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CD19: Lowering the Threshold for Antigen Receptor Stimulation of B Lymphocytes

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Lymphocytes must proliferate and differentiate in response to low concentrations of a vast array of antigens. The requirements of broad specificity and sensitivity conflict because the former is met by low-affinity antigen receptors, which precludes achieving the latter with high-affinity receptors. Coligation of the membrane protein CD19 with the antigen receptor of B lymphocytes decreased the threshold for antigen receptor–dependent stimulation by two orders of magnitude. B lymphocytes proliferated when approximately 100 antigen receptors per cell, 0.03 percent of the total, were coligated with CD19. The B cell resolves its dilemma by having an accessory protein that enables activation when few antigen receptors are occupied.

The immune system must respond to low concentrations of antigen for the efficient elimination of infections. The growth and differentiation of lymphocytes are mediated by antigen receptors that have low affinity for their ligands because they are products of recombinatorial gene rearrangement that takes place in the absence of selection by antigen. Therefore, lymphocytes must have mechanisms that enable them to be stimulated when relatively few receptors have bound antigen. The T lymphocyte has the accessory membrane proteins CD4 and CD8, which, when coligated with the T cell antigen receptor (TCR) by the major histocompatibility complex (MHC)–peptide complex, decrease the number of TCRs that must be ligated (*1*). No membrane protein of the B lymphocyte that is analogous to CD4 and CD8 has been identified.

The CD19 membrane protein, a member of the immunoglobulin (Ig) superfamily, is B cell–specific and is expressed at each developmental stage except that of the terminally differentiated plasma cell (*2*). It is a component of a complex that contains at least two other membrane proteins, complement receptor type 2 (CR2, also called CD21) and TAPA-1 (*3*). The CR2 protein mediates the capacity of the complement system to enhance the production of antibody in response to low concentrations of antigen in vivo (*4*). Both CD19 and CR2 augment activation of phospholipase C in B cells when coligated with membrane Ig (mIg), but independent ligation of CD19 suppresses B cell activation (*5*, *6*).

To determine whether ligating CD19 alters the capacity of mIgM to induce DNA synthesis, replicate samples of human B cells and mitomycin-treated murine L cells that express human $Fc_\gamma RII$ ($Fc_\gamma RII$–L cells) (*7*) were cultured for 2.5 days in the presence of incremental concentrations of a monoclonal antibody (MAb) to IgM, a saturating concentration of MAb to CD19, or an equal concentration of an irrelevant MAb, MOPC-21, and interleukin-4 (IL-4). In the absence of anti-CD19, the lowest concentration of anti-IgM that induced incorporation of [^{3}H]thymidine by B cells above the background incorporation was 6.7×10^{-11} M. In the presence of anti-CD19, this was reduced to 6.7×10^{-13} M (Fig. 1) (*8*, *9*). At higher concentrations of anti-IgM, anti-CD19 also increased the magnitude of [^{3}H]thymidine incorporation to twice that induced by anti-IgM alone. Anti-CD19 alone did not induce proliferation. The ligation of CD19 both lowered the threshold for B cell activation by mIgM and increased the magnitude of the B cell response to optimal levels of mIgM stimulation.

In a parallel experiment to assess the binding characteristics of the monoclonal anti-IgM, replicate samples of B cells and $Fc_\gamma RII$–L cells were incubated with incremental concentrations of ^{125}I-labeled anti-IgM in the presence or absence of 2×10^{-7} M unlabeled anti-IgM. Cell-bound and free antibody were separated, and specific binding was calculated. The L cells alone did not specifically bind the radiolabeled antibody. Scatchard analysis showed that the anti-IgM bound to the mixture of B cells and L cells with a dissociation constant (K_d) of 1.8×10^{-9} M, and to 2.7×10^5 sites per cell at saturation. The presence of anti-CD19 did not alter the K_d of the anti-IgM for B cells in the presence of $Fc_\gamma RII$–L cells (*10*). Therefore, in Fig. 1, the threshold concentration of anti-IgM that induced B cell proliferation in the presence of anti-CD19 bound to only 92 mIgM molecules per cell, or 0.03% of the total mIgM expressed per cell.

