Synthetic Peptide Homologous to the Envelope Proteins of Retroviruses Shares a Cross-Reacting Epitope with the CD4 Receptor†

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A synthetic peptide (CKS-17) homologous to a highly conserved region of the retroviral transmembrane protein p15E was tested for its effect on receptor expression on monocytes. The CKS-17 amino acid sequence is present in several retroviruses including human T-cell lymphotropic virus types I and II and human immunodeficiency virus. The CKS-17 peptide has been previously shown to inhibit monocyte superoxide production, natural killer cell activity, polyclonal B-cell activation, and monocyte-mediated killing by inactivation of interleukin-1. In this study, we demonstrated that the anti-CD4 monoclonal antibody OKT4 binds strongly in vitro to CKS-17-treated human blood monocytes, whereas other antibodies tested were not reactive. This observed binding was the result of direct interaction of OKT4 antibody with the CKS-17 peptide. Moreover, a partial homology was found in amino acid sequence analysis of the CD4 epitope and the CKS-17 peptide.

Several in vivo and in vitro studies have shown that retroviruses can produce immunosuppression in different animal hosts. Different components of the virus are believed to be responsible for these immunosuppressive effects. The retroviral transmembrane envelope protein p15E, a 26-amino-acid peptide, inhibits several immune functions of macrophages and lymphocytes (7). This transmembrane sequence is conserved among several murine, feline, simian, and human retroviruses including human T-cell lymphotropic virus types I and II and human immunodeficiency virus (HIV) (12).

CKS-17 peptide is a synthetic 17-amino-acid peptide sequence which represents the first 16 amino acids of the p15E envelope protein. This peptide has several major effects on cells of the immune system. Its effects on monocytes and lymphocytes include the inhibition of mitogen- and alloantigen-stimulated proliferation of human lymphocytes (2), proliferation of a cloned murine cytotoxic T-cell line (2), immunoglobulin synthesis in B cells (9), the respiratory burst of human monocytes (5), and the inhibition of human natural killer cell-mediated cytotoxicity (6). Recently, inhibition of gamma interferon production by human peripheral mononuclear cells (MNC) has been demonstrated (11). We investigated the effect of CKS-17 coupled to human serum albumin (HSA) on the modulation of CD4 membrane receptor expression on monocytes.

MATERIALS AND METHODS

CKS-17 peptides. CKS-17 and HSA-coupled CKS-17 peptides were the generous gift from George J. Cianciolo and

Ralph Snyderman, Genentech Inc., South San Francisco, Calif. The methods for peptide synthesis and its coupling to carrier protein have been described previously (5). Briefly, CKS-17 (LQNRRLDLLFLKEGGL) was prepared by Merrifield solid-phase synthesis techniques and purified by using ion-exchange and reverse-phase high-pressure liquid chromatography, and its sequence was verified by high-pressure liquid chromatography and amino acid analysis (5). CKS-17 was coupled to the carrier protein HSA by the following method. Peptide (1.0 mM) and HSA (0.030 mM) were mixed in 0.1 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride for 10 h at 22°C at pH 5.0. An equal volume of 1.0 M glycine was then added, and the mixture was rotated overnight at 4°C and then extensively dialyzed against Hanks balanced salt solution. Efficiency of coupling was estimated to be 30% by using radiolabeled peptide, resulting in conjugated material with a final peptide concentration of 0.15 mM and a final HSA concentration of 0.015 mM. HSA alone was processed in an identical manner and used as an additional control termed HSA.

Isolation of blood MNC and culture of EBV-transformed B cells. Blood was drawn from healthy donors in preservative-free heparinized plastic syringes, and MNC were isolated by centrifugation over Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) (1). MNC were washed twice with cold Hanks balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 4.2 mM sodium bicarbonate, and 0.1% glucose at pH 7. The cells were then counted and maintained on ice until used. Epstein-Barr virus (EBV)-transformed B cells (provided by M. E. Conley) were prepared from normal human blood lymphocytes infected with EBV and maintained in RPMI 1640 medium supplemented with 10% fetal calf serum in in vitro culture (3). Cells were passaged twice a week and maintained at 37°C in in vitro culture in a humidified atmosphere with 5% CO₂.

