disks each were collected at each time interval. The tissue was placed in absolute methanol for 18 hours. The methanol extract was removed, the tissue was washed with methanol, and the methanol fractions were combined. The final extract was dried under nitrogen and adjusted to 100 μl with absolute methanol. Samples (50 μl) were separated by HPLC on a C-18 reverse-phase column as described in (*2*).

9. H. A. Stafford, in *The Biochemistry of Plants*, E. E. Conn, Ed. (Academic Press, New York, 1981), vol. 7, pp. 118–137; G. Hrazdina and G. J. Wagner, *Arch. Biochem. Biophys.* **237**, 88 (1985).
10. R. C. Pecket and C. J. Small, *Phytochemistry* **19**, 2571 (1980); C. J. Small and R. C. Pecket, *Planta* **154**, 97 (1982); M. Nozue and H. Yasuda, *Plant Cell Rep.* **4**, 252 (1985).
11. L. M. V. Jonsson, W. E. Donker-Koopman, P. Uitslager, A. W. Schram, *Plant Physiol.* **72**, 287 (1983).

12. Journal article 12356 of the Purdue University Agricultural Experiment Station (AES). Use of the AES Electron Microscopy Center is acknowledged. Supported in part by NSF grant DCB 89-05121 and the Indiana Corporation for Science and Technology.

19 January 1990; accepted 18 April 1990

Human CD4 Binds Immunoglobulins

PETAR LENERT, DANIEL KROON, HANS SPIEGELBERG, EDWARD S. GOLUB, MAURIZIO ZANETTI*

T cell glycoprotein CD4 binds to class II major histocompatibility molecules and to the human immunodeficiency virus (HIV) envelope protein gp120. Recombinant CD4 (rCD4) bound to polyclonal immunoglobulin (Ig) and 39 of 50 (78%) human myeloma proteins. This binding depended on the Fab and not the Fc portion of Ig and was independent of the light chain. Soluble rCD4, HIV gp120, and sulfated dextrans inhibited the CD4-Ig interaction. With the use of a panel of synthetic peptides, the region critical for binding to Ig was localized to amino acids 21 to 38 of the first extracellular domain of CD4. CD4-bound antibody (Ab) complexed with antigen approximately 100 times better than Ab alone. This activity may contribute to the Ab-mediated enhancement of cellular HIV interaction that appears to depend on a trimolecular complex of HIV, antibodies to gp120, and CD4.

THE GLYCOPROTEIN CD4 PARTICIPATES in adhesion of T lymphocytes to target cells (*1*), thymic development (*2*), and transmission of intracellular signals during T cell activation (*3*). CD4 is also the high-affinity receptor for HIV gp120 (*4*), an activity of amino acids 42 to 55 of the NH_2-terminal domain (*5*). This domain has an Ig-like fold, and residues 40 to 55 correspond to the second complementarity-determining region of human Ig κ light (L) chain (*5*).

In preliminary experiments, we observed that serum Ig from normal subjects bound soluble recombinant CD4 (rCD4) and CD4 solubilized from the $CD4^+$ CEM cell line in enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA). We therefore tested 50 human myeloma proteins of unknown antigen reactivity representing each class and subclass (Table 1). Thirty-nine of 50 (78%) myeloma proteins devoid of rheumatoid factor activity bound CD4 regardless of their heavy (H) or L chain isotype. The binding values varied among myeloma proteins of the same class, subclass, and L chain type. Although all classes bound, none of the six myeloma proteins in the IgG4 subclass tested were positive, even at relatively high concentration (200 μg/ml). Myeloma proteins representative of each Ig class were labeled with ^{125}I, and they displayed a single band of 46 to 50 kD in a protein immunoblot of soluble rCD4 (Fig. 1A). This was the same size as the band identified by Leu3a, a murine monoclonal antibody (MAb) to CD4. Whereas ^{125}I-labeled Ig binding was unaffected by electrophoresis of CD4 under reducing conditions, MAb Leu3a binding decreased after reduction. This suggests that the Ig binding site, unlike the Leu3a epitope (*6*), is less affected by the intactness of the three-dimensional conformation of the molecule. However, because it is generally accepted that partial renaturation takes place after blotting, the extent to which the tertiary structure of CD4 still influences Ig binding cannot be assessed with precision. Human Ig also bound $CD4^+$ T cells. In flow cytometry, CEM cells were stained by polyclonal Ig with a unimodal peak, yielding a reproducible and consistent (~5 ×) shift in the mean fluorescence intensity above that of control cells (Fig. 1B). By comparison, MAb Leu3a gave a mean fluorescence intensity ten times as great.

The relation of the Ig and gp120 binding sites of CD4 was analyzed in three ways. Soluble recombinant gp120 inhibited (50% inhibition at <1 μg of gp120 per milliliter) the binding of a ^{125}I-labeled human myeloma protein to rCD4 in solid-phase RIA (Fig. 2A). Dextran sulfate, which blocks the binding of HIV to $CD4^+$ cells and prevents infection in vitro (*7*), inhibited the CD4-Ig interaction (50% inhibition at <0.5 μg of dextran per milliliter), an effect apparently on CD4 because incubation with dextran sulfate followed by washing was equally

