associated with the amyloid syndrome (*15*). Similarly, FAP type III is associated with amyloid deposits that contain a peptide of apolipoprotein A1 with a single amino acid substitution (Gly to Arg) (*12*). In Gerstmann-Sträussler-Scheinker disease (GSS), a hereditary adult-onset dementia with cerebral amyloid plaques composed of a fragment of the prion protein, every GSS family studied to date has a mutation in the gene coding for this protein (*16–18*). Hereditary cerebral hemorrhage with amyloidosis in Icelandic kindreds (HCHWA-I) is associated with a single amino acid substitution in cystatin C (*19*), and a similar syndrome in Dutch kindreds (HCHWA-D) is associated with a single amino acid substitution of Gln for Glu in APP (*20*).

Although other single amino acid substitutions in APP may be associated with amyloid deposits and Alzheimer's disease, it is unlikely that single amino acid substitutions in APP can explain all sporadic forms of Alzheimer's disease. Recently it has been proposed that in some cases of FAP, normal transthyretin may form amyloid deposits in association with unknown aging factors (*21*). Similarly, in Alzheimer's disease, different pathogenic processes may lead to a common clinical presentation.

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Regulation of Transendothelial Neutrophil Migration by Endogenous Interleukin-8

ANDREAS R. HUBER, STEVEN L. KUNKEL, ROBERT F. TODD, III, STEPHEN J. WEISS*

Movement of neutrophils from the bloodstream to inflamed tissue depends on the activation of both the neutrophil and the endothelial cell. Endothelial cells lining the postcapillary venule respond to proinflammatory mediators by expressing adhesion molecules and synthesizing a variety of neutrophil-activating factors. Endothelial cell production of a 77–amino acid variant of interleukin-8 (IL-8) was found to be a requirement for the invasion of neutrophils through a vessel wall model. IL-8 secreted by cytokine- or lipopolysaccharide-stimulated endothelial cells induced the rapid shedding of neutrophil lectin adhesion molecule–1, the up-regulation of leukocyte β_2 integrins, and the attachment and transmigration of the neutrophils. Thus, endogenous endothelial IL-8 regulates transvenular traffic during acute inflammatory responses.

IN VIVO, INTERLEUKIN-1β (IL-1β), TUMOR necrosis factor α (TNFα), or lipopolysaccharide (LPS) trigger the endothelial cells that line the postcapillary venule to express adhesion molecules that can bind to circulating neutrophils (*1*). This initial adhesive interaction is regulated by a member of the lectin-cellular adhesion molecule family (lectin adhesion molecule–1; LECAM-1) that is displayed on the surface of unstimulated neutrophils (*1, 2*). Binding of the neutrophils to the inflamed endothelium then initiates an orchestrated series of events in which the neutrophils shed LECAM-1 and engage a second set of adhesion-promoting glycoproteins, the leukocyte β_2 integrins (*1, 2*). The adherent neutrophils then penetrate the vessel wall by moving between interendothelial-cell junctions and proceeding through the underlying basement membrane into the interstitium (*1–3*).

The initial interaction between LECAM-1 on the circulating neutrophil and the surface of stimulated endothelial cells is presumed to be a passive event (*1, 2*). However, the source and identity of the factors responsible for the initiation of the subsequent shedding of LECAM-1, the up-regulation of the β_2 integrins, and neutrophil diapedesis is unclear. We now show, in a model of the postcapillary venular wall (*3*), that IL-1β–, TNFα-, or LPS-stimulated endothelial cells actively promote neutrophil transvenular migration by the synthesis and release of a 77–amino acid variant of interleukin-8 ($IL\text{-}8_{77}$) (*4*). Although endothelial $IL\text{-}8_{77}$ is thought to be a specific inhibitor of neutrophil-endothelial cell interactions (*5*), we show that this molecule is a primary promoter of neutrophil diapedesis by virtue of its ability to both regulate LECAM-1 and β_2 integrin expression and to form a transendothelial cell chemotactic gradient.

To duplicate the conditions under which endothelial cells promote transvenular neutrophil migration in vivo, we added IL-1β, TNFα, or LPS to the basilar compartment of the blood vessel wall construct, and after a 4-hour incubation, 1×10^6 neutrophils were added in fresh media to the apical compartment, and invasion was monitored. Under these conditions, 79 ± 7%, 81 ± 6%, and 85 ± 8% (± SEM, $n = 4$) of the added neutrophils were associated with IL-1β–, TNFα-, and LPS-stimulated vessel wall constructs, whereas only 9 ± 3% were associated with untreated, control cultures (*6*). After 90 min, almost all of the vessel wall–associated neutrophils had successfully penetrated the endothelial layer and invaded the under-

A. R. Huber, R. F. Todd, III, S. J. Weiss, Department of Internal Medicine, Division of Hematology and Oncology, University of Michigan, Ann Arbor, MI 48109.
S. L. Kunkel, Department of Pathology, University of Michigan, Ann Arbor, MI 48109.

