TCR-Induced Transmembrane Signaling by Peptide/MHC Class II Via Associated Ig-α/β Dimers

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Previous findings suggest that during cognate T cell–B cell interactions, major histocompatability complex (MHC) class II molecules transduce signals, leading to Src-family kinase activation, Ca^{2+} mobilization, and proliferation. Here, we show that antigen stimulation of resting B cells induces MHC class II molecules to associate with Immunoglobulin (Ig)-α/Ig-β (CD79a/CD79b) heterodimers, which function as signal transducers upon MHC class II aggregation by the T cell receptor (TCR). The B cell receptor (BCR) and MHC class II/Ig-α/Ig-β are distinct complexes, yet class II–associated Ig-α/β appears to be derived from BCR. Hence, Ig-α/β are used in a sequential fashion for transduction of antigen and cognate T cell help signals.

Humoral immune responses to most protein antigens require direct interaction between antigen-specific B lymphocytes and CD4-positive helper T cells. Within these T-cell–B cell conjugates, paired molecules on opposing cells mediate adhesion and signal transduction (*1*). Central among these interactions is MHC class II/antigenic peptide engagement by TCR. Although signals transduced by the TCR and coreceptors lead to T cell activation, events that occur in the opposing B cell during this interaction are undefined.

Several studies have suggested that MHC class II molecules may play a role in transducing signals to B cells (*2–5*). For example, antibody-mediated aggregation of MHC class II on B cells that were previously stimulated by antibodies against the BCR induces calcium mobilization, as well as the protein tyrosyl phosphorylation of a wide spectrum of proteins by Src-family kinases (*3*, *5*, *6*). Transduction of these signals does not require the MHC class II cytoplasmic domains (*7*). On the basis of these findings, we hypothesized that during immune responses, antigen binding to B cell antigen receptors may initiate not only antigen processing, but also priming of the B cell, so that subsequent MHC class II aggregation by the TCR initiates transduction of B cell–activating signals. Further, priming may involve the induced association of MHC class II with a transducer.

To examine mechanisms of tyrosine kinase activation after TCR–MHC class II interactions, we used resting B cells from 3-83 μδ Ig transgenic mice, which express membrane IgM and IgD specific for H-$2K^{k/b}$ (*8*). Soluble, *Drosophila*-expressed H-$2K^b$–conjugated dexamine (7:1 molar ratio) was used as an antigen [K^b_7-Dex (*9*, *10*)] to stimulate 3-83 μδ B cells in vitro before analysis of MHC class II signaling. Unlike resting cells, antigen-stimulated B cells mobilized calcium upon subsequent MHC class II aggregation (*11*). Protein tyrosyl phosphorylation was observed when resting B cells were stimulated via BCR, but not upon MHC class II engagement; whereas in antigen-primed B cells, both MHC class II and BCR aggregation led to the tyrosyl phosphorylation of proteins (Fig. 1A). Thus, stimulation of resting B cells by antigen leads to a qualitative alteration in MHC class II coupling to systems that generate second messengers.

Because aggregation of MHC class II in antigen-primed B cells elicited protein tyrosyl phosphorylation and calcium mobilization responses that were strikingly similar to those mediated by the BCR, and considering that MHC class II signaling is reportedly coupled to activation of Src- and Syk-family kinases (*4*, *12*), it seemed possible that BCR and MHC class II share a common transducer. BCR signals are transduced via the membrane Ig associated heterodimer Ig-α and Ig-β (CD79a and CD79b, respectively), which become tyrosyl-phosphorylated upon antigen stimulation (*13*). To test the possibility that Ig-α and Ig-β act as the MHC class II signal transducers, 3-83 μδ B lymphocytes were stimulated through antigen receptors, MHC class II, or MHC class I, and the tyrosine phosphorylation of Ig-α/β dimers was analyzed. Ig-α and Ig-β phosphorylation was detected in resting cells upon aggregation of membrane Ig, but not MHC class II. However, aggregation of MHC class II on antigen-primed B cells induced phosphorylation of Ig-α, as well as a second species, identifiable as Ig-β on the basis of size and association with Ig-α (Fig. 1B), suggesting that the molecules function as MHC class II transducers.

Previous studies have demonstrated that BCR signaling leads to tyrosyl phosphorylation of only those Ig-α and Ig-β dimers that are associated with the aggregated antigen receptors (*14*). In view of these findings, results shown in Fig. 1B suggested that on primed cells, MHC class II may be physically associated with the Ig-α/β that is tyrosyl-phosphorylated upon class II aggregation. To test this possibility, MHC class II molecules were immunoprecipitated, and their association with Ig-α was assessed by Ig-α–specific immunoblotting. Results demonstrate that Ig-α is associated with MHC class II in primed, but not resting, B cells [Fig. 1C and (*11*)]. The association was specific, because Ig-α was not detected in immunoprecipitates antibodies against CD22. To further confirm the specificity of this interaction, we assessed association by reciprocal coimmunoprecipitation. Primed cells were lysed and immunoprecipitates were prepared by using antibodies against Ig-β (or antibodies directed against the MHC class II molecules I-A^d and I-E^d) before fractionation and blotting by using a polyclonal antibody against the MHC class II (β chain) cytoplasmic tail (*15*) (or an antibody against Ig-α). As shown in Fig. 1D, MHC class II was found in Ig-β precipitates of primed cells, and the inverse was also true. Thus, antigen stimulation of resting B cells leads to MHC class II association with Ig-α/β dimers demonstrable by reciprocal coimmunoprecipitation.

B cell priming requires 5 to 12 hours of incubation with antigen (*3*), and thus may require transcriptional activation of protein expression. To address this possibility, 3-83 μδ B cells were treated for 9 hours with K^b_7-Dex in the presence of actinomycin D before stimulation through BCR or MHC class II. Although antigen receptors remained functional, no induction of protein tyrosyl phosphorylation was seen upon engagement of MHC class II on cells primed in the presence of actinomycin D (Figs. 1, A and B). Similarly, the association of Ig-α/β with MHC class II induced by antigen priming is blocked in the presence of actinomycin D (Fig. 1C). Collectively, these data indicate that the priming of MHC class II and association with Ig-

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α/β requires gene transcription. This dependence may indicate that only class II that is newly synthesized after antigen stimulation can associate with Ig-α/β dimers or that de novo synthesis of proteins that mediate this association is required.

If MHC class II–associated Ig-α/β functions equivalently to BCR-associated Ig-α/β, i.e., as its transducer, it is likely that MHC class II aggregation would only lead to tyrosyl phosphorylation of its associated Ig-α and Ig-β. To address this possibility, fractionated MHC class II immunoprecipitates, generated as described in Fig. 1C, were probed sequentially with phosphotyrosine- and Ig-α–specific antibodies (Fig. 2A). Tyrosyl phosphorylation of MHC class II–associated Ig-α and Ig-β was observed upon engagement of MHC class II on primed cells, indicating that MHC class II molecules use associated Ig-α/β for signal transduction.

Experiments described above indicate that membrane Ig and MHC class II are associated with unique pools of Ig-α/β. To assess whether fidelity is seen in the ability of the two receptors to mediate phosphorylation of only associated Ig-α/β, the following experiment was undertaken. K46μ lymphoma cells (*16*), which express a nitrophenol (NP)-specific antigen receptor (*17*), were stimulated with antigen (NP_7BSA) or antibodies directed against MHC class II. After stimulation, membrane Ig or MHC class II immunoprecipitates were fractionated and immunoblotted using phosphotyrosine- and Ig-α–specific antibodies. As shown in Fig. 2B, membrane Ig–associated Ig-α/β heterodimers were tyrosyl-phosphorylated only in response to BCR stimulation. Conversely, MHC class II aggregation led to phosphorylation of only Ig-α/β associated with MHC class II. These data provide further evidence that membrane Ig and MHC class II use distinct pools of Ig-α/β heterodimers in signal transduction.

