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11. Rabbit antiserum to human IL-8 [R. M. Strieter *et al.*, *ibid.* **264**, 10621 (1989)] reacted with IL-8_{69}, IL-8_{72}, and IL-8_{77}, but not with other members of the β-thromboglobulin supergene family as determined by protein immunoblot analysis (*8*). The antiserum completely neutralized the neutrophil chemotactic activity of all forms of IL-8 tested without altering the chemotactic activities of the other members of the supergene family (*8*). In selected experiments, results were confirmed with murine monoclonal antibodies to IL-8 (8C4 or 46E5) [M. Sticherling, J.-M. Schröder, E. Christophers, *J. Immunol.* **143**, 1628 (1989)].
12. Although the endothelial surface was only washed gently, the LECAM-1–dependent adherence of unstimulated neutrophils to stimulated endothelial cells can only be monitored readily under conditions of flow (*2*).
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14. The IL-8 gradient established by the stimulated endothelial cells is shallow by necessity because the highest concentration of the cytokine would be found in the zone immediately subjacent to the endothelium. Consistent with this fact, neutrophils infiltrated no deeper than 200 μm below the endothelial cell surface. If, however, IL-8 was introduced into the basilar compartment of the constructs, neutrophils migrated completely through the collagen matrix into the lower chamber (*8*).
15. We have found that IL-1β–, TNFα-, or LPS-dependent transmigration was significantly reduced when endogenous IL-8 was allowed to equilibrate between the two compartments of the vessel wall construct. Because the contents of the luminal compartment of patent blood vessels are constantly exchanged in vivo by flowing blood, a concentration gradient of IL-8 leading into the subendothelial matrix would always be favored, thereby assuring efficient neutrophil transmigration. The collapse of an IL-8 gradient across the vessel wall surface and the consequent inhibition of neutrophil invasion would be predicted to occur only under those conditions in which normal blood flow was interrupted. Similarly, after this manuscript was submitted, Hechtman *et al.* reported that IL-8_{77} promotes neutrophil accumulation in vivo after its extravascular administration but inhibits neutrophil accumulation when administered intravenously [D. H. Hechtman *et al.*, *J. Immunol.* **147**, 883 (1991)].
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22. Endothelial cell–derived IL-8 associated with the type I collagen matrix was lost as a consequence of the multiple washes required for the immunohistochemical analyses. If, however, the endothelial cells were cultured on the more dense and physiologic substratum provided by the stromal surface of human amniotic tissue [M. B. Furie and D. D. McHugh, *J. Immunol.* **143**, 3309 (1989)], matrix-associated IL-8 remained localized within the interstitium.
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Suppression of the Immune Response by a Soluble Complement Receptor of B Lymphocytes

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The CD19-CR2 complex of B lymphocytes contains proteins that participate in two host-defense systems, the immune and complement systems. The ligand for the subunit of the immune system, CD19, is not known, but the complement receptor subunit, CR2 (CD21), binds activation fragments of the C3 component of the complement system and may mediate immunopotentiating effects of complement. A recombinant, soluble CR2 was prepared by fusing the C3-binding region of the receptor to immunoglobulin G1 (IgG1). The $(CR2)_2$-IgG1 chimera competed with cellular CR2 for C3 binding and suppressed the antibody response to a T cell–dependent antigen when administered to mice at the time of immunization. This inhibitory effect of $(CR2)_2$-IgG1 demonstrates the B cell–activating function of the CD19-CR2 complex and suggests a new method for humoral immunosuppression.

A MEMBRANE PROTEIN COMPLEX ON B lymphocytes contains CD19, a member of the immunoglobulin (Ig) superfamily, CR2 (also known as CD21), a receptor of the complement system, and several unidentified components (*1*). Treatment of such cells with antibody to CD19 results in the activation of both a protein tyrosine kinase (*2*) and a phospholipase C (*2, 3*). The protein tyrosine kinase linked to the CD19-CR2 complex may differ from that activated by the membrane Ig because activation of the CD19-CR2 complex does not induce phosphorylation of phospholipase C–γ1, which is phosphorylated on tyrosine in response to ligation of membrane Ig (*4*). Thus, the CD19-CR2 complex initiates a pathway of B cell signaling that is distinct from that triggered by the membrane Ig complex. Despite this distinction, activation of B cells by this complex can be synergistically enhanced by the membrane IgM complex (*1, 2, 5, 6*).

The iC3b and C3dg fragments of C3 and the Epstein-Barr virus (*7*) are the only known ligands for the CD19-CR2 complex, although, one may exist for CD19, and an inability to activate C3 by the classical complement pathway is associated with impaired antibody responses in animals and humans to antigens that are T dependent and independent (*8–10*). Individuals with inherited classical pathway deficiencies also have constitutively low serum levels of IgG4 (*11*) and frequently have autoimmune diseases (*12*). C3, however, is the parental molecule for ligands that interact with four complement receptors, CR1 through CR4, of which only CR2 associates with CD19 (*1, 13*). Thus, these immunoregulatory effects of the complement system could not be ascribed to the CD19-CR2 complex. We now demonstrate

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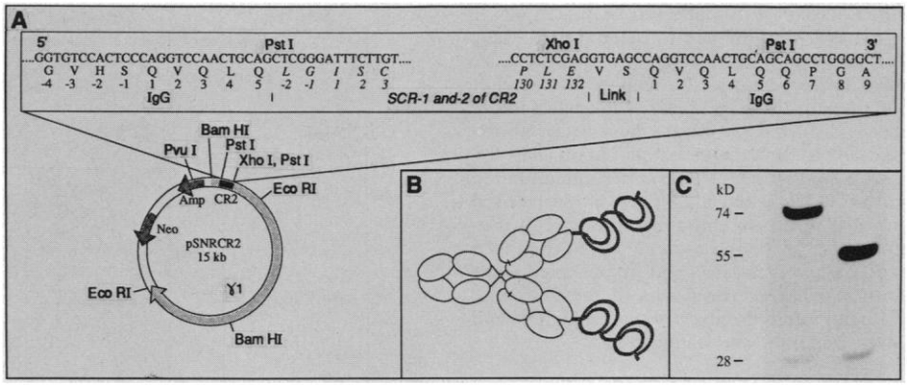


Fig. 1. Construction and expression of the $(CR2)_2$-IgG1 chimera (*25*). (**A**) Map of the pSNRCR2 plasmid encoding the CR2-γ1 chimeric construct. SCR-1 and -2 of CR2 (in italic) were inserted into a Pst I site of pSNR021, which contains the gene encoding a γ1 heavy chain specific for the hapten NP (gray region). The black region is the SCR insert. We placed linkers (link) at the 3′-end of the insert to encode valine, serine, and the first five amino acids of the heavy chain. The numbers refer to the positions of each amino acid in the γ1 and CR2 polypeptides, with negative numbers indicating leader sequences. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (**B**) Model of $(CR2)_2$-IgG1 showing attachment of the SCRs (right) of CR2 to the heavy chains of the Ig molecule (left). (**C**) SDS–polyacrylamide gels of purified, recombinant $(CR2)_2$-IgG1 (left lane) and IgG1 (right lane). Molecular size standards are shown at left margin in kilodaltons.

that CR2 is the receptor that mediates these effects of the complement system by demonstrating that a recombinant, soluble form of CR2 that competes with cellular receptor for ligand suppresses the antibody response in mice. Therefore, one function of the CD19-CR2 complex is to enhance the primary B cell response to antigens.

Three considerations led to the preparation of a soluble $(CR2)_2$-IgG1 chimera (Fig. 1B). (i) The dissociation constant (K_d) for a monovalent interaction between CR2 and iC3b or C3dg is in the micromolar range (*14, 15*), indicating a requirement for a multivalent soluble receptor. (ii) The ligand-binding site of CR2 functions after transfer to another protein (*16*). (iii) Chimeric Ig molecules can express ligand-binding domains of other receptors (*17*). Of the 15 tandemly aligned, short consensus repeats (SCRs) of the extracytoplasmic region of CR2, only the two NH_2-terminal repeats, SCR-1 and -2, are required for the binding of iC3b, C3dg, and Epstein-Barr virus (*16, 18*). A plasmid encoding these two SCRs fused to the NH_2-terminus of a murine γ-1 gene specific for the hapten 4-hydroxy-3-nitrophenacetyl (NP) (Fig. 1A) and the unmodified plasmid encoding the Ig gene (*19*) were transfected into J558L cells, which synthesize a lambda light chain (*20*). Transfectants were screened by an enzyme-linked immunosorbent assay (ELISA) for the production of $(CR2)_2$-IgG1 and IgG1, clones producing high amounts of the antibodies were expanded in bulk culture, and the two antibodies were purified from culture supernatants by affinity chromatography (*21*). Analysis of the purified proteins by SDS–polyacrylamide gel electrophoresis showed that the light chains were identical in size and that the heavy chain of the $(CR2)_2$-IgG1 chimera was 19 kD larger than that of IgG1, reflecting the presence of the two SCRs (Fig. 1C).

We assayed the binding of polymeric C3dg by the $(CR2)_2$-IgG1 chimera by incubating K562 cells expressing recombinant full-length CR2 with ^{125}I-labeled polymeric C3dg alone and in the presence of incremental concentrations of $(CR2)_2$-IgG1 or IgG1. The $(CR2)_2$-IgG1 caused a dose-related inhibition of the binding of polymeric C3dg to the K562 cells, with 50% inhibition occurring at 7 nM; IgG1 had no effect (Fig. 2). Therefore, the soluble receptor competes

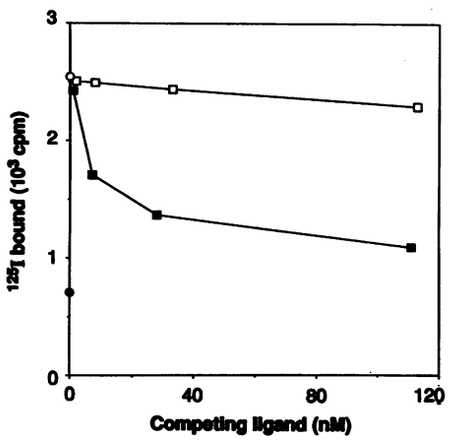


Fig. 2. Inhibition of binding of ^{125}I-labeled polymerized C3dg (1.9×10^5 cpm/μg) (*5*) to cellular CR2 by chimeric $(CR2)_2$-IgG1. Replicate samples of K562 cells stably expressing recombinant CR2 (*1*) were incubated with ^{125}I-labeled polymeric C3dg alone (○), with the indicated concentrations of IgG1 (□), with the indicated concentrations of $(CR2)_2$-IgG1 (■), or with monoclonal antibody OKB7 (10 μg/ml) (●) (Ortho Diagnostics Systems), which blocks binding of C3dg to CR2 (*26*). The cells were centrifuged through dibutyl-dinonyl phthalate, and the amount of ^{125}I bound to the cells was determined. The ^{125}I-labeled polymeric C3dg bound to 25% of the available CR2 sites on the K562 cells.

