Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5933–5937, August 1989
Immunology

Haplotype-specific differences in signaling by transfected class II molecules to a Ly-1$^+$ B-cell clone

(T-cell–B-cell interaction/immunoglobulin secretion/B-cell activation)

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Communicated by Donald C. Shreffler, May 19, 1989

ABSTRACT **CH12.LX B cells are responsive to antigen-dependent differentiative signals transmitted through their surface E^k molecules. Although CH12.LX cells express surface A^k molecules with normal protein sequence, the A^k molecules do not deliver differentiative signals to these B cells. To determine whether introduction of a new A molecule into CH12.LX cells would correct this deficiency, CH12.LX cells were transfected with the genes encoding new A_α and/or A_β molecules. It was found that transfected cells responded to antigen-specific signals delivered via the $E_\alpha^kE_\beta^k$, $A_\alpha^bA_\beta^b$, or $A_\alpha^kA_\beta^b$ molecule. However, the B cells did not respond to signals generated by the molecule $A_\alpha^kA_\beta^k$, $A_\alpha^bA_\beta^k$, $A_\alpha^kA_\beta^d$, or $A_\alpha^kA_\beta^u$. Comparison of these sequences suggests that two A_β residues, His-47 and Trp-197, are important to the transmission of differentiative signals to B cells.**

CH12.LX, an Ia^k, Ly-1$^+$, μ^+, δ^+ murine B-cell clone, can be induced to differentiate by antigen (sheep erythrocytes, SRBCs) and signals mediated through its E^k, but not its A^k, molecules (1). Although E^k-specific T helper (Th) cells can serve this function, their soluble products are not necessary; binding of the E^k molecules of CH12.LX by anti-E^k monoclonal antibodies (mAbs) is sufficient to deliver the Ia-mediated signal (1). These findings demonstrate that B-cell class II molecules, together with antigen, can effectively transmit differentiative signals to the B cell. Similar findings implicating class II molecules in signal transduction have been described by other laboratories (2–4).

A major question raised by these data is which structural features of class II molecules are important to the function of signal transduction to B lymphocytes. Recently, we have demonstrated that the failure of the A^k molecules of CH12.LX to transmit differentiative signals is not due to coding sequence alterations in the genes encoding the A_α or A_β chain of A^k (5). CH12.LX A^k molecules may be unique in their inability to transduce a differentiative signal. Alternatively, CH12.LX may respond only to signals transduced through E, and not A, molecules. To distinguish between these possibilities, we transfected CH12.LX with DNA encoding A molecules of various haplotypes.

Cell lines expressing surface products of the transfected genes were isolated and could be induced by lipopolysaccharide to secrete SRBC-specific IgM. These cell lines responded similarly to CH12.LX to antigen-specific, E^k-mediated differentiative signals. In addition, transfected cells were able to respond to antigen-specific, major histocompatibility complex (MHC)-restricted signals mediated via molecules of A_β^b paired with either A_α^k or A_α^b. The cells were not responsive to binding of mAbs to transfected A_β^d or A_β^u molecules, however. Possible explanations of these findings, and the relation of signal transmission to protein sequence of the class II molecule, are discussed.

MATERIALS AND METHODS

Cells. CH12.LX, an Ia^k, μ^+, δ^+, Ly-1$^+$ murine B-cell clone described previously (1), was used in all experiments. CH12.LX cells were maintained as described (1) in B-cell medium (BCM). Transfected cells were grown in BCM supplemented with 400 μg of the antibiotic G418 per ml (BCM-G; Sigma).

Preparation of Transfectants. The A_β^b gene was transferred into CH12.LX cells using cosmid Aβ19, containing the genes for A_β^b and neomycin resistance (*neo*^{r}) (ref. 6; provided by G. Widera). The plasmid pKAB (ref. 7; provided by L. Schook) was used to transfer the A_α^b gene into CH12.LX cells. The A_β^d gene was transferred using the plasmid pCA52 (ref. 8; provided by B. Malissen), and the A_β^u gene was introduced via the plasmid pA_β^uLXR (ref. 9; provided by P. Jones). Cosmid Aβ19 allowed direct selection; plasmids pKAB, pCA52, and pA_β^uLXR were cotransfected in 3- to 5-fold molar excess with a plasmid containing the *neo*^{r} gene, pSV2neo (10). Plasmid and cosmid DNAs were prepared by standard procedures and were linearized by digestion with appropriate restriction enzymes prior to transfection. CH12.LX cells were washed in RPMI 1640 medium and resuspended at a density of 2×10^7 per ml. DNA in 10–20 μl of sterile Tris/EDTA was mixed with 0.5 ml of cells. The cell/DNA mixture was electroporated using a Bio-Rad gene pulser at 2500–2700 V/cm, 500 μF. Cells were incubated on ice for 10 min following electroporation and then incubated overnight at 37°C in 10 ml of BCM. The next day, surviving cells (usually 30–50% of the starting population) were plated in BCM-G at a concentration of 5×10^4 cells per ml in 2-ml wells of 24-well culture plates. Cultures were fed at 3- to 4-day intervals, and clones of G418-resistant cells were established within 3–5 weeks.