Preparation of coated microbeads. Uniform latex carboxy-

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TABLE 1. Effect of CKS-17-HSA on antigen expression of monocytes^a

Type of monoclonal antibody	Log mean channel fluorescence $\pm$ SD	
	HSA-treated cells	CKS-17-HSA-treated cells
None	7.10 $\pm$ 0.68	16.14 $\pm$ 1.90
Mac-1	31.91 $\pm$ 0.84	42.14 $\pm$ 2.68
LeuM3	69.53 $\pm$ 1.96	66.33 $\pm$ 3.23
OKT4	16.95 $\pm$ 1.51	62.13 $\pm$ 2.81
OKT4A	13.68 $\pm$ 1.56	26.82 $\pm$ 3.00
OKIa	25.81 $\pm$ 0.81	38.39 $\pm$ 0.95

^a Peripheral blood MNCs from normal human donors were incubated with HSA or CKS-17-HSA (0.15 μ M HSA per 10^6 cells or 1.5 μ M CKS-17 bound to 0.15 μ M HSA per 10^6 cells) at 4°C for 30 min. After the incubation, the cells were washed and stained with monoclonal antibodies and polyclonal goat anti-mouse F(ab')₂ conjugated with rhodamine. Mean channel fluorescence was determined with an EPICS C flow cytometer. Data represent log mean channel fluorescence $\pm$ standard deviations from four different experiments.

lated microbeads (2.39 μ m in diameter) (Seragen Diagnostics, Indianapolis, Ind.) were coated with different peptides by the method of Nathan and Cohn (10) with minor modifications. The carboxylated microbeads were washed three times with carbonate buffer (pH 9.6) and then three times with phosphate buffer (pH 4.5). Microbeads were then incubated with 2% 1-cyclohexyl-3(2-morpholinoethyl)-carbodiimide metho-*p*-toluene sulfonate for 3 h at room temperature and then washed with borate buffer (pH 8.5) and subsequently incubated overnight on a rotary shaker with the peptide to be bound at room temperature. The next day, the microbeads were treated with 1 M glycine for 60 min to block unbound ligand, washed, and kept in phosphate buffer (pH 7.4) with 5% glycerol at 4°C until used.

Effects of CKS-17 on monoclonal antibodies binding to monocytes or coated microbeads. MNC were incubated with 15 μ M CKS-17-HSA per ml for 20 min at 37 or 4°C. Following treatment, cells were washed once with Hanks balanced salt solution and stained with different types of monoclonal antibodies. Surface receptor expression was studied with the following murine monoclonal antibodies: anti-LeuM3 (Becton Dickinson and Co., Mountain View, Calif.), Mac-1 (gift from Donald Anderson, Baylor University, Houston, Tex.), and OKIa, OKT4, and OKT4A (Ortho Diagnostics, Inc., Raritan, N.J.). Fluorescein- or rhodamine-labeled F(ab')₂ goat anti-mouse antibodies (Tago Inc., Burlingame, Calif.) were used as second antibodies. MNC surface receptors and monoclonal antibody binding to

TABLE 2. OKT4 antibody binding to HSA- and CKS-17-HSA-treated EBV-transformed B lymphocytes^a

Treatment	Log mean channel fluorescence $\pm$ SD
None.....	6.7 $\pm$ 0.2
HSA.....	5.7 $\pm$ 0.6
CKS-17-HSA.....	32.1 $\pm$ 3.5

^a EBV-transformed cells were incubated for 30 min at 4°C with CKS-17-HSA (15 μ M/ml) or with HSA as a control. After incubation, cells were washed and treated with OKT4 and rhodamine-conjugated sheep anti-mouse immunoglobulin G. Log mean channel fluorescence was determined with an EPICS C flow cytometer. Data represent mean values from four different experiments $\pm$ standard deviations.

coated microbeads were assayed as follows. Cells were initially washed with cold phosphate-buffered saline (GIBCO Laboratories, Chagrin Falls, Ohio) supplemented with 0.1% bovine serum albumin (Behring Diagnostics, La Jolla, Calif.) and 0.01% sodium azide (Fisher Scientific Co., Pittsburgh, Pa.); beads were washed with phosphate-buffered saline only. Cells or beads were then incubated with monoclonal antibody for 30 min at 4°C and then washed once and incubated with fluorescein- or rhodamine-labeled goat anti-mouse antibodies for 30 min at 4°C. Cells or beads were then washed, and fluorescence was determined with an EPICS C flow cytometer (Coulter Immunology, Hialeah, Fla.). The results were expressed as the mean of the logarithm of green or red fluorescence.

