Phagocytic Function of Monocytes in Children with Human Immunodeficiency Virus Type 1 Infection

GRATIELA TARDEI,1* DAN DUCULESCU,2 CHRISTIAN CAPO,3 CARMEN-CRISTINA DIACONU,1 ADRIAN MUTIU,1 JEAN-LOUIS MEGE,3 AND COSTIN EUGEN CERNESCU1

St. S. Nicolau Institute of Virology1 and Victor Babes Hospital of Infectious and Tropical Diseases,2 Bucharest, Romania, and Unité des Rickettsies, CNRS UPRESA 6020, Faculté de Médecine, Marseille, France3

Received 22 July 1999/Returned for modification 30 September 1999/Accepted 14 December 1999

We investigated the phagocytic function of monocytes in 7- to 10-year-old children horizontally infected with human immunodeficiency virus type 1 (HIV-1) in comparison to that in healthy sex- and age-matched controls. CR3-mediated phagocytosis was increased in patients with HIV-associated pulmonary tuberculosis, independently of CD4 counts and p24 antigenemia.

Human immunodeficiency virus (HIV) infection significantly increases susceptibility to common pathogens and favors the occurrence of opportunistic infections. The increased susceptibility to common infections has been related to abnormal antibody response to specific antigens (5, 15), while the occurrence of opportunistic infections is associated with mononuclear phagocyte system dysfunction (4). Indeed, monocytes and macrophages are known to play an essential role in the defense against intracellular microorganisms (10). These cells also represent targets and long-term hosts for HIV replication with subsequent cell function dysregulation (12, 14). Phagocytosis is one of the primary antimicrobial functions of monocytes and macrophages. Unlike the case of Fc receptors, phagocytosis mediated by complement receptors seems to facilitate the intracellular survival of invading pathogens (9).

Pediatric HIV infection with a particular subtype of HIV type 1 (HIV-1), subtype F, is characteristic of the Romanian AIDS epidemic (1, 7). A high rate of tuberculosis (TB) is noted among HIV-infected Romanian children with moderate and severe immune deficiency. A diagnosis of TB in HIV-infected children is difficult, and the occurrence of TB significantly worsens the clinical evolution, suggesting that the Mycobacterium tuberculosis infection is critical in the outcome of pediatric HIV disease.

In the present study, we addressed the question whether monocyte phagocytosis, dependent or not on opsonins, is modified in HIV-1-infected Romanian children. We also investigated the relationship between the clinical, immunological, and virological status of patients and potential monocyte phagocytosis abnormalities.

Forty-one HIV-1-infected children (24 boys and 17 girls), aged 7 to 10 years, and naive of antiretroviral and intravenous immunoglobulin therapy, were studied. Ten sex- and age-matched healthy subjects presenting for hepatitis B virus vaccination were enrolled as controls. Informed consent was obtained from the parents of each child. HIV-1 infection was horizontally acquired in all studied patients, most probably before 2 years of age. The clinical status of HIV-infected children was evaluated in accordance with the 1994 revised Centers for Disease Control (CDC) classification (6). At the moment of testing, 24 of these children were devoid of intercurrent infections (ICI), while 8 had pulmonary TB and 9 had non-TB ICI, i.e., bacterial pneumonia, oropharyngeal candidiasis, otitis media, or dysentery. CD4 counts were determined with the Manual CD4 Count Kit (Coulter, Miami, Fla.), and p24 antigenemia was measured after immune complex dissociation (ICD) with an HIV-1 p24 antigen enzyme-linked immunosorbent assay and ICD Prep Kit (Coulter). Monocyte phagocytosis was assessed by an in vitro test as previously described (13).

Briefly, monocytes were isolated by Ficoll gradient centrifugation followed by adherence to Lab-Tek chamber slides (Nunc, Naperville, Ill.). Zymosan was extemporaneously labeled with fluorescein isothiocyanate and then opsonized with autologous serum. Duplicate wells of adherent monocytes were incubated for 30 min with a 1-mg/ml concentration of fluorescein isothiocyanate-zymosan in RPMI 1640 medium and then washed with cold phosphate-buffered saline to remove unattached particles, fixed with formaldehyde, and counterstained with Evans blue dye. Slides were examined with a fluorescence microscope by an investigator unaware of the clinical status of the tested subjects. At least 150 cells were counted for each test well. Cells having internalized at least one particle were considered positive and were counted along with their associated zymosan particles. The phagocytosis index (PI) was defined as the number of particles internalized by 100 positive cells. Statistical analysis was conducted with a two-tailed Student t test and Pearson correlation.

