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The Interferon Antagonist Sarcolectin in the Progress of HIV-1 Infection and in AIDS

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ABSTRACT

Sarcolectin (SCL) is a nonspecific stimulator of cellular DNA synthesis that was found in all animal sera tested to date. It inhibits the established interferon (IFN)-dependent antiviral state, restoring cells to their normal status. In this study, we examined the excretion/secretion of the IFN antagonist SCL in sera from healthy donors and in sera collected during different periods of human immunodeficiency virus type 1 (HIV-1) infection. We followed HIV-1-infected patients during all stages of development (seroconversion, initial and advanced phases of AIDS) and found a significant increase in SCL in sera of HIV-infected patients compared with seronegative subjects used as controls. This increase was established during seroconversion, and then the titers leveled off. In the final stage of the disease, the SCL titer increased again very significantly. We attribute this rapid rise to the virus-dependent destruction of T cells that can no longer be repaired. The high SCL level observed at this final stage, which is most predictive of the disease's progression, suggests that the action, rather than the production, of IFN is impaired.

INTRODUCTION

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) infection elicits a broad range of host responses and is associated with dysregulation of cytokine production.[1] Thus, a number of cytokines—interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), interferon-α (IFN-α), and IFN-γ—are produced at an increased level *in vivo*.[2]

IFNs are pleiotropic cytokines that exhibit multiple biologic effects on cells and tissues, and they have long been recognized as antiviral agents. Further investigations into the role of these cytokines are underway. Their action involves binding to the IFN cell membrane receptor and initiation of an intracellular signal transduction cascade. As an antagonist of IFNs, sarcolectin (SCL) blocks the synthesis of secondary proteins promoted by IFN, which restores to the cells the capacity to stimulate DNA synthesis. Thus, virus sensitivity in the cells reappears.[3]

We show here that SCL may play a role in HIV-1 pathogenesis by taking into account the repeated immune challenges to which HIV-1-infected patients are repeatedly exposed. We hypothesize that an SCL–IL-2–IFN cycle operates during normal T cell clonal expansion. The study of this cycle could help to improve our understanding of the HIV-1-induced pathologic processes. This, in turn, would put us on the path to therapy for restoring the balance between these three different proteins. A preliminary report on this subject was published earlier.[4]

MATERIALS AND METHODS

Polyclonal monospecific, monoclonal, and oligopeptide antibodies

The specific antibodies raised against the N-acetyl neuraminic acid (NANA) chromatography-purified 55-kDa SCL, as described in previous studies, were used.[5] We also employed antibodies to oligopeptides SCL 41–55, 81–95, and 455–469 synthesized by Neosystem (Strasbourg, France). In some experiments, commercial K2C7 monoclonal antibodies (mAb) were used (Sigma C-6417) because of the close structural relationship between SCL and mesothelial cytokeratin K2C7 as control.[6]

ELISA assay

ELISA assay was performed by the routine procedure. The antigen was found to 96-well EIA/RIA flat-bottom plates (Costar, Cambridge, MA) by incubating the preparation overnight at 4°C or 2 h at ambient temperature. Unbound material was discarded, and the plates were washed three times

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with 0.05 M carbonate-bicarbonate buffer, pH 9.6. To eliminate unspecific binding, wells were blocked with 5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) added at 37°C for 2 h. After washing three times with PBS containing 0.05% Tween 20, primary antibodies at 1:100 were added and incubated 1 h at 37°C. Bound antibody was probed with a secondary antibody (Amersham antirabbit Ig F(ab′) at a 1:2000 dilution) (Amersham, Arlington Heights, IL) for 1 h after three washings. For controls, three preparations were employed, a negative control at the highest concentration of the antigen for which the specific 55-kDa was omitted or replaced by normal rabbit serum and two positive controls: (1) serial dilutions of BSA used as reference antigen to exclude nonspecific binding due to SCL and (2) a known positive reference SCL control antigen from a pool of normal sera. The plates were washed three times, and enzymatic reactions were carried out at ambient temperature by adding H_2O_2 and *o*-phenylenediamine (Abbott S.A., France). Reactions were stopped after 4 min by addition of 100 μl of 2 N H_2SO_4. Color development was measured bichromatically at 490 and 630 nm.