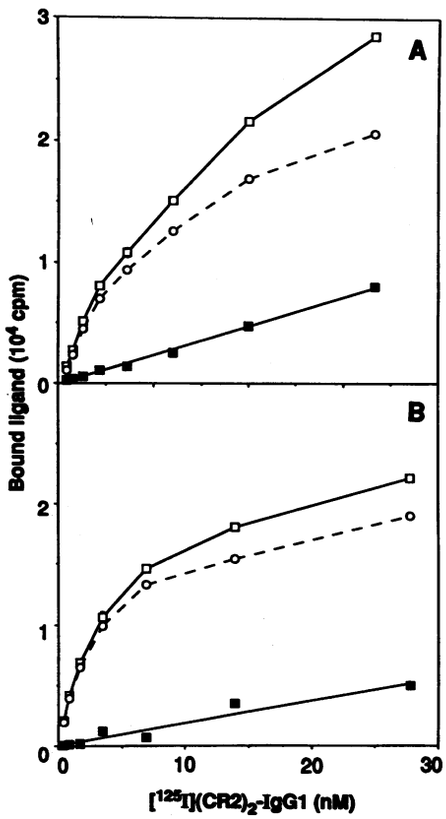


Fig. 3. Binding of ^{125}I-labeled $(CR2)_2$-IgG1 to zymosan particles bearing human or murine C3 fragments (*27*). (**A**) We incubated the indicated concentrations of ^{125}I-labeled $(CR2)_2$-IgG1 for 30 min at 0°C with 1.3×10^7 zymosan particles that had been reacted with human serum in the presence of Ca^{2+} and Mg^{2+} to permit activation of the alternative pathway (□) or with zymosan particles with serum in the presence of EDTA (■). Bound and free ligand were separated by centrifugation of the particles through a solution of 10% bovine serum albumin. Specific binding of ^{125}I-labeled $(CR2)_2$-IgG1 to C3 fragments (○) was calculated as the difference between the amount bound to particles that had been reacted with serum in the presence of cations and the amount bound to serum-reacted particles in EDTA. The data represent the mean of duplicate determinations. (**B**) We assessed ^{125}I-labeled $(CR2)_2$-IgG1 in parallel for its capacity to bind to zymosan particles that had been reacted with murine serum in the presence of divalent cations (□) or EDTA (■), and specific binding (○) was calculated as described in (A).

with cellular CR2 in a concentration range that indicates bivalent interaction with polymeric ligand.

The interaction of ^{125}I-labeled $(CR2)_2$-IgG1 with the iC3b fragment of murine and human C3, respectively, was measured; CR2 binds the iC3b fragment with the same affinity as it does C3dg (*15*). The $(CR2)_2$-IgG1 bound to the zymosan particles that had been coated with human and mouse iC3b with K_d's of 10.0 ± 3.7 nM (n = 5, mean ± SD), and 3.2 ± 1.6 nM (n = 4), respectively (Fig. 3). Therefore, $(CR2)_2$-IgG1 can compete with cellular CR2 for complement ligands in the mouse.

BALB/c mice were assessed for their immune response to sheep erythrocytes (E). Mice in one group received recombinant IgG1 and were immunized with either 4 × 10^5 or 4 × 10^6 E. Mice in a second group received $(CR2)_2$-IgG1 instead of the IgG1; and mice in a third group were depleted of C3 by treatment with cobra venom factor (CVF) for 24 hours before immunization. A fourth group of mice received only phosphate-buffered saline (PBS) and was not immunized with sheep E. On day 5 after immunization, mice were assessed for the number of splenic plaque-forming cells (PFC) secreting IgM antibody specific for sheep E and for serum levels of the IgM, IgG1, IgG2a, IgG2b, and IgG3 isotypes of antibodies to sheep E (anti-E). The control mice given the recombinant IgG1 responded in a dose-related manner to sheep E with increased splenic PFC and serum concentrations of specific antibody of each isotype, except IgG1 (Fig. 4). Depletion of C3 abolished the specific antibody response (*8, 22*), indicating that, at these concentrations of antigen, the B cell response was dependent on the activation of this complement protein. The mice that had received $(CR2)_2$-IgG1 also had low antibody responses, suggesting that cellular CR2 mediated the complement dependency of this B cell response.

