

Distinct Structural Compartmentalization of the Signal Transducing Functions of Major Histocompatibility Complex Class II (Ia) Molecules

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Summary

Class II major histocompatibility complex encoded proteins (MHC class II or Ia molecules) are principal plasma membrane proteins involved in activation of both B and T cells during antigen-driven immune responses. Recent data indicate that class II molecules are more than simply recognition elements that provide a ligand for the T cell antigen receptor. Changes in B cell physiology that follow class II binding are now recognized as being required not only for the induction of T cell activation, but also for B cell activation and proliferation. It is interesting to note that class II molecules appear to transduce signals via two distinct mechanisms depending upon the differentiative state of the B cell on which they are expressed. While one of these pathways, involving cAMP generation and protein kinase C localization in the cytoskeletal/nuclear compartment, is seen in resting B cells, the second is seen in primed B cells and involves tyrosine kinase activation, inositol lipid hydrolysis, and Ca²⁺ mobilization. Use of this pathway is correlated with ability of class II to transduce signals leading to B cell proliferation. To begin to address the molecular basis of this unique, activation-dependent, differential coupling of class II to signaling pathways, we conducted mutational analysis of class II structural requirements for signal transduction. Here we report that the cytoplasmic (Cy) domains of I-A^k class II molecules are not required for either receptor-mediated activation of protein tyrosine phosphorylation or Ca²⁺ mobilization. This is in contrast to the requirement of the Cy domain of beta chain of class II for the alternate signaling pathway and efficient antigen presentation to autoreactive T cell lines. Disparate distribution of functional motifs within the MHC class II molecules may reflect use of distinct receptor associated effector molecules to sustain different modes of signal transduction in various class II-expressing cells.

The evolution and maintenance of the polymorphisms of MHC class II molecules for the singular role of binding peptide antigen for presentation to T cells may have been complemented by the strict conservation of amino acids (monomorphisms) in the transmembrane (TM) and cytoplasmic (Cy) domains in order to maintain the ability to exercise multiple signaling functions. With respect to signaling via class II molecules (1), it is appreciated that the amino acids of the TM and Cy domains are as critical to function of the class II molecule as are the amino acids that comprise its peptide binding site. The six COOH-terminal amino acids of the α chain are implicated in controlling the translational diffusion (2) of class II molecules while the Cy domain of both α and β determine its efficiency in antigen presentation (3–5, and Wade, W.F., personal observation). The basis of the later effect is not known, but it is thought to reflect a

necessity that the molecule transmit a transmembrane signal for effective antigen presentation or that interaction of these molecules with other components at the plasma membrane is needed for the correct, optimal orientation of the class II molecule for T cell recognition (6–8). While the 18-amino acid beta chain Cy domain of class II has been found to control the translocation of protein kinase C to the cytoskeletal/nuclear compartment after class II receptor engagement, it has also been implicated in contributing to antigen presentation efficiency, especially presentation to autoreactive T cells (3, 4, 9, 10). Recently studies of several groups have revealed that MHC class II molecules alternately use an additional signaling mechanism involving rapid activation of a protein tyrosine kinase, Ca²⁺ mobilization, and hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdInsP2) yielding diacylglycerol (DAG), and inositol 1,4,5-trisphosphate (InsP3)

(11–13). In the mouse, coupling to this pathway is only seen in cells that have been activated by antigen surrogate anti-Ig antibodies, IL-4 receptor ligation (11, 14), or CD40 ligand stimulation (André, P., and J. C. Cambier, manuscript in preparation). The use by the same receptor of alternate signaling pathways depending upon the differentiative context is novel in biology, and of likely importance in the immune response. This prompted us to undertake structure/function analysis of the Cy domains of class II to determine if the amino acids that control previously defined function overlap with the amino acids that control the ability of the receptor to mediate and activate tyrosine phosphorylation Ca^{2+} mobilization.