Immunoblotting analysis

Specimens for the detection of SCL were submitted to 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using standard methods. Proteins were transferred onto nitrocellulose membranes via electroblotting. Membranes were blocked 2 h with PBS containing 0.05% Tween 20 and 5% skim milk at ambient temperature and then incubated with primary antibodies overnight at 4°C (or 2 h at ambient temperature). The following antibodies were used: antibodies raised against NANA-purified SCL antigens, oligopeptide antibodies synthesized according to the SCL sequences listed above, or commercial mAb K2C7 (Sigma C-6417) as reported[6] (all diluted 1:200). Bound antibody was probed with a second antibody combined with horseradish peroxidase (HRP) monkey antirabbit IgG (Amersham Pharmacia Biotech RPN 1064, Paris, France) at a final dilution of 1:20,000 in PBS-Tween for 1 h. Immunoreactive proteins were visualized using an enhanced chemoluminescence (ECL) detection kit (Amersham Pharmacia Biotech) according to the supplier's instructions.

RESULTS

Study subjects

Patients from the Hôpital Saint Antoine in Paris, who form the majority of the study population, were divided in these early studies into four categories according to the state of development of the HIV infection: (A) 60 HIV-1-negative control patients from the same hospital and in comparable age groups, (B) 13 seroconverted patients (CDC stage I–II), (C) 15 with symptoms usually observed during this phase (CDC stage III), and (D) 16 more advanced cases (CDC stage IV).[7] For all study groups, we calculated the mean value and the standard deviation (SD) for each SCL titration by dose-response assay. Interpretation of the results was supported by statistical analysis using the *r* correlation coefficient.[8] The majority of the sera were obtained from the service of Medecine Interne, Hôpital Saint Antoine in Paris. Some others, from patients in the advanced stages of AIDS, were provided by the service of Professor Lurmuha at the Clinique Universitaire, Kinshasa (R.D.C.).

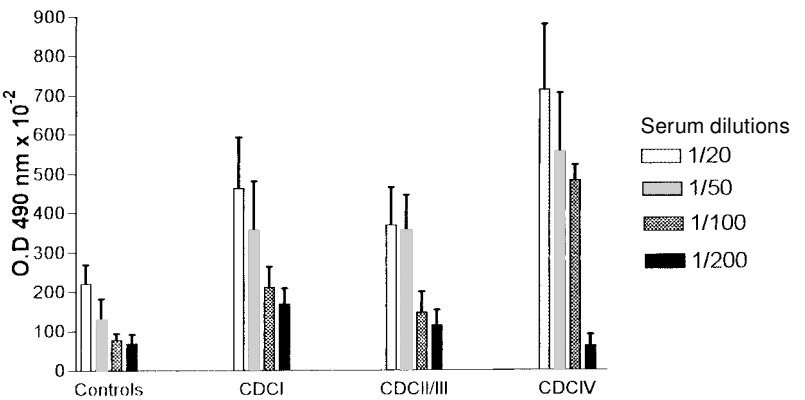

FIG. 1. Serum SCL titers in control subjects and AIDS patients. SCL titers in sera during different phases of the disease. The different stages follow the progression of the disease and are classified as follows: 60 controls; 13 seroconverted patients stage (CDC I–II); 15 CDC stage III patients with generalized lymphadenopathy with or without common symptoms; 16 CDC stage IV patients with opportunistic infections or secondary tumors.[5] The assay was performed using antisera developed against a NANA affinity chromatography highly purified SCL antigen. The SCL concentration increases during seroconversion and progresses with the disease.

Statistical analysis

Stage	*Correlation coefficients*	*Significance*	*Stage*	*Correlation coefficients*	*Significance*
Control/CDC I–II	0.9702	$p < 0.01$	CDC III/CDC IV	0.8175	$p < 0.01$
CDC I–II/CDC III	0.9575	$p < 0.01$	CDC I–II/CDC IV	0.8595	$p < 0.01$

ELISA assay using monospecific antibody developed against the highly purified SCL 55-kDa antigen in rabbits

The results (Fig. 1) show that (1) between primary infection and CDC stage I–II, a highly significant increase in SCL antigen can be detected in all sera of HIV-1-infected patients compared with the control HIV-1-seronegative individuals, (2) throughout CDC I–II and the first phase of CDC III, the SCL titers are stable, (3) during the latter phase of CDC III they decrease slightly, and (4) during CDC IV, the SCL titers increase significantly, progressing with the severity of the disease.

We completed these first experiments with sera raised against the full-length SCL-NANA affinity chromatography-purified antigen, with other tests aimed at increasing immune specificity and defining the different immune reactions. For this purpose, we raised oligopeptide antibodies to selected domains of the cloned gene, as described in Materials and Methods.

Selection, identification, and SCL specificity of polyclonal oligopeptide antibodies directed against three domains of the cloned protein

The structure of the cloned SCL cDNA was reported in a previous publication.[6] Briefly, the protein consists of four α-helices with a β sheet on each extremity. On the amino-end, the β sheet carries most of the lectinic properties, mainly in amino acids 41–55. On the carboxyl-end, the β sheet is very short and has a highly antigenic segment in 455–469. In between, oligopeptides 81–95 are located on the first α-helix. All sera were assayed separately with each of the antibodies raised to the three oligopeptides provided by the cloned gene. As reference, commercial mAb to the monomer of K7 cytokeratin was employed (Table 1).

TABLE 1. ESTIMATION OF SPECIFICITY AND ANTIGENICITY OF ANTIBODIES RAISED AGAINST CLONED SCL OLIGOPEPTIDES IN RABBITS[a]

	Oligopeptides (3 μg/ml)		
Antibody	*41–55*	*81–96*	*455–469*
Oligoantibodies[b]			
41–55	6400	<200	<200
81–96	<200	12800	<200
455–469	<200	<200	12800
Monoclonal	400–800	<200	<200

[a]The ELISA assay and the reference commercial mAb SCL[1] used are described in Materials and Methods. The mouse mAb recognizes only the 41–55 epitope of the cloned protein (OD 490 nm).

[b]Cross-reactivities of 3 different SCL specific oligopeptide antibodies:

41–55 Gly Leu Gly Ala Ser Arg Pro Arg Val Ala Val Arg Ser Ala Tyr

81–95 Asp Pro Phe Ser Gln Arg Val Arg Gln Glu Glu Ser Glu Gln Ile

455–469 Tyr Ser Ile Arg Thr Ala Ser Ala Ser Arg Arg Ser Thr Arg Asp

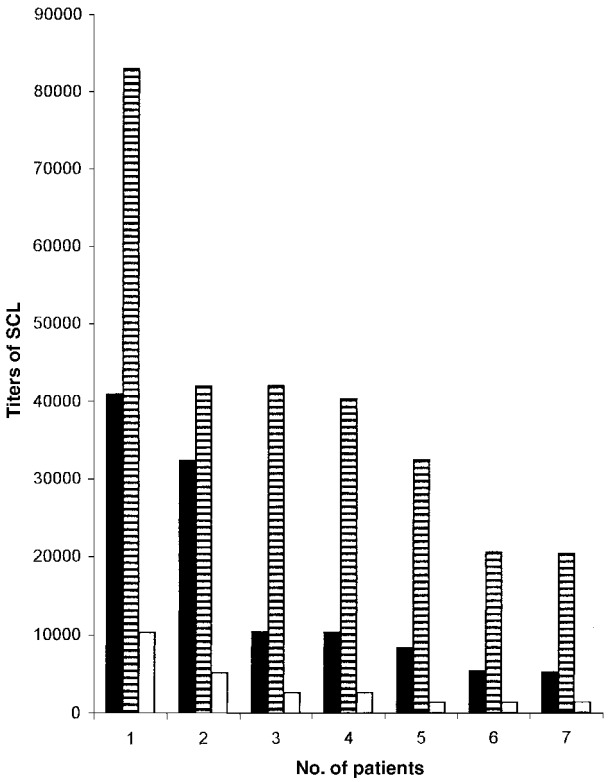

FIG. 2. Affinity of the three different oligopeptide antibodies to seven randomly selected HIV-1-positive sera. Assay of antisera raised against oligopeptides selected from three different domains of the cloned gene: black bars, SCL amino acid end 41–55; open bars, early portion of the α-helical domain, SCL 81–95; striped bars, the last 15 amino acids at the carboxyl-end of the gene 455–469. The three antibodies are assayed with each of the seven HIV-1-positive sera from randomly selected patients. All antibodies react with the SCL protein, but the most antigenic domain is on the carboxyl-end. As shown in another report,[9] these antibodies are also identified with known SCL-positive commercial mAb and SCL-positive serum from an identified osteosarcoma patient.

Evaluation of oligopeptide SCL polyclonal antibodies using seven randomly selected HIV-1-positive patient sera

To evaluate the affinity of the antibodies raised to the different oligopeptide antigens, we studied in an ELISA assay seven randomly selected HIV-1-positive sera. Each was assayed with the three different SCL oligopeptide antibodies. The results are shown in Figure 2. All the antibodies not only recognized their specific oligopeptide domain of the cloned molecule but also reacted with the whole SCL protein, but, as expected, with variable affinity. In all cases, the antibody raised against the terminal oligopeptide 455–469 responded significantly better in the ELISA assay than did the 41–55 antibody. The antibodies directed against amino acids 81–95 to the first helical antigen performed poorly. We, therefore, selected for further studies the highly specific 455–469 terminal amino acid.

