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Soluble human CD4 elicits an antibody response in rhesus monkeys that inhibits simian immunodeficiency virus replication

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ABSTRACT **Rhesus monkeys infected with the simian immunodeficiency virus of macaques (SIV_{mac}) demonstrate significant virologic and clinical improvement as a result of treatment with human recombinant soluble CD4 (rsCD4). We show that human rsCD4 does not efficiently inhibit SIV_{mac} replication in bone marrow macrophages of rhesus monkeys and does not significantly augment bone marrow hematopoietic colony formation *in vitro*. However, plasma of human rsCD4-treated rhesus monkeys does exhibit significant anti-SIV_{mac} activity *in vitro*. Plasma of these animals efficiently blocks SIV_{mac} replication in peripheral blood lymphocytes and bone marrow macrophages. It also increases granulocyte/macrophage colony formation *in vitro* by bone marrow cells of SIV_{mac}-infected monkeys. This plasma and the IgG fraction of plasma from a rhesus monkey immunized with human rsCD4 in adjuvant demonstrate reactivity with a soluble form of the rhesus monkey CD4 molecule, exhibit binding to $CD4^+$ but not $CD8^+$ concanavalin A-activated rhesus monkey peripheral blood lymphocytes, and precipitate the CD4 molecule from surface-labeled activated rhesus monkey peripheral blood lymphocytes. Moreover, anti-viral activity is demonstrable in the IgG fraction of plasma from a human rsCD4-immunized monkey. These studies raise the possibility that a modified human CD4 molecule serving as an immunogen might elicit an antibody response that could potentially induce a beneficial therapeutic response in human immunodeficiency virus-infected individuals.**

The human CD4 molecule is a high-affinity receptor for the human immunodeficiency virus (HIV) envelope glycoprotein (1). This has been demonstrated by a number of experimental approaches. Monoclonal anti-CD4 antibodies block HIV infection of target cells *in vitro* (2, 3). Cells not expressing the CD4 molecule may become susceptible to HIV infection after transfection with the CD4 gene (4). Moreover, it has been shown (5–9) that a recombinantly produced soluble form of the extracellular portion of the CD4 molecule can block HIV infection of cells *in vitro*.

These findings led to the suggestion that a soluble form of CD4 may prove valuable in the therapy of HIV-infected individuals. Recombinant soluble CD4 (rsCD4) might be employed for the targeting of toxins to cells infected with HIV that express the HIV envelope glycoprotein on their surfaces (10–12). rsCD4 might also inhibit virus infection by directly competing with cell surface CD4 molecules for the binding of HIV. It was this possibility that led us to assess the therapeutic value of rsCD4 in rhesus monkeys infected with the simian immunodeficiency virus of macaques (SIV_{mac}). These studies demonstrated significant virologic and clinical improvement in the SIV_{mac}-infected monkeys as a result of treatment with human rsCD4 (13).

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In the present studies, we demonstrate that human rsCD4 is inefficient *in vitro* at mediating many of the antiviral effects observed in the rsCD4-treated SIV_{mac}-infected rhesus monkeys. However, we show that rhesus monkeys develop an antibody response after treatment with human rsCD4, and this antiserum binds to monkey CD4 and inhibits SIV_{mac} replication in monkey cells.

MATERIALS AND METHODS

Animals. Two normal rhesus monkeys (*Macaca mulatta*) were treated for 50 days with human rsCD4, each receiving 2 mg intramuscularly (i.m.) daily. Four normal rhesus monkeys were immunized subcutaneously, two with 1 mg of human rsCD4 and two with 1 mg of human serum albumin (HSA) in an emulsion with complete Freund's adjuvant (Sigma) and again by the same route 34 days later with 1 mg of human rsCD4 or 1 mg of HSA in an emulsion with incomplete Freund's adjuvant (Sigma). The animals were maintained in accordance with the guidelines of the Committee on Animals for the Harvard Medical School and the National Institutes of Health (14).

rsCD4. Human rsCD4 (Biogen) was produced as described (5). Rhesus rsCD4, consisting of the N-terminal 375 amino acids of the rhesus monkey CD4 molecule, was secreted from Chinese hamster ovary (CHO) cells and purified by a combination of ion-exchange and immunoaffinity chromatographic procedures (W. Meier, personal communication). This resulted in >90% pure soluble rhesus monkey CD4, by Coomassie stained SDS/PAGE and Western blot analysis.