RESULTS

Binding of CKS-17-HSA to monocytes. CKS-17-HSA-treated MNC were stained with different types of monoclonal antibodies, and monocytes were identified by forward and right-angle scatter on the flow cytometer. No significant changes were observed in the binding of the monoclonal antibodies OKT4A, OKIa, LeuM3, and Mac-1 to the CKS-17-HSA-treated monocytes or to the controls. However, a significant increase in the binding of monoclonal antibody OKT4 was observed for the CKS-17-HSA-treated monocytes (Table 1). OKT4 binding to monocytes incubated with CKS-17-HSA was similar at 37 or 4°C (data not shown), and therefore this enhancement is not the consequence of upregulation of the receptor. Marginal increase in binding of other monoclonal antibodies also occurred in the incubation control and is due to nonspecific binding of the second antibody (goat anti-mouse fluorescein isothiocyanate conjugate).

FIG. 1. Flow cytometric determination of OKT4 binding by CKS-17-HSA-covered microspheres. HSA, CKS-17, and CKS-17-HSA were bound covalently to carboxylated latex microbeads. The coated microbeads were tested for their ability to bind mouse monoclonal anti-human OKT4 antibody by using a rhodamine-conjugated sheep anti-mouse immunoglobulin G antibody. Determination of binding capacity was performed by an EPICS C flow cytometer. Panel A shows no binding of OKT4 to microbeads coated with HSA or CKS-17 (exactly identical). Panel B shows positive binding of OKT4 to microbeads coated with CKS-17-HSA.

TABLE 3. Antibody binding to HSA-, CKS-17-, and CKS-17-HSA-coated microspheres^a

Type of antibody	Log mean channel fluorescence $\pm$ SD		
	HSA-coated spheres	CKS-17-coated spheres	CKS-17-HSA-coated spheres
None	0.15 $\pm$ 0.03	0.11 $\pm$ 0.01	0.16 $\pm$ 0.02
OKT4	0.29 $\pm$ 0.12	0.88 $\pm$ 0.16	76.28 $\pm$ 11.15
OKT4A	0.21 $\pm$ 0.06	0.34 $\pm$ 0.02	0.17 $\pm$ 0.03
Mac-1	0.45 $\pm$ 0.30	0.80 $\pm$ 0.18	13.49 $\pm$ 7.57
LeuM3	0.28 $\pm$ 0.02	0.69 $\pm$ 0.35	0.37 $\pm$ 0.10
OKIa	0.23 $\pm$ 0.06	1.26 $\pm$ 0.85	0.33 $\pm$ 0.08

^a Uniform carboxylated latex particles (diameter, 2.39 μ m) were covalently bound with HSA, CKS-17, or CKS-17-HSA. The coated microspheres were incubated with monoclonal antibody and polyclonal goat anti-mouse F(ab')₂ conjugated to fluorescein isothiocyanate. Fluorescence was determined with an EPICS C flow cytometer. Data represent log mean channel fluorescence $\pm$ standard deviations from four different experiments.

Binding of CKS-17-HSA to EBV-transformed B cells and carboxylated microbeads. To determine whether the increase in OKT4 binding to CKS-17-treated cells was due to upregulation of CD4 by CKS-17-HSA or whether monoclonal antibody OKT4 can bind directly to CKS-17-HSA, we studied the OKT4 binding to CKS-17-HSA-treated EBV-transformed B cells and CKS-17-HSA-coated microbeads. Since EBV-transformed B lymphocytes lack the CD4 receptor, OKT4 would not be expected to bind to them. After incubation with CKS-17-HSA for 20 min, however, OKT4 bound significantly to the cells, while no binding was seen with the HSA treatment alone (Table 2). The direct binding of OKT4 to CKS-17-HSA peptide was also tested by using microbeads which were coated with three different peptides, CKS-17-HSA, CKS-17, and HSA; the last two peptides were used as controls. Monoclonal antibody OKT4 bound significantly to CKS-17-HSA-treated microbeads (Fig. 1), while no binding occurred with the other controls or with the monoclonal antibodies OKT4A, Mac-1, LeuM3, and OKIa (Table 3).