TABLE 2. SCL ELISA TITERS IN SERA OF NORMAL DONORS AND OF HIV-INFECTED PATIENTS AT DIFFERENT PHASES OF DISEASE[a]

	Control		*CDC I–II*		*CDC III*		*CDC IV*	
	Donor	*Titer*	n *Patient*	*Titer*	n *Patient*	*Titer*	n *Patient*	*Titer*
1 Experiment 1	AA	640	7	10280	60	2560		
2	31	320	10	10280	80	2560		
3	35	640	12	10280	79	5120		
4	34	1280	19	10280	61	2560		
5	37	1280	31	10280	63	2560		
6 Experiment 2	AA	1280	88	1280	280	2520		
7	31	1280	137	5120	286	5120		
8	33	1280	163	5120	370	5120		
9	35	1280	194	10240	407	20240		
10	37	1280	202	10240	433	10240		
11 Experiment 3	31	1280	8	10240			DD	41960
12	32	1280	13	10240			2	41960
13	33	1280	23	2560			9	10240
14	34	1280	34	1280			10	83920
15	37	1280	42	5120			18	20240
16 Experiment 4	BB	2560	280	5120			47	20240
17	416	650	286	2560			761	2560
18	404	2560	360	5120			763	5120
19	39	650	407	5120			EE	5120
20	CC	650	433	5120			FF	20240
21	633	650	437	10240			12	5120

[a]Four independent experiments are presented. ELISA titers are expressed using dose-response assays. The linearity for each experimental value is controlled. The titer is the dilution that contains at least 100 OD units of SCL. BSA is used as negative control, and the positive control is highly purified SCL oligopeptides. At the 1:6400 dilution, the antibody 455–469 recognizes 0.37 ng SCL antigen. Each experimental set was performed at the same time with the same reagents.

Study of sera from control donors and patients in CDC groups I–II, III, or IV using the antibody raised to oligopeptide 455–469 aa

In four further experiments on sera of individual patients, 21 HIV-1-seronegative controls and 21 HIV-1-seropositive individuals were studied using the antibody raised to oligopeptide 455–469. The four experiments were performed in parallel under the same conditions and using the same antibody preparation (Table 2). The patients were classified into groups CDC I–II, CDC III, and CDC IV.

In the four experiments, compared with controls, phase CDC I–II sera show a highly significant increase in SCL production. The levels stabilize after seroconversion. The results of exper-

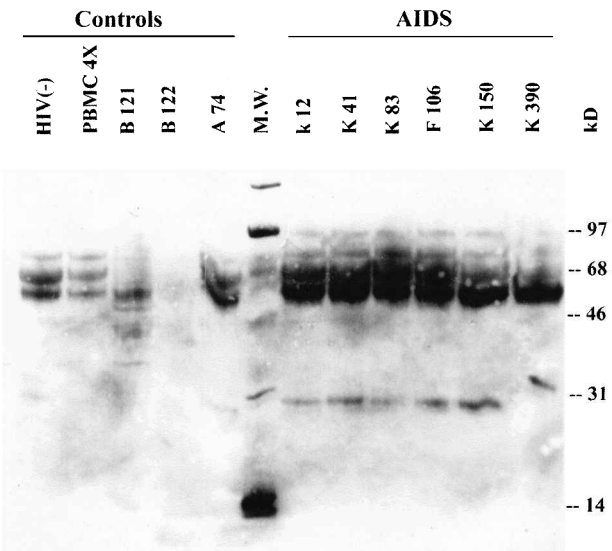

FIG. 3. SCL proteins in sera of controls and CDC III–IV patients studied by Western blot, using commercial mAb.[7] From left to right: Lane 1, 1:100 HIV-1-negative serum; lane 2, PBMC media from conditioned culture; the third passage contains 2% serum. We use the first serum-free passage in the presence of IL-2 (100 UI/ml). The 55-kDa SCL band is seen as well as the 65-kDa band, considered to be a result of binding of SCL fragments or molecules on residual albumin.[7] No other band can be seen in the corresponding SDS gel. Thus, the only visible protein detected in the serum-free medium is SCL (data to be published in more detail); lanes 3–5, biopsy specimen from osteosarcoma patients, maintained in serum-free medium; lane 4, control medium; lane 6, molecular weight markers; lanes 7–12, 1:100 sera from advanced cases of AIDS. In all specimens, the 55-kDa band is very intense when compared to the HIV-1-negative serum. The 65-kDa band is practically absent in lane 10, and the 55-kDa band is predominant. This case was considered to be very severe. In all AIDS cases, the 30-kDa band seen only in the lanes provided by the severe cases, suggests cross-reactivity with a dimer of galactose-binding lectin (galectin).

