Translational Diffusion of Class II Major Histocompatibility Complex Molecules Is Constrained by Their Cytoplasmic Domains

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Abstract. Site-directed mutagenesis in vitro was used to introduce stop codons in the genomic DNA of the α and β chains of the murine class II major histocompatibility complex antigen, I-A^k. Mutated DNA was transfected into B lