Prognostic Value of Soluble Intercellular Adhesion Molecule-1 (s-ICAM-1) in HIV-Infected Children

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Central events in the host defence system and immune-mediated damage are tightly regulated by cell adhesion molecules. Sera from 28 human immunodeficiency virus (HIV)-1 infected children divided into groups according to disease severity, six seroreverting (SR) children and 25 healthy controls were studied to detect the presence of soluble intercellular adhesion molecule-1 (s-ICAM-1). Soluble ICAM-1 levels were found to be significantly increased in HIV-infected children in comparison with SR children or healthy controls. Levels of soluble ICAM-1 were higher in patients with severe forms of HIV-infection than in those with a milder form of the disease. Significant differences in titers of s-ICAM-1 were recorded between SR children and HIV-infected children with mild disease or healthy controls. There was a significant correlation between s-ICAM-1 levels and the concentrations of beta 2 microglobulin (β2m) and, to a lesser extend, immunoglobulin A levels (IgA). Soluble ICAM-1 levels didn't change considerably in HIV-infected children in stable clinical conditions, independently of their clinical stage of the disease, during a follow-up period of 9–12 months. Conversely, s-ICAM-1 levels increased simultaneously with the appearance of new well-defined clinical disorders or decreased during the improvement of clinical conditions. A significant negative correlation was recorded between the titers of the s-ICAM-1 and the $CD4^+$ T-cell levels. These results suggest that the s-ICAM-1 might be another useful tool to evaluate disease progression.

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INTRODUCTION

The intercellular adhesion molecule 1 (ICAM-1), a member of the immunoglobulin superfamily, is expressed on endothelial cells, some epithelial cells, and is present in an intracellular pool in monocytes [1–3]. Activation of lymphocytes results in an increase in ICAM-1 on T and B lymphocytes [4]. Treatment of fibroblasts and endothelial cells with inflammatory mediators such as interleukin (IL)-1, tumour necrosis factor (TNF)-α, interferon (IFN)-γ and lipopolysaccharide results in a dramatic up-regulation of ICAM-1 on the surface of these cells [5]. During immune and inflammatory responses ICAM-1 promotes a leukocyte adhesion by binding to its ligands the leukocyte-function associated antigen-1 (LFA-1), macrophage-1 antigen (MAC-1) and CD43 [6–8].

ICAM-1 is not only present in a membrane-bound, but also in a soluble form which contains all five extracellular domains of membrane-bound ICAM-1 [9]. Soluble ICAM-1 (s-ICAM-1) may thus result from a proteolytic cleavage of cell-bound ICAM-1 close to the cell membrane.

In vitro stimulation of human umbilical vein endothelial cells with cytokines not only increases the cell membrane expression of ICAM-1 but also results in the release of s-ICAM-1, which increases in parallel with the ICAM-1 cell-surface expression [10]. The release of s-ICAM-1 is not restricted to endothelial cells of different vascular beds, but has

Table 1. Clinical characterization of HIV-infected children

Patient	Age (years)	Sex	Disease category	Clinical conditions	Antiretroviral therapy
1	6	M	MD	no symptoms	–
2	2	M	MD	no symptoms	–
3	8	F	MD	no symptoms	–
4	1	M	MD	no symptoms	–
5	6	F	MD	no symptoms	–
6	2	M	MD	Diarrhoea	–
7	5	F	MD	lymphadenopathy	AZT
8	2	F	MD	lymphadenopathy	AZT
9	4	F	MD	no symptoms	AZT
10	1	F	MD	asthma, diarrhoea	AZT
11	2	M	MD	no symptoms	AZT
12	7	M	MD	no symptoms	–
13	7	F	MD	no symptoms	AZT
14	7	F	MD	flu	nevirapine, ddC, nelfinavir
15	10	M	MD	no symptoms	d4T, ritonavir, ddC
16	9	M	MD	no symptoms	ddC, nelfinavir, nevirapine
17	1	F	MD	no symptoms	AZT, ddI, nevirapine
18	7	M	MD	no symptoms	AZT
19	2	M	SD	hepatomegaly	AZT
20	9	M	SD	undernourishment, candidiasis	AZT
21	3	M	SD	pneumonia	AZT
22	3	F	SD	undernourishment, LIP	AZT
23	5	F	SD	pneumonia, *Herpes zoster*, undernourishment	AZT
24	1	F	SD	Chronic Hepatitis	AZT, ddI
25	2	M	SD	disseminated bacillus Calmette Guerin infection	AZT, ddI
26	10	M	SD	Cryptosporidiosis	ritonavir, indinavir
27	7	M	SD	microangiopathy	AZT, ddI, indinavir, ritonavir
28	7	F	SD	undernourishment, *Mycobacterium avium*	AZT, ddI, indinavir, efavirenz

F, female; M, male; MD, mild disease; SD, severe disease; AZT, zidovudine; ddI, didanosine, ddC, dideoxycytidine; d4T, stavudine.

also been reported to occur *in vitro* in human peripheral blood mononuclear cells (PBMC) [9] and various types of human nonhaematopoietic cells, such as synovial cells [11], hepatocytes [12], and several carcinoma cells [13], as well as melanoma cells [14].

Comparison of levels of specific s-CAMs might provide a better understanding of a particular pathology. While s-ICAM-1 can be detected in the circulation of normal human individuals [9], there is now quite a large number of studies to demonstrate that levels of s-ICAM-1 in various human body fluids are elevated in a wide variety of inflammatory and infectious diseases, as well as in cancers [15–21].

Several studies have reported changes in s-ICAM-1 in adult HIV-1 infected patients [22–25] but, to the author's knowledge, there have been no reports in children.

In the study presented herein, s-ICAM-1 levels were measured in HIV-infected children with different degrees of disease evolution.

The s-ICAM-1 levels were associated with parameters of immune activation and disease progression in order to determine if s-ICAM-1 could be of any predictive value in the course of the disease.

PATIENTS AND METHODS

Study population. Serum samples were collected from paediatric HIV-1 infected patients treated at the authors' College Hospital. The group studied comprised 28 HIV-1 infected patients (13 males, 15 females, aged 9 months to 7 years) and six children born from HIV-infected mothers that have later become HIV-antibody negative (SR children aged 6–11 months). HIV-infection was confirmed by ELISA and Western blot analysis; vertical transmission was observed in all subjects. Patients were selected on the basis of their clinical status according to the CDC 1994 paediatric classification [26]. HIV-1 infected children were divided into the following groups: children with nonsymptomatic or mildly symptomatic disease (category MD, $n = 18$) and children with severely symptomatic disease (category SD, $n = 10$). All symptomatic HIV-infected children were under antiretroviral therapy with reverse transcriptase inhibitors and/or protease inhibitors. The clinical features of HIV-infected children studied are shown in Table 1. Control samples were obtained from 25 HIV-1 seronegative healthy children, aged 1–8 years, among the population coming to the hospital for vaccination. Informed consent was obtained from the parents of all children included in the study.

Determinations of levels of s-ICAM-1, IgA and β2 m. Serum levels of s-ICAM-1 were measured by a two site enzyme-linked immunosorbent assay kit (R & D System, Minneapolis, USA), according to the manufacturers instructions. Serum IgA levels were evaluated by nephelometry (QM 300, Sanofi Pasteur, Marne la Coquette, France), whereas β2m concentrations were determined by a radial immunodiffusion assay (The Binding Site, Birmingham, UK).

CD4+ T-cell count in HIV-infected children. Relative values of $CD4^+$ and $CD8^+$ were evaluated by double-labelling studies. One hundred μl of heparinezed whole blood were incubated with CD4-PE and CD3-fluorescein isothiocyanate (FITC) or CD8-PE and CD3-FITC for 30 min at room temperature. Staining with nonrelated isotype controls showed the nonspecific fluorescence. All monoclonal antibodies (MoAbs) were obtained from Becton Dickinson (Mountain View, CA, USA). After incubation the samples were lysed with FASCS lysing solution. Cells were fixed with 1% paraformaldehyde, 1% sodium cacodylate-NaCl for at least 15 min and 10 000 cells per tube were analyzed with a Becton-Dickinson FACScan flow cytometer (Becton-Dickinson). Data were analyzed by using the Simulset Software (Becton Dickinson). The percentage of $CD3^+CD4^+$ or $CD3^+CD8^+$ T-cells was determined in the patients studied.

Statistical analysis. The Mann–Whitney *U*-test was used to compare levels of s-ICAM-1, IgA and β2m in the different groups evaluated. A correlation analysis was obtained by Spearman rank method. $P < 0.05$ was considered statistically significant.