iments 1 and 2 indicate that during the transition from CDC I–II to CDC III, the serum SCL titers remain high but decrease slightly over time. The results from experiments 3 and 4 show that between CDC I–II and CDC IV phases, the serum SCL titers increases very significantly. The results from phase CDC III in experiments 1 and 2 compared with phase CDC IV in experiments 3 and 4 show the same increased serum SCL titers. This burst of SCL release is established during the final phase of the disease, when the plasma viral load is high and the $CD4^+$ T cell count is collapsed.

Western blotting analysis

The highly specific quantitative data obtained enabled us to incriminate SCL in the HIV-1-dependent infectious process. It was, therefore, important to identify SCL in sera using Western blots to estimate the size and the specificity of the SCL molecule.

The results of Western blots of 6 patients with advanced AIDS are shown in Figure 3. Five of them were sera from Kinshasa, and one was from Paris. For controls we used (1) one seronegative serum from an adult, (2) 4-fold concentrated conditioned medium from a healthy adult (peripheral blood mononuclear cells [PBMC] grown in the absence of serum), (3) two HIV-1-seronegative biopsy specimens from a Children's Hospital in Paris, (4) control medium, (5) serum from a patient ill with osteogenic sarcoma (from the same hospital), (6) protein size markers, and (7–12) sera from 6 advanced AIDS cases.

All sera were assayed with commercial K7 mAb. In all severe cases of AIDS, the 55-kDa bands are far more intensively labeled than in HIV-1-negative sera or other controls. The 65-kDa label is complex, resulting from the binding of some 55-kDa scattered SCL protein molecule remains to albumin. The dimer 97–110 kDa proteins were poorly recognized, as was the 67-kDa protein. This suggests a possible regulatory interaction between SCL and albumin that could behave as a transport molecule.[5]

It is of interest that these antibodies react intensively with the 55-kDa bands from AIDS. The 30-kDa bands correspond to a 14-kDa galactose-binding lectin (galectin) dimer.

DISCUSSION

It is important to stress that two independent sets of experiments, one using monospecific antibodies raised to the 55-kDa SCL protein and the other using the 455–469 oligopeptide antibody raised to the cloned protein, gave comparable results and generated similar conclusions. They show that during HIV-1 seroconversion a significant increase in SCL excretion/secretion in the patients' sera can be observed, followed by a leveling off. This is clearly established by the highly sensitive ELISA assays with three different SCL antibodies: (1) monospecific, raised against the NANA chromatography-purified 55-kDa SCL, (2) directed against the terminal oligopeptide of the cloned protein, and (3) commercial mouse mAb. The oligopeptide antibodies are of special interest for studying both the sensitivity and the specificity of the SCL immune assays.

During the CDC IV phase, the serum SCL titers increase in parallel with the loss of IFN sensitivity of the cells, as SCL inhibits the functions of the IFN-dependent secondary proteins, as previously suggested.[10] SCL can promote a loss of physiologic refractoriness to repeated IFN inductions.[11] This might explain, at least in part, the ineffectiveness of even the high IFN titers during this final period of AIDS. In parallel, the HIV virus load augments, and the $CD4^+$ T cell count collapses. As reported by Hendel et al.,[10] sera from "fast progressor" patients (CDC III–IV) contain more IFN than sera from long-term "no progressors" (CDC I–II). The excess amount of these ineffective IFNs, which can accumulate in the serum, could damage some cells.

It is of interest to connect these data to other pathologic disorders. An excess of such circulating ineffective IFNs has already been reported in systemic lupus erythematosus[12] and various autoimmune diseases, in which large amounts of circulating IFNs are regularly detected. These IFNs seemingly aggravate the pathologic process, and this might be connected to comparable excesses of SCL.[13] Our data suggest that the imbalance created in the normal SCL–IL-2–IFN cycle[9,14,15] HIV-1 infection is very difficult to overcome.

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