appreciated by all specialists working in the field of pediatric infectious diseases. Furthermore, as the editors point out in their preface to this outstanding textbook, the rapid progress of molecular biology and the development of a new "molecular medicine" have provided much information for the understanding of infectious diseases. New diagnostic techniques have been established, and they have, for example, led to the creation of recombinant hepatitis B vaccines, thus improving the prevention of a potentially severe viral infectious disease in children and adults.

All medical progress in pediatric infectious diseases is documented by the contributions of 185 authors who wrote chapters on their specialty. The book is divided into seven major parts that contain 209 individual chapters of variable length. The first part, encompassing eight chapters, deals with general aspects of *Host-Parasite Relationships and the Pathogenesis of Infectious Diseases.* The extensive second part on the *Infection of Specific Organ Systems* is divided into 15 sections according to the anatomical site or general appearance of infections. Starting with the discussion of upper and lower airway infections, this part ends with sections on opportunistic infections and the frequent clinical situation of fever of unknown origin. In part three, the most extensive and central component of this textbook, all relevant *specific microorganisms* that cause infections in children are presented in alphabetical order, in distinct chapters of eight sections (bacterial infections, viral infections, chlamydial infections, rickettsial diseases, mycoplasma, fungal diseases, parasitic diseases and unclassified infectious disease).

The other four parts of the book are shorter, but nevertheless very important and useful for clinical practice. Part four is titled *Infection Control* and is divided into two concise chapters on nosocomial infections and the control of infections in the pediatric hospital. This important part of the book puts emphasis on the existence of active infection control teams in pediatric hospitals, as they are recommended by the Centers for Disease Control and widely accepted in the United States. The chapter on the control of infections contains an excellent comprehensive table on the required hospital precautions for patients with infectious diseases that may be used as a practical guideline.

Part five consists of two instructive chapters on *antimicrobial and antiviral agents* used for the therapy of pediatric infectious diseases. Part six introduces concepts for the *Prevention of Infectious Diseases* and explains the development and practice of active immunizing agents and passive immunization. The final part of this outstanding textbook features *Aids to the Diagnosis of Infection* with rather short chapters on the use of bacteriology, serology and virology laboratories, and two closing chapters on rapid diagnostic techniques.

The book is organized in a systematic manner and is easy to handle when looking for specific information (despite the weight of the two volumes). It is very helpful that the extensive index appears at the end of both volumes. Each chapter of the book provides up to hundreds of relevant current and historical references for further study of the literature.

It may be unnecessary to stress that the third edition of this well-established textbook may serve as an invaluable companion for all pediatricians and physicians whose main interest is focused on the broad field of pediatric infectious diseases. Taking into account the practical value of this two-volume set as a desk reference book, even the high price seems justifiable.

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Infection 22 (1994) No. 3 © MMV Medizin Verlag GmbH München, München 1994