Materials and Methods

Preparation of Transfectants. K46J, an I-A^d positive B cell lymphoma was maintained in IMDM medium supplemented with 5% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mM sodium pyruvate, 2 mM glutamine, and 5×10^{-5} M 2-ME. Transfection was accomplished via electroporation with an electroporator (GIBCO BRL, Gaithersburg, MD) using previously described constructs (10) (Table 1). Briefly, 5×10^6 cells were pulsed in the presence of 20 µg each of linearized genomic DNA for the A_α and A_β chains, as well as 5 µg of pSV2-neo. The settings for the pulse were 50 µF, low impedance, and 300 V. After electroporation, cells were cultured (10^4 – 10^5 /ml) in 24-well plates for 16 h before the addition of G418 to a final concentration of 1 mg/ml. Resistant cells from wells exhibiting clonal growth were subcloned and analyzed for surface expression levels of I-A^k by exposing cells to biotinylated anti-I-A^k (mAb) 39J (20 µg/ml per 10^7 cells), and then to fluoresceinated avidin (1/100; Tago, Inc., Burlingame, CA) before analysis by flow cytometry (10). Cells that were used in these

studies expressed various phenotypes of MHC class II that had been mutated by the addition of premature stop codons (10): K46J 17.4 (wt/wt, wild type α and β chains). The α chain phenotype is always listed first. K46J 73.35 (αCT12/βCT12, CT refers to a COOH-terminal truncation and the number indicates how many amino acids are missing from the COOH terminus) K46J 25.1 (αCT12/wt), K46J 1.6.24 (wt/βCT12), K46J 7.1 (αCT12/βCT18), and K46J 7.5 (αCT12/βCT18). As predicted by studies of Germain et al. (15), immunochemical analysis of these cells revealed no evidence of mixed molecule I-A^k/I-A^d formation (data not shown).

Analysis of Intracellular Free Calcium [Ca^{2+}]_i. [Ca^{2+}]_i analysis was performed as described previously (11). K46J were loaded with Indo-1 AM (Molecular Probes Inc., Eugene, OR) coated with biotinylated antibodies including anti-I-A^{db} (20 µg D3.137/ 10^7 cells/ml), anti-I-A^k (20 µg 39J/ 10^7 cells/ml), anti-H-2K^d (25 µg 34.5.8s/ 10^7 cells/ml), or anti-B220 (12 µg RA3.3A1/ 10^7 cells/ml), and washed and resuspended at 10^6 cells/ml. [Ca^{2+}]_i levels were analyzed by flow cytometry before and after stimulation with avidin (10 µg/ml; Sigma Chemical Co., St. Louis, MO). D3.137 mAb was a gift from Dr. Sue Tonkonogy, North Carolina State University School of Veterinary Medicine (Raleigh, NC). Other antibodies were obtained from the American Type Culture Collection (Rockville, MD). Analyses were conducted using a flow cytometer (model 50H; Ortho Diagnostic Systems, Westwood, MA) with an appended data acquisition system and MTIME software (both from Phoenix Flow Systems, San Diego, CA).

Analysis of Induction of Protein Tyrosine Phosphorylation. B lymphoma cells were washed twice and resuspended in IMDM at 10^7 cells/ml. Saturating concentrations of biotinylated anti-I-A^k (20 µg 39J/ml) were added and cells were incubated for 15 min at room temperature. Unbound antibody was removed by washing one time before cells were resuspended at 5×10^6 cells/50 µl per sample. Primary antibodies were cross-linked by addition of 4 µg avidin/sample. After incubation for varied time periods at 37°C, reactions were terminated by addition of an equal volume of 2× ice-cold

Table 1. Primary Amino Acid Sequence of the Connecting Peptide, Transmembrane, and Cytoplasmic Domains of I-A^k

CP	TM	Cy
...	<u>MSELTETVVCALGLSVGLVGIVVGTIFII</u>	IQGRSGGTSRHPGPL
...	<u>RAQSESARSKMLSGIGGCVLGVI</u>	FLGLGLFIRHRSQKGRGPPAGLLQ
...	<u>MSELTETVVCALGLSVGLVGIVVGTIFII</u>	IQG
...	<u>RAQSESARSKMLSGIGGCVLGVI</u>	FLGLGLFIRHRSQKGRGPPAGLLQ
...	<u>MSELTETVVCALGLSVGLVGIVVGTIFII</u>	IQGRSGGTSRHPGPL
...	<u>RAQSESARSKMLSGIGGCVLGVI</u>	FLGLGLFIRHRSQK
...	<u>MSELTETVVCALGLSVGLVGIVVGTIFII</u>	IQG
...	<u>RAQSESARSKMLSGIGGCVLGVI</u>	FLGLGLFIRHRSQK
...	<u>MSELTETVVCALGLSVGLVGIVVGTIFII</u>	IQG
...	<u>RAQSESARSKMLSGIGGCVLGVI</u>	FLGLGLFI
		α wt
		β wt
		α CT12
		β wt
		α wt
		β CT12
		α CT12
		β CT12
		α CT12
		β CT18