Table 1. Human myeloma proteins of different isotype bind rCD4. Binding of human myeloma proteins to soluble rCD4 in ELISA. Fifty purified human myeloma proteins representing all Ig classes and subclasses were tested. Polyvinyl chloride microtiter plates (Dynatech) were coated with soluble rCD4 (Receptin, Biogen) (3 μg/ml) in 0.9% NaCl by incubating at 4°C overnight. Human myeloma proteins diluted (40 μg/ml) in phosphate-buffered saline (PBS) containing 1% bovine serium albumin (BSA) and 0.5% Tween 20 (PBSA), were incubated for 3 hours at room temperature. Bound antibodies were revealed with a goat antibody to human Ig (H chain specific) conjugated to horseradish peroxidase (HRP) (Sigma) in PBSA containing 15% newborn calf serum. Detection of IgE and IgD required an intermediate incubation with goat antibodies to IgE or IgD, respectively, followed by HRP-conjugated swine antibody to goat Ig (Tago). Values represent the mean absorbance (A_{492}), which shows binding of myeloma proteins grouped by H chain isotype. SEM, <15%. The 40 μg/ml concentration was chosen on the basis of pilot experiments in which the saturation points were determined with myeloma proteins of different classes. Saturation of binding was obtained at 40 μg/ml for IgG, IgM, IgD, and IgE, and at 80 μg/ml for IgA. By comparison, MAb Leu3a (Becton-Dickinson) specific for CD4 reached plateau at ~4 μg/ml. A_{492} of the second antibody alone (1:1500) was less than 0.050. Values of A_{492} less than 0.2 were considered positive. By comparison, the binding value (A_{492}) of polyclonal human Ig human γ-globulins fraction II, Pentex, Miles) (40 μg/ml) was 1.5 (see Table 2). Binding to a recombinant MAb (IgG2b, κ) (*23*) and to BSA (molecular weight 65,000) are specificity controls.

Myeloma protein	No. positive	Absorbance (A_{492})		
		rCD4	rIg	BSA
IgG1	4/6	0.61	0.13	0.14
IgG2	5/6	0.48	0.09	0.1
IgG3	6/6	0.58	0.15	0.15
IgG4	0/6	0.18	0.16	0.14
IgM	6/6	0.81	0.12	0.13
IgA1	3/5	0.36	0.11	0.12
IgA2	3/3	0.38	0.11	0.09
IgD	7/7	0.85	0.11	0.19
IgE	5/5	1.10	0.10	0.13

P. Lenart and M. Zanetti, Division of Dermatology, Department of Medicine; University of California, San Diego, San Diego, CA 92103.
D. Kroon, The R. W. Johnson Pharmaceutical Research Institute, Raritan, NJ 08869–0602.
H. Spiegelberg, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.
E. S. Golub, Johnson & Johnson Laboratories, Research Institute of Scripps Clinic, La Jolla, CA 92037.

*To whom correspondence should be addressed.

inhibitory (Fig. 2B). A series of synthetic peptides encompassing the first two extracellular domains of CD4 showed that peptide p16-49 and p21-49 in the first extracellular domain bound Ig comparably with rCD4 (Fig. 2C). Peptide 39-60 gave no detectable signal, whereas low binding occurred on peptides comprising the region 66 to 82 (p58-82 and p66-90). Therefore, amino acid residues 21 to 38 appeared to be critical for the CD4-Ig interaction. To directly test this and to better delineate the minimal peptide sequence both sufficient and necessary to mediate binding to Ig, we synthesized and tested the p21-38 and a series of truncated peptides (Fig. 3). Peptides 22-38, 23-38, 24-38, and 21-37 showed binding comparable to that of p21-38, p21-49, and rCD4. From the NH_2-terminus a slight decrease in binding occurred with p25-38, followed by a marked drop with p29-43. Peptides shortened at the COOH-terminus showed a progressive drop in their ability to bind Ig. Collectively, these data point to a putative minimal Ig-binding peptidic region of CD4 within amino acid residues 25 to 37. That peptides considerably shorter than p21-49 bound Ig suggests that this property is not a function of the length of the peptide. Sulfated dextrans, unlike soluble gp120, also inhibited the binding of Ig to synthetic p21-49.

Table 2. Recombinant CD4 binds the Fab and not the Fc fragment of immunoglobulins $F(ab')_2$ fragments of human polyclonal IgG (HGG) and two myeloma IgG1, κ proteins, Mag and Gil, were prepared by pepsin digestion (18 hours at 37°C) in 0.12 M sodium acetate buffer, pH 4.0 (*26*). The digestion was terminatd by addition of 3 M tris-base (Trizma) buffer, pH 8.0, followed by separation on a Sephacryl S-300 column. Fab and Fc fragments were prepared by papain digestion at an enzyme-to-Ig ratio of 1:200. The digest was dialyzed against 0.02 M tris-HCl, pH 8.0, followed by chromatography on DEAE-cellulose and CM (carboxymethyl cellulose) solumns. The purity and the molecular weight of $F(ab')_2$, Fab, and Fc fragments were checked by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). The assay was performed as in Table 1. Values for A_{492} represent means of triplicate wells, which differed by <5%. Proteins were tested at 40 μg/ml. Intact Ig and Fc fragments were detected with an HRP-conjugated goat antibody to human Ig (H chain specific) (Sigma); $F(ab')_2$ and Fab fragments were detected with HRP-conjugated goat antibody to human κ and λ L chain (Caltag). The working dilution of HRP-conjugated reagents was normalized on intact Ig for equivalent binding. The A_{492} binding of HRP-conjugated goat antibody to human Ig to Fc fragments coated (2 μg/ml) on microtiter plates was >2.0. The binding of ^{125}I-labeled C1q (Cytotech) to microtiter wells coated with with Fab and Fc fragments (2 μg/ml) as determined by RIA is indicated. Values refer to the mean counts per minute of triplicate wells which differed by <5%; by comparison, binding to BSA was 623 cpm. ND, not determined.

Proteins	rCD4 (A_{492})				p21-49 (A_{492})				C1q (cpm)	
	Ig	$F(ab')_2$	Fab	Fc	Ig	$F(ab')_2$	Fab	Fc	Fab	Fc
HGG	1.5	0.8	0.8	0.1	1.5	0.9	0.8	0.2	512	2928
IgG1, κ	0.8	ND	1.2	0.1	1.1	ND	1.5	0.1	396	2428
IgG1, κ	0.9	ND	1.8	0.1	0.8	ND	2.0	0.1	485	3636

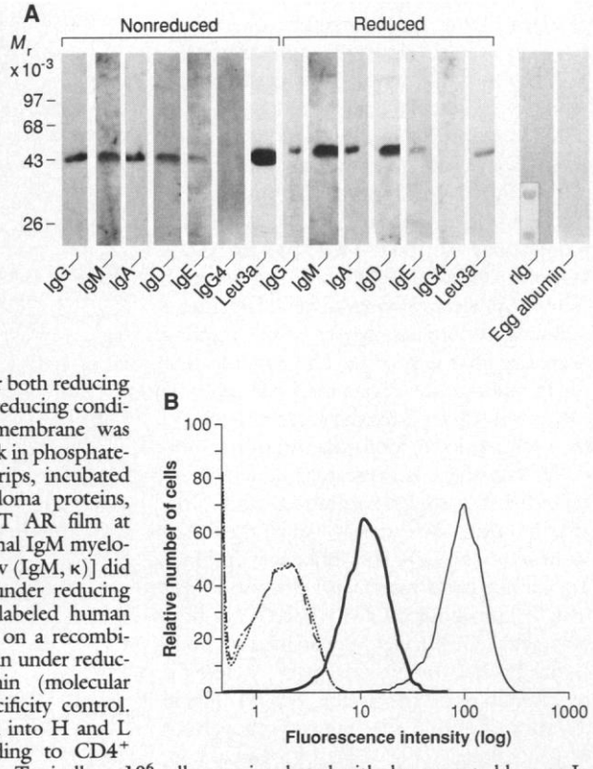

Fig. 1. (**A**) The binding of ^{125}I-labeled human myeloma proteins to rCD4 in an immunoblot. Purified human myeloma of each class [Mag (IgG1, κ); Wil (IgM, κ); May (IgD, λ); Put (IgA1, λ); PS (IgE, κ); and Heb (IgG4, λ)] and murine MAb Leu3a (Becton-Dickinson), were labeled by the chloramine-T method (*24*). Electrophoresis with 10% SDS and polyacrylamide gel, then protein transfer of rCD4 (5 μg per lane) to 0.45-μm Nitro Screen West protein transfer membranes (Du Pont) were done under both reducing (5% β-mercaptoethanol) and nonreducing conditions (*25*). After blotting, the membrane was blocked by soaking in 10% dry milk in phosphate-buffered saline (PBS), cut into strips, incubated with individual ^{125}I-labeled myeloma proteins, and exposed to Kodak X-OMAT AR film at −70°C for 18 hours. Two additional IgM myeloma proteins [Vin (IgM, κ) and Bew (IgM, κ)] did not show a more intense band under reducing conditions. The binding of ^{125}I-labeled human myeloma protein Mag (IgG1, κ) on a recombinant Ig (IgG1, λ) (*10*) that was run under reducing conditions and egg albumin (molecular weight 45,000) is shown as specificity control. The inset shows that rIg resolved into H and L chain. (**B**) Flow cytometry binding to $CD4^+$ CEM cells by human polyclonal Ig. Typically, ~10^6 cells were incubated with deaggregated human Ig (100 μg/ml) (▬) in Hanks' balanced salt solution containing 2% bovine serum albumin (BSA) for 90 min at 37°C. After washing, the cells were incubated with a goat antibody [$F(ab')_2$] to human IgG (H and L chain specific) conjugated with biotin (Caltag) at 4°C for 30 min, followed by a second incubation with streptavidin conjugated to R-phycoerythrin (Caltag). The binding of MAb Leu3a (20 mg/ml) (——) was similarly revealed using a goat antibody to mouse Ig conjugated with biotin (Caltag). Goat antibody to human Ig (− · −) and to mouse Ig (· · · ·) followed by the streptavidin conjugate served as controls. At least 10,000 cells per sample were analyzed in an Ortho Cytofluorograf IIs. Dead cells were excluded on the basis of low, forward, and 90° light-scattering properties.

We assessed the relative importance of the Fab versus the Fc region in binding rCD4 using human polyclonal Ig, two IgG1 myeloma proteins, and their respective $F(ab')_2$, Fab, and Fc fragments. Comparable binding by the Fab but not by the Fc fragments occurred both on rCD4 and p21-49 (Table 2). Conversely, the Fc but not the Fab fragments bound ^{125}I-labeled C1q, a ligand of the CH2 domain (*8*). This shows that the Fc fragments still have structural integrity. Thus, rCD4 interacts with the Fab region [the variable (V) domain of the H and L chains plus the CH1 domain] of Ig. That the Fc fragment is apparently not involved was confirmed by the lack of reactivity of five human heavy chain disease (HCD) proteins with either rCD4 or p21-49. The HCD proteins are molecules of variable length consisting of isolated H chain that lacks the V region (natural deletion mutants) (*9*) (Table 3). Therefore, the Fc region does not mediate binding to CD4. The fact that the human myeloma proteins that bound rCD4 were both of κ and λ chain indicated that the L chain isotype did not affect binding. To directly test this, we used the H chain of a murine MAb that binds rCD4 associated with either a κ or a λ chain as a result of a chain recombination (*10*). The recombinant molecules bound comparably to both rCD4 and p21-49, and the isolated H but not L chain bound to rCD4 and p21-49 (Table 4). Thus binding to CD4 is probably mediated by the Fd region (V_H plus CH1) of the Ig molecule.

