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# Overlapping But Nonidentical Binding Sites on CD2 for CD58 and a Second Ligand CD59

William C. Hahn, Elisabeth Menu, Alfred L. M. Bothwell, Peter J. Sims, Barbara E. Bierer*

**The interaction of the T cell glycoprotein CD2 with one ligand, CD58, contributes to T cell function. We have identified CD59, a glycoprotein with complement-inhibitory function, as a second physiological ligand for CD2. Antibodies to CD59 inhibit CD2-dependent T cell activation in murine T cell hybridomas expressing human CD2. In an in vitro binding assay with purified CD58 and CD59, $CD2^+$ cells bind not only immobilized CD58 but also CD59. With two complementary approaches, it was demonstrated that the binding sites on CD2 for CD58 and CD59 are overlapping but nonidentical. These observations suggest that direct interactions between CD2 and both CD58 and CD59 contribute to T cell activation and adhesion.**

Antigen-specific T cell immune responses depend on the expression of the T cell receptor (TCR)–CD3 complex; however, a number of other T cell–specific molecules play important roles in T cell activation and function (*1, 2*). Accessory molecules, such as CD2, function by facilitating adhesion, transducing signals that synergize with or modulate signals produced by the TCR-CD3 complex, or both. CD2 binds to lymphocyte function–associated antigen-3 (LFA-3, CD58), a broadly expressed 40- to 70-kD glycoprotein, and this receptor-ligand pair contributes both to T cell activation and to T cell adhesion with thymic epithelium, antigen-presenting cells, and erythrocytes (*1–3*). Although the interaction of CD2 with CD58 is important for T cell function, monoclonal antibody (MAb) inhibition studies have shown that at least one other erythrocyte protein, CD59, contributes to the phenomenon of spontaneous lymphocyte-erythrocyte rosetting, implying that a receptor for CD59 exists on the T cell (*4, 5*).

CD59 (protectin, HRF20, membrane inhibitor of reactive lysis) is a broadly distributed 18- to 20-kD membrane glycoprotein that restricts the lysis of human erythrocytes and leukocytes by human serum complement (*6–10*). To determine whether CD59 participates in T cell function by interacting with the CD2 molecule, we stimulated an antigen-specific murine T cell hybridoma that expresses the human CD2 molecule (*11*) with the human $CD58^+CD59^+$ Burkitt's lymphoma–derived B cell line Daudi. Expression of human CD2 in this hybridoma (16CD2-XH14) greatly enhanced the ability of these cells to respond to stimulation by Daudi as compared to the parent hybridoma (By155.16) (Fig. 1), and this enhancement was partially inhibited by either a purified, monospecific rabbit immunoglobulin G (IgG) to CD59 or an MAb to CD58 (Fig. 1). The simultaneous addition of antibodies to CD58 and CD59 resulted in complete inhibition of interleukin-2 (IL-2) production, suggesting that CD58 and CD59 each bind to CD2. In contrast, the addition of antibodies specific for either CD58 or CD59 did not inhibit the IL-2 response of a $CD2^-$ hybridoma that expresses the human CD4 molecule (16CD4-13) (*12*) (Fig. 1).

To assess whether $CD2^+$ cells bind CD59 directly, we tested the ability of

W. C. Hahn and E. Menu, Division of Pediatric Oncology, Dana-Farber Cancer Institute, and Harvard Medical School, Boston, MA 02115.
A. L. M. Bothwell, Section of Immunobiology, Yale Medical School, New Haven, CT 06510.
P. J. Sims, Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation and Department of Microbiology and Immunology, Oklahoma University Health Sciences Center, Oklahoma City, OK 73104.
B. E. Bierer, Division of Pediatric Oncology, Dana-Farber Cancer Institute, and Harvard Medical School, and Hematology-Oncology Division, Department of Medicine, Brigham and Women's Hospital, and Department of Medicine, Harvard Medical School, Boston, MA 02115.
*To whom correspondence should be addressed.

$CD2^+$ hybridomas to bind plate-bound, purified human CD58 or CD59 in an in vitro binding assay. The parental $CD2^-$ hybridoma (By155.16) was unable to bind either CD58 or CD59 (Fig. 2A). In contrast, $CD2^+$ hybridomas bound both CD58 and CD59 but did not bind purified human major histocompatibility complex (MHC) class I proteins. Binding to CD58 and CD59 was concentration-dependent (Fig. 2B). To compare CD58 and CD59 binding, we used concentrations of CD58 (25 pg per well) and CD59 (1 ng per well) that gave equivalent binding for subsequent experiments. The specificity of the observed binding was confirmed by first incubating plate-bound protein with a mouse antibody to CD58 (CD58 MAb), purified rabbit IgG to CD59 (anti-CD59), or nonimmune rabbit IgG. CD58 MAb abrogated binding of $CD2^+$ cells to CD58 without affecting the binding to CD59 (Fig. 2C). Reciprocally, anti-CD59 inhibited binding of $CD2^+$ cells to CD59 without affecting binding to CD58. The nonimmune rabbit IgG had no effect on the binding of these cells to either CD58 or CD59. To determine the effect of coimmobilization of purified CD58 and CD59 on the binding of $CD2^+$ cells, we tested the binding of $CD2^+$ hybridoma cells on combinations of CD58 and CD59 over a range of concentrations. In each case, the combination of CD58 and CD59 had additive effects on the binding of these cells (*13, 14*). Taken together, these observations establish that $CD2^+$ cells bind CD59 in the absence of other proteins.

Previous studies have identified three extracellular regions to which a number of murine MAbs directed against human CD2 bind (*15*). The first region (region I) centers around amino acids 43 to 46, whereas the second region (region II) centers around amino acids 86 and 87. Antibody blocking and transfection experiments have shown that both region I and region II are critical for the interaction of CD2 with CD58 (*1, 11, 15, 16*). The expression of the third MAb binding region (CD2R) is induced upon T cell activation (*1*). With a panel of defined Mabs to CD2 (CD2 MAbs) (*17*), we examined the ability of these to inhibit the binding of $CD2^+$ cells to CD59 (Fig. 3A). Each of these MAbs bound the human CD2 expressed in these cells (*13*). Whereas MAb directed against region I and II abrogated CD2 binding to purified CD58, region I MAbs did not inhibit the CD2-CD59 interaction; remarkably, only MAb to region II inhibited binding of $CD2^+$ cells to purified CD59 (Fig. 3A). CD2R MAb did not affect the binding of $CD2^+$ cells to either CD58 or to CD59 (Fig. 3A). These observations suggest that the binding site on CD2 for CD59 can be differentiated from that of CD58.

To confirm these observations, we tested the ability of hybridomas expressing CD2 molecules with single amino acid substitutions in region I and in region II to bind CD58 and CD59. The hybridoma cell lines 16CD2-K43N (*1*) and 16CD2-Q46L (*11*) express CD2 molecules with single amino acid mutations at positions 43 (Lys to Arg) and 46 (Glu to Lys), respectively, whereas the 16CD2-Y86D and 16CD2-D87H cell lines (*16*) express CD2 molecules with single amino acid substitutions at positions 86 (Tyr to Asp) and 87 (Asp to His). These mutant cell lines are able to bind certain, but not all, MAbs to CD2 and are able to produce IL-2 when stimulated with particular pairs of CD2 MAbs (*11, 16*). None of these cell lines are able to bind to or be activated by CD58 (*11, 16*). Confirming the MAb data, both cell lines expressing epitope-loss mutations in region I (16CD2-K43N and 16CD2-Q46L) were unable to bind plate-bound CD58 but retained the ability to bind CD59 (Fig. 3B). This binding to CD59 was specifically inhibited by the addition of antibodies to CD59 (*13*). In contrast, the hybridoma cell lines expressing CD2 region II mutants (16CD2-Y86D and 16CD2-D87H) were unable to bind either CD58 or CD59 (Fig. 3B). For each CD2-expressing cell line at least two cell lines expressing equivalent concentrations

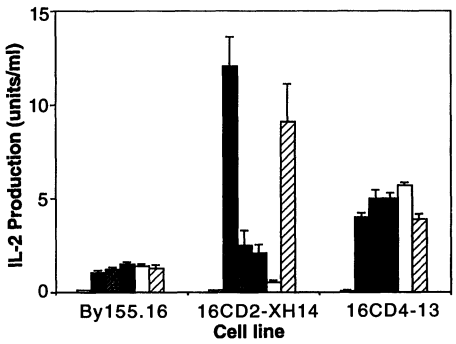


**Fig. 1.** Inhibition of CD2-dependent T cell activation by CD58 MAb and anti-CD59. Murine T cell hybridomas ($5 \times 10^4$) expressing no human molecules (By155.16), human CD2 (16CD2-XH14), or human CD4 (16CD4-13) were stimulated with media alone (horizontal stripes) or with $4 \times 10^5$ human Burkitt's lymphoma–derived B cell line Daudi in the absence (black) or presence of the CD58 MAb TS2/9 (*26*) (dark striped), an IgG fraction of monospecific rabbit anti-CD59 (*8, 27*) (gray), CD58 MAb plus anti-CD59 (open), or the ICAM-1 MAb LB.2 (light striped). Antibodies were used at 1 μg/ml. Hybridoma cells were cultured in 48-well plates under the conditions listed above. At 24 hours, IL-2 production was measured by the ability of culture supernatants to support the proliferation of the IL-2–dependent murine T cell line, CTLL-20 (*28*), as described (*29*). Mean ± SE is shown. The experiment pictured is representative of five independent experiments.

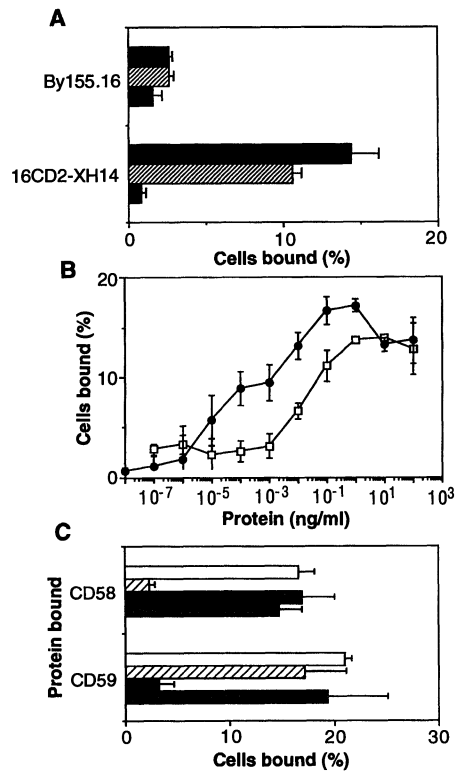


**Fig. 2.** Human $CD2^+$ cells bind purified human CD58 and CD59. (**A**) Human $CD2^+$ (16CD2-XH14) but not $CD2^-$ murine hybridoma cells (By155.16) bind purified CD58 (gray), and CD59 (striped). Neither cell line bound purified MHC class I proteins (black). The mean ± SE for a single experiment, representative of five independent experiments, is shown. CD58 was immunoaffinity purified from lysates of the human lymphoblastoid cell line JY with a TS2/9 MAb–conjugated Sepharose 4B column as described (*29*). CD59 was purified from human erythrocytes as described (*8, 27*). Binding assays using these purified proteins were performed as described (*29*) with minor modifications (*30*). For the experiments in (A) and (**C**), we used protein concentrations of 25 pg per well for CD58 and 1 ng per well for CD59 were used. (**B**) The binding of $CD2^+$ cells to purified CD58 and CD59 is dose-dependent. CD58 (circles) and CD59 (squares) were plated at the indicated concentrations, and the binding of $CD2^+$ hybridoma cells was determined. The experiment shown is representative of three independent experiments. (**C**) Specific inhibition of $CD2^+$ cell binding to CD58 and CD59 by antibodies to CD58 and CD59. Cells bound either CD58 or CD59 in the absence of antibodies (white) or in the presence of a nonimmune rabbit IgG preparation (dark striped). The CD58 MAb TS2/9 (1 μg/ml) (light striped) or rabbit anti-CD59 (1 μg/ml) (black) was added to protein-coated wells 1 hour before the addition of cells. The experiment shown is representative of three independent experiments.

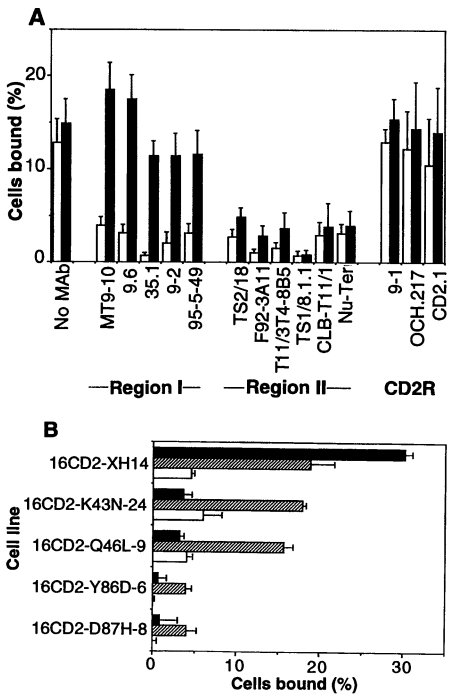


**Fig. 3.** The CD2 binding sites for CD58 and CD59 are partially overlapping. (**A**) Inhibition of $CD2^+$ cell binding to CD58 and CD59 by a panel of CD2 MAbs. The binding of $CD2^+$ hybridoma cells to CD58 (white) and CD59 (black) was determined as described (*30*) except that CD2 MAbs (1 μg/ml) were added to $CD2^+$ hybridoma cells for 10 min before plating onto purified proteins. The CD2 MAbs were obtained from the Third International Conference on Leukocyte Typing (*17*). The MAbs were grouped according to their binding sites as described (*15*). The experiment pictured represents the mean ± SE for seven experiments. (**B**) Binding of cell lines expressing CD2 extracellular point mutants to CD58 and CD59. Wild-type CD2-, CD2-K43N–, CD2-Q46L–, CD2-Y86D-, and CD2-D87H–expressing hybridomas were plated onto purified human CD58 (black), CD59 (striped), and MHC class I proteins (white), and specific binding was determined. The experiment shown is representative of five independent experiments. CD58 was used at 25 pg per well, and CD59 was used at 1 ng per well. These hybridomas are named according to the wild-type amino acid, its position in the human CD2 sequence, and the mutated amino acid. The nomenclature of these CD2 mutants has been changed (*1, 11, 16*) to correspond with the numbering used for the CD2 NMR structure (*18*).

of CD2 were tested, each giving identical results (*13*). Taken together, these observations identify at least one region of the CD2 molecule required for contact with CD59, and establish that the binding sites for CD58 and CD59 on CD2 are overlapping but nonidentical.

In the recently reported nuclear magnetic resonance solution structure of the first Ig-like domain of rat CD2 (*18*), human CD2 MAb binding regions I and II are predicted to share the same face of the β sheet made up of β strands C, C′, C″, F, and G. Widely separated from each other, region I is predicted to span the loop between strands C′ and C″, whereas region II spans the loop between strands F and G. These regions localize to the periphery of the predicted Ig-like structure of CD2, well positioned to interact with putative ligands. The CD2 epitope-loss mutants studied here map within these predicted regions of interaction. Based on this structure, CD58 and CD59 may bind the same or distinct CD2 molecules.

Like intercellular adhesion molecule-1 (ICAM-1) (*19*), MHC class II proteins (*2*), CD22 (*20*), and several of the integrins (*2*), CD2 appears to have multiple ligands that bind to distinct portions of the extracellular domain. CD58 and CD59 are structurally unrelated although both molecules can be attached to the membrane through a glycophosphatidylinositol linkage (*6, 9, 21, 22*). CD58, like CD2, is a member of the immunoglobulin superfamily (*21*), whereas CD59 shares homology with the murine Ly-6 antigens (*7, 22*). These observations demonstrate that the immunoglobulin superfamily member CD2 can interact with two structurally diverse ligand families. Because the interaction of CD2 with CD58 and with CD59 enhances T cell antigen responsiveness (Fig. 1), these observations suggest that the interaction of these distinct molecules with CD2 plays a role in T cell function.

Moreover, CD59 and CD58 serve as physiological signaling receptors. MAbs to CD59 have been reported to initiate signal transduction events in T cells (*22, 23*), and CD59 is associated with the lymphoid-restricted phosphoprotein $p56^{lck}$, an Src-like tyrosine kinase implicated in T cell signaling (*24*). The interaction of CD2 with CD59 may therefore play a role in T–T interactions. MAbs to CD58 induce cytokine secretion from antigen-presenting cells (*25*). The interaction of CD2 with CD58 and CD59 may thus serve to regulate the activity of T cells and, coordinately, antigen-presenting cells.

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30. Proteins were bound to polystyrene microtiter plates (Titertek, ICN-Flow Laboratories, McLean, VA) in 0.3% octyl-β-glucoside, 0.1 M sodium bicarbonate buffer, pH 9.2, for 12 hours at 37°C. Remaining protein binding sites were blocked with 1% heat-denatured bovine serum albumin for 2 to 4 hours at 37°C. The plates were washed extensively to remove unbound proteins, and in experiments using antibodies, antibodies (1 μg/ml) were added to wells for 1 hour at 37°C. Hybridoma cells previously labeled with 30 μCi of $^3$H-labeled thymidine for 16 hours were added to the wells at a concentration of $2 \times 10^5$ cells per well. Plates were spun for 1 min at 50*g* and incubated for 30 min at 37°C. We removed unbound cells by aspirating the fluid contents of each well through a 22-gauge needle and carefully rinsing each well four times with 100 μl of RPMI medium. The radioactivity remaining in each well was determined, and the number of cells bound calculated by: Percent cells bound = [(sample cpm − nonspecific cpm)/(input cpm − nonspecific cpm)] × 100.


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