We used unopsonized and opsonized zymosan to investigate mainly the complement receptor 3 (CR3)-mediated monocyte phagocytosis since these particles bind to distinct domains of CR3. The β-glucans, which are the major carbohydrate component of zymosan, bind to the lectin-binding domain of the CR3 α chain, while opsonized zymosan particles also bind the α chain of CR3 to its interactive domain (I domain) (16). Phagocytosis of zymosan particles by monocytes was studied in HIV-infected children and healthy controls, and no significant differences were seen when they were globally analyzed. Opsonization increased zymosan phagocytosis by monocytes similarly in HIV-infected children and controls. We did not find significant differences between either PI values or percentages of monocytes ingesting zymosan particles among patients and controls. The CD4 counts significantly correlated with p24 antigenemia (r = −0.3497, P < 0.05) and...
TABLE 1. CD4 counts and ICD p24 antigenemia in HIV-infected children and controls

| Group of subjects | CD4 count (10^6 cells/liter) | ICD p24 Ag (pg/ml) |
|-------------------|-----------------------------|-------------------|
| HIV infected (n = 41) split by: | | |
| CDC category | | |
| N + A (n = 5) | 1,004 (333) | 23 (4) |
| B (n = 23) | 324 (239) | 42 (25) |
| C (n = 13) | 105 (123) | 145 (188) |
| ICI | | |
| Free of ICI (n = 24) | 382 (396) | 119 (177) |
| With non-TB ICI (n = 9) | 367 (300) | 58 (24) |
| With TB (n = 8) | 173 (144) | 62 (60) |
| Control (n = 10) (healthy children) | 1,232 (294) | |

* Values represent the means of each category followed by standard deviations in parentheses.

b Antigen.

with the CDC clinical category (Table 1), but there was no significant correlation between phagocytosis parameters and either CD4 counts or p24 antigenemia.

The relationship between the ICI status of patients and monocyte phagocytosis was then investigated. Phagocytosis parameters for unopsonized and opsonized zymosan were similar in children without ICI, children with non-TB ICI, and healthy controls (Fig. 1). In contrast, we found a significant increase in the uptake of unopsonized and opsonized zymosan in HIV-infected children with TB in comparison to that in controls and HIV-infected children either with non TB-ICI or without ICI (Fig. 1). The fact that six of these children fell into clinical category C and two fell into the clinical category B suggests that pulmonary TB rather than the clinical stage of HIV infection affects the phagocytic ability of monocytes.

Our study shows that CR3-mediated monocyte phagocytosis is not significantly affected in Romanian children infected with HIV-1 who are naive of antiretroviral and intravenous immunoglobulin therapy, provided that they do not experience TB infection. A pathway-specific increase in CR3-mediated monocyte phagocytosis was seen only in HIV-infected children experiencing pulmonary TB. Previous reports show that monocyte phagocytosis in HIV-infected adults is normal or reduced compared to that in healthy controls (3, 8, 11). The monocyte phagocytosis has been found to be amplified in less-symptomatic HIV-infected adults (2, 8). The CD4 count of adults with AIDS (8) and the viral load of HIV-infected individuals (3) have been shown to correlate with the phagocytic activity of monocytes. However, our results did not show a significant correlation between phagocytosis parameters and the virological and immunological markers. The mechanisms of the increased CR3-mediated phagocytosis observed in HIV-infected children with TB could be explained by CR3 overexpression and/or by a conformation-dependent increase of CR3 avidity. These situations might be induced by cytokines released in response to M. tuberculosis infection. Since M. tuberculosis enters monocytes and macrophages via complement receptors, it is predictable that the increase in CR3-mediated phagocytosis could favor the intracellular uptake of bacteria and the subsequent development or dissemination of TB. We believe that our results should trigger further assessment of monocyte phagocytosis as a potential tool in predicting and managing opportunistic infections during pediatric HIV infection.

This work was supported by the Romanian Academy’s grant GAR 106/1997.