Because of the involvement of the Fab, we investigated the effect of antigen (Ag) on the CD4-Ig interaction. Murine MAbs to thyro-

Table 3. Human heavy chain disease (HCD) proteins did not bind rCD4 and p21-49. The following HCD proteins were tested: Cra$^{12-216}$ (γ1; hinge region + CH2 and CH3); Zuc$^{18-216}$ (γ3; hinge region + CH2 and CH3); Hal$^{10-252}$ (γ4; CH2 and CH3); Bur$^{7-338}$ (μ; CH3 and CH4); and Mey (γ1). The superscript numbers accompanying the protein name identify the corresponding amino acid residues in the HCD protein and in normal Ig of the same H chain isotype (*9*). Proteins were tested (40 μg/ml) for binding to rCD4 and p21-49 as in Table 2. Wells coated with p39-60 and goat antibody to human Ig (anti-Ig) served as negative and positive controls, respectively. The assay was performed as in Table 1. Values for A_{492} represent means of triplicate wells, which differed by <5%. Bound HCD proteins were detected using an HRP-conjugated goat antibody to human Ig (H chain–specific) (Sigma).

Well coating	Absorbance (A_{492})				
	Cra	Zuc	Hal	Bur	Mey
rCD4	0.1	0.1	0.2	0.2	0.2
p21-49	0.2	0.2	0.2	0.4	0.3
p39-60	0.1	0.1	0.1	0.1	0.1
Anti-Ig (Fc)	1.4	1.2	1.5	1.4	1.5

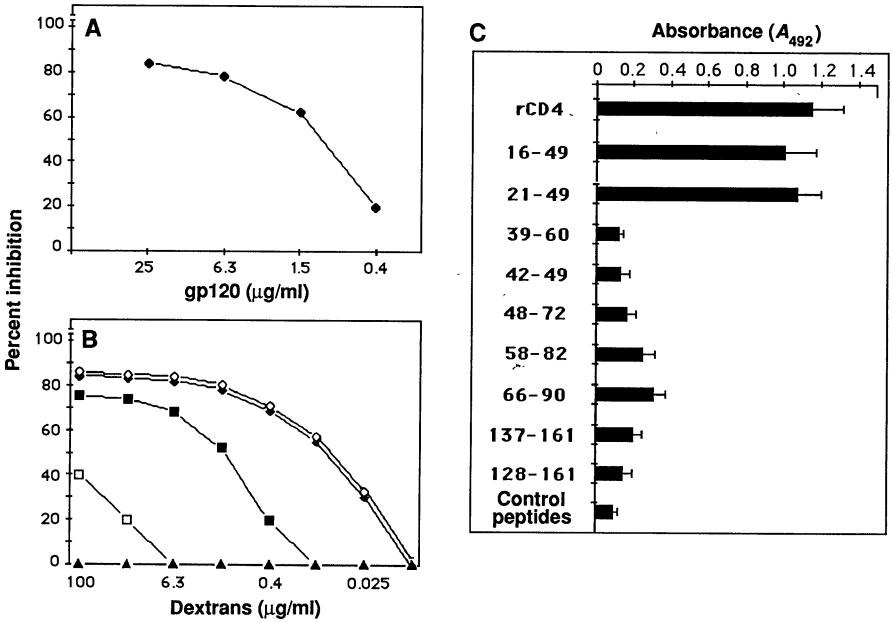

Fig. 2. (**A**) Inhibition of the CD4-Ig interaction by recombinant gp120. Soluble recombinant gp120 (Chiron) inhibits the RIA binding of a ^{125}I-labeled IgE, κ myeloma protein (PS) to rCD4. (**B**) Inhibition of the CD4-Ig interaction by dextrans. Binding to rCD4 of an IgG1, κ myeloma protein (10 μg/ml) is inhibited by sulfated dextran [(◇) 8 kD, (◇) 5 kD, and (■) 500 kD] and nonsulfated dextran [(□) 10 kD and (▲) 506 kD] (Sigma). (**C**) Soluble CD4-derived synthetic peptides (25 μg/ml) and rCD4 (25 μg/ml) block the binding to rCD4 immobilized on microtiter wells of preformed complexes of thyroglobulin (60 μg/ml) and MAb to thyroglobulin (5 μg/ml). Assay conditions were as described in Table 1 and Fig. 2, with the exception that thyroglobulin–thyroglobulin mAb complexes were first incubated with rCD4 or CD4 synthetic peptides for 1 hour at +37°C. Results are the means of triplicate wells, which differed by <5%. (Maximum binding value, ~1.0 A_{492}.)