The association of additional proteins with MHC class II was investigated by cell-surface–labeling experiments. K46μ and M12.g3r lymphoma cells (*16*), which constitutively exhibit the primed and resting MHC class II signaling phenotypes (*7*, *18*), respectively, were used in this endeavor. The cells were labeled with biotin before lysis, immunoprecipitation of membrane Ig and MHC class II, and fractionation by nonreducing SDS–polyacrylamide gel electrophoresis (SDS-PAGE) to allow visualization of Ig-α/β as disulfide-linked dimers rather than dissociated chains, which have mobility similar to that of class II α and β chains. Streptavidin–horseradish peroxidase (streptavidin-HRP) blotting revealed the expected alpha and beta chains of class II (35 and 30 kD, respectively) in class II immunoprecipitates from both cell types (Fig. 3A). In addition, the nonreduced Ig-α and Ig-β heterodimer (approximately 70 kD) was observed only in K46μ cell precipitates. Coprecipitating Ig-α/β was also seen in BCR immunoprecipitates from K46μ cells, along with membrane IgM. It is noteworthy that, despite similar Ig-α/β levels, membrane IgM was not detected in class II immunoprecipitates from K46μ cells, indicating that MHC class II–associated Ig-α/β is not due to a direct association of MHC class II with the BCR complex. The reciprocal was also observed. Furthermore, no additional surface proteins were found to coprecipitate specifically with MHC class II in K46μ cells. However, such proteins could exist yet escape detection if they were poorly biotinylated. The species that migrates immediately below Ig-α/β was seen even in samples that were not subjected to biotinylation (*19*). These analyses demonstrate that the

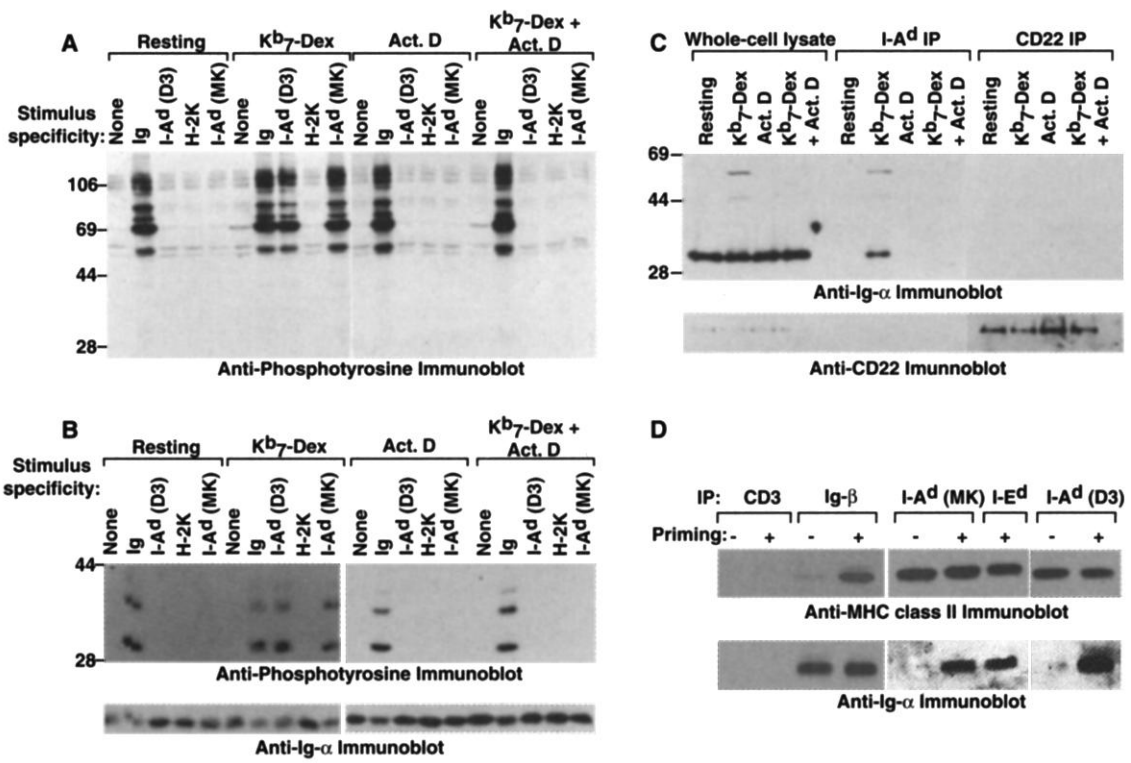

Fig. 1. Antigen induces priming of resting B cells and the association of MHC class II with Ig-α/β (*25*). (**A**) MHC class II aggregation induces protein tyrosyl phosphorylation in antigen-prestimulated B cells. 3-83 μδ splenic B lymphocytes were cultured for 9 hours without stimulus (resting), or with K^b_7-Dex, actinomycin D, or K^b_7-Dex + actinomycin D. Cells were then washed and stimulated for 2 min through their BCR (antibodies against Ig), MHC class II (I-A^d, D3.137, or MKD6), or MHC class I (H-2K). After CHAPS lysis, postnuclear supernatants were subjected to SDS-PAGE fractionation and phosphotyrosine-specific immunoblotting (*26*). Positions of the molecular size markers are indicated in kilodaltons. (**B**) MHC class II aggregation induces Ig-α and Ig-β phosphorylation in antigen-prestimulated B cells. 3-83 μδ splenic B cells were primed and stimulated as in (A). Cells were then lysed, subjected to Ig-α immunoprecipitation, fractionated by SDS-PAGE, and analyzed by phosphotyrosine- and Ig-α–specific immunoblotting. (**C**) MHC class II is associated with Ig-α in antigen-primed cells. 3-83 μδ splenic B cells were primed as in (A). Cells were then lysed, and immunoprecipitation was performed by using antibodies against I-A$^{d/b}$ (D3.137) or against CD22 (Cy34.1.2) coupled to Sepharose. Immunoprecipitates and whole-cell lysates were fractionated by SDS-PAGE and subjected to Ig-α– and CD22-specific immunoblotting. (**D**) Reciprocal coimmunoprecipitation of MHC class II with Ig-α/β. Splenic B cells were primed and lysed as above. Immunoprecipitation was performed with antibodies against Ig-β (HM79), isotype-matched antibodies against CD3 (145.2C11), and antibodies against I-A$^{d/b}$ (D3.137) or I-E (14-4-4) coupled to Sepharose. SDS-PAGE fractionated immunoprecipitates were then immunoblotted with antibodies specific for MHC class II and Ig-α.

major MHC class II–associated proteins in B cells with the primed phenotype are Ig-α and Ig-β, and, further, that MHC class II signaling is not the result of association with the BCR in primed cells.

It is tempting to speculate that antigen stimulation leads to internalization of BCR complexes that, like associated antigen, are delivered to the class II intracellular compartment, and, further, that this is the mechanism by which MHC class II molecules acquire Ig-α/β dimers. In support of this, Ig-α/β have been found in the class II intracellular compartment after BCR stimulation (*20*). Alternatively, priming may lead to MHC class II association with newly synthesized Ig-α/β. If the former hypothesis is correct, BCR must recycle spontaneously in K46μ cells to provide Ig-α/β for newly synthesized MHC class II. To begin to address this possibility, we isolated, by immunoselection, spontaneous variants of K46 cells that do not express cell-surface Ig yet express levels of class II comparable to membrane Ig–expressing K46 cells. It was surprising that these cells also do not express detectable surface Ig-β, indicating that their cell-surface MHC class II is not associated with Ig-α/β dimers (*11*). However, these membrane Ig–negative cells were found by immunoblotting to contain nearly normal levels of total cellular Ig-α and Ig-β (Fig. 3C). Membrane Ig–negative cells failed to mobilize calcium in response to MHC class II stimulation (Fig. 3B). Similarly, class II aggregation on membrane Ig–negative cells did not induce the tyrosyl phosphorylation of Ig-α or Ig-β (Fig. 3C). When membrane Ig expression is restored to membrane Ig–negative K46 cells (*27*), however, aggregation of MHC class II again induces a calcium response (Fig. 3B). These findings suggest that MHC class II–associated Ig-α/β dimers are derived from cell-surface or recycling BCR.

The data presented above suggest that TCR/CD4 binding to MHC class II during cognate T cell/B cell interactions leads to transduction of signals via Ig-α/β leading to tyrosine kinase activation. To address this question in a more physiological context, we coupled soluble DO.11.10 TCR monomers [specific for I-A^d complexed with ovalbumin (OVA) peptide] to beads and used these to stimulate OVA-pulsed K46μ and M12.g3r cells (*11*). Antibody staining and digital imaging of the resultant conjugates demonstrated that class II aggregation by DO.11.10 TCR induced tyrosyl phosphorylation of proteins at bead-cell contact points, but only in primed phenotype cells (Fig. 4A). We noted that, at later time points, the primed phenotype cells engulfed the TCR-coated beads and exhibited a halo of tyrosyl phosphoproteins surrounding the beads. Furthermore, TCR beads induce a proportion of Ig-α/β to cluster at bead-cell contact points. As expected, only a small proportion of Ig-α/β, presumably representing that associated with OVA peptide–loaded MHC class II, is

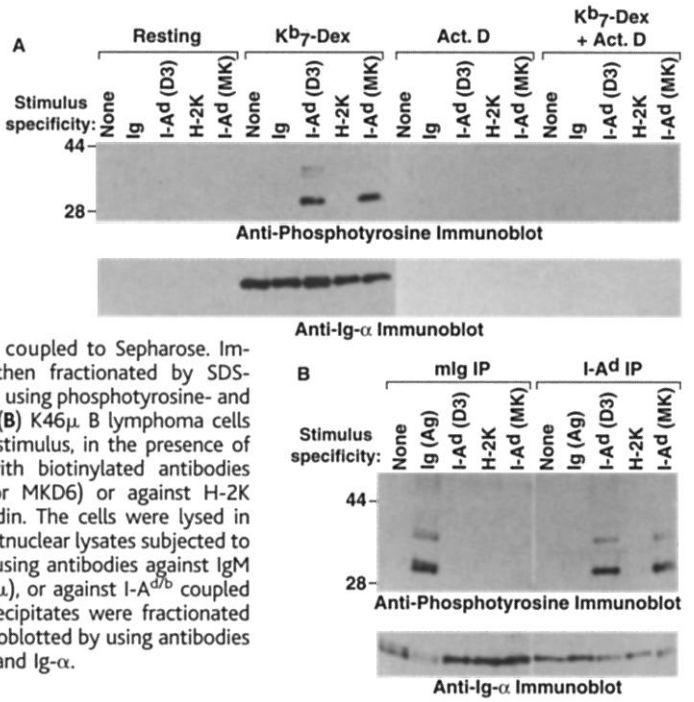

Fig. 2. MHC class II aggregation leads to tyrosyl phosphorylation of MHC class II–associated but not membrane Ig–associated Ig-α. (**A**) 3-83 μδ splenic B cells were primed and stimulated as described in Fig. 1A, lysed, and MHC class II–immunoprecipitated by using either antibodies against avidin or against I-$A^{d/b}$ (D3.137) coupled to Sepharose. Immunoprecipitates were then fractionated by SDS-PAGE and immunoblotted using phosphotyrosine- and Ig-α–specific antibodies. (**B**) K46μ B lymphoma cells were incubated without stimulus, in the presence of antigen (NP_7BSA), or with biotinylated antibodies against I-$A^{d/b}$ (D3.137 or MKD6) or against H-2K (M1/42) followed by avidin. The cells were lysed in CHAPS buffer and the postnuclear lysates subjected to immunoprecipitation by using antibodies against IgM (b-7-6, antibody against μ), or against I-$A^{d/b}$ coupled to Sepharose. Immunoprecipitates were fractionated by SDS-PAGE and immunoblotted by using antibodies against phosphotyrosine and Ig-α.

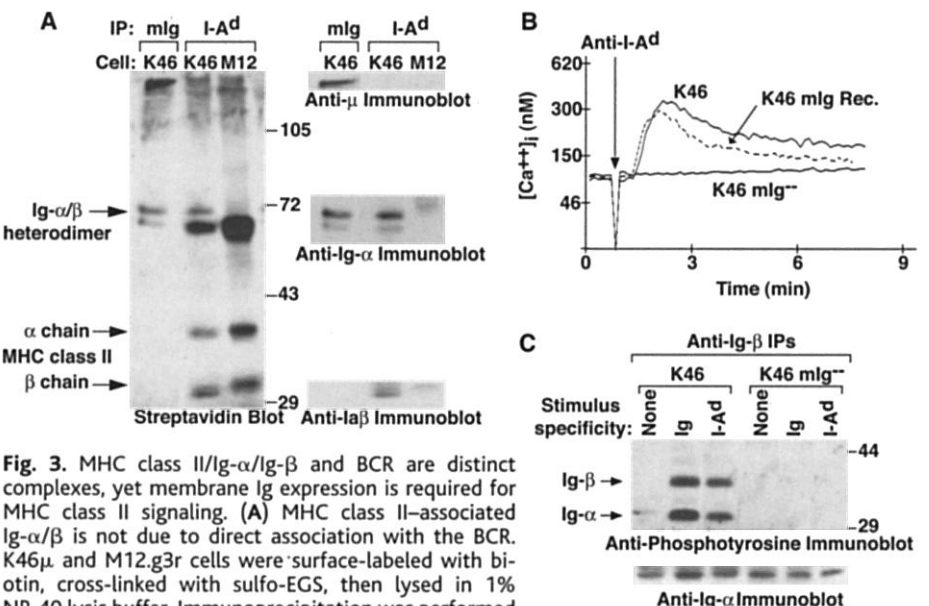

Fig. 3. MHC class II/Ig-α/Ig-β and BCR are distinct complexes, yet membrane Ig expression is required for MHC class II signaling. (**A**) MHC class II–associated Ig-α/β is not due to direct association with the BCR. K46μ and M12.g3r cells were surface-labeled with biotin, cross-linked with sulfo-EGS, then lysed in 1% NP-40 lysis buffer. Immunoprecipitation was performed by using antibodies against IgM (b-7-6) or against I-$A^{d/b}$ (D3.137) coupled to Sepharose. Immunoprecipitates were washed and the cross-linker was disrupted by mild reduction before fractionation by nonreducing SDS-PAGE. Polyvinlyidene difluoride transfers were then probed with streptavidin-HRP followed by immunoblotting with antibodies specific for IgM, Ig-α, and the MHC class II β chain (*11*). (**B**) Membrane Ig–negative K46 cells do not mobilize calcium upon MHC class II aggregation. K46, K46 membrane Ig–negative, and K46 membrane Ig–negative cells reconstituted with membrane Ig [K46 mIg Rec. (*27*)] were loaded with Indo1-AM and stimulated through their MHC class II (*26*). Intracellular free calcium levels were monitored by flow cytometry before and during stimulation. Mean $[Ca^{2+}]_i$ is shown as a function of time with the addition of stimuli denoted by the arrow. (**C**) MHC class II aggregation does not induce the tyrosyl phosphorylation of Ig-α/β in membrane Ig–negative K46 cells. K46 and K46 membrane Ig–negative cells were incubated for 2 min at 37°C without stimulus, with antibodies against Ig or against I-$A^{d/b}$ as in Fig. 2B. Postnuclear lysates were subjected to immunoprecipitation with antibodies against Ig-β (HM79) coupled to Sepharose. Immunoprecipitates were fractionated by SDS-PAGE and analyzed by phosphotyrosine- and Ig-α–specific immunoblotting.

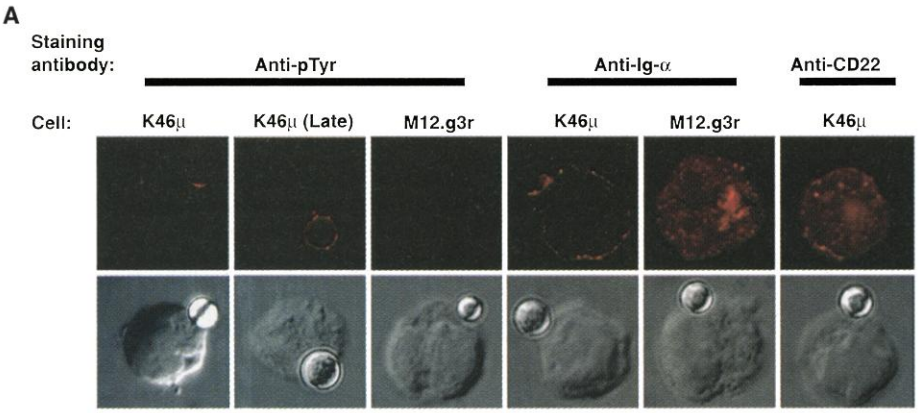

Fig. 4. TCR binding to MHC class II/peptide complexes triggers redistribution of tyrosyl-phosphorylated proteins and Ig-α and induces the tyrosyl phosphorylation of Ig-α/β in primed B cells. (**A**) K46μ and M12.g3r cells were pulsed with OVA and then incubated with beads that were previously coupled with biotinylated DO.11.10 TCR monomers (*11*). Indicated proteins were detected by immunofluorescence microscopy after staining with antibodies specific for phosphotyrosine (Transduction Laboratories), Ig-α, and CD22 followed by a Cy3-conjugated donkey antibody against rabbit Ig (Jackson Laboratories). Nomarski views are located below each digitally deconvolved immunofluorescence image (*11*). (**B**) K46μ and M12.g3r cells were pulsed with OVA as above before incubation with beads coupled or not coupled with TCR or antibodies against MHC class II (D3.137). M12.g3r cells were stimulated with antibodies against Ig as a positive control. The cells were lysed in 1% NP-40 and subjected to immunoprecipitation with antibodies against Ig-β coupled to Sepharose. Immunoprecipitates were then fractionated by SDS-PAGE and sequentially immunoblotted with phosphotyrosine- and Ig-α–specific antibodies.

seen at the contact. The translocation of these proteins appeared specific, as CD22 clustering was not observed under these conditions. Additionally, TCR binding to MHC class II/peptide complexes stimulated the tyrosyl phosphorylation of Ig-α/β in primed phenotype cells (Fig. 4B). This response was not observed in K46μ cells cultured without OVA (*19*) and is thus antigen-specific. The amount of phosphorylated Ig-α/β stimulated by TCR binding was less than that induced by beads coated with antibodies against MHC class II molecules, consistent with TCR aggregation of only the fraction of MHC class II molecules loaded with OVA peptide. M12.g3r cells were competent to transduce signals leading to Ig-α/β phosphorylation as indicated by their response to BCR aggregation. These data demonstrate that tyrosine kinases are activated and Ig-α/β phosphorylation is induced by TCR engagement of MHC class II–peptide complexes during physiological responses.

MHC class II–mediated transduction of activating signals is most likely of importance in class II–restricted, T cell contact–dependent B cell signaling, a very complex phenomenon involving the engagement of multiple receptor pairs (*5*). CD40 has generally been considered the principal transducer of the B cell–activating signals during this interaction (*21*), but recent studies using CD40 knockout mice demonstrate that germinal center formation and class switching are not absolutely dependent on CD40 signaling (*22*, *23*). Further, provided B cell antigen receptors are stimulated, T cells and CD40-negative B cells can collaborate to mount thymus-dependent responses (*24*). Thus, alternate mechanisms, perhaps involving MHC class II signal transduction (*3*), provide signals during physiological, cognate T cell–B cell interactions. It seems most likely that these signaling events are important early in the immune response when antigen stimulated B cells seek specific T cells, especially CD40L-negative naïve T cells, in the T cell–rich cortical regions of the lymph nodes and spleen.

References and Notes

1. J. C. Cambier, K. R. Lehmann, *J. Exp. Med.* **170**, 877 (1989).
2. N. A. Mooney, C. Grillot-Courvalin, C. Hivroz, L. Ju, D. Charron, *J. Immunol.* **145**, 2070 (1990).
3. J. C. Cambier, D. C. Morrison, M. M. Chien, K. R. Lehmann, *J. Immunol.* **146**, 2075 (1991).
4. T. Morio, R. S. Geha, T. A. Chatila, *Eur. J. Immunol.* **24**, 651 (1994).
5. D. C. Parker, *Annu. Rev. Immunol.* **11**, 331 (1993).
6. P. J. L. Lane, F. M. McConnell, G. L. Schieven, E. A. Clark, J. A. Ledbetter, *J. Immunol.* **144**, 3684 (1990).
7. P. André, J. C. Cambier, T. K. Wade, T. Raetz, W. F. Wade, *J. Exp. Med.* **179**, 763 (1994).
8. D. M. Russell *et al.*, *Nature* **354**, 308 (1991).
9. J. C. Cheronis *et al.*, *J. Med. Chem.* **37**, 348 (1994).
10. M. R. Jackson, E. S. Song, Y. Yang, P. A. Peterson, *Proc. Natl. Acad. Sci. U.SA.* **89**, 12117 (1992).
11. Supplementary data and information on experimental protocols are available on *Science* Online at www.sciencemag.org/cgi/content/full/291/5508/1537/DC1.
12. S. B. Kanner *et al.*, *Tissue Antigens* **46**, 145 (1995).
13. J. Cambier, *J. Immunol.* **155**, 3281 (1995).
14. M. R. Gold, L. Matsuuchi, R. B. Kelly, A. L. DeFranco, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3436 (1991).
15. P. Pierre *et al.*, *Nature* **388**, 787 (1997).
16. K. J. Kim, C. Kanellopoulos-Langevin, R. M. Merwin, D. H. Sachs, R. Asofsky, *J. Immunol.* **122**, 549 (1979).
17. M. E. Reth, P. Petrac, L. Wiese, L. Lobel, F. W. Alt, *EMBO J.* **6**, 3299 (1987).
18. W. F. Wade *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6297 (1989).
19. J. C. Cambier *et al.*, unpublished observations.
20. J. R. Drake, P. Webster, J. C. Cambier, I. Mellman, *J. Exp. Med.* **186**, 1299 (1997).
21. P. Lane, *Curr. Opin. Immunol.* **8**, 331 (1996).
22. D. van Essen, H. Kikutani, D. Gray, *Nature* **378**, 620 (1995).
23. N. Chirmule, J. Tazelaar, J. M. Wilson, *J. Immunol.* **164**, 248 (2000).
24. C. E. Schrader, J. Stavnezer, H. Kikutani, D. C. Parker, *J. Immunol.* **158**, 153 (1997).
25. The 3-83 μδ splenic B cells were isolated via Percoll ($\rho > 1.079$) and primed by incubation (2×10^6 cells ml^{-1}) at 37°C for 9 to 12 hours with K^b_7-Dex (0.2 μg ml^{-1}), with or without actinomycin D (1.5 μg ml^{-1}, Sigma).
26. Analyses of intracellular free calcium as well as immunoblotting with antibodies against phosphotyrosine and the effector proteins have been described previously [B. J. Vilen, T. Nakamura, J. C. Cambier, *Immunity* **10**, 239 (1999); R. J. Benschop, D. Melamed, D. Nemazee, J. C. Cambier, *J. Exp. Med.* **190**, 749 (1999)]. Blotting reagents used were antibodies against Ptyr (Ab-2, Oncogene); rabbit antisera specific for full-length Ig-α, CD22 cytoplasmic tail, and Ia β chain cytoplasmic tail; streptavidin-HRP (Pierce); goat antibodies against mouse μ heavy chain–HRP (Southern Biotechnology); rat antibodies against mouse γ1–HRP (Zymed); and Protein A–HRP (Zymed).
27. K46 membrane Ig–negative cells were retrovirally infected with a heavy and light chain [A. R. Venkitaraman, G. T. Williams, P. Dariavach, M. S. Neuberger, *Nature* **352**, 777 (1991)] containing a construct as described [I. Tamir *et al.*, *Immunity* **12**, 347 (2000)] and sorted for uniform membrane Ig expression before analysis.

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