Nomenclature of Transfected Cell Lines. Transfectants of CH12.LX are labeled with the prefix CH12, followed by a designation listing the new Ia molecule and its haplotype, and the number of each cell line. Thus, CH12.LX cells expressing the complete A^b molecule are designated CH12.AB. If only a single chain of a new Ia molecule is expressed, the chain is specified (by A for α or B for β), prior to the haplotype designation. Cell lines expressing the A_β^b molecule are thus CH12.ABB, and those expressing the A_α^b molecule are CH12.AAB. Cells expressing the A_β^d or A_β^u molecules are called CH12.ABD or CH12.ABU, respectively. For the sake

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Abbreviations: BCM, B-cell medium; BCM-G, BCM containing G418; MHC, major histocompatibility complex; mAb, monoclonal antibody; pfc, plaque-forming cell(s); SRBC, sheep erythrocyte; Th, T helper.

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of brevity, we omit the prefix "CH12" in discussing the transfected lines.

Cell-Surface Antigen Phenotyping of Transfectants. Cell-surface Ia molecules were detected on transfectants by using immunofluorescence with mAbs. All mAbs used in phenotyping and differentiation experiments in this study are described in Table 1. Staining was performed as described (20). Stained cells were analyzed on an EPICS V cell sorter (Coulter), using the 488-nm line from an argon-ion laser. The A_β^b transfectant ABB1 also tested positively for cell-surface A_β^b by microcytotoxicity, as described (21), using the IgM anti-A_β^b mAb 28-16-8S (15). All mAbs were prepared as hybridoma tissue culture supernatants in our laboratory. Specific immunoglobulin concentrations for each mAb were determined by using an isotype-specific ELISA, as described elsewhere (20). Representative transfected ABB, ABD, and ABU cell lines were also tested, and found to be effective, as stimulators in a one-way mixed-lymphocyte reaction.

To determine the relative levels of expression of cell-surface products of endogenous and transfected genes, individual lines of G418-resistant CH12.LX cells were examined by immunofluorescence flow cytometry. CH12.LX and the transfected cell lines expressed high levels of cell-surface IgM (mean channel shifts of 130–138) as well as the endogenous $A_\alpha^k A_\beta^k$ molecule (mean channel shifts of 86–118). Cell-surface expression of the transfected Ia molecules was lower than that of the endogenous Ia molecules in all of the transfectants (mean channel shifts of 30–60 for A_α^b and A_β^b, 15–40 for A_β^d, and 7–15 for A_β^u), consistent with previous observations of Ia transfectants (22). However, the expression of Ia molecules produced by transfected genes was a consistent and stable characteristic of each transfected cell line. mAbs specific for A_α^b, A_β^b/A_β^d, or A_β^u do not bind parental CH12.LX cells (no channel shift; used as a control in all experiments). All of the transfected cell lines reported here could be induced to secrete IgM by culture in 50 μg of lipopolysaccharide per ml.

Assays for B-Cell Differentiation. Induced differentiation of CH12.LX cells and transfectants was as described (1). Briefly, 2×10^5 B cells were cultured in BCM, in 2-ml cultures containing varying concentrations of mAbs or T cells. For cultures containing antigen, 10^6 SRBCs were included per culture. For cultures containing T cells, 5000–50,000 irradiated (1500 R; 1 R = 0.258 mC/kg) cells of the Th cell hybridoma 2h405.2 (anti-A^k alloreactive) or AODH3.4 (anti-A^b alloreactive), provided by P. Marrack, were included per culture. Cultures were incubated for 72 hr at 37°C in 5% CO_2 in air. All cultures were performed in duplicate. Direct hemolytic plaque-forming cells (pfc) were measured as described (23).

RESULTS

Induction of Differentiation of Cells Expressing A_β^b. Transfected lines ABB1 (A_β^b), AAB1 (A_α^b), and AB1 ($A_\alpha^b + A_\beta^b$) were tested for antigen-specific, MHC-restricted induced differentiation. The parental clone CH12.LX is unresponsive to differentiative signals delivered by binding of A^k-alloreactive Th cells or mAbs specific for A_α^k or A_β^k (1, 5). We wished to determine if this reflects an inability of CH12.LX cells to respond to signals delivered via any A molecule or if only the A^k molecules of CH12.LX cannot deliver differentiative signals.