SIV_{mac} Replication in Peripheral Blood Lymphocytes (PBLs) and Bone Marrow Macrophages. PBLs and mononuclear cells from bone marrow were isolated by Ficoll/diatrizoate density gradient centrifugation. A SIV_{mac} stock was prepared from a cell-free supernatant of a culture of isolate 251 maintained in concanavalin A (Con A; Sigma)-activated normal human PBLs. For studies of *in vitro* SIV_{mac} infection, Con A-activated recombinant human interleukin 2 (IL-2) (kindly provided by Hoffmann–La Roche) expanded rhesus monkey PBLs were first incubated with plasma, fractionated plasma, or an anti-CD4 monoclonal antibody (mAb) (19Thy5D7; kindly provided by S. Schlossman, Dana–Farber Cancer Institute) for 1 hr at 37°C. They were then incubated for 2 hr with 85 infectious doses (IDs) of the SIV_{mac} stock (1 ID is defined as the minimum dilution of this stock virus-containing supernatant that infects rhesus monkey PBLs under these culture conditions).

Bone marrow cells from SIV_{mac}-infected or normal rhesus monkeys were placed in culture in Lab-Tek chamber slides

Abbreviations: CFU-GM, granulocyte–macrophage colony-forming unit; Con A, concanavalin A; HIV, human immunodeficiency virus; ID, infectious dose; IL-2, interleukin 2; mAb, monoclonal antibody; PBL, peripheral blood lymphocyte; rsCD4, recombinant soluble CD4; SIV_{mac}, simian immunodeficiency virus of macaques; HSA, human serum albumin.

(Nunc) without lectin stimulation or cytokines. After 7 days of culture, the adherent cells were incubated with medium alone, plasma, fractions of plasma, or anti-CD4 mAb for 1 hr at 37°C. Cells from uninfected monkeys were then incubated for 2 hr with 256 IDs of the SIV_{mac} stock. In blocking experiments with rsCD4, adherent bone marrow cells from SIV_{mac}-infected monkeys were maintained in the continuous presence of human rsCD4, plasma, or anti-CD4 mAb. Reverse transcriptase activity in culture supernatants was measured as an indication of *in vitro* SIV_{mac} replication (15).

Hematopoietic Colony Formation. A two-layer culture was established for the quantitation of granulocyte/macrophage colonies [granulocyte–macrophage colony-forming units (CFU-GM)] from bone marrow cells, as described (16). In some experiments, human rsCD4, plasma, fractions of plasma, or anti-CD4 mAb were added to the underlayer at the initiation of the cultures.

Flow Cytometric Analysis. For two-color flow cytometric analyses, Con A-activated IL-2-expanded PBLs from a rhesus monkey treated with human rsCD4 were incubated with a 1:16 dilution of plasma from a human rsCD4-treated rhesus monkey for 40 min at 4°C, washed, and then incubated with fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin (which reacts with rhesus monkey immunoglobulin) (Tago) and either phycoerythrin-conjugated anti-CD4 (OKT4; Ortho) or anti-CD8 (DAKO-T8; DAKO, Carpinperia, CA) for 40 min at 4°C. After cells were fixed, flow cytometric analyses were performed on an EPICS-CS (Coulter).

Detection of Anti-Human and Anti-Rhesus Monkey rsCD4 Antibody. Anti-rsCD4 antibody titers were determined by ELISA. Ninety-six-well plates (Nunc) were coated overnight with human or rhesus monkey rsCD4 or HSA at 0.3 μg per well. Plasma samples were added to wells at various dilutions and incubated for 1 hr at room temperature. Horseradish peroxidase-conjugated goat anti-human immunoglobulin (1:2000 dilution, Zymed) was then added and incubated for 2 hr. After washing, 100 μl of 3,3',5,5'-tetramethylbenzidine peroxidase substrate solution (Kirkegaard and Perry, Gaithersburg, MD) was added to each well. After 10 min the reaction was terminated and light absorbance was read at 450 nm on an ELISA reader. In this anti-rsCD4 antibody assay, pooled normal rhesus monkey plasma served as a negative control. A positive ELISA reading was defined as a binding level of twice the mean or mean + 4 SDs above the negative control levels in each assay.

Surface Labeling, Immunoprecipitation, and SDS/PAGE. Lymphoblasts were surface labeled using a water-soluble Bolton–Hunter reagent (17). Cells were resuspended with 35 mM sodium borate (pH 9.2) and then incubated with sulfosuccinimidyl-3-(4-hydroxy-phenyl)propionate, a water-soluble Bolton–Hunter reagent (Pierce). After a 30-min incubation at 4°C, cells were incubated with 0.5 mCi of ^{125}INa (Amersham; 1 Ci = 37 GBq) for 20 min in a vial coated with 100 μg of Iodo-Gen (Pierce). After washing, cells were incubated on ice for 30 min in lysis buffer. The resulting supernatant was precleared and incubated with either a mAb or rhesus monkey plasma. Immunoprecipitation was performed using 3 mg of goat anti-mouse or goat anti-human immunoglobulin bound to microspheres (Kirkegaard and Perry). The microspheres were washed and resuspended in 80 μl of SDS/PAGE sample buffer. SDS/PAGE was carried out by using the method of Laemmli (18).

Plasma Fractionation. IgG and non-IgG plasma fractions were prepared from rhesus monkeys immunized with human rsCD4 by using a protein A-Sepharose column. Plasma was applied to a protein A-Sepharose CL-4B (Sigma) column equilibrated in 100 mM Tris·HCl/3 M NaCl, pH 8.9. The column was washed with the same buffer and the non-IgG fraction was recovered. The column was then eluted with 100 mM acetic acid/150 mM NaCl, pH 2.9, and the eluate was dialyzed and concentrated. The IgG fractions of the eluate were identified by absorbance at 280 nM.

RESULTS

We have demonstrated a clinical improvement in SIV_{mac}-infected rhesus monkeys treated with human rsCD4 (13). During and immediately after a 50-day parenteral course of human rsCD4, these animals exhibited decreased virus replication in their bone marrow macrophages and PBLs. Cultures established from bone marrow macrophages as well as PBLs co-cultivated with the SIV_{mac}-permissive cell line H9 during this period of treatment did not yield virus. The SIV_{mac}-infected rhesus monkeys also showed improvement in their bone marrow hematopoietic function during rsCD4 treatment. This improvement, an increase in bone marrow granulocyte/macrophage and erythrocyte progenitor cell colonies, probably resulted from the inhibition of SIV_{mac} replication in bone marrow macrophages (16).

A number of observations made during the course of these studies suggested that rsCD4 may have mediated at least

Table 1. Plasma of a human-rsCD4-treated rhesus monkey inhibits SIV_{mac} replication in monkey PBLs and bone marrow macrophages

		Reverse transcriptase activity, cpm × 10^{-3}/ml							
		PBLs				Bone marrow macrophages			
Addition	Dilution	Day 3	Day 8	Day 13	Day 16	Day 26	Day 35	Day 44	Day 55
None (control)						0.8	0.6	6.5	14.6
rsCD4 (200 μg/ml)						0.8	5.5	9.3	15.7
Plasma (prior to treatment)	1:5	0.7	9.8	18.6	1.4				
Plasma (after 47 days of treatment)	1:1	0.4	1.1	2.6	0.8				
	1:5	0.6	1.0	2.5	0.5				
	1:10					0.2	0.3	0.8	0.7
	1:25	0.4	5.8	11.9	1.5				
	1:50					0.2	1.4	0.6	1.7
	1:125	0.6	11.0	17.6	1.7				
Anti-CD4 mAb		0.5	0.7	2.7	1.9	0.2	0.4	0.9	0.7

Con A-activated IL-2-expanded PBLs from a normal rhesus monkey were preincubated with plasma (at the noted dilutions) or anti-CD4 mAb (19Thy5D7, 1:100 dilution of ascites fluid) for 1 hr at 37°C. Supernatants were then aspirated and cells were incubated for 2 hr with 85 IDs of SIV_{mac} stock. Bone marrow cells from a SIV_{mac}-infected rhesus monkey were placed in culture without lectin stimulation or cytokines. After 7 days of culture, nonadherent cells were removed. Cultures were maintained in the continuous presence of rsCD4, plasma (at the noted dilutions), or anti-CD4 mAb (1:100 dilution of ascites fluid). The plasma was obtained from a normal rhesus monkey prior to or 47 days after initiating a 50-day course of treatment with daily i.m. injections of human rsCD4. Periodically, tissue culture supernatant was assessed for reverse transcriptase activity as a measure of SIV_{mac} replication.

Table 2. Plasma of a human rsCD4-treated rhesus monkey augments CFU-GM *in vitro* in bone marrow cells from SIV_{mac}-infected rhesus monkeys

Addition	Amount or dilution	CFU-GM, no./5 × 10^4 cells							
		Normal rhesus monkey				SIV_{mac}-infected rhesus monkey			
		Mm120	Mm122	Mm220	Mm157	Mm129	Mm335	Mm156	Mm202
None (control)		254	165	200	221	102	24	16	92
rsCD4	200 μg/ml					135	34	21	120
	100 μg/ml	256	157	165	231	134	34	13	108
	10 μg/ml	254	175	171	212	97	22	13	108
Plasma (prior to treatment)	1:5	244	169	207	200	82	41	25	104
	1:20					97	26		
	1:100					97	22		
Plasma (after 47 days of treatment)	1:5	246	170	203	218	182	149	75	224
	1:20					187	76		
	1:100					98	30		

Two-layer cultures were established for the quantitation of CFU-GM colonies. An overlayer of 1 × 10^5 bone marrow cells in 0.3% agar was layered onto an underlayer of 0.5% agar containing recombinant GM-CSF (60 ng/ml). Human rsCD4 or plasma was added to the underlayer at the initiation of culture. Results are expressed as the mean of duplicate cultures counted on day 14 of culture. For more information on plasma, see Table 1.

some of the clinical improvement in the monkeys by a mechanism other than direct competition for virus binding. Although human rsCD4 inhibits the replication of SIV_{mac} in rhesus monkey PBLs *in vitro* (13), the concentration of human rsCD4 required to inhibit this viral replication *in vitro* was not maintained in the rsCD4-treated monkeys during their course of treatment. Human rsCD4 at >100 μg/ml is required to inhibit significantly an *in vitro* SIV_{mac} infection of rhesus monkey PBLs (13). During the initial 24 hr of treatment, peak rsCD4 plasma levels of ≈1.4 μg/ml could be detected in these monkeys; by day 47 of treatment, a peak plasma level of only ≈250 ng/ml was reached. Moreover, human rsCD4 was quite inefficient at inhibiting SIV_{mac} replication in rhesus monkey bone marrow macrophages *in vitro* (Table 1) and caused only a modest augmentation of rhesus monkey bone marrow hematopoietic colony formation *in vitro*, even at very high concentrations (Table 2).

Interestingly, the plasma of a rsCD4-treated normal rhesus monkey, which contained rsCD4 at <250 ng/ml, inhibited SIV_{mac} replication in both rhesus monkey PBLs and bone marrow macrophages. As shown in Table 1, a 2-hr preincubation of normal rhesus monkey PBLs with this plasma inhibited the replication of SIV_{mac} in a concentration-dependent fashion. Moreover, whereas rsCD4 at 200 μg/ml caused no significant inhibition of SIV_{mac} replication in bone marrow macrophages derived from a SIV_{mac}-infected rhesus monkey, this plasma blocked virus replication. Similarly, the plasma of this rsCD4-treated rhesus monkey markedly augmented numbers of CFU-GM colonies from the bone marrow of SIV_{mac}-infected rhesus monkeys (Table 2). Plasma from the same monkey taken prior to initiating treatment did not increase colony formation in these bone marrow cells. Plasma of the rsCD4-treated animal did not cause an increase in CFU-GM in bone marrow cells of normal rhesus monkeys.

We sought to determine what component of the plasma of the rsCD4-treated rhesus monkey contained this antiviral activity. The extracellular portion of the rhesus monkey CD4 molecule differs from its human homologue at 33 of 375 amino acids (R. Fisher, personal communication). One might, therefore, expect that rhesus monkeys treated with human rsCD4 should develop antibody responses to the foreign epitopes of this protein defined by these amino acid differences. In fact, as shown in Fig. 1, two normal rhesus monkeys treated with daily 2-mg i.m. inoculations of rsCD4 for 50 days clearly developed an antibody response to the human rsCD4 protein. Surprisingly, however, plasma of these animals also demonstrated reactivity with a soluble form of the extracellular portion of the rhesus monkey CD4 molecule (Fig. 1). Moreover, whereas antibody in the plasma of a rhesus monkey prior to initiating treatment did not bind to rhesus monkey PBLs, antibody in the plasma of this monkey after initiation of rsCD4 treatment clearly bound to $CD4^+$ but not $CD8^+$ Con A-activated rhesus monkey PBLs (Fig. 2). Such plasma also precipitated the CD4 molecule from surface-labeled Con-A-activated rhesus monkey PBLs (Fig. 3). Monkeys treated by a similar schedule with HSA never developed an anti-rsCD4 antibody response (Figs. 1 and 3). Thus, the immunologic tolerance that the monkey might have been expected to demonstrate to its own CD4 molecules expressed on the surface of its own cells appears to have been broken by the daily administration of human rsCD4.

We then sought to determine whether antibodies raised in a rhesus monkey conventionally immunized with rsCD4

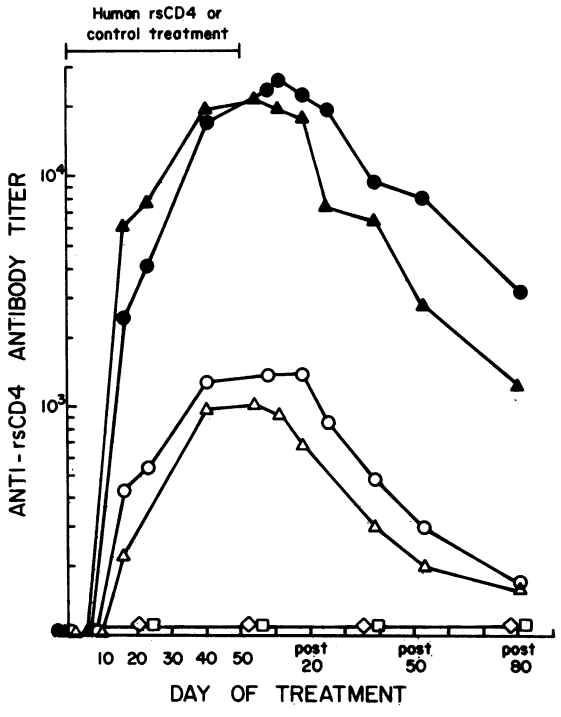

FIG. 1. Human rsCD4-treated rhesus monkeys develop antibody responses to human rsCD4 (●, ▲) and rhesus rsCD4 (○, △). HSA-treated monkeys do not generate anti-rhesus rsCD4 antibody responses (◇, □). The anti-rsCD4 antibody titers represent the reciprocal of the final dilution of plasma that resulted in a positive ELISA reading.

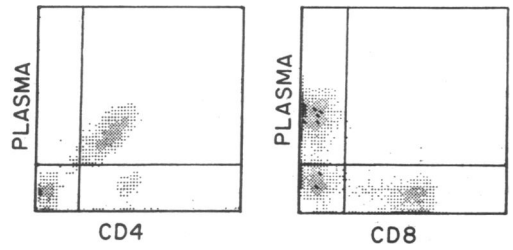

FIG. 2. Two-color flow cytometric analysis demonstrating that antibody in the plasma of a human rsCD4-treated rhesus monkey binds to rhesus monkey $CD4^+$ but not $CD8^+$ PBLs. Con A-activated IL-2-expanded rhesus monkey PBLs from a rhesus monkey previously treated with rsCD4 were incubated with plasma from a rsCD4-treated rhesus monkey taken on day 47 of a 50-day course of treatment. These cells were then incubated with fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin and finally with either phycoerythrin-conjugated anti-CD4 or anti-CD8 mAbs. The horizontal and vertical scales indicate a $\log_{10}$ relative fluorescence.