*To whom correspondence should be addressed.

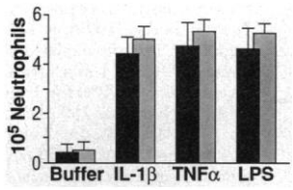

Fig. 1. Identification of neutrophil chemotactic activity in stimulated vessel wall constructs. Primary or secondary passages of human umbilical vein endothelial cells were cultured on matrices of type I collagen for 21 days in double-chamber culture dishes as described (*3*). Constructs were incubated in HBSS containing 20% heat-inactivated human plasma alone (buffer) or with recombinant human IL-1β (5 units/ml, 3×10^7 units/mg, Upjohn), recombinant human TNFα (100 units/ml, 2.2×10^7 units/mg, Cetus), or LPS (100 ng/ml, *Escherichia coli*/055:B5, Difco). After a 4-hour incubation with IL-1β, TNFα, or LPS, conditioned media were removed from the type I collagen matrix underlying the endothelial monolayer and pooled with media from the basilar compartment. Aliquots of the media were then tested for chemotactic potential across matrices of type I collagen alone (shaded bars) or untreated vessel wall constructs (black bars). The number of neutrophils that migrated into the lower compartment of the double-chamber culture dishes after a 12-hour incubation was determined by electronic cell counting (*3*). Results are expressed as the mean ± 1 SEM of at least four experiments.

lying type I collagen matrix to an average depth of ~200 μm. This response was dependent on a biosynthetically active endothelium because neither IL-1β, TNFα, nor LPS could induce neutrophils to invade either naked matrices of type I collagen or cycloheximide-treated vessel wall constructs.

To determine if direct-acting neutrophil chemotactic factors were synthesized and deposited by the overlying endothelium, we incubated vessel wall constructs with IL-1β, TNFα, or LPS, and the conditioned medium underlying the endothelial cells was tested for chemotactic activity. The pooled media from stimulated, but not control, vessel wall constructs induced significant neutrophil migration through matrices of type I collagen or unstimulated vessel wall constructs (Fig. 1).

IL-1β–, TNFα-, or LPS-stimulated endothelial cells can synthesize a number of factors capable of affecting neutrophil adhesivity or motility, including platelet-activating factor, leukotriene C_4, IL-6, granulocyte–colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), melanoma-growth stimulating activity (MGSA/gro), and IL-8 (*4, 7*). However, the chemotactic activity detected in the endothelial cell–conditioned medium was not diminished after either extensive dialysis (molecular size cutoff of 3 kD) or the addition of neutralizing antisera to IL-6, G-CSF, or GM-CSF (*8, 9*). The cytokine IL-8 was initially characterized as a 72–amino acid neutrophil chemoattractant synthesized by human monocytes; subsequent studies have identified a variety of cell types capable of generating IL-8 variants including 79–, 77–, and 69–amino acid forms ($IL\text{-}8_{79}$, $IL\text{-}8_{77}$, and $IL\text{-}8_{69}$, respectively) (*4*). Researchers reported that IL-1β–stimulated endothelial cells synthesized $IL\text{-}8_{77}$, but this variant acted exclusively as a powerful inhibitor of neutrophil-endothelial cell interactions (*5*). Because our vessel wall construct primarily generated a neutrophil chemoattractant, we considered the possibility that the transcription, translation, processing, or activity of endothelial cell–derived IL-8 was altered in our system. Vessel wall constructs treated with IL-1β, TNFα, or LPS rapidly responded with significant increases in IL-8–specific mRNA (Fig. 2A).