RESULTS

Levels of s-ICAM-1 were measured in sera from HIV-1 infected children, either with a mild or a severe disease, and compared with levels either in aged-matched noninfected children, or SR children.

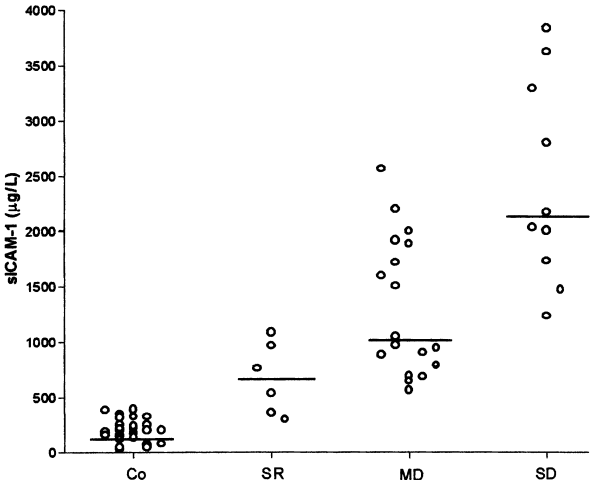

Fig. 1. Levels of s-ICAM-1 in sera from children with mildly symptomatic (MD, $n = 18$), or severely symptomatic (SD, $n = 10$) HIV-infection. The levels of s-ICAM-1 were determined in sera from HIV-infected children, seroreverting (SR) children ($n = 6$, see Patients and Methods) or noninfected, age-matched controls (C0, $n = 25$). Each point represents one patient; the groups median are shown as well. Statistical analysis was performed using the Mann–Whitney *U*-test. Levels of s-ICAM-1 were higher in HIV-infected children compared with SR children or healthy controls ($P < 0.05$). s-ICAM-1 levels were significantly higher in SD compared with MD ($P < 0.05$) and SR ($P < 0.05$). Differences in titers of s-ICAM-1 between SR children and MD ($P < 0.05$) or healthy controls ($P < 0.01$) were statistically significant.

Soluble ICAM-1 levels were found significantly increased in HIV-infected children (median s-ICAM-1 = 1162 μg/l), in comparison with healthy controls (median s-ICAM-1 = 200.5 μg/L; $P < 0.0001$) or SR children (median s-ICAM-1 = 706 μg/L; $P < 0.05$). Levels of s-ICAM-1 were higher in patients with severe forms of HIV-infection than in a milder form of the disease (Fig. 1). Significant differences in titers of s-ICAM-1 were recorded between SR children and HIV-infected children with mild disease or healthy controls (Fig. 1).

The relationship between the levels of s-ICAM-1 and parameters of immune activation has also been examined. HIV-infected children showed significantly higher ($P < 0.05$) IgA (median = 1190 mg/l) and β2m (median = 3.66 mg/l) levels compared with noninfected controls (IgA = 593 mg/L; β2m = 1,90 mg/l). A significant correlation was found between s-ICAM-1 levels and the concentrations of β2m ($r = 0.48$; $P < 0.05$) whereas a low correlation was found with IgA levels ($r = 0.35$; $P < 0.05$) (Fig. 2).

In order to evaluate the usefulness of s-ICAM-1 as prognostic indicator, s-ICAM-1 levels were determined in nine HIV-infected children and compared with $CD4^+$ T cells levels every 3 months during a follow-up period of 9–12 months.

Soluble ICAM-1 levels didn't change considerably in HIV-infected children in stable clinical conditions, independently of their clinical stage of the disease (Fig. 3A and B). In contrast, levels of s-ICAM-1 increased at the same time as the appearance

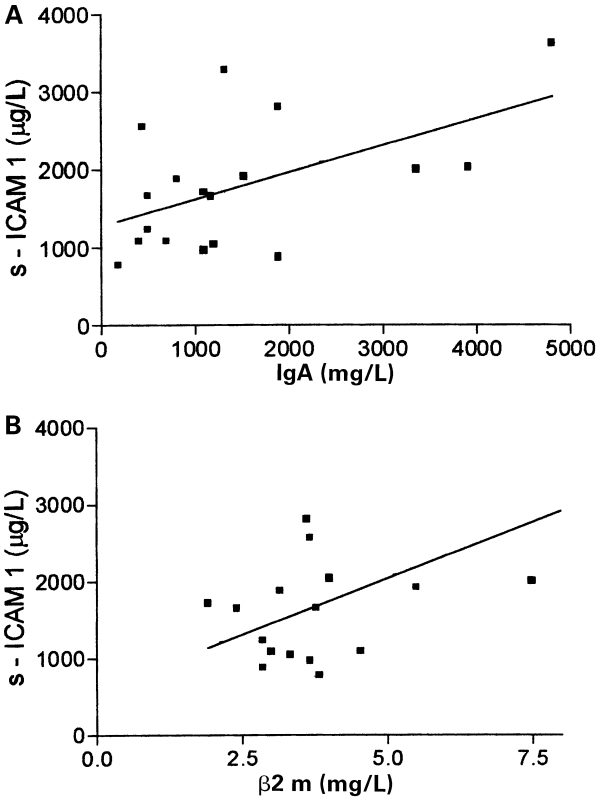

Fig. 2. Correlative analysis of s-ICAM-1, immunoglobulin (Ig)A and beta 2 microglobulin (β2m) serum levels. The Spearman rank test was performed to establish the correlation between serum s-ICAM-1, IgA and β2m levels in 28 HIV-1 infected children. There was a significant correlation between serum s-ICAM-1 and both IgA ($r = 0.35$; $P < 0.05$) and β2m ($r = 0.48$; $P < 0.05$) levels.

of a hepatitis C infection. Later on, levels of s-ICAM-1 decreased along with disease activity. In the same way, in another case with severely symptomatic disease, s-ICAM-1 levels increased simultaneously with the development of microangiopathy (Fig. 3B). Patients showing an improvement in clinical conditions considerably decreased s-ICAM-1 levels over time (Fig. 3A).

A negative correlation was recorded between the titers of s-ICAM-1 and $CD4^+$ T cells levels in HIV-infected children under follow-up ($r = -0.63$; $P < 0.001$).

DISCUSSION

Data are presented showing that serum levels of s-ICAM-1 are elevated in HIV-1 infected children and correlated with disease severity. These results are in accordance with a previous report showing increased levels of s-ICAM-1 in adult patients with a severe form of HIV-1 infection [27–29]. However, HIV-associated immune activation and the course of infection may differ in HIV-infected children with regard to adults, because most children are infected perinatally, HIV being introduced into a naive and developing immune system. The child immune system is generally less responsive to viruses and shows immunophenotypic evidence of immaturity. Then, information obtained from studies performed in adults cannot be extrapolated to the paediatric population [30].

Soluble forms of CAMs are released into serum, either because of shedding from the endothelial cell surface or differential mRNA splicing to form a truncated, soluble form with noncytoplasmic anchoring sequence [10]. The amounts of soluble vascular cell adhesion molecule-1 (s-VCAM-1), s-ICAM-1, s-E-selectin and soluble platelet selectin (s-P-selectin) released showed a direct correlation with cell surface expression of these molecules [21,31]. Thus, high levels of s-ICAM-1 recorded in sera from HIV-1 infected children suggest the increased expression of such molecule on the cell surface. Alternatively, s-ICAM-1 might be released from damaged or inflamed tissue [9].

An issue of major importance when determining levels of soluble adhesion molecules is whether or not these soluble adhesion molecules detected are biologically active. Furthermore, there may be a difference in the systemic and local biological activity of shed adhesion molecules.

With regard to the possible physiological roles of circulating adhesion molecules, two major concepts have emerged, which are not mutually exclusive. First, if the shed molecules retain