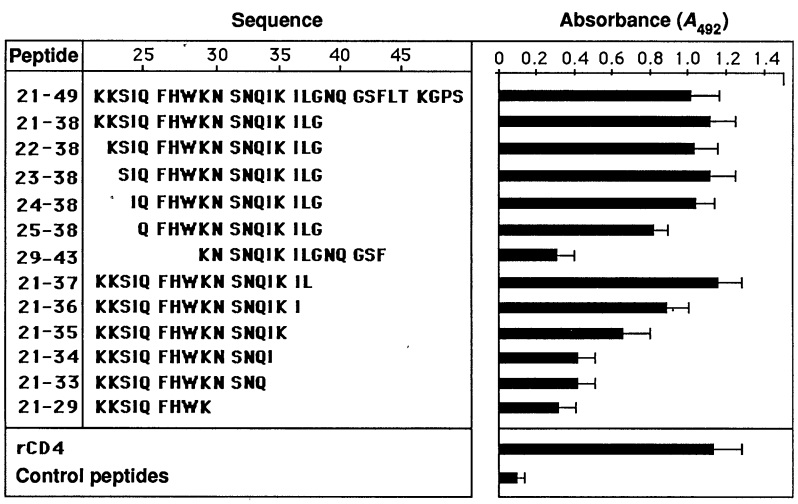

Fig. 3. Mapping of the Ig binding site on CD4 with synthetic peptides spanning from amino acid residue 21 to 38 of the first extracellular domain. The binding to rCD4 and p21-49 is shown as positive controls. Performance of the assay and control peptides are as in Fig. 2. Values refer to means + SEM. Tests were done in triplicate.

globulin that were complexed with Ag bound CD4 several times better than antibody (Ab) alone (Fig. 4A). Considering the molecular weight of Ab 160,000 and that of the Ag 660,000, we estimated that maximum binding on rCD4 occurred at a molar Ab/Ag ratio of less than 0.5, a value characteristic of soluble complexes. Although this is compatible with enhanced binding in Ag excess, and cross-linking to rCD4 through the Ag could be excluded, low level aggregation due to the valence of the antigen cannot be ruled out completely. Similar results were obtained using F(ab′)$_2$ fragments. The degree of enhanced binding varied somewhat with different Ab-Ag pairs. The specificity of the binding of Ab-Ag complexes to rCD4 was demonstrated in a subsequent experiment in which soluble rCD4 inhibited binding in a concentration-dependent manner (Fig. 4B). Because 50% inhibition was obtained with ~5 μg/ml, the relative avidity of the interaction between Ab-Ag complexes and rCD4 can be estimated (*11*) in the order of 10^7 M^{-1}, about two orders of magnitude as great as that of Ab alone. Soluble p21-49, unlike other CD4-derived and control peptides, also inhibited the binding of preformed Ab-Ag complexes to rCD4 (Fig. 4C). The partial inhibition by p58-82 raises the question of whether these residues may form a separate binding site. A computer-generated model of CD4 based on sequence homology and alignment with the REI Bence-Jones protein (*6*) indicates that residues 34 to 38 and 74 to 82, albeit distant in the primary structure, could be contiguous in the three-dimensional folding of the native molecule, hence forming a complex Ig binding site.

The structural homology between the NH_2-terminal domain of CD4 and the V region of Ig L chain suggests that the CD4-Ig interaction reported here is of the domain-domain type interaction between polypeptides with an Ig fold. This type of interaction traditionally of a noncovalent nature is well known for the V-V association in the Ig molecule. It has been suggested that it occurs between various members of the Ig gene superfamily (*12*), including between Ig and T cell receptor V regions (*13*). Since synthetic segments of an Ig V_H have been shown to associate with a natural L chain to form an antigen binding site (*14*), our use of synthetic peptides as surrogate of CD4 (an L chain–homologous polypeptide) is plausible. The relative affinity of interaction be-

tween V_H and V_L is also low ($\sim 10^6\ M^{-1}$) (*15*). Finally, it is intriguing to speculate that the Ig binding peptide may be mimicking the natural domain association between V_H and V_L. When one aligns the sequences of CD4 and human V κ chains (*6*) and examines the residues in CD4 that bind the H chain, they are seen to be in the same region where V κ contacts the H chain (*6, 16*).

An interaction between CD4 and Ig suggests a mechanism for enhancement of HIV infection by antibodies. CD4, HIV gp120, and antibodies to gp120 form a trimolecular complex that modulates the CD4 molecule and, by means of cross-linking, delivers a down-regulatory signal to T cells (*17*) similar to the effect of some antibodies to CD4 (*18*). Enhancement of HIV infection by antibodies (*19*) may be dependent on CD4 (*20*). Therefore, CD4 may also participate in a phenomenon commonly believed to depend on Fc receptors at the surface of monocytes and macrophages (*21*). Our data suggest that the Ig and gp120 binding sites on CD4 are proximal but probably not identical, a view supported by the fact that Ig, unlike gp120 (*22*), bound reduced CD4. Although the ability of CD4 to bind Ab-Ag complexes may be sufficient for internalization by receptor-mediated endocytosis with consequent enhancement of infection (*4*), the contribution of Abs to particular sites of the virus cannot be ruled out. Our finding that CD4 and synthetic p21-49 bind Ab-Ag complexes tightly and specifically offers a mechanism that might account for Ab-mediated enhancement of HIV infection.