Data presented in Fig. 1*A* show that the A_β^b-expressing transfectant ABB1 can be induced to secrete IgM hemolytic for SRBCs (a 10- to 15-fold increase in pfc frequency) by coculture in the presence of SRBCs and mAbs specific for

Table 1. mAbs used in phenotyping and differentiation studies

Antibody	Specificity	Isotype	Ref(s).
10-3.6.2	A_β^k	IgG2a	11, 12
10-2.16	A_β^k	IgG2b	11, 12
11-5.2.1.9	A_α^k	IgG2b	11, 12
39F	A_α^k	IgG2a	12, 13
34-5-3S	$A_\beta^{b,d}$	IgG2a	12, 14
28-16-8S	$A_\beta^{b,d}$	IgM	12, 15
4D5	A_α^b	IgG2a	16
3B9	A_α^b	IgG2a	16
14-4-4S	E_α^k	IgG2a	17
BP107.2.2	A_β^u	IgG3	18
MKS4	A_β^u	IgG2b	19

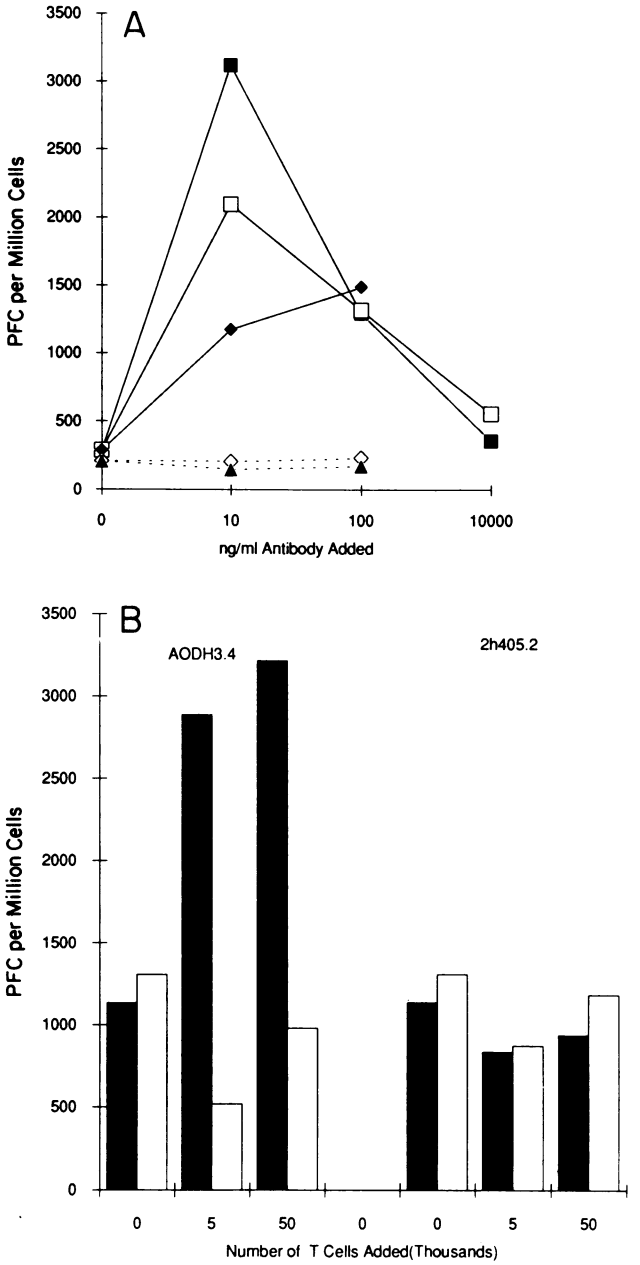

FIG. 1. Stimulation of differentiation in an A_β^b transfectant (ABB1) by anti-A_β^b mAbs or Th cells. (*A*) Cells of the A_β^b transfectant ABB1 (solid lines) were cultured with SRBCs and various concentrations of anti-A_β^b mAb 34-5-3S (■) or 28-16-8S (□) or the anti-E^k mAb 14-4-4S (◆). Dashed lines represent untransfected CH12.LX cells cultured with SRBCs and 34-5-3S (▲) or 28-16-8S (◇). (*B*) Induced differentiation of ABB1 cells by Th cells and antigen. Cells were cultured for 72 hr with (solid bars) or without (open bars) SRBCs and 5000 or 50,000 irradiated Th cells. AODH3.4 Th cells are A^b-specific; 2h405.2 Th cells are A^k-specific.