The primary amino acid code for the truncation phenotypes of I-A^k that were expressed in K46J, a B cell lymphoma. The transmembrane (TM) sequence is underlined; the cytoplasmic (Cy) domain amino acids are to the right of the underlined sequences and the connecting peptide (CP) is to the left. CT and then a number indicates a COOH-terminal truncation of the indicated number of amino acids. The generation of the mutant I-A^k-encoding genomic clones has been described elsewhere (6). Genomic constructs for the various combinations of the α and β chains, along with the construct pSV2-neo were transfected into K46J cells. G418-resistant clones were isolated and screened secondarily by immunofluorescence staining with FITC-anti-I-A^k antibodies.

lysis buffer (1% NP-40, 150 mM NaCl, 2 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 10 mM Tris-HCl (pH 7.3), 1 mM phenylmethylsulfonyl fluoride, 0.4 mM EDTA, 10 mM sodium fluoride, 2 $\mu\text{g}/\text{ml}$ aprotinin, 2 $\mu\text{g}/\text{ml}$ leupeptin, and α -1-antitrypsin (2 $\mu\text{g}/\text{ml}$). After lysis at 4°C, nuclei were pelleted and post-nuclear supernatants were boiled in SDS sample buffer, subjected to SDS-PAGE on a 8% gel, and transferred onto Immobilon (Millipore Corp., Bedford, MA). Membranes were blocked overnight at 4°C with 5% bovine albumin (Cohn crystallized; ICN Biomedicals, Costa Mesa, CA) in 10 mM Tris (pH 8) and 150 mM NaCl (Tris buffered saline [TBS]), and then probed with an anti-phosphotyrosine antibody (Ab-2; Oncogene Science Inc., Manhasset, NY) for 2 h at room temperature. After washing, the immunoblots were incubated with a rabbit anti-mouse IgG1 antibody (Zymed Labs., Inc., S. San Francisco, CA) for 1 h at room temperature, then washed before exposure to ^{125}I protein A for 1 h at room temperature, washed again, and then subjected to autoradiography.

Results

The analysis of MHC class II structural requirements for Ca^{2+} mobilization after class II ligation was made possible by the discovery (see Fig. 2) that in the B cell lymphoma K46J, cross-linking of endogenous MHC class II (I-A^d) molecules triggers the Ca^{2+} mobilization pathway seen both in human and mouse peripheral B cells (11, 12, 13). We therefore constructed transfectants of K46J that express previously described mutant I-A^k molecules (10) with various combinations of truncated α or β chains, or both. The mutants are defined in Table 1. These transfectants were selected with 1 mg/ml G418, cloned by limiting-cell dilution and analyzed for surface expression levels of I-A^k, Fig. 1. It can be seen that the relative expression of mutant I-A^k on individual transfectants is within a twofold of range of wild type and there is considerable overlap in expression between the transfectant 17.4 (wt/wt) with that of the most extreme truncation phenotype clone, 7.5, which lacked the COOH-terminal cytoplasmic 12 amino acids of the α chain and 18 amino acids of β chain ($\alpha\text{CT12}/\beta\text{CT18}$). No staining of K46J parents was seen, verifying their lack of expression of I-A^k and

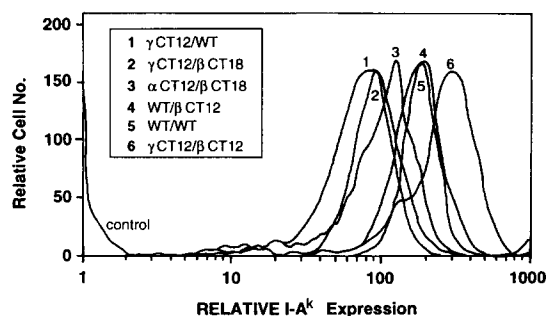


Figure 1. Analysis for surface expression levels of I-A^k in transfected K46J B lymphoma. Cells were exposed to biotinylated anti-I-A^k (mAb) and then to fluoresceinated avidin before analysis of flow cytometry. Shown are the immunofluorescence histograms of K46J parent cells (control), K46J 17.4 (wt/wt) (5), K46J 73.35 ($\alpha\text{CT12}/\beta\text{CT12}$) (6), K46J 25.1 ($\alpha\text{CT12}/\text{wt}$) (1), K46J 1.6-24 (wt/ βCT12) (4), K46J 7.1 ($\alpha\text{CT12}/\beta\text{CT18}$) (3), and K46J 7.5 ($\alpha\text{CT12}/\beta\text{CT18}$) (2).

crossreactive antigens. All K46J transfectants expressed endogenous I-A^d at the same level when analyzed by the mAb D3.137 (data not shown). The I-A^d and I-A^k expressed by transfectants were insensitive to PLC digestion indicating that none of the transfected gene products was expressed in phosphatidylinositol-glycan linked form (data not shown).

The K46J transfectants were tested for their ability to mobilize Ca^{2+} through either the endogenous wild type I-A^d or the transfected genes' product, I-A^k. It can be seen in Fig. 2 that three transfectants 17.4 (wt/wt), 73.35 ($\alpha\text{CT12}/\beta\text{CT12}$), and 7.1 ($\alpha\text{CT12}/\beta\text{CT18}$) undergo Ca^{2+} mobilization response after cross-linking of I-A^k by the anti-I-A^k mAb 39J (IgG2a anti- α chain). These responses appeared equivalent to that mediated through their endogenous I-A^d. Similar responses were made by transfectants that express I-A^k with truncation phenotypes $\alpha\text{CT12}/\text{wt}$ or $\text{wt}/\beta\text{CT12}$ (data not shown). Equivalent responses were seen when the anti-I-A^k antibody 10.2.16 (IgG2b anti- β chain) was used as stimulus but cells did not respond to the anti-H2K^d antibody (34.5.8s, IgG2a) or to anti-B220 (RA3-3A1) demonstrating specificity of the response to anti-Ia (data not shown).

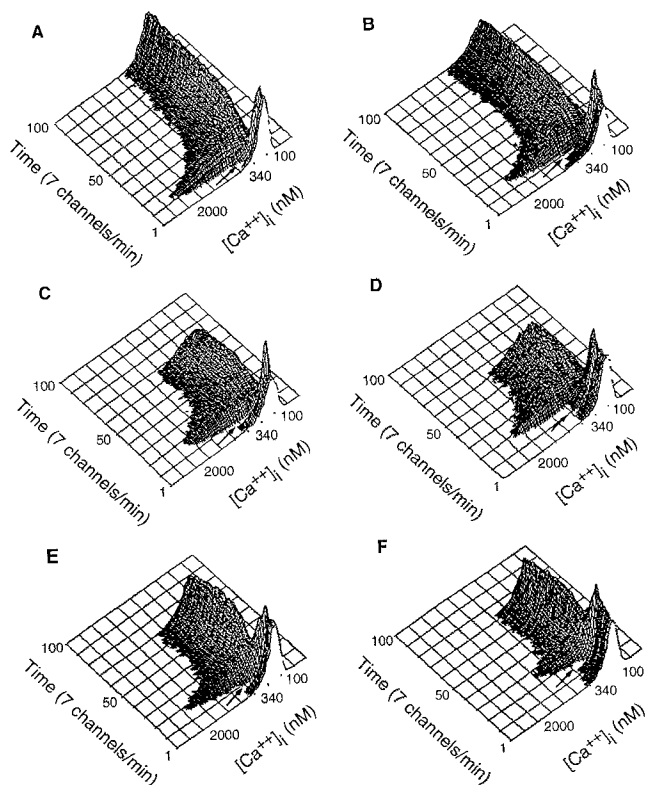


Figure 2. Cross-linking of wild type or truncated I-A^k molecules induce Ca^{2+} mobilization in K46J. K46J (wt/wt) (A and B), K46J 73.35 ($\alpha\text{CT12}/\beta\text{CT12}$) (C and D) and K46J 7.1 ($\alpha\text{CT12}/\beta\text{CT18}$) (E and F) were loaded with Indo-1 as described (11), coated with biotinylated anti-I-A^{d,b} (20 μg D3.137/ 10^7 cells/ml) (A, C, and E) or biotinylated anti-I-A^k (20 μg 39J/ 10^7 cells/ml) (B, D, and F), washed and resuspended at 10^6 cells/ml. $[\text{Ca}^{2+}]_i$ levels were analyzed by flow cytometry before and after stimulation with avidin (10 $\mu\text{g}/\text{ml}$, Sigma Chemical Co.) added at arrow. Data shown in this and subsequent figures are representative of more than three replicate experiments.

While it was clear that the truncated I-A^k molecules could mediate a Ca²⁺ mobilization after their ligation, the isometric analysis of the data indicated that maximal responses and response kinetics differed somewhat among subclones. Therefore we conducted a more quantitative analysis of class II-mediated Ca²⁺ fluxes in the subclones.

Quantitative comparison of the ability of mutant receptors expressed by subclones of K46 to transduce signals leading to Ca²⁺ mobilization required that we correct for (a) potential variations in intrinsic ability of individual subclones to signal through Ia and (b) clonal differences in I-A^k expression that might limit signal transduction. We normalized for the intrinsic ability of clones to signal through Ia based on relative responses to cross-linking of their endogenous I-A^d (Table 2). The maximum mean intracellular free calcium concentration (m[Ca²⁺]_i) seen in response to cross-linking of the endogenous I-A^d was determined for each subclone. We then established the proportionality between this response and that of the K46J17.4 subclone expressing wild-type I-A^k, e.g., (m[Ca²⁺]_i of anti-I-A^d-stimulated K46J7.1)/(m[Ca²⁺]_i of anti-I-A^d-stimulated K46J17.4) = 230/340 = 0.68. Using this factor we normalized the intrinsic responsiveness of each subclone to K46J17.4. To then normalize the I-A^k responses of each subclone, e.g., K46J7.1, to that of the wild-type I-A^k subclone (K46J17.4), we divided the normalization factor for each subclone, in this case, 0.68, into the particular subclone's m[Ca²⁺]_i response to anti-I-A^k. This corrected value is referred to as normalized m[Ca²⁺]_i (see Table 2). It should be noted that expression of I-A^d was essentially identical in all clones and the m[Ca²⁺]_i response of clones to anti-I-A^d did not vary by more than 20%.

To accommodate potential effects of differential I-A^k expression on the responses observed, we first established the relationship between the relative number wild-type I-A^k molecules ligated and the magnitude of the subsequent Ca²⁺ response. Saturating and subsaturating doses of anti-I-A^k mAb (39J) were used to define this relationship in K46J 17.4

(wt/wt). Indo-1-loaded cells were exposed to varied concentrations of biotinylated anti-I-A^k. Cells were then washed and samples were divided and subjected to analysis of [Ca²⁺]_i before and after cross-linking of biotinylated anti-I-A^k with excess avidin. Alternatively, relative I-A^k molecules ligated on cells were quantitated by exposure of the parallel samples to fluoresceinated avidin, followed by analysis of immunofluorescence by flow cytometry. As shown in Fig. 3, ligation of as few as 20% of wild-type I-A^k molecules on K46J17.4 cells led to a detectable [Ca²⁺]_i response. The magnitude of the response was directly correlated with levels of receptor ligation when 20–60% of I-A^k were ligated. Above this level of receptor ligation the response was maximal.

Based on this relationship, it was possible to predict what response should result from ligation of any number of normally functional I-A^k molecules on clones that expressed mutant I-A^k. For each mutant clone we simply plotted the normalized m[Ca²⁺]_i seen after I-A^k stimulation against I-A^k bound using saturated staining of K46J17.4 (wt/wt) I-A^k as 100% (as above). Mutants whose response equaled those predicted for wild-type I-A^k were considered not to be defective. As can be seen in Fig. 3, normalized [Ca²⁺]_i responses of mutant I-A^k-bearing cells were similar in magnitude to those of wild-type I-A^k-bearing cells under conditions of comparable I-A^k ligation. Thus the Cy domains of class II molecules are not required for receptor mediation of Ca²⁺ mobilization after class II ligation.

The earliest detectable event in Ia-mediated signaling in normal murine B cells and K46 cells is inductive protein tyrosine phosphorylation (13). Further, previous studies have demonstrated that the Ia-mediated [Ca²⁺]_i responses are dependent on protein tyrosine kinase activity indicating that these events are causally related (11). To determine whether mutant I-A^k molecules are normal in terms of their ability to activate protein tyrosine phosphorylation, we stimulated K46J17.4 (wt/wt) and K46J7.1 (αCT12/βCT8) with biotinylated anti-I-A^k antibody and avidin for varied time periods and analyzed changes in whole cell tyrosine phos-

Table 2. Normalization of Anti-I-A^k Responses for the Clone's Intrinsic Ability to Mobilize Calcium After I-A Cross-linking

Subclone	m[Ca ²⁺] _i * to anti-I-A ^d <i>nM</i>	(subclone m[Ca ²⁺] _i to anti-I-A ^d)/ (K46J17.4 m[Ca ²⁺] _i to anti-I-A ^d)	m[Ca ²⁺] _i to anti-I-A ^k <i>nM</i>	Normalized I-A ^k response† <i>nM</i>	Relative I-A ^k expression (percent 17.4)
7.1	230	0.68	160	235	43
7.5	230	0.68	135	198.5	31.5
73.35	270	0.79	255	323	96
25.1	280	0.82	130	158.5	31
1.6-24	270	0.79	235	297	91
17.4	340	1	320	320	100

* Maximum mean intracellular free Ca²⁺ concentration, resting [Ca²⁺]_i was 70 nM.

† (Subclone X m[Ca²⁺]_i to anti-I-A^k)/[(subclone X m[Ca²⁺]_i to anti-I-A^d)/(K46J17.4 m[Ca²⁺]_i to anti-I-A^d)] = normalized I-A^k response.

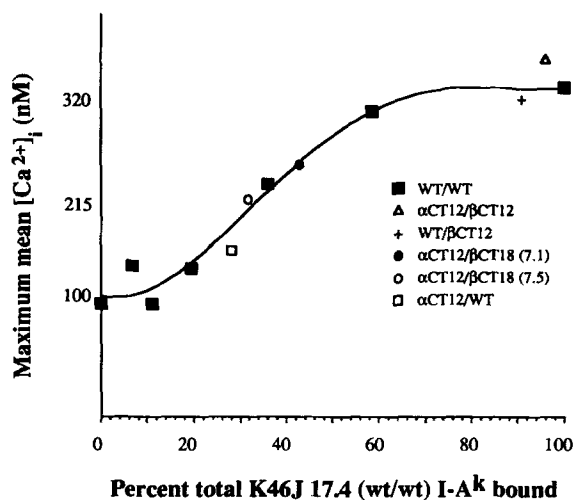


Figure 3. Tailless I-A molecules are competent “Ca²⁺” signal transducers. The anti-I-A^k mAb, 39J, was titrated with K46J 17.4 (*wt/wt*). Some cells were loaded with Indo-1 before exposure to decreasing concentrations of biotinylated anti-I-A^k, washed, and then subjected to analysis of [Ca²⁺]_i before and after cross-linking of biotinylated anti-I-A^k with excess avidin. α CT12/ β CT18 (7.1) and α CT12/ β CT18 (7.5) designate two distinct clones, 2 and 3, respectively, in Fig. 1. The maximum mean change in [Ca²⁺]_i was determined as described (11).

phoproteins patterns by anti-phosphotyrosine immunoblotting of electrophoretic transfers of SDS-PAGE fractionated whole cell lysates. As shown in Fig. 4, the wild-type and α CT12/ β CT18 I-A^k molecules transduced signal leading to apparently equivalent protein tyrosine phosphorylation. The same tyrosine phosphoprotein patterns were found by cross-linking I-A^d on K46J (data not shown). Notable is inducible phosphorylation of three dominant substrates *M_r* ~93k, 64k, and 60k (see *arrows*) through both *wt/wt* and tailless α CT12/ β CT18 I-A^k molecules. Surprisingly, in the experiment shown, stimulation lead to an apparent reduction in phosphorylation of proteins of ~106 and 75 k. This effect was not seen reproducibly. Thus deletion of the entire cytoplasmic tails of α and β does not affect the coupling of I-A to protein tyrosine kinase activation.

Discussion

Data presented demonstrate that the cytoplasmic tails of I-A _{α} and I-A _{β} chains are not required for MHC class II-mediated transmembrane signal transduction leading to protein tyrosine phosphorylation and calcium mobilization. Other receptor systems including mIg, CD2, TCR- ζ , PDGF, EGF, and CD3 differ from class II in that sequences responsible for receptor-mediated Ca²⁺ mobilization are found within Cy domains (16–20). It should be noted that the Cy domains of these molecules are appreciably larger than those of either the α or β chain of class II. Class II is similar to

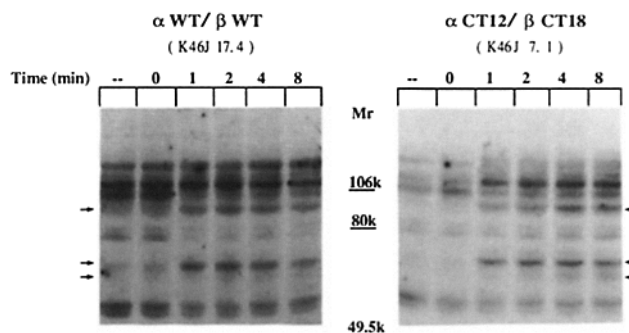


Figure 4. Kinetics of I-A^k cross-linking-triggered induction of protein tyrosine phosphorylation in K46J 17.4 (*wt/wt*) and K46J 7.1 (α CT12/ β CT18). The cells were coated with biotinylated 39J and then stimulated with avidin for 0–8 min as indicated. Reactions were stopped with ice cold lysis buffer and total cell lysate proteins were resolved by SDS-PAGE electrophoresis, transferred to immobilon, and immunoblotted with antiphosphotyrosine, followed by ¹²⁵I-protein A and autoradiography, as described in Materials and Methods. The mol wt markers are as indicated. (*Arrows*) Position of pp93, pp64, pp60. (Lane -) Uncoated cells.

the class I MHC molecule in not requiring a Cy domain in order to signal Ca²⁺ mobilization (21). A conceptual underpinning of the fact that MHC class II and class I molecules can mediate Ca²⁺ fluxes without Cy domain amino acids is provided by the recent observation that glycoposphoinositol-linked plasma membrane proteins such as CD55 and CD58, Ly6, and Thy 1 mediate tyrosine kinase activation and in some cases Ca²⁺ mobilization in the absence of cytoplasmic structure (22, 23). Thus there is no a priori requirement that tyrosine kinase coupled receptors such as I-A possess cytoplasmic domains. Presumably these molecules activate cytoplasmic effector pathways by virtue of interaction with other cell surface molecules which in turn transduce protein tyrosine kinase-activating signals.

Perhaps most importantly, the findings reported here indicate that distinct sequence motifs confer distinct functional potential upon MHC class II molecules. Both α and β chains contain sequences that are required for efficient antigen presentation (3, 4). The individual chains also have unique functions: the COOH-terminal 6 Cy domain amino acids of α chains appear to interact with cytoskeletal elements that determine the rate of translation diffusion of class II in the plasma membrane (2), while the sequence that mediates protein kinase C cytoskeletal/nuclear interaction is localized in a very basic six-amino acid motif adjacent to the plasma membrane in the beta chain Cy domain (5, 10). In view of the fact that these multiple functions are encoded in relatively short Cy domains, 12 and 18 amino acids for the α and β chains, respectively, it is perhaps not surprising that the amino acid sequences that mediate protein tyrosine kinase activation and Ca²⁺ mobilization response are in either the TM or ecto domains of the α and/or β chains.

We thank Judy Franconi for assistance in preparation of the manuscript.

The studies described were supported by United States Public Health Service grants AI-21768 and AI-20519 (John Cambier), and AI-31160 (William F. Wade). William F. Wade was a Leukemia Society Special Fellow during these studies.

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Received for publication 8 November 1992 and in revised form 9 July 1992.

References

1. Cambier, J.C., K.L. Lehmann, and W.F. Wade. 1991. The emerging receptor function of MHC class II molecules. *In* Blood Cell Biochemistry, Lymphocytes and Granulocytes, Vol. 3. J.R. Harris, editor. Plenum Publishing, New York. 99-114.
2. Wade, W.F., J.H. Freed, and M. Edidin. 1989. Translation diffusion of class II major histocompatibility complex molecules is constrained by their cytoplasmic domains. *J. Cell Biol.* 109:3325.
3. Griffith, I.J., Z. Ghogawala, N. Nabavi, A. Myer, D.E. Goan, D.J. McKean, and L.H. Glimcher. 1988. Cytoplasmic domain affects membrane expression and function of an Ia molecule. *Proc. Natl. Acad. Sci. USA.* 85:4847.
4. St.-Pierre, Y., N. Nabavi, Z. Ghogawala, L.H. Glimcher, and T.H. Watts. 1989. A functional role for signal transduction via the cytoplasmic domains of MHC class II proteins. *J. Immunol.* 143:808.
5. Nabavi, N., Z. Ghogawala, A. Myer, I.J. Griffith, W.F. Wade, Z.Z. Chen, D.J. McKean, and L.H. Glimcher. 1989. Antigen presentation abrogated in cells expressing truncated Ia molecules. *J. Immunol.* 142:1444.
6. St.-Pierre, Y., and T.H. Watts. 1991. Characterization of the signaling function of MHC class II molecules during antigen presentation by B cells. *J. Immunol.* 147:2875.
7. Jenkins, M.K., E. Burrell, and J.D. Ashwell. 1990. Antigen presentation by resting B cells: effectiveness at inducing T cell proliferation is determined by costimulatory signals, not T cell receptor occupancy. *J. Immunol.* 144:1585.
8. Mueller, D.L., M.K. Jenkins, and R.H. Schwartz. 1989. Clonal expansion versus functional clonal inactivation: a costimulatory signaling pathway determines the outcome of T cell antigen receptor occupancy. *Annu. Rev. Immunol.* 7:445.
9. Cambier, J.C., M.K. Newell, L.B. Justement, J.C. McGuire, K.L. Leach, and Z.Z. Chen. 1986. Ia binding ligand and cAMP stimulate translocation of PKC in B lymphocytes. *Nature (Lond.)* 146:2075.
10. Wade, W.F., Z.Z. Chen, R. Maki, S. McKercher, E. Palmer, J.C. Cambier, and J.H. Freed. 1989. Altered I-A-mediated transmembrane signaling in B cells that express truncated I-A^t protein. *Proc. Natl. Acad. Sci. USA.* 86:6297.
11. Cambier, J.C., D.C. Morrison, M.M. Chien, and K.R. Lehmann. 1991. Modeling of T cell contact-dependent B cell activation: IL-4 and antigen ligation primes quiescent B cells to mobilize calcium in response to Ia cross-linking. *J. Immunol.* 146:2075.
12. Mooney, N., C. Grillot-Courvalin, C. Hivroz, L.Y. Ju, and D. Charron. 1990. Early biochemical events following MHC class II mediated signaling on human B lymphocytes. *J. Immunol.* 145:2075.
13. Lane, P.J., L.F.M. McConnel, F.L. Scieven, E.A. Clark, and J.A. Ledbetter. 1990. The role of Class II molecules in human B cell activation: association with phosphatidyl inositol turnover, protein tyrosine phosphorylation, and proliferation. *J. Immunol.* 146:715.
14. Cambier, J.C., and K. Lehmann. 1989. Ia-mediated signal transduction leads to proliferation of primed B lymphocytes. *J. Exp. Med.* 170:877.
15. Germain, R.N., D.M. Bentley, and H. Quill. 1985. Influence of allelic polymorphism on the assembly and surface expression of class II MHC (Ia) molecules. *Cell.* 43:233.
16. Beyers, A.D., S.J. Davis, D.A. Cantrell, M. Izquierdo, and A.F. Williams. 1991. Autonomous roles for the cytoplasmic domains of the CD2 and CD4 T cell surface antigens. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:77.
17. Wegener, A.M.K., F. Letourneur, A. Hoeveler, T. Borcker, F. Luton, and B. Malissen. 1992. The T cell receptor/CD3 complex is composed of at least two autonomous transduction nodules. *Cell.* 68:83.
18. Cantley, L.C., K.R. Auger, C. Carpenter, B. Duckworth, A. Graziani, R. Kapeller, and S. Soltoff. 1991. Oncogenes and signal transduction. *Cell.* 64:281.
19. Ullrich, A., and J. Schlessinger. 1990. Signal transduction by receptors with tyrosine kinase activity. *Cell.* 61:203.
20. Letourneur, F., and R.D. Klausner. 1992. Activation of T cells by a tyrosine kinase activation domain in the cytoplasmic tail of CD3 ϵ . *Science (Wash. DC).* 255:79.
21. Gur, H., R. El-Zaatari, T.D. Geppert, M.C. Wacholtz, J.D. Taurog, and P.E. Lipsky. 1990. Analysis of T cell signaling by class I MHC molecules: the cytoplasmic domain is not required for signal transduction. *J. Exp. Med.* 172:1267.
22. Stefanova, I., V. Horejsi, I.J. Ansotegui, W. Knapp, and H. Stockinger. 1991. GPI-anchored cell-surface molecules complexed to protein tyrosine kinases. *Science (Wash. DC).* 254:1016.
23. Thomas, P.M., and L.E. Samelson. 1992. The glycoposphatidylinositol-anchored Thy-1 molecule interacts with the p60^{src} protein tyrosine kinase on T cells. *J. Biol. Chem.* 267:12317.