In conclusion, this report describes a new type of interaction between two members of the Ig gene superfamily, Ig and CD4. This is mediated directly by the NH_2-domain of CD4 between amino acids 21 to 38 and the Fd region of Ig. Thus, the CD4-Ig interaction is independent of an Fc receptor or receptors. Although the relevance of this finding in immune physiology remains to be established, its importance is best understood in light of the reports indicating that in HIV-infected individuals antibodies alone, or as Ab-Ag complexes, may cause enhancement of infection by means of a CD4-mediated mechanism. This and similar considerations are relevant to strategies of immunotherapy with soluble CD4 and immunization with gp120 subunit vaccines.

Table 4. Binding of Ig to rCD4 and p21-49 is independent of the L chain. Recombinant antibodies $\gamma_1 62$ (IgG1, λ) and $\gamma_1 V_k M$ (IgG1, κ) are L chain recombinant molecules of MAb 62 (IgG1, κ) produced by transfection of the cloned V_H gene (a member of the $V_H 7183$ gene family) into J558L or P3×63-Ag8.653 cells to yield a λ- or κ-bearing Ig, respectively (*10*). H and L chains of the native MAb were isolated as described (*27*). Proteins were tested (20 μg/ml) for binding to rCD4 and p21-49. Goat antibody to mouse Ig (anti-Ig) and p39-60 served as positive and negative controls, respectively. The assay was performed as in Table 1. Values for A_{492} represent means of triplicate wells which differed by <5%. Bound Ig were detected using an HRP-conjugated goat antibody to mouse Ig (H chain specific) (Cappel).

Well coating	Absorbance (A_{492})				
	IgG1, κ	rIgG1, κ	rIgG1, λ	H chain	L chain
rCD4	0.6	0.8	0.8	0.8	0.1
p21-49	0.9	0.8	0.8	0.9	0.2
p39-60	0.1	0.1	0.1	0.1	0.1
Anti-Ig	>2.0	>2.0	>2.0	>2.0	>2.0*

*The L chain was detected with a rat antibody to mouse κ L chain.

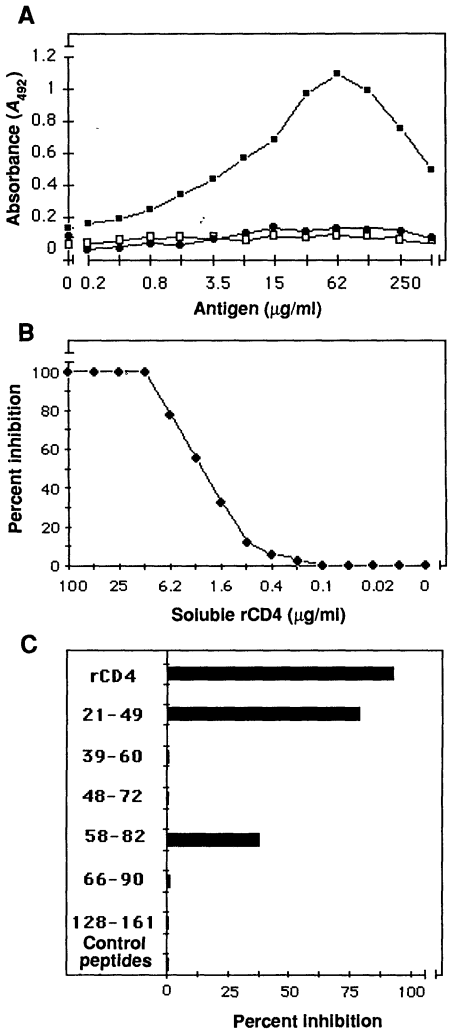

Fig. 4. (**A**) Binding of preformed complexes of thyroglobulin and thyroglobulin MAb to rCD4 in ELISA. A standard concentration (5 μg/ml) of MAb 62 (*10*) was incubated overnight with increasing amounts of Ag (thyroglobulin). The mixture was then transferred to a microtiter plate coated with rCD4 (3 μg/ml). After incubation (3 hours at room temperature), bound Ab-Ag complexes (■) were revealed by a horseradish peroxidase (HRP)–conjugated goat antibody to mouse Ig (1 hour at room temperature) as described in Table 1. In experiments in which the molar ratio between Ab and Ag was kept constant, saturable binding was observed at an Ab concentration 1/10th that for monomeric or heat-aggregated Ig. We could exclude the possibility that enhanced binding was due to cross-linking to rCD4 through Ag because ^{125}I-labeled thyroglobulin did not bind to rCD4 (rCD4, 812 cpm; BSA, 700 cpm; and thyroglobulin MAb, 5236 cpm). Thyroglobulin–thyroglobulin MAb complexes (□) and thyroglobulin mixed with a MAb to dinitrophenol (●) did not bind to BSA- or rCD4-coated microtiter wells, respectively. (**B**) Competitive inhibition by soluble rCD4 of the binding of a preformed complex of thyroglobulin (60 μg/ml) and thyroglobulin MAb (5 μg/ml) to rCD4 (3 μg/ml). Assay conditions were as described above, with the exception that Ab-Ag complexes were first incubated with rCD4 for 1 hour at 37°C. Results are the means of triplicate wells which differed by <5%. (**C**) Mapping of the Ig binding site on CD4 with the use of synthetic peptides. Eight human IgG myeloma proteins (three IgG1, three IgG2, and two IgG3) were used as serological probes for binding to rCD4, 9 CD4-derived peptides, and 20 CD4-unrelated control peptides, respectively. All peptides were made on an automated peptide synthesizer with a *p*-methylbenzhydrylamine resin (US Biochemicals), and purified by either ion-exchange chromatography or high-performance liquid chromatography (HPLC). Polyvinyl microtiter plates were coated with individual peptide (5 μg/ml) by overnight incubation at 4°C. Myeloma proteins (40 μg/ml) were incubated for 3 hours at room temperature. Bound Igs were revealed as in Table 1. Values refer to means + SEM. Tests were done in triplicate.