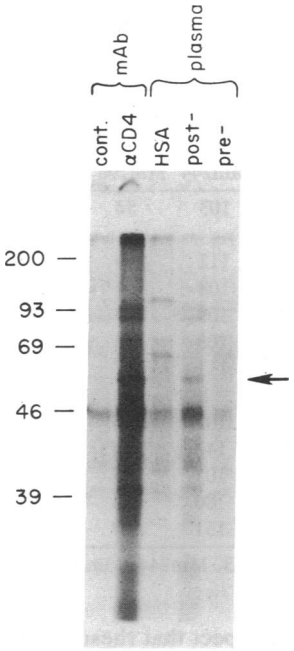

FIG. 3. Plasma immunoglobulin from a human rsCD4-treated rhesus monkey precipitates rhesus monkey CD4 from surface-labeled rhesus monkey PBLs. Con-A-activated IL-2-expanded rhesus monkey PBLs were surface labeled, a lysate was prepared, and immunoprecipitations were performed with a control mAb (lane cont.), an anti-CD4 mAb (αCD4), plasma from a HSA-treated monkey, plasma from a human rsCD4-treated rhesus monkey (lane post-), and pre-treatment plasma from the same animal (lane pre-). Immunoprecipitates were then subjected to SDS/PAGE. The arrow denotes the molecular mass of CD4. Molecular masses are indicated to the left in kDa.

might also inhibit SIV_{mac} replication. A normal rhesus monkey was immunized with human rsCD4 in an emulsion in complete Freund's adjuvant. Plasma taken from this animal before and after immunization was separated into IgG and non-IgG fractions by protein A column passage. As shown in Table 3, antibodies in the preimmune plasma of this animal demonstrated no binding to rhesus monkey rsCD4 in an ELISA. This plasma also did not contain antibody that bound to lectin-activated rhesus monkey PBLs. However, immune plasma demonstrated significant anti-rhesus monkey CD4 binding both to lectin-activated monkey PBLs and in an ELISA. This activity was present in the IgG fraction of the immune plasma; the non-IgG fraction of the plasma demonstrated no such reactivity.

The plasma of the immunized monkey but not the monkey's preimmune plasma inhibited SIV_{mac} replication in rhesus monkey PBLs and bone marrow macrophages (Table 3). This anti-SIV_{mac} activity was contained in the IgG but not in the non-IgG fraction of the immune plasma. The plasma of the animal after immunization but not prior to immunization also augmented CFU-GM in bone marrow cells of SIV_{mac}-infected rhesus monkeys (Table 4). Moreover, the IgG but not the non-IgG fraction of the immune plasma from this monkey mediated this augmentation of colony formation. Therefore, an antibody raised in the rhesus monkey by immunization with human rsCD4 appears to inhibit SIV_{mac} replication in monkey cells.

DISCUSSION

An individual's immune system is usually tolerant to self proteins. Thus, it is not surprising that humans receiving as much as 210 mg of human rsCD4 weekly by i.m. inoculation for as long as 4 weeks have developed only a very low-titer antibody response to that rsCD4 (19). Plasma from these individuals could not be shown to bind to activated human

Table 3. IgG fraction of plasma from a rhesus monkey immunized with human rsCD4 inhibits SIV_{mac} infection of PBLs and bone marrow macrophages

Addition	Plasma reactivity with rhesus monkey CD4		Reverse transcriptase activity, cpm × 10^{-3}/ml						
			PBLs				Bone marrow macrophages		
	ELISA titer	Cell staining	Day 10	Day 13	Day 17	Day 20	Day 18	Day 31	Day 45
None (control)			0.4	4.0	9.6	4.1	0.8	1.1	3.8
Plasma (prior to immunization)									
Unfractionated	<60	<4	0.7	4.1	5.6	3.9	0.9	1.2	4.1
Non-IgG fraction	<60	<4	0.4	1.9	9.0	4.3	0.6	0.9	6.4
IgG fraction	<60	<4	0.4	3.3	8.2	3.4	0.5	1.3	3.2
Plasma (after immunization)									
Unfractionated	7146	23.7	0.3	0.3	0.6	0.7	0.5	0.4	0.3
Non-IgG fraction	<60	<4	0.4	2.3	6.2	2.5	0.8	2.8	13.6
IgG fraction	8073	22.3	0.3	0.3	0.5	0.5	0.5	0.6	0.4
Anti-CD4 mAb			0.4	0.3	0.5	0.6	0.5	0.5	0.4