Amino acid sequence similarity of CD4 glycoprotein and CKS-17 peptide. Using the Bionet resource, we studied a computer-assisted comparison of CKS-17 and CD4 to determine the presence of possible homologies between the two peptides which may explain the binding of anti-CD4 antibody to CKS-17. Analysis of both sequences revealed a partial homology between CKS-17 peptide and amino acids 202 to 218 and 408 to 424 in the CD4 protein (Fig. 2). The first region is present in the binding epitope of the OKT4 and not of the OKT4A antibody (8). The sequence homology between the CKS-17 peptide and the HIV envelope protein gp120 is also shown in Fig. 2.

DISCUSSION

We have investigated the antibody-binding capacity of a well-conserved region of oncogenic retrovirus transmembrane envelope protein p15E. p15E and CKS-17 (a synthetic 17-amino-acid peptide present in p15E) are known to mediate several *in vitro* inhibitory effects on different cell types (2, 5, 6, 9, 11). Moreover, CKS-17 peptide is also present in several human retrovirus envelope proteins, including human T-cell lymphotropic virus types I and II and HIV (12). CKS-17 mediates its action more efficiently when bound to a carrier protein, bovine or human serum albumin. HIV has been shown to infect primarily CD4-positive cells, namely, T-helper lymphocytes and monocytes. Downregulation of the CD4 surface receptor usually occurs after infection with HIV. We therefore examined the possible role of CKS-17 peptide in inhibiting the expression of CD4 glycoprotein on the surface of monocytes. Treatment of the monocytes with CKS-17-HSA did not decrease the binding of the anti-CD4 antibody OKT4 to the cells but instead increased its binding (Table 1). We further demonstrated binding of the OKT4 antibody to EBV-transformed B lymphocytes (CD4 negative) after their incubation with CKS-17-HSA (Table 2). Moreover, coupling of carboxylated microbeads with CKS-17-HSA increased their binding to the anti-CD4 antibody OKT4 (Table 3). The binding of OKT4 to CKS-17-HSA appeared to be antibody specific, since binding did not occur with other monoclonal antibodies.

A computer-assisted comparison of CKS-17, HIV proteins, and CD4 antigen with the Bionet resource revealed a region between amino acids 427 and 443 of the gp120 of HIV and between 408 and 424 of the CD4 molecule that have the highest homology to CKS-17 (Fig. 2). However, as amino acids 408 to 424 are present in the intracytoplasmic part of the CD4 protein sequence, they should not play a significant role in the binding of CKS-17 to OKT4 antibody. Binding was not observed between OKT4 antibody and microbeads coated with a mixture of HIV proteins (data not shown); this may be due to the blocking of the binding site by the proteins. Further studies are required with purified gp120. A recent report has demonstrated high homology between gp41 of HIV and the β 1 domain of HLA class II molecules, a surface receptor on mononuclear phagocytes which plays an important role in antigen presentation (4). Sera tested from 36% of patients with acquired immunodeficiency syndrome contained antibodies that reacted with class II molecules; the role and significance of these antibodies are unknown (4).

In summary, we presented data that suggest mimicry between CD4 receptors on monocytes and CKS-17, a synthetic peptide that corresponds to a conserved region of

HIV gp120	427	R C S S N I T G L L L T R D G G N	443	
		. X : . . . : X		
CKS-17		L Q N R R G L D L L F L K E G G L		
CD4	408	L L L F I G L G I F F C V R C R H	424	50% identity in
		: X : . . X		
CKS-17		L Q N R R G L D L L F L K E G G L		6-amino-acid overlap
		: X : X		
CD4	202	L A F Q K A S S I V Y K K E G E Q	218	26.7% identity in
				15-amino-acid overlap

FIG. 2. Computer-assisted comparison of CKS-17, HIV envelope peptide gp120, and CD4.

retroviral envelopes. Delineation of the role of this mimicry in HIV infection of monocytes requires further investigation.

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