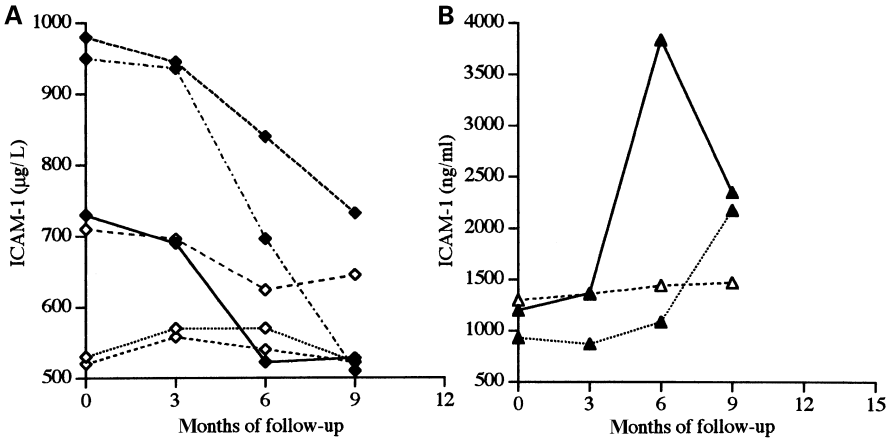

Fig. 3. Fluctuation of the levels of s-ICAM-1 in the clinical course of HIV-infection. Serum levels of s-ICAM-1 were evaluated in six HIV-infected children with mild disease (A) and three children with severe disease (B) every 3 months during a follow-up period of 9–12 months. Open symbols represent HIV-infected children in stable clinical conditions whereas children showing new clinical disorders or health improvement during the time of follow-up are represented by the closed symbols.

their ability to bind their specific ligands on leukocytes, the release of these adhesion molecules may induce a decrease in the potential adhesiveness of leukocytes by competing with the membrane-bound receptors for their ligands. Indeed, it has been suggested that s-ICAM-1 may play a role in downregulating adhesive interactions between the leukocytes and the membrane-bound form of this adhesion molecule [11]. In this context, increased s-ICAM-1 levels in HIV-1 infected patients might have a beneficial effect by regulating adhesion. Secondly, soluble adhesion molecules can act as costimulatory factors, triggering a response in a ligand-bearing cell. s-ICAM-1 has been demonstrated to deliver chemokinetic signals to lymphocytes and to enhance cytokine production and T-cell proliferative responses stimulated by alloantigen in mixed lymphocyte cultures [32]. From a clinical perspective, the issue whether elevated circulating levels of soluble adhesion molecules in pathological inflammatory conditions are either of benefit for, or detrimental to patients, is still open.

ICAM-1 may be relevant in the pathogenesis of HIV-1 infection because it was claimed that the ICAM-1/LFA-1 adhesion pathway might be involved in a HIV-1 cytopathic mechanism of syncytia formation, as well as in the spread of the virus by cell–cell transmission [33–35]. In addition, LFA-1/ICAM−1 interaction has been reported to be a potent costimulus for antigen-specific activation of resting T cells and to mediate lymphocyte sequestration in lymphoid tissue [25, 36].

Increased levels of s-ICAM-1 were found in SR children. Considering that the SR children were younger than 1 year, the s-ICAM-1 levels could have been transferred by the placenta from the mother. However, little is known about the clearance of circulating adhesion molecules.

Our findings concerning the correlation between levels of s-ICAM-1 and β2m, points to a role of s-ICAM-1 as a marker of immune activation, because β2m appeared to be a well-established marker of immune activation in HIV-infection [37–39]. However, the relatively poor correlation observed, in particular with IgA, suggests that the expression of these molecules could be affected by different stimuli.

There are conflicting reports regarding the correlation between ICAM-1 levels and CD4 cell counts in adults [23, 24, 28, 29]. The association between the levels of s-ICAM-1 and $CD4^+$ counts as well as the fact that the s-ICAM-1 increased along with the appearance of concurrent diseases suggest that s-ICAM-1 might be a valuable prognostic indicator in confirmed paediatric HIV-infection. Moreover, two out of the three children with severe disease whose s-ICAM-1 levels were evaluated during a follow-up period, died at the end of this study. Conversely, three patients showing improving clinical conditions during the follow-up showed decreased s-ICAM-1 levels.

It has been shown that the expression of ICAM-1 was dramatically reduced during antiretroviral therapy [25] suggesting that the ICAM-1 levels are increased because of the HIV infection. Likewise, the three children showing a striking decrease in s-ICAM-1 during follow-up were under treatment with three different antiretroviral drugs. However, increased s-ICAM-1 levels recorded in SR children suggests that the s-ICAM-1 could be useful in confirmed HIV-infection.

Both adult and paediatric studies have during the last 5 years clearly demonstrated the prognostic significance of plasma RNA and CD4 [39–42] levels in the course of HIV-infection. Indeed, these markers have become the cornerstones of our understanding of HIV pathogenesis and the basis of current treatment strategies. However, the use of s-ICAM-1, might be another useful tool to evaluate disease progression.

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