Normal rhesus monkey was immunized with rsCD4 (1 mg) in an emulsion with complete Freund's adjuvant and immunized again 34 days later with rsCD4 (1 mg) in an emulsion with incomplete Freund's adjuvant. Plasma taken prior to immunization or 70–77 days after the first immunization was fractionated into IgG and non-IgG fractions by using a protein A column. Anti-rhesus monkey CD4 antibody was measured by ELISA using rhesus monkey rsCD4 as an antigen. Results are expressed as the reciprocal of the final dilution of plasma that resulted in a positive reading. Con A-activated IL-2-expanded PBLs from a human rsCD4-treated rhesus monkey were incubated with a 1:16 dilution of the plasma fraction noted, then with a 1:50 dilution of fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin, and finally analyzed by flow cytometry. Results are expressed as percent fluorescence-positive cells. For more information on PBLs, see footnotes to Table 1. Bone marrow cells from a normal rhesus monkey were placed in culture without lectin stimulation or cytokines. After 7 days of culture, nonadherent cells were removed. Adherent cells were incubated with the indicated plasma fractions for 1 hr at 37°C. Cells were then washed and incubated with 256 IDs of SIV_{mac} stock.

Table 4. IgG fraction of plasma from a human rsCD4-immunized rhesus monkey augments CFU-GM *in vitro* in bone marrow cells from SIV_{mac}-infected monkeys

Addition	Dilution	CFU-GM, no. per 5×10^4 cells		
		Mm244	Mm108	Mm169
None (control)		103	54	95
Plasma (prior to immunization)				
Unfractionated	1:25	112	59	95
	1:125	108	57	98
Non-IgG fraction	1:125	109	57	97
IgG fraction	1:25	106	55	91
	1:125	111	57	96
Plasma (after immunization)				
Unfractionated	1:25	185	116	145
	1:125	142	90	123
Non-IgG fraction	1:125	105	57	96
IgG fraction	1:25	180	114	148
	1:125	151	87	133

For more information, see Tables 2 and 3. Mm244, Mm108, and Mm169 are SIV_{mac}-infected rhesus monkeys.

PBLs (data not shown). One might expect that rhesus monkeys treated parenterally with human rsCD4 would develop antibody responses with specificity for the determinants of the human CD4 molecule which the rhesus monkey recognizes as foreign. Surprisingly, however, human rsCD4-treated rhesus monkeys generated antibody responses that recognize at least portions of their autologous CD4 molecule. The antigenicity of a poorly immunogenic molecule can be significantly enhanced by attaching it to a highly immunogenic carrier protein. Thus, the rhesus monkeys may have developed an anti-self CD4 antibody response because their immune cells were presented with autologous CD4 epitopes in direct continuity with foreign antigenic determinants. It is also possible that a component of the rsCD4-elicited anti-CD4 antibody response of the monkeys was induced by self CD4 amino acid sequences presented to the immune system in a nonphysiologic tertiary conformation.

At high concentrations *in vitro*, human rsCD4 can inhibit SIV_{mac} infection of rhesus monkey PBLs (13, 20). It is, therefore, possible that some of the clinical improvement demonstrated in the rsCD4-treated SIV_{mac}-infected rhesus monkeys in our earlier studies may have been mediated by the direct interference by rsCD4 with SIV_{mac} binding to CD4-bearing cells *in vivo* (20). However, these SIV_{mac}-infected monkeys also developed anti-rsCD4 antibody responses during the course of their treatment (data not shown). Moreover, plasma from these animals, taken during and within 2 weeks after cessation of treatment with rsCD4, inhibited SIV_{mac} replication in rhesus monkey cells *in vitro* (data not shown). These observations raise the distinct possibility that the antibody response generated by these monkeys may have contributed to the anti-viral effect of this treatment.

The use of specific antibodies as therapeutic agents has been explored in many clinical settings. In the preantibiotic era, specific serotherapy was utilized with some success in the treatment of bacterial illnesses. T-lymphocyte-specific mAbs are currently used in the treatment of acute renal allograft rejections (21). However, the use of mAbs as therapeutic agents has been limited by their elicitation of anti-mouse immunoglobulin responses (22). The use of a soluble protein such as rsCD4 to elicit and maintain a specific antibody response might harness the potential therapeutic utility of antibodies while circumventing the problem of "neutralizing" anti-immunoglobulin responses.

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