We measured the fraction of CD19 that must be ligated to augment B cell proliferation by incubating replicate samples of B cells and $Fc_\gamma RII$–L cells in the presence of suboptimal anti-mIgM, IL-4, and incremental concentrations of anti-CD19 for 2.5 days, after which incorporation of [^{3}H]thymidine was assayed. The enhancing effect of anti-CD19 increased incrementally from 6.7×10^{-12} M, which bound <0.2% of CD19, up to a saturating concentration of 6.7×10^{-8} M (Fig. 2A). To determine

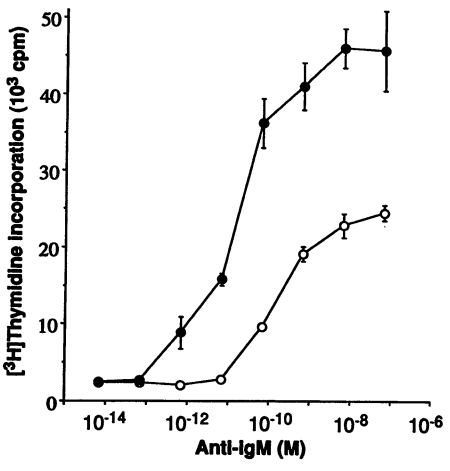

Fig. 1. Enhancement of DNA synthesis in B lymphocytes by the coaggregation of mIgM and CD19. Replicate samples of 5×10^4 peripheral blood B cells (*8*) were cultured in 0.2 ml of RPMI-1640 with 10% fetal calf serum in flat-bottom culture plates with 2×10^4 mitomycin-treated fibroblastic L cells expressing human CDw32/$Fc_\gamma RII$ and recombinant IL-4 (200 units/ml, Genzyme). The B cells were stimulated with a range of concentrations of MAb DA4.4 anti-IgM (*9*) together with either control antibody MOPC-21 (○) or HD37 anti-CD19 (●). Cells were pulsed with [^{3}H]thymidine for the last 16 hours of a 60-hour culture. Results are means ± SD of triplicates. Cells cultured with either control antibody or anti-CD19 alone incorporated 2726 ± 256 and 2475 ± 255 counts per minute (cpm), respectively. Representative of four experiments.

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whether coaggregation of CD19 with mIgM on the B cells was necessary for augmented cellular proliferation, in replicate samples of cells, $F(ab')_2$ anti-CD19 was substituted for IgG anti-CD19. The $F(ab')_2$ form of anti-CD19, which could not interact with the $Fc_\gamma RII$, suppressed rather than enhanced mIgM-induced B cell proliferation (Fig. 2B). Thus, CD19 augments signaling by mIgM only when the two membrane proteins are coaggregated, probably reflecting a requirement for their proximity.

Anti-CD19 enhanced mIgM-dependent stimulation both in the absence and presence of IL-4 and did not alter the dose response characteristics of the B cells to IL-4. Anti-CD19 also did not induce long-term proliferation of B cells stimulated with anti-IgM or change the time at which peak synthesis occurred (*10*).

To determine whether ligation of other members of the CD19 complex also enhances mIgM signaling, we incubated B cells with the $Fc_\gamma RII$–L cells in the presence of saturating concentrations of anti-CD19, anti–TAPA-1, anti-CR2, or control MOPC antibody in combination with IL-4 and incremental concentrations of anti-IgM, and we assessed the cells for [^{3}H]thymidine incorporation after 2.5 days. The anti-CD19 again decreased the concentration of anti-IgM required for B cell proliferation by approximately two orders of magnitude (Fig. 3).

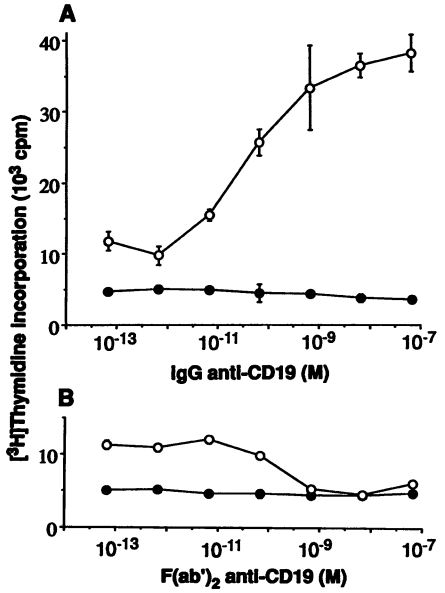

Fig. 2. The requirement for coligation of CD19 with mIgM. B cells were cultured with mitomycin-treated $Fc_\gamma RII$–L cells and IL-4 (200 units/ml), were stimulated with a range of concentrations of either (**A**) IgG or (**B**) $F(ab')_2$ HD37 anti-CD19 alone (●) or in combination with 10 ng/ml of IgG DA4.4 anti-IgM (○), and were assessed for their capacity to incorporate [^{3}H]thymidine. Representative of three independent replications of the experiment.

Anti–TAPA-1, an antibody selected for its capacity to suppress the growth of a human B cell lymphoma (*11*), augmented proliferation when coligated with higher concentrations of anti-IgM, and anti-CR2 modestly augmented the B cell response. The greater activity of anti-CD19 relative to that of anti–TAPA-1 or anti-CR2 suggests that, among the components of the complex, CD19 is the primary mediator of augmented signaling through the mIgM complex. The other components may be less effective because they are reversibly associated with CD19, as demonstrated for CR2, which also forms a complex with CD35 (*12*).

Table 1. Comparison of the effect of anti-CD19 with that of antibodies to other B cell surface proteins. B cells were cultured with mitomycin-treated $Fc_\gamma RII$–L cells, 200 units/ml IL-4, and 5 μg/ml of each IgG1 monoclonal antibody either alone or together with 10 ng/ml of DA4.4 anti-IgM, and they were assessed for incorporation of [^{3}H]thymidine. Anti-IgM at 1.0 μg/ml induced 19,835 ± 732 cpm. Replicate samples of B cells were incubated with the same antibody preparations at 0°C for 30 min, stained with fluorescein-labeled anti-mouse IgG, and analyzed for fluorescence by flow cytometry. The mean fluorescence channel (MFC) for each population is given.

MAb	CD	[^{3}H]Thymidine incorporation (10^3 cpm)		MFC
		Alone	Anti-IgM added	
None		2.6±0.2	7.7±0.6	42
MHM24	11a	2.3±0.2	7.4±0.3	362
HD37	19	3.9±0.7	22.3±1.4	222
L27	20	2.1±0.8	4.4±0.1	886
4KB128	22	2.5±0.2	7.9±0.7	289
BU38	23	3.0±0.3	9.1±0.3	90
Yz-1	35	2.5±0.1	9.3±0.7	273
BU25p	Cl II*	3.1±0.8	13.9±0.8	145

*MHC class II.

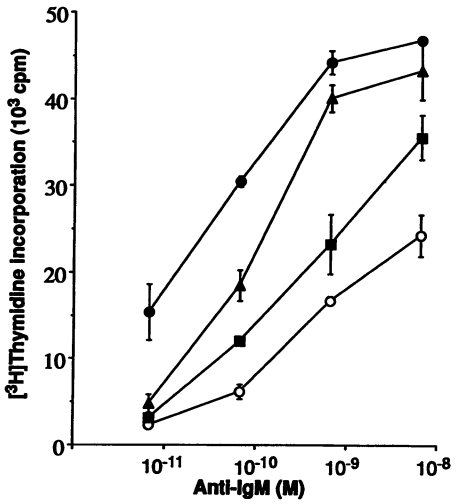

Fig. 3. Enhancement of DNA synthesis induced in B cells by the coaggregation of CD19, CR2, or TAPA-1 with mIgM. B cells were cultured with mitomycin-treated $Fc_\gamma RII$–L cells and IL-4 (200 units/ml) and were stimulated with incremental concentrations of DA4.4 anti-IgM alone (○) or together with 1 μg/ml of HD37 anti-CD19 (●), BU32 anti-CR2 (■), or 5A6 anti–TAPA-1 (▲). Cells cultured without added antibody or stimulated with antibodies to CD19, CR2, or TAPA-1 alone incorporated 2450 ± 155, 2556 ± 180, 2205 ± 103, and 1776 ± 329 cpm, respectively. Representative of three experiments.

These data show that CD19 participates in a fundamental problem of the immune system, the competing requirements for sensitivity and broad specificity. Coligation of CD19 with mIgM lowered the threshold for effective mIgM signaling so that ligation of only 0.03% of antigen receptors was sufficient to induce cellular proliferation. This function was unique to CD19 and was dependent on coligation with mIgM, linking CD19 with antigen-specific stimulation of the B cell.

This function of CD19 is consistent with its large, conserved cytoplasmic domain and the cocapping of CD19 with mIg (*13*). The enhancing effect of CD19 also parallels the function of the T cell accessory proteins, CD4 and CD8. Each is a member of the Ig superfamily, each is physically or functionally coupled to a protein tyrosine kinase, and each decreases the threshold for antigen receptor–mediated increases in free intracellular Ca^{2+} concentration and for lymphocyte proliferation when coligated with the antigen receptor; moreover, independent ligation of CD19, CD4, or CD8 suppresses signaling through the antigen receptors of B and T cells (*1, 6, 14*) (Fig. 2). This analogy predicts that there is a ligand for CD19 to permit complement-independent stimulation of the complex and that an antigen-presenting cell for B cells exists on which both antigen and the CD19 ligand reside.

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9. The IgG1 MAbs used were DA4.4 anti-IgM (American Type Culture Collection), MOPC-21 control antibody (Organon Teknika), HD37 anti-CD19 (Dako, Carpentaria, CA, and G. Moldenhauer), BU32 anti-CR2/CD21 (Binding Site), 5A6 anti–TAPA-1 (*11*), MHM24 anti–LFA-1/CD11a (Dako), L27 anti-CD20 (Becton-Dickinson), 4KB128 anti-CD22 (Dako), BU38 anti-CD23 (Binding Site), Yz-1 anti-CR1/CD35 [P. S. Changelian, R. M. Jack, L. A. Collins, D. T. Fearon, *J. Immunol.* **134**, 1851 (1985)], and BU25 anti-MHC class II (Binding Site).
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Participation of Non–Zinc Finger Residues in DNA Binding by Two Nuclear Orphan Receptors

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Steroid-thyroid hormone receptors typically bind as dimers to DNA sequences that contain repeated elements termed half-sites. NGFI-B, an early response protein and orphan member of this receptor superfamily, binds to a DNA sequence that contains only one half-site (5′-AAAGGTCA-3′). A domain separate from the NGFI-B zinc fingers, termed the A box, was identified and is required for recognition of the two adenine-thymidine (A-T) base pairs at the 5′ end of the NGFI-B DNA binding element. In addition, a domain downstream of the zinc fingers of the orphan receptor H-2 region II binding protein, termed the T box, determined binding to tandem repeats of the estrogen receptor half-site (5′-AGGTCA-3′).

The steroid-thyroid hormone receptor superfamily is a class of ligand-activated transcription factors that mediates cellular response to hydrophobic ligands (*1*). Much of our knowledge concerning the DNA binding function of these nuclear receptors comes from studies of the estrogen and glucocorticoid receptors. The DNA recognition sites of these proteins contain two copies of protein-specific six-base sequences termed half-sites that are oriented as inverted repeats (*2*, *3*). Mutagenesis (*4*, *5*), nuclear magnetic resonance (*6*), and x-ray crystallography (*7*) studies show that characteristic residues within the Cys_2-Cys_2 Zn finger domains of these receptors make base-specific contacts with the half-sites. High binding affinity and a further degree of sequence specificity based on half-site spacing are achieved by dimerization of two protein monomers, which is a function of the Zn finger (*4*, *7*) and COOH-terminal (*2*, *8*) domains. This model is generally applicable, except that several receptors bind preferentially to promoter elements that contain direct rather than inverted repeats of half-site sequences (*9*, *10*), with the specificity of binding determined by the nucleotide spacing between half-sites (*11*, *12*).

The mammalian protein NGFI-B [(*13*), also called nur77 (*14*)] is an orphan receptor because it shares the structural features of nuclear receptors but has no known ligand specificity. An NGFI-B response element (NBRE) has been identified by genetic selection in yeast (*15*) that confers NGFI-B–dependent transcriptional activity to a heterologous promoter and consists of the 9-nucleotide sequence 5′-AAAAGGTCA-3′. The NBRE contains an estrogen receptor element–thyroid hormone receptor element (ERE-TRE) half-site (5′-AGGTCA-3′). It is likely that the NGFI-B Zn fingers recognize the ERE-TRE half-site like an estrogen or thyroid hormone receptor monomer through the conserved base-contacting residues in these proteins (*4*, *13*). The precise mechanism of binding must be distinct, however, because there is no half-site repeat in the NBRE and nucleotides outside of the half-site are required for binding (*15*).

To characterize nucleotide requirements in the sequence adjacent to the half-site in the NBRE, we synthesized three oligonucleotide pools in which each of the three positions nearest the 5′ end of the sequence 5′-AAAAGGTCA-3′ were replaced with equimolar mixtures of C, G, and T (*16*). These oligonucleotide pools were then used as probes in an electrophoretic mobility shift assay with NGFI-B protein synthesized in Chinese hamster ovary (CHO) cells (*15*). NGFI-B recognized the oligonucleotide pool with C, G, and T at position 1 and the NBRE itself with approximately equal affinity (Fig. 1A). In contrast, replacement of the A at either position 2 or 3 resulted in a reduction of the shifted complex, suggesting that NGFI-B makes base-specific contacts at these nucleotides. To assess the magnitude of this effect, we used the electrophoretic mobility shift assay to perform a Scatchard binding analysis (*17*). NGFI-B bound to a probe that contained the NBRE sequence with a dissociation constant (K_d) of 1.0 nM (Fig. 1B), a physiologically relevant affinity similar to that of estrogen and thyroid hormone receptors for their DNA elements (*8*, *18*). In contrast, a probe that had a substitution of G for A at position 2 of the NBRE was bound by NGFI-B with a K_d of 3.3 nM (Fig. 1B).

Most nuclear receptors do not bind with high affinity to DNA sequences that contain a single half-site because the interaction of one monomer with one half-site does not provide sufficient free energy to stabilize the protein-DNA complex. In contrast, three results support the model that NGFI-B binds to the NBRE as a monomer. (i) The high-affinity NBRE is limited to eight contiguous nucleotides with a single half-site [(*15*); Fig. 1]. (ii) When full-length and truncated NGFI-B

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