REFERENCES

1. Apetrei, C., A. Necula, C. Holm-Hansen, I. Loussert-Ajakia, I. Pandrea, C. Cozmi, A. Streinu-Cercel, F. R. Pascau, E. Negut, G. Molnar, M. Duca, M. Pece, F. Brun-Vezinet, and F. Simon. 1998. HIV-1 diversity in Romania. AIDS 12:1079–1085.

2. Bandres, J. C., J. Trial, D. M. Musher, and R. D. Rossen. 1993. Increased phagocytosis and generation of reactive oxygen products by neutrophils and monocytes of men with stage 1 human immunodeficiency virus infection. J. Infect. Dis. 168:75–83.

3. Baqui, A. A., T. F. Meiller, M. Zhang, and W. A. Falkler, Jr. 1999. The effects of HIV viral load on the phagocytic activity of monocytes activated with lipopolysaccharide from oral microorganisms. Immunopharmacol. Immunotoxicol. 21:421–438.

4. Bender, B. S., B. L. Davidson, R. Klein, C. Brown, and T. C. Quinn. 1988. Role of the mononuclear phagocyte system in the immunopathogenesis of human immunodeficiency virus infection and the acquired immunodeficiency syndrome. Rev. Infect. Dis. 10:1142–1154.

5. Bernstein, L. J., H. D. Ochs, R. J. Wedgwood, and A. Rubinstein. 1985. Defective humoral immunity in pediatric acquired immune deficiency syndrome. J. Pediatr. 107:352–357.

6. Centers for Disease Control and Prevention. 1994. 1994 revised classification system for human immunodeficiency virus infection in children less than 13 years of age. Morbid. Mortal. Weekly Rep. 43:1–7.

7. Cernescu, C. E., G. Tardei, A. Necula, S. M. Ruta, and C.-P. Pau. 1994. The serologic significance of F viral genotype for human immunodeficiency virus type 1 epidemic. J. Infect. Dis. 170:1043–1044.

8. Dohmeyer, T. S., B. Raffel, J. M. Dobmeyer, S. Findhammer, S. A. Klein, D. Bolte, D. Roether, R. Ross, and R. H. Ross. 1995. Decreased function of monocytes and granulocytes during HIV-1 infection correlates with CD4 cell counts. Eur. J. Med. Res. 1:9–15.

9. Kaufmann, S. H. 1993. Immunity to intracellular bacteria. Annu. Rev. Immunol. 11:29–163.

10. Langemans, J. M. Hazenbos, and R. Van Furth. 1994. Antimicrobial functions of mononuclear phagocytes. J. Immunol. Methods 174:185–194.

11. Roilides, E., A. Holmes, C. Blake, P. A. Pizzo, and T. J. Walsh. 1993. Defective antifungal activity of monocyte-derived macrophages from human immunodeficiency virus-infected children against Aspergillus fumigatus. J. Infect. Dis. 168:562–565.

12. Roulston, A., R. Lin, P. Beauparlant, M. A. Wainberg, and J. Hiscott. 1995. Regulation of human immunodeficiency virus type 1 and cytokine gene expression in myeloid cells by NF-κB/Rol transcription factors. Microb. Infect. 5:481–505.

13. Sanguedolce, M. V., C. Capo, B. Rongrand, and J. L. Mege. 1992. Zymosan-stimulated tumor necrosis factor-α production by human monocytes. Down-modulation by phorbol ester. J. Immunol. 140:2229–2236.

14. Schuitmaker, H., R. Matthijs, and L. Thomsen. 1996. Viral and cellular determinants of HIV-1 replication in macrophages. AIDS 10(Suppl. A):S25–S32.

15. Szczek, K. M., C. Mitcheltree, L. R. Roberts, and E. R. Stiehm. 1992. Deficient polymorphonuclear cell and mononuclear cell antibody-dependent cellular cytotoxicity in pediatric and adult human immunodeficiency virus infection. J. Infect. Dis. 166:486–493.

16. Thornton, B. P., V. Vetvicka, D. M. Pitman, R. C. Goldman, and G. D. Ross. 1996. Analysis of the sugar specificity and molecular location of the β-glucan binding lectin site of complement receptor type 3 (CD11b/CD18). J. Immunol. 156:1235–1246.