The effect of the $(CR2)_2$-IgG1 chimera on the expression of anti-E was assessed over a period of 3 to 5 weeks after immunization with 4 × 10^6 E. Two groups of BALB/c mice received IgG1 and $(CR2)_2$-IgG1; a third group received PBS and was not immunized. The $(CR2)_2$-IgG1 completely suppressed IgM anti-E at day 5 (Fig. 5). The occurrence of anti-E among the IgG isotypes, which persisted for up to 40 days in the IgG1-treated mice, was diminished by 50 to 70% in the mice treated with $(CR2)_2$-IgG1. Therefore, soluble CR2 inhibits the primary response to a T-dependent antigen and subsequent isotype switching. The experiment was repeated in the C3H/HeJ strain, which is resistant to lipopolysaccharide, to exclude effects of possible lipopolysaccharide contamination of the recombinant proteins. The suppression of antibody

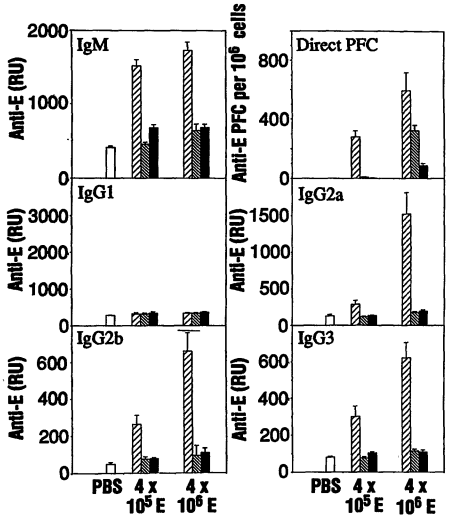
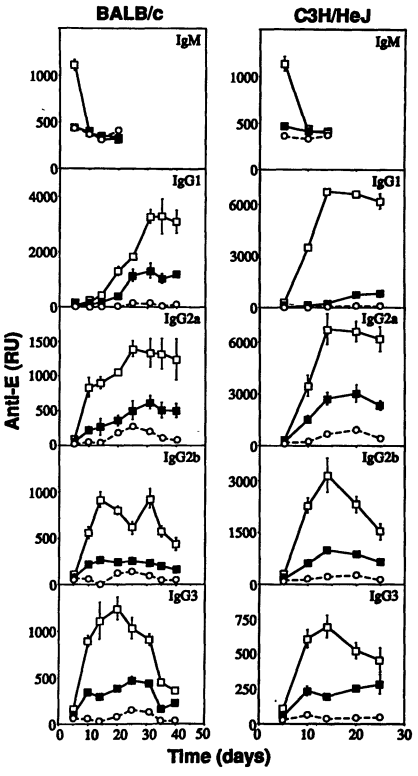


Fig. 4. Comparison of the effects of $(CR2)_2$-IgG1 and CVF in mice immunized with sheep E. One group (black boxes) of eight female BALB/c mice between 6 and 8 weeks of age was depleted of C3 by treatment with four doses of 5 μg of CVF 24 hours before intravenous immunization with 4 × 10^5 or 4 × 10^6 E. Two groups of mice received a total of 800 μg of $(CR2)_2$-IgG1 (boxes with narrow white stripes) or IgG1 (boxes with wide white stripes) in four equally divided doses at the time of immunization and at 0.5 hour, 3 hours, and 17 hours later. A fourth group of mice received only PBS and was not immunized with sheep E (white boxes). After 5 days, the number of PFC specific for sheep E was assayed (direct PFC), and the serum concentrations of the five indicated isotypes of anti-E were measured by ELISA and reported in relative units (RU) (*28*). The data represent mean ± SEM.

Fig. 5. Prolonged suppression by $(CR2)_2$-IgG1 of the antibody response to sheep E in BALB/c and C3H/HeJ mice. Two groups of six BALB/c and C3H/HeJ mice were administered a total of 800 μg of $(CR2)_2$-IgG1 (■) or IgG1 (□) in five equally divided doses given 1 hour before immunization; at the time of immunization; and 0.5 hour, 3 hours, and 17 hours after immunization with 4 × 10^6 sheep E. A third group received only PBS (○). Concentrations of the five indicated isotypes of specific antibody were determined by ELISA every five days and are presented as the mean ± SEM of the results of the four mice remaining after the mice with the highest and lowest responses were eliminated.