REFERENCES AND NOTES

1. P. Marrack *et al.*, *J. Exp. Med.* **158**, 1077 (1983); C. Doyle and J. L. Strominger, *Nature* **330**, 256 (1987); Q. J. Sattentau and R. A. Weiss, *Cell* **52**, 631 (1988); F. Emmrich, *Immunol. Today* **9**, 296 (1988); B. E. Bierer and S. J. Burakoff, *FASEB J.* **2**, 2584 (1988).
2. H. R. MacDonald, R. K. Lees, R. Schneider, R. M. Zinkernagel, H. Hengartner, *Nature* **336**, 471 (1988).
3. D. Gay *et al.*, *ibid.* **328**, 626 (1987); B. P. Sleckman *et al.*, *ibid.*, p. 351; A. Veillette, M. A. Bookman, E. M. Horak, L. E. Samelson, J. B. Bolen *ibid.* **338**, 257 (1989).
4. A. G. Dalgleish *et al.*, *ibid.* **312**, 763 (1984); D. Klatzmann *et al.*, *ibid.*, p. 767; J. S. McDougal *et al.*, *Science* **231**, 382 (1986); P. J. Maddon *et al.*, *Cell* **47**, 333 (1986); N. R. Landau, M. Warton, D. R. Littman, *Nature* **334**, 159 (1988).
5. L. K. Clayton *et al.*, *Nature* **335**, 363 (1988); A. Peterson and B. Seed, *Cell* **54**, 65 (1988); J. Arthos *et al.*, *ibid.* **57**, 469 (1989).
6. P. A. Bates, M. J. McGregor, S. A. Islam, Q. J. Sattentau, M. J. E. Sternberg, *Protein Eng.* **3**, 13 (1989); Q. J. Sattentau *et al.*, *J. Exp. Med.* **170**, 1319 (1989).

7. H. Mitsuya *et al.*, *Science* **240**, 646 (1988).
8. A. R. Duncan and G. Winter, *Nature* **332**, 738 (1988).
9. E. C. Franklin and B. Frangione, *Contemp. Top. Mol. Immunol.* **4**, 89 (1975).
10. M. Sollazzo *et al.*, *Protein Eng.*, in press.
11. A. Nieto, A. Gaya, M. Jansa, C. Moreno, J. Vives, *Mol. Immunol.* **21**, 537(1984); S. C. Wallick, E. A. Kabat, S. L. Morrison, *J. Exp. Med.* **168**, 1099 (1988). The relative avidity of the interaction was calculated by means of a solid-phase assay in which the binding of Ig or Ab-Ag complexes to rCD4 on polyvinyl microtiter wells is competed for by soluble rCD4. For the binding of preformed thyroglobulin:thyroglobulin MAb complexes (Fig. 4B), a relative K_a value of $\sim 10^{-7}$ was estimated. By comparison, the K_a of the same noncomplexed MAb was $\sim 3 \times 10^{-5}$.
12. A. F. Williams and A. N. Barclay, *Annu. Rev. Immunol.* **6**, 381 (1988).
13. M. L. B. Becker *et al.*, *Cell* **58**, 911 (1989); J. Goverman *et al.*, *ibid.* **60**, 929 (1990).
14. T. Kubiak, D. B. Whitney, R. B. Merrefield, *Biochemistry* **26**, 7849 (1987).
15. M. Klein *et al.*, *ibid.* **18**, 1473 (1979).
16. O. Epp *et al.*, *Eur. J. Biochem.* **45**, 513 (1974).
17. R. S. Mittler and M. K. Hoffmann, *Science* **245**, 1380 (1989).
18. I. Bank and L. Chess, *J. Exp. Med.* **162**, 1294 (1985).
19. W. E. Robinson, Jr., D. C. Montefiori, W. M. Mitchell, *Lancet* **i**, 790 (1988); A. Takeda, C. U. Tuazon, F. A. Ennis, *Science* **242**, 580 (1988); J. Homsy, M. Meyer, M. Tateno, S. Clarkson, J. A. Levy, *ibid.* **244**, 1357 (1989).
20. W. E. Robinson, Jr., D. C. Montefiori, D. H. Gillespie, W. M. Mitchell, *J. Acquired Immune Defic. Syndr.* **2**, 33 (1989); S. Matsuda *et al.*, *Scand. J. Immunol.* **30**, 425 (1989); A. Takeda, R. W. Sweet, F. A. Ennis, *Second Annual National Coop. Vaccine Groups for AIDS* (abstr.) (15 to 18 October 1989).
21. S. C. Kliks and S. B. Halstead, *Nature* **285**, 504 (1980).
22. N. E. Richardson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6102 (1988); C. C. Ibegbu *et al.*, *J. Immunol.* **142**, 2250 (1989).
23. rIg is a recombinant Ig molecule whose H chain derives from the fusion of the V_H62 gene (*10*) with a murine IgG2b C region gene, and is associated with a murine λ_1 L chain. The rIg is produced by J558L cells.
24. P. L. McConahey and F. J. Dixon, *Methods Enzymol.* **70A**, 210 (1980).
25. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
26. L. A. Steiner and P. M. Blumberg, *Biochemistry* **10**, 4725 (1971).
27. M. Zanetti, F.-T. Liu, J. Rogers, D. H. Katz, *J. Immunol.* **135**, 1245 (1985).
28. Supported by grants AI 23871 from the National Institute of Allergy and International Research and Exchange Board (P.L.). We thank L. Steiner and B. Frangione for making available the human myeloma proteins, Biogen for the gift of soluble rCD4 (Receptin), Chiron for supplying soluble recombinant gp120, and D. Young for the flow cytometry analysis.