A_β^b (34-5-3S and 28-16-8S). The decrease in pfc seen at higher concentrations of mAb is due to complement-mediated lysis of the CH12.LX B cells that occurs during the plaque assay. The data in Fig. 1*A* also demonstrate that the anti-E^k mAb 14-4-4S induces differentiation of ABB1 cells, as it does of CH12.LX cells (1). Anti-A_β^b mAbs do not induce nonspecific differentiation of the parental CH12.LX cells, since no stimulation of pfc formation is seen.

Antigen-Dependent Differentiation Induced via A_β^b. The MHC-restricted, antigen-specific induced differentiation of CH12.LX requires two signals: one delivered by binding of the E^k molecule by T cells or mAbs, and one by binding of the surface IgM molecules by antigen (SRBCs; ref. 1). To determine whether the differentiation of ABB1 cells induced by anti-A_β^b mAbs was similarly antigen-dependent, ABB1 cells were cultured with 0.1 or 1.0 μg of each of two A_β^b-specific mAbs per ml in either the presence or the absence of SRBCs. Differentiation induced by mAbs specific for A_β^b was dependent upon the presence of antigen; no increase in pfc was seen if SRBCs were omitted from the cultures (data not shown). Thus, molecules produced by transfected class II genes appear to function as antigen-dependent signal transducers in transfected subclones of CH12.LX.

Parental CH12.LX cells, like the *in vivo* CH12 lymphoma from which they were derived, can be induced to differentiate in the presence of antigen and E^k-specific Th cells (1, 24). To determine if the differentiation of ABB1 cells can be mediated by T cells, ABB1 cells were cocultured with the alloreactive Th cell hybridoma AODH3.4 (anti-A^b) or 2h405.2 (anti-A^k). Data presented in Fig. 1*B* show that A^b-specific Th cells induced antigen-dependent differentiation of ABB1 cells. A significant increase in pfc frequency was seen in the presence of Th and SRBCs; no increase was seen if SRBCs were omitted. A^k-specific Th cells (2h405.2) were ineffective in inducing IgM secretion, either in the presence or the absence of SRBCs.

The Endogenous A_α^k Molecule of CH12.LX Can Deliver a Differentiative Signal. Since single chains of class II molecules are not expressed on cell-surface membranes as isolated chains (25), the A_β^b chains produced in ABB1 cells must pair with endogenous A_α^k chains to produce a cell-surface molecule that can be recognized by A_β^b-specific mAbs. Pairing of A_β^b with E_α^k is possible, but previous studies (8, 26, 27) show that this is a rare event. In addition, immunoprecipitation and two-dimensional gel electrophoresis of the A_β^b molecule of ABB1 cells shows that A_β^b coprecipitates with A_α^k in these cells (G.A.B., M. S. McMillan, and J.A.F., unpublished). Neither A_α^k nor A_β^k-specific mAbs can induce differentiation of untransfected CH12.LX cells, (1, 5). The successful transmission of differentiative signals through the $A_\alpha^k A_\beta^b$ molecules of ABB1 cells suggests that the endogenous A_α^k molecules of CH12.LX may be capable of delivering a differentiative signal, if the endogenous A_β^k chain is replaced by A_β^b. To determine whether this is correct, we cultured ABB1 cells in the presence of SRBCs and mAbs specific for either A_α^k or A_β^k. The results of this experiment, shown in Fig. 2*A*, demonstrate that two anti-A_α^k mAbs (11-5.2.1.9 and 39F) were able to stimulate differentiation in ABB1 cells, although both are ineffective in doing so in CH12.LX cells (5). As before, two anti-A_β^k mAbs (10-3.6.2 and 10-2.16) do not stimulate differentiation in either CH12.LX (1) or ABB1 cells (Fig. 2*A*). Thus, the endogenous A_α^k chains of ABB1 cells are capable of delivering differentiative signals when paired with A_β^b but not when paired with endogenous A_β^k chains.

It was possible that A_β^k might transduce a differentiative signal if it was paired with a different A_α chain. To test this hypothesis, the cDNA encoding A_α^b was introduced into CH12.LX cells. In AAB1 cells, the A_α^b molecule must pair with the endogenous A_β^k molecule to be expressed on the cell surface. Data presented in Fig. 2*B* show that culture of AAB1 cells in the presence of SRBCs and either of two mAbs (3B9 and 4D5) specific for A_α^b did not result in antigen-specific B-cell differentiation. These cells were capable of responding to class II-mediated signals, since culture with SRBCs and a mAb specific for E^k (14-4-4S) did induce IgM secretion.

Fig. 2*C* demonstrates that anti-A_α^b mAbs 3B9 and 4D5 were able to deliver class II-mediated signals to the $A_\alpha^b + A_\beta^b$ transfectant clone AB1. In parallel with results obtained with parental CH12.LX cells and the ABB1 transfectant (ref. 1; Fig. 2*A*), AB1 cells were not stimulated to differentiate in the presence of SRBCs and an anti-A_β^k mAb (10-3.6.2). Fig. 2*C* shows that the inability of 3B9 and 4D5 to stimulate AAB1 cells through their $A_\alpha^b A_\beta^k$ molecules is not due to either a defect in the A_α^b molecule encoded by pKAB or an intrinsic deficiency of these mAbs to stimulate B cells, since AB1 cells are stimulated by anti-A_α^b mAbs. Thus, it appears that AAB1 cells cannot respond to anti-A_α^b mAbs because the A_β^k chain with which A_α^b is paired is an ineffective partner for signal transduction.

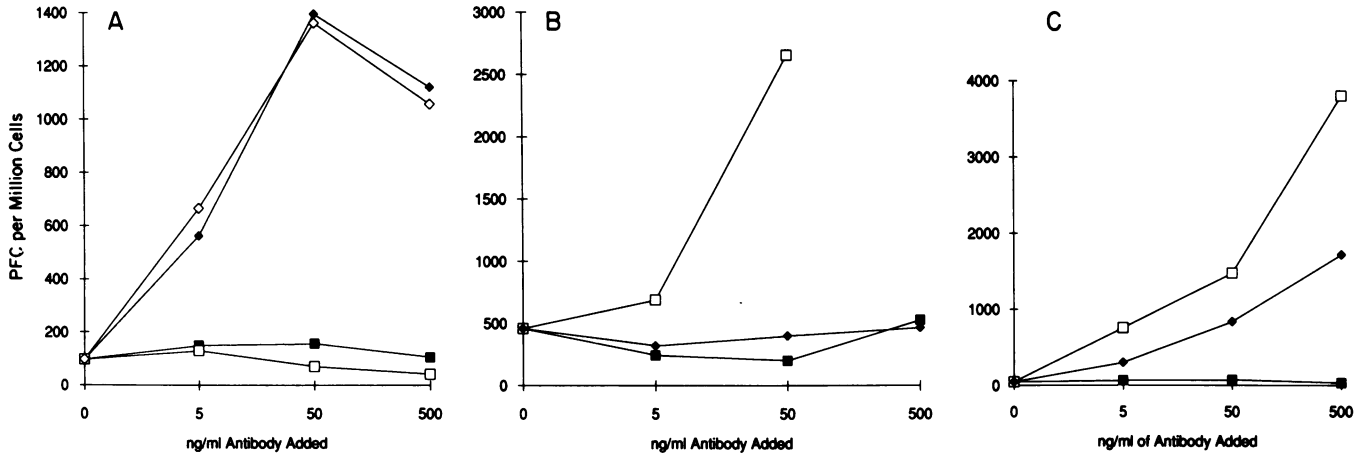

FIG. 2. (*A*) Induced differentiation of ABB1 cells by anti-A_α^k, but not anti-A_β^k, mAbs. ABB1 cells were cultured with SRBCs and varying concentrations of anti-A_α^k mAb 11-5.2.1.9 (◇) or 39F (◆) or anti-A_β^k mAb 10-3.6.2 (□) or 10-2.16 (■). (*B*) Failure to induce differentiation in an A_α^b transfectant (AAB1) using anti-A_α^b mAbs. AAB1 cells were cultured for 72 hr in the presence of SRBCs and varying concentrations of anti-E_α^k (14-4-4S, □) or anti-A_α^b (3B9, ◆; 4D5, ■) mAbs. (*C*) Induced differentiation by anti-A_α^b mAbs of a transfectant expressing both A_α^b and A_β^b (AB1). AB1 cells were cultured with SRBCs and varying concentrations of mAbs specific for A_α^b (3B9, □; 4D5, ◆) or A_β^k (10-3.6.2, ■).

Inability of A_β^d and A_β^u Molecules to Transduce a Differentiative Signal to CH12.LX. To determine whether A molecules of other haplotypes could transmit signals to CH12.LX cells, the cells were transfected with the genes encoding A_β^d or A_β^u. The results, summarized in Table 2, show that 10 independent cell lines expressing A_β^d and 5 expressing A_β^u were unresponsive to signals delivered through their A molecules, although all responded to E-mediated signals. This is in contrast to cell lines expressing A_β^b, as presented in Figs. 1 and 2 and summarized in Table 2.

The protein sequences of A_β^k, A_β^b, A_β^d, and A_β^u (refs. 9, 28; numbered as in ref. 29) were compared to determine if any sequence differences between these molecules exist that correlate with their ability to deliver differentiative signals to CH12.LX cells. Residues that were identical in haplotypes k, d, and u but differed in haplotype b were candidates. This analysis, presented in Fig. 3, identified four positions (amino acids 12, 47, 89, and 197) at which A_β^b differs in sequence from the three haplotypes (k, d, and u) that could not signal CH12.LX. In two of these positions (amino acids 47 and 197), the k, d, and u haplotypes share the same residues (tyrosine at position 47, arginine at position 197), which differ from the b haplotype (histidine at position 47, tryptophan at position 197). At position 12, haplotypes k, d, u, and b have residues glutamine, lysine, glutamine, and methionine, respectively; at position 89 the residues are proline, serine, proline, and histidine. Thus, these protein sequence differences may contribute to the ability of a given A molecule to directly signal CH12.LX B cells. This hypothesis may be tested by constructing the appropriate site-directed mutants.

DISCUSSION

Although B-cell class II molecules have been primarily studied as recognition structures for antigen presentation to T cells (30, 31), evidence is accumulating that Ia antigens can also transmit differentiative signals to B cells (1–4). Earlier investigations in murine (2) and human (32) systems suggested that signals delivered via class II molecules can regulate B-cell activation. More recently, it has been shown that cells of the murine B-cell clone CH12.LX can be induced to differentiate to IgM secretion by signals delivered through their surface IgM and Ia molecules (1). Previous studies demonstrated that CH12.LX cells differentiate in response to antigen plus the binding of Th cells or mAbs specific for their E^k, but not A^k, molecules (1). If the structure of the A^k molecule is responsible for the failure of CH12.LX A^k to deliver differentiative signals, the introduction of A molecules with different sequences into CH12.LX should permit the transmission of A-specific signals. If, however, CH12.LX cells are only capable of responding to E-specific differentiative signals, introduced A molecules will be ineffective in signal transduction. To discriminate between these two possibilities, CH12.LX cells were transfected with genes encoding A molecules of other haplotypes. Following transfection, cells were examined for the ability to receive antigen-specific differentiative signals via the introduced A molecules.

Table 2. Response of transfected cell lines to mAbs specific for A_β

Cell type	*n*	Control	mAb1	mAb2
CH12.LX	4	11.9 ± 7.1	11.0 ± 1.4	9.3 ± 38
CH12.ABB	2	6.2 ± 5.4	110.5 ± 5.2	103.3 ± 12.8
CH12.ABD	10	9.5 ± 5.7	13.0 ± 5.7	11.9 ± 6.0
CH12.ABU	5	13.2 ± 11.3	12.7 ± 11.4	11.6 ± 9.5

Values represent the percentage of the response (pfc per million cells, mean ± SD) obtained with SRBCs and anti-E^k mAb (500 ng/ml). n, Number of experiments (CH12.LX) or number of cell lines tested (transfectants). Control, cells incubated with SRBCs alone (no mAb). mAb1, 10-2.16 (CH12.LX), 28-16-8S (ABB and ABD), or BP107.2.2 (ABU). mAb2, 10-3.6.2 (CH12.LX), 34-5-3S (ABB and ABD), or MKS4 (ABU).

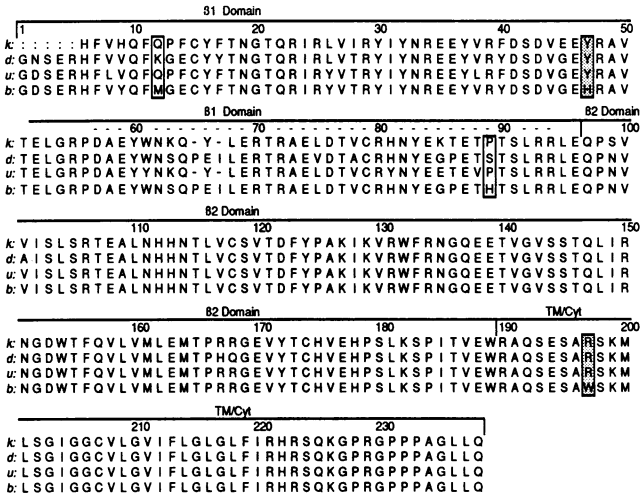

FIG. 3. Comparison of the amino acid sequences of A_β^k, A_β^d, A_β^u, and A_β^b. Open boxes identify positions at which the sequence of A_β^b differs from that of the other three haplotypes. Shaded boxes mark positions at which A_β^k, A_β^d, and A_β^u all share the same sequence, which differs from that of A_β^b.

The results presented here demonstrate that it is the A_β^k chain of CH12.LX A^k molecules that is responsible for their failure to transmit differentiative signals. If this chain is replaced with the A_β^b chain, the resultant $A_\alpha^k A_\beta^b$ hybrid molecule delivers differentiative signals to CH12.LX cells. In contrast, A_β^d and A_β^u are ineffective in complementing A_β^k in this function. These data indicate that CH12.LX cells are not inherently incapable of responding to antigen-specific differentiative signals delivered through A molecules.

We note that the A^k molecule has been reported to send a signal for the translocation of protein kinase C from the cytoplasm to the nucleus (3). This study is not directly comparable to ours, since a heterogeneous population of splenic B cells was used, whereas we are studying a single B-cell clone. In addition, CH12.LX cells are Ly-1^+, and it is possible that Ly-1^+ B cells differ somewhat in their activation requirements from Ly-1^- B cells. It should be noted that protein kinase C translocation measures a very early biochemical event, which has not yet been shown to be predictive for B-cell differentiation. In contrast, we are measuring immunoglobulin secretion, a terminal differentiation event with direct biological relevance. Findings from the two types of studies could be reconciled if the class II molecule could transmit two types of signal. One signal could result in protein kinase C translocation, but a second signal, dependent upon the protein sequences described here, might also be required for immunoglobulin secretion.

We transfected CH12.LX cells with the genes encoding either A_β^d or A_β^u molecules, which share the A_β^k transmembrane/cytoplasmic protein sequence. We found that neither A_β^d nor A_β^u was able to transduce detectable differentiative signals to CH12.LX (Table 2). Analysis of the entire protein sequences of these four molecules identifies four amino acids that differ between A_β^b and the other haplotypes. Thus, the sequence at any or all of these residues could affect the efficiency of these A molecules as signal transducers.

At position 47, the tyrosine residue of the k, d, and u haplotypes is replaced by histidine in the b haplotype. This exchanges an easily phosphorylated amino acid with an aromatic side chain (tyrosine) for a residue (histidine) with neither of these structural features. At position 197, k, d, and u have an arginine, the most hydrophilic of the amino acids.

In contrast, the b haplotype has the highly hydrophobic amino acid tryptophan. Site-directed mutagenesis may be used to directly examine the effect of various amino acid substitutions at these positions.

Other features of protein sequence may affect the ability of a class II molecule to serve as a B-cell signal receptor. It is possible that the combination of a certain α and β chain affects the efficiency of the complete class II molecule to serve as a signal transducer. Such an effect could operate separately from combinatorial effects on levels of class II surface expression (22). Thus, although A_β^d and A_β^u chains cannot signal well when paired with A_α^k, it is possible that these β chains could serve this function if paired with an α chain other than A_α^k.

The immunofluorescent staining of $A_\alpha^k A_\beta^d$ and $A_\alpha^k A_\beta^u$ molecules on the ABD and ABU cell lines was, on the average, lower than staining of $A_\alpha^k A_\beta^b$, $A_\alpha^b A_\beta^k$, or $A_\alpha^b A_\beta^b$ on the ABB, AAB, and AB cell lines, as described in *Materials and Methods*. Although this indicates fewer hybrid class II molecules on the surface of ABD and ABU cells, we do not believe that this is sufficient to explain the failure of A_β^d and A_β^u molecules to transmit differentiative signals. First, as stated in *Materials and Methods*, ABD and ABU cell lines are fully capable of stimulating a mixed lymphocyte reaction by H-2^a lymph node cells (data not shown). Further, the endogenous A_β^k molecules of CH12.LX and the transfected cell lines do not transmit differentiative signals, although they are present in abundant amounts on the cell surface. All AB and ABB cell lines were induced to differentiate by A_β^b-specific mAbs, independent of variation in the level of A_β^b expression of the individual cell lines. The range of mAb staining of A_β^d, which did not transmit a detectable differentiative signal, overlapped the range of A_β^b staining on ABB cell lines. Thus, we feel that the level of expression of hybrid molecules on ABD and ABU cell lines is not sufficient to explain their inability to transmit class II-specific differentiative signals to CH12.LX cells.

The role of class II as a B-cell signal transducer has been recently confirmed in normal murine splenic B cells, where it was found that anti-Ia antibodies induce elevated cAMP levels and the translocation of protein kinase C from the cell nucleus to the cytoplasm (3). The role of B-cell Ia molecules as signal transmitters to the B cell must be investigated in detail, using CH12.LX cells as a clonal model. The successful transfection of CH12.LX cells with genes encoding class II molecules of various haplotypes allows the use of this B-cell clone as a model system in which to study the role of Ia as a B-cell signal transducer. Introduced class II genes can be genetically manipulated to produce alterations in the structure of the class II molecule. This approach should permit a detailed analysis of the structural features of Ia that are important in determining its role as a transmitter of differentiative signals to the B cell.

We are grateful to Drs. R. Germain, P. Jones, D. McKean, and D. Sachs for providing some of the B-cell hybridomas used in this study and to Dr. John Frelinger for critical review of the manuscript. This study was supported by NIH Grant CA 420771. G.A.B. is an Investigator of the National Arthritis Foundation.

1. Bishop, G. A. & Haughton, G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7410–7414.
2. Niederhuber, J. E., Frelinger, J. A., Dugan, E., Coutinho, A. & Shreffler, D. C. (1975) *J. Immunol.* **115,** 1672–1675.
3. Cambier, J. C., Newell, M. K., Justement, L. B., McGuire, J. C., Leach, K. L. & Chen, Z. Z. (1987) *Nature (London)* **327,** 629–632.
4. Snow, E. C. & Noelle, R. J. (1987) *Immunol. Rev.* **99,** 173–192.
5. Bishop, G. A., McMillan, M. S., Haughton, G. & Frelinger, J. A. (1988) *Immunogenetics* **28,** 184–192.
6. Widera, G. & Flavell, R. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5500–5504.
7. Landais, D., Matthes, H., Benoist, C. & Mathis, D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2930–2934.
8. Malissen, B. N., Shastri, N. & Hood, L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3958–3962.
9. Estess, P., Begovich, A. B., Koo, M., Jones, P. P. & McDevitt, H. O. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3594–3598.
10. Southern, P. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1,** 327–341.
11. Oi, V. T., Jones, P. P., Goding, J. W., Herzenberg, L. A. & Herzenberg, L. A. (1978) *Curr. Top. Microbiol. Immunol.* **81,** 115–129.
12. Landais, D., Beck, B. N., Buerstedde, J.-M., Degraw, S., Klein, D., Koch, N., Murphy, D., Pierres, M., Tada, T., Yamamoto, K., Benoist, C. & Mathis, D. (1986) *J. Immunol.* **137,** 3002–3005.
13. Pierres, M., Devaux, C., Dosseto, M. & Marchetto, S. (1981) *Immunogenetics* **14,** 481–495.
14. Ozato, K., Mayer, N. & Sachs, D. (1982) *Transplantation* **34,** 113–120.
15. Ozato, K. & Sachs, D. (1981) *J. Immunol.* **126,** 317–321.
16. Beck, B. N., Buerstedde, J. M., Krco, C. J., Nilson, A. E., Chase, C. G. & McKean, D. J. (1986) *J. Immunol.* **136,** 2953–2961.
17. Ozato, K., Mayer, N. & Sachs, D. H. (1980) *J. Immunol.* **124,** 533–540.
18. Symington, F. W. & Sprent, J. (1981) *Immunogenetics* **14,** 53–61.
19. Kappler, J. W., Skidmore, B., White, J. & Marrack, P. (1981) *J. Exp. Med.* **153,** 1198–1214.
20. Bishop, G. A. & Haughton, G. (1987) *J. Immunol.* **138,** 3308–3313.
21. Frelinger, J. A., Niederhuber, J. E., David, C. S. & Shreffler, D. C. (1974) *J. Exp. Med.* **140,** 1273–1284.
22. Braunstein, N. S. & Germain, R. N. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2921–2925.
23. Cunningham, A. J. & Szenberg, A. (1968) *Immunology* **14,** 599–600.
24. LoCascio, N. J., Arnold, L. W., Corley, R. B. & Haughton, G. (1984) *J. Mol. Cell. Immunol.* **1,** 177–187.
25. Jones, P. P., Murphy, D. B. & McDevitt, H. O. (1981) *Immunogenetics* **12,** 321–337.
26. Germain, R. N. & Quill, H. (1986) *Nature (London)* **320,** 72–75.
27. Lotteau, V., Teyton, L., Burroughs, D. & Charron, D. (1987) *Nature (London)* **329,** 339–341.
28. Choi, E., McIntyre, K., Germain, R. & Seidman, J. G. (1983) *Science* **221,** 283–286.
29. Klein, J. (1986) *Natural History of the Major Histocompatibility Complex* (Wiley, New York), p. 362.
30. Schwartz, R. H. (1985) *Annu. Rev. Immunol.* **3,** 237–261.
31. Germain, R. N. & Malissen, B. (1986) *Annu. Rev. Immunol.* **4,** 281–315.
32. Palacios, R., Maza-Martinez, O. & Guy, K. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3456–3460.