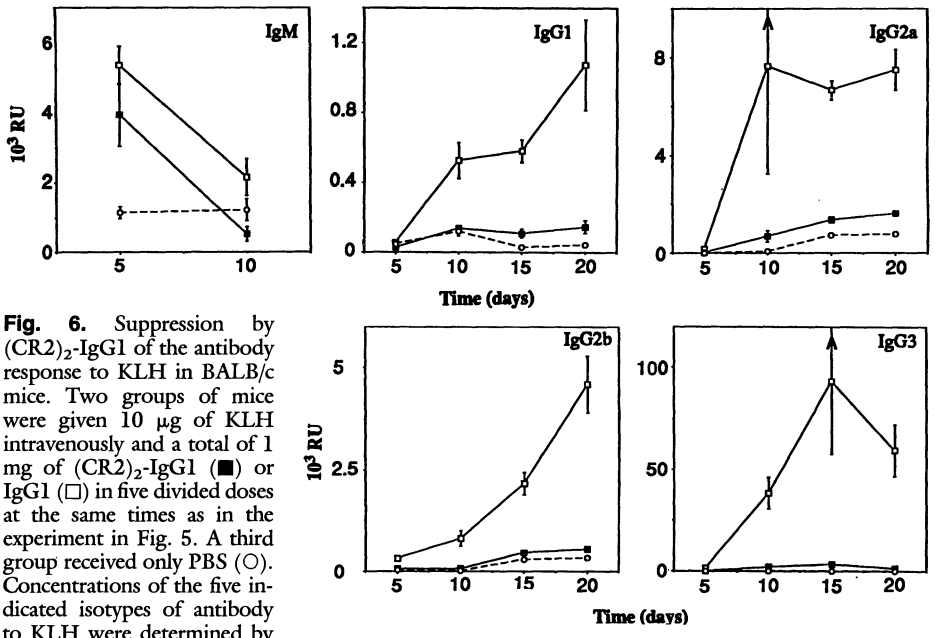


Fig. 6. Suppression by $(CR2)_2$-IgG1 of the antibody response to KLH in BALB/c mice. Two groups of mice were given 10 μg of KLH intravenously and a total of 1 mg of $(CR2)_2$-IgG1 (■) or IgG1 (□) in five divided doses at the same times as in the experiment in Fig. 5. A third group received only PBS (○). Concentrations of the five indicated isotypes of antibody to KLH were determined by ELISA. The data are reported as the mean ± SEM for four mice per group.

responses to sheep E in the C3H/HeJ mice by $(CR2)_2$-IgG1 was equivalent to that in the BALB/c mice (Fig. 5).

We determined the effects of $(CR2)_2$-IgG1 on the response to a soluble T-dependent antigen by immunizing mice with 10 μg of keyhole limpet hemocyanin (KLH) in the presence of 1 mg of $(CR2)_2$-IgG1 or IgG1. The amounts of KLH-specific IgG1, IgG2a, IgG2b, and IgG3 in serum were almost completely suppressed for at least 20 days by $(CR2)_2$-IgG1, whereas the IgM response was not significantly diminished (Fig. 6). These results contrast with the enhancing effect of specific IgG for soluble antigens (*23*). It is thus unlikely that the Fc region of the chimera participates in suppression.

These experiments show that a soluble form of a B lymphocyte receptor can cause immunosuppression in vivo, and they identify CR2 as the complement receptor that mediates the capacity of C3 to enhance the immune response. This finding indicates that the signal-transducing function of the CD19-CR2 complex potentiates the response of B cells to antigen in vivo, confirms studies suggesting a role for CR2 in B cell activation in vitro (7), and clarifies the observation that a monoclonal antibody to murine CR1 that was cross-reactive with CR2 inhibited antibody responses in mice (*24*). An application of the inhibitory activity of $(CR2)_2$-IgG1 may be to prevent primary antibody responses to immunogenic agents used for immunosuppression and cancer therapy, such as xenogeneic monoclonal antibodies.

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25. The Eae I–Xho I fragment encoding SCR-1 and -2 of CR2 [J. J. Weis *et al.*, *ibid.* **167**, 1047 (1988)] was inserted into the 5′ Pst I site of pSNR021 (*19*) with oligonucleotide linkers. J558L myeloma cells were transfected by electroporation at 960 μF and 350V with pSNR021 and pSNRCR2 that had been linearized with Pvu I and selected in medium containing G418 (Gibco) (1 mg/ml). Recombinant proteins were purified from culture supernatants of appropriate clones by affinity chromatography on 5-iodo-NP-Sepharose (*21*), reduced with dithiothreitol, separated on a SDS–polyacrylamide gel (5 to 15% gel), and stained with Coomassie blue.
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27. A suspension of zymosan (Sigma) (10 mg/ml) in PBS was autoclaved, washed three times with sterile PBS, and resuspended in PBS to a concentration of 10 mg/ml, which corresponds to 1.3×10^9 particles per milliliter. Zymosan suspension (5 ml) was centrifuged, and the sediment resuspended with human or mouse serum (2 ml) and Hanks' balanced salt solution, with Ca^{2+} and Mg^{2+} (Gibco) or 10 mM EDTA (8 ml). After incubation for 1 hour at 37°C, the particles were washed three times in PBS containing EDTA (5 mM) and stored at 4°C in this buffer. The $(CR2)_2$-IgG1 was radioiodinated [P. J. Fraker and J. C. Speck, *Biochem. Biophys. Res. Commun.* **80**, 849 (1978)] to a specific activity of 7.3×10^6 cpm/μg.
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Neutralization of Divergent HIV-1 Isolates by Conformation-Dependent Human Antibodies to Gp120

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The spectrum of human immunodeficiency virus type 1 (HIV-1) isolates neutralized by antibodies from HIV-1–infected humans is broader than the spectrum of isolates neutralized by sera from animals immunized with purified gp120 subunits. This broader neutralization was due, in part, to the presence of antibodies to conserved gp120 conformational epitopes. Purified conformation-dependent gp120-specific human antibodies neutralized a wider range of virus isolates than human antibodies directed to linear determinants in gp120 and were also responsible for the majority of the gp120-specific CD4-blocking activity of HIV-1–infected human sera. A gp120 subunit vaccine that effectively presents these conformation-dependent neutralization epitopes should protect against a broader range of HIV-1 variants than a vaccine that presents exclusively linear determinants.

AN HIV-1 VACCINE MUST ELICIT AN immune response that protects against infection by the numerous genetic variants that characterize this virus (*1*). The envelope glycoprotein gp120 of HIV-1 elicits virus neutralizing antibodies (*2*) and protects chimpanzees from a live virus challenge (*3*). However, the neutralizing antibodies induced by subunit immunization with gp120 from a single isolate typically lack the ability to neutralize other HIV-1 isolates (*4–6*). These neutralizing antibodies primarily recognize linear epitopes in the central area of the third hypervariable region (V3) of gp120 (*5*), the principal neutralizing determinant (PND) (7). However, sera from HIV-1–infected humans can neutralize a broad spectrum of virus isolates (*6, 8*), which cannot be explained on the basis of reactivity to linear epitopes in the V3 region (*9*). The cross-reactive neutralizing activity of human sera may be due, in part, to antibodies directed to conformational epitopes in HIV-1 gp120 that have not been efficiently presented by the gp120 vaccine formulations tested to date in animals. To test this hypothesis, we have purified and characterized such antibodies from HIV-1–infected human sera.

We purified gp120-specific, conformation-dependent antibodies from the immunoglobulin (Ig) fraction of pooled human sera positive for HIV antibody (Ig^{HIV}) by affinity chromatography using two versions of recombinant gp120 from the HIV-SF2 virus isolate [ARV-2 (*10*)] sequentially: env $2\text{-}3_{SF2}$, a nonglycosylated, denatured version produced in yeast (*11, 12*); and

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