11 December 1989; accepted 27 April 1990

Induction of Neonatal Tolerance to Mls^a Antigens by CD8$^+$ T Cells

SUSAN R. WEBB AND JONATHAN SPRENT

Antigen-specific tolerance of T cells to minor lymphocyte stimulatory (Mls) antigens can be induced in mice by neonatal injection of foreign lymphohematopoietic cells. Although immune responses to Mls^a antigens are controlled by B cells, CD8$^+$ T cells were the most effective cell type for induction of Mls^a tolerance. Tolerance was evident in both thymus and lymph nodes and could be induced by as few as 2×10^4 CD8$^+$ T cells; these cells were 50 to 100 times as potent as CD4$^+$ cells or B cells in causing functional tolerance and deletion of $V_\beta 6^+$ T cells. Thus, intrathymic contact with antigens expressed on CD8$^+$ T cells may play an important role in controlling the normal development of tolerance.

THE SELECTIVE CAPACITY OF T LYMPHOCYTES to respond to foreign antigens while maintaining tolerance to self is one of the hallmarks of the immune system. Although self tolerance has generated much investigation, the mechanisms involved are not well understood, and it is unclear which cell types are responsible for presenting antigen in tolerogenic form. The prevailing view is that tolerance is induced intrathymically and reflects T cell contact with specialized antigen-presenting cells (APCs) such as macrophages and dendritic cells (*1*). This question is most easily addressed with the model of Billingham *et al.* for neonatal tolerance induction (*2*). We investigated the cell types controlling the induction of neonatal tolerance to Mls antigens in mice. The high precursor frequency of T cells reactive to Mls antigens and the availability of antibodies specific for Mls-reactive T cells makes the Mls system useful for probing tolerance induction.

Mls antigens are poorly characterized cell surface molecules that are immunogenic for unprimed T cells (*3, 4*). These antigens show limited polymorphism, and only two stimulatory forms, Mls^a and Mls^c, are known to exist. T cell reactivity to Mls antigens (anti-Mls response) correlates with expression of particular T cell receptor (TCR) molecules (*5, 6*). Thus, anti-Mls^a responses are controlled mainly by T cells expressing $V_\beta 6$ and $V_\beta 8.1$ TCRs, whereas anti-Mls^c responses involve $V_\beta 3^+$ T cells. Conversely, *Mls^a* and *Mls^c* mice show selective deletion of $V_\beta 6^+$ plus $V_\beta 8.1^+$ cells and $V_\beta 3^+$ cells, respectively.

Because Mls molecules cannot be detected serologically (*3, 4*), information on the tissue distribution of Mls molecules has depended on defining the cell types capable of stimulating Mls-reactive T cells and T hybridomas. Mls antigens are presented effectively by B cells but not by T cells or typical APCs such as macrophages and dendritic cells (*7–9*). In the case of T cells, these data have to be viewed with caution, however, because recognition of Mls antigens requires

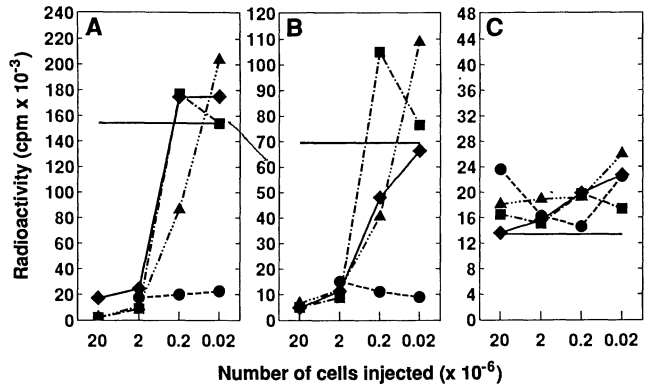

Fig. 1. Relative efficiency of different cell types in inducing functional tolerance to (**A**) Mls^a and (**B**) Mls^c antigens. (**C**) Response to H-2^p. B10.BR (*H-2^k*, *Mls^b*) neonates were intravenously injected with various numbers of (B10.BR × CBA/J)-F_1 (*H-2^k*, *Mls^b* × *H-2^k*, *Mls$^{a/c}$*) cells within 24 hr of birth. T cell–depleted spleen cells (■) (T$^-$ spleen), CD4$^+$ T cells (◆), and CD8$^+$ T cells (●) were purified as described in Table 1. B cells (▲) were purified from T$^-$ spleen by subsequent passage over two sequential G10 columns followed by 2 hr of adherence to plastic tissue culture dishes to remove adherent cells. MLR were carried out 6 weeks later as described for Table 1 with CD4$^+$-enriched T cells (LN cells treated with anti-Ia, J11d, anti-CD8, anti–Lyt 1.1, anti–Lyt 2.1, and complement) as responders (1×10^5 cells per well). Spleen stimulators (5×10^5 cells per well) were mitomycin C–treated before culture. The response of normal (uninjected) B10.BR mice is indicated by a horizontal line on each panel.

Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.