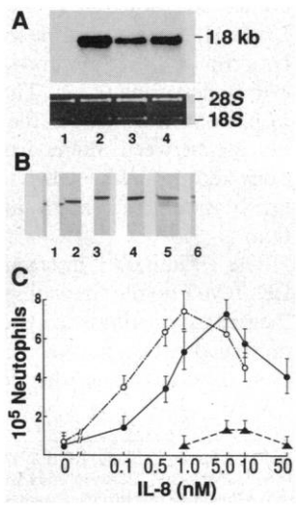

Fig. 2. (**A**) Expression of IL-8 mRNA by vessel wall constructs was assessed by Northern (RNA) blot analysis. (Top) Total cellular RNA from control (lane 1), IL-1β– (lane 2), TNFα- (lane 3), and LPS- (lane 4) treated constructs was isolated, separated by agarose gel electrophoresis, transblotted to nitrocellulose, and hybridized with a ^{32}P 5′ end-labeled 30-nucleotide probe as described (*19*). (Bottom) We confirmed equivalent amounts of total RNA per gel by monitoring 28*S* and 18*S* ribosomal RNA. (**B**) Identification of IL-8 variants secreted by stimulated vessel wall constructs. After stimulation with IL-1β, TNFα, or LPS, conditioned media were collected and IL-8 variants concentrated by immunoaffinity chromatography (*20*). The variants were resolved by electrophoresis in an SDS-polyacrylamide gel-Tricine system, transblotted to Immobilon (Millipore), and immunolocalized with rabbit antiserum to human IL-8 (*20*). Recombinant $IL\text{-}8_{69}$, $IL\text{-}8_{72}$, and $IL\text{-}8_{77}$ standards (Biosource International, Camarillo, California) are shown in lanes 1, 2, and 3, respectively; the IL-8 variants synthesized by IL-1β–, TNFα-, or LPS-stimulated constructs are shown in lanes 4, 5, and 6, respectively. (**C**) Chemotactic activity of recombinant IL-8 variants in vessel wall constructs. $IL\text{-}8_{77}$ (●) or $IL\text{-}8_{72}$ (○) at the indicated concentrations were added to the lower compartment of vessel wall constructs immediately before the addition of 1×10^6 neutrophils to the upper compartment. The number of invading neutrophils was assessed after a 90-min incubation as in Fig. 1. Minimal invasion was observed when $IL\text{-}8_{77}$ was added simultaneously to the upper and lower chambers (▲). Results are expressed as the mean ± 1 SEM of five or more experiments.

Conditioned media that were analyzed by protein immunoblots with IL-8–specific polyclonal antisera contained primarily $IL\text{-}8_{77}$ (Fig. 2B), which was verified by amino-terminal sequence analysis (NH_2-Ala-Val-Leu-Pro-Arg) (*5, 10*). To determine whether $IL\text{-}8_{77}$ may act as a chemoattractant, rather than an inhibitor in the vessel wall model, we added increasing concentrations of recombinant $IL\text{-}8_{77}$ to the basilar compartment of untreated constructs (Fig. 2C). Under these conditions, a chemoattractant response similar to that of $IL\text{-}8_{72}$ was elicited (*4*). $IL\text{-}8_{77}$ did not display chemokinetic activity; only minimal invasion was observed when equal concentrations of the recombinant cytokine were added simultaneously to the apical and basilar compartments (Fig. 2C).

Because a correlation existed between the ability of vessel wall constructs to synthesize IL-8 and their ability to promote neutrophil transmigration, we examined the effect of IL-8–specific neutralizing antiserum on the invasive response (*11*). In the presence of the antiserum, neutrophil transmigration was markedly inhibited (Fig. 3, A to C). Quantitative analyses of neutrophil invasion revealed that the antiserum to IL-8 inhibited IL-1β–, TNFα-, and LPS-dependent transmigration by 77 ± 9%, 71 ± 6% and 63 ± 8%, respectively (± SEM, $n = 5$). The subsequent addition of an alternative chemotactic stimulus (C5a) to the basilar compartment of the antiserum-treated constructs induced normal invasion (*8*); thus, the inhibitory action of antiserum was not a nonspecific depressive effect on neutrophil or endothelial cell function.

Given the ability of the IL-8 antisera to inhibit invasion, endogenous IL-8 could regulate neutrophil attachment, neutrophil transmigration, or both. Neutrophils shed LECAM-1 and up-regulate β_2 integrins in the course of their adhesive interaction with the endothelial cells ligands, intercellular adhesion molecule–1 (ICAM-1) and endothelial-leukocyte adhesion molecule–1 (ELAM-1) (*1, 2*). Thus, using flow cytometry, we assessed the ability of endogenous IL-8 to regulate attachment directly by altering the expression of the neutrophil adhesion molecules. Conditioned media from IL-1β–stimulated vessel wall constructs induced LECAM-1 shedding and increased β_2 integrin surface expression in a manner inhibitable by the antiserum to IL-8 (Fig. 4). In contrast, the antiserum to IL-8 did not affect the expression of either ICAM-1 or ELAM-1 on the endothelial cell surface (*8*). To determine whether the IL-8–mediated changes in LECAM-1 and β_2 integrin expression were critical to the strong adhesive interactions necessary for invasion, we

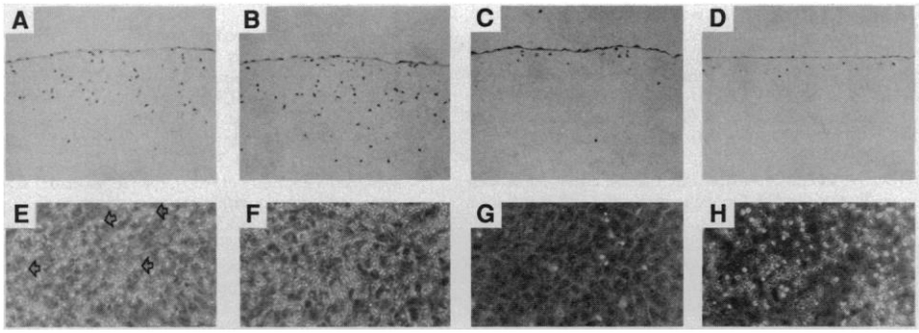

Fig. 3. Effect of neutralizing antiserum to IL-8 on neutrophil–vessel wall interactions. After a 4-hour incubation with IL-1β, media in the apical compartment was removed, and neutrophils (1×10^6) suspended in fresh media were incubated with the stimulated constructs alone (**A** and **E**) or with IL-1β–stimulated constructs to which a 1:250 dilution of preimmune sera (**B** and **F**) or a 1:250 dilution of antiserum to IL-8 (**C** and **G**) were added 30 min before the addition of the neutrophils. We then assessed neutrophil–vessel wall interactions after washing either by preparing cross sections after a 90-min incubation (A to C) or by phase-contrast microscopy after a 15-min incubation (E to G). In panels (**D**) and (**H**), neutrophils were treated with 10 nM recombinant $IL\text{-}8_{77}$ for 25 min and washed before their addition to IL-1β–stimulated constructs incubated with antiserum to IL-8. Cross sections and phase-contrast micrographs were taken at ×100 (printed at ×51) and ×250 (printed at ×127.5), respectively, with arrows pointing to adherent neutrophils. Similar results were obtained with either TNFα- or LPS-stimulated constructs.

incubated neutrophils with stimulated vessel wall constructs and monitored the effect of the antiserum to IL-8 on adhesion by phase-contrast microscopy. In IL-1β–treated constructs, neutrophils were tightly associated with the apical face of the endothelium in the absence or presence of the preimmune sera (Fig. 3, E and F). In contrast, neutrophils were almost completely prevented from adhering to the IL-1β–treated endothelium in the presence of the antiserum to IL-8 (Fig. 3G) (*12*). We confirmed the ability of IL-8 alone to regulate adherence by first treating resting neutrophils with 10 nM recombinant $IL\text{-}8_{77}$. These stimulated cells tightly bound to IL-1β–stimulated vessel wall constructs despite the presence of the antiserum to IL-8 (Fig. 3H), but few transmigrated the monolayer under these conditions (Fig. 3D).

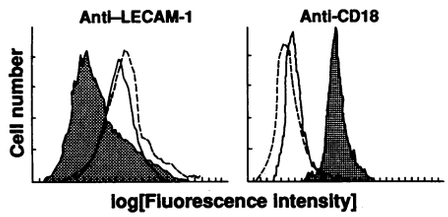

Fig. 4. Regulation of surface LECAM-1 and β_2 integrin expression on neutrophils by endothelial cell–derived IL-8. Neutrophils (3×10^6) were incubated for 30 min with conditioned media from unstimulated vessel wall constructs (dashed line), IL-1β–stimulated constructs (shaded area), or IL-1β–stimulated constructs to which antiserum to IL-8 was added 15 min before the addition of neutrophils (solid line). LECAM-1 (left panel) and β_2 integrin expression (right panel) were monitored by flow cytometric analysis with monoclonal antibodies LAM 1.2 (anti–LECAM-1) and IB4 (anti-CD18), respectively (*2, 21*).

Because exogenous IL-8 up-regulated adhesion but did not induce transmigration in the presence of the antiserum to IL-8, additional signals were apparently required for invasion. IL-8 is a basic polypeptide with heparin-binding activity, and a variety of bioactive molecules with similar characteristics can be found in association with the subendothelial matrix (*4, 13*). Thus, we considered the possibility that a chemotactic gradient of matrix-associated IL-8 might be required for neutrophil transmigration. Indeed, immunohistochemical analysis of IL-1β–stimulated vessel wall constructs revealed immunoreactive IL-8 in association with both the endothelial cell monolayer and the underlying interstitium (Fig. 5). If, however, we extensively washed the basilar compartment of the vessel wall construct to deplete IL-8 from the subendothelial matrix, neither resting nor neutrophils first stimulated with $IL\text{-}8_{77}$ displayed invasive activity unless exogenous IL-8 was again added to the basilar compartment (*8*).

IL-1β–, TNFα-, or LPS-stimulated endothelial cells use endogenous IL-8 to regulate neutrophil transmigration. When endogenous IL-8 is bound by a neutralizing antiserum, the expression of LECAM-1 on resting neutrophils or ICAM-1 and ELAM-1 on the surface of stimulated endothelial cells is not sufficient to mediate a maximal invasive response. Endothelial cell–derived IL-8 appears to function as a necessary cofactor in neutrophil transmigration by regulating the expression of leukocyte adhesion molecules, as well as by generating the required chemotactic gradient (*14*). A requirement for $IL\text{-}8_{77}$ in transendothelial cell migration of IL-1β–stimulated constructs is in apparent contradiction with the report that the 77–amino acid variant acts as a specific inhibitor of neutrophil-endothelial cell interactions (*5*). However, $IL\text{-}8_{77}$, like all chemoattractants, is an ineffective promoter of invasion if the stimulus distributes equally between apical and basilar compartments (Fig. 2C) (*4*). In the experimental model used by Gimbrone and colleagues (*5*), endothelial cell monolayers not only secrete IL-8 into the medium but also deposit IL-8 into the subendothelial matrix (*8*). Thus, the apparent inhibitory effect exerted by $IL\text{-}8_{77}$ under these conditions may be attributed to the collapse of the endogenous chemotactic gradient (*15*).

Our study has focused on neutrophil-endothelial cell interactions, but the ability of the inflamed endothelium to regulate transmigration by the cooperative display of adhesive ligands and the synthesis of specific chemoattractants should serve as a useful paradigm for a variety of other tissue-invasive processes. Cytokine- or LPS-stimulated endothelial cells are not only able to elicit lymphocyte, eosinophil, monocyte, and perhaps even tumor cell transmigration (*16*) but are also able to synthesize a superfamily of cell-specific chemoattractants (*17, 18*). Our findings suggest that the characterization of these factors and the generation of appropriate inhibitors could lead to the development of powerful and specific anti-inflammatory or antimetastatic therapeutic interventions.

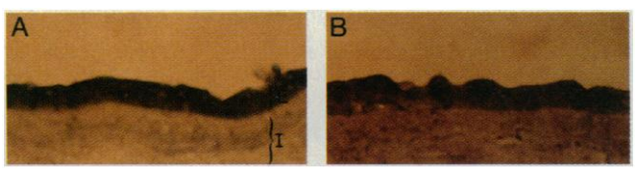

Fig. 5. Immunolocalization of IL-8 in vessel wall constructs. Endothelial cells were cultured on acellular amniotic membranes (*22*) for 7 days and subsequently incubated with either buffer alone (**A**) or IL-1β (5 units/ml) for 4 hours (**B**). The constructs were then washed, cryosectioned, and stained for IL-8 with rabbit antiserum to IL-8, followed by biotinylated goat antibody to rabbit immunoglobulin, streptavidin-conjugated peroxidase, and 3-amino-9-ethylcarabazole (*19*). Immunolocalized IL-8 stains red under these conditions. Almost no staining was obtained when the antiserum was replaced with preimmune sera. E and I indicate endothelial cells and interstitium, respectively. Magnification ×260.

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10. IL-8, purified by immunoaffinity chromatography as described in Fig. 2, was sequenced as described [R. W. Hewick, M. W. Hunkapiller, L. E. Hood, W. J. Dreyer, *J. Biol. Chem.* **256**, 7990 (1981)].
11. Rabbit antiserum to human IL-8 [R. M. Strieter *et al.*, *ibid.* **264**, 10621 (1989)] reacted with $IL\text{-}8_{69}$, $IL\text{-}8_{72}$, and $IL\text{-}8_{77}$, 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)].
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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 $IL\text{-}8_{77}$ 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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18. Given that antisera to IL-8 did not completely inhibit neutrophil transmigration, despite the presence of antibody excess, and that stimulated endothelium generate the chemotaxin MGSA/gro [D. Wen *et al.*, *EMBO J.* **8**, 1761 (1989)], we postulate that MGSA/gro may play a secondary role in transmigration under the conditions studied.
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Suppression of the Immune Response by a Soluble Complement Receptor of B Lymphocytes

THOMAS HEBELL, JOSEPH M. AHEARN, DOUGLAS T. FEARON*

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

Division of Molecular and Clinical Rheumatology, Department of Medicine, and the Graduate Program in Immunology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

*To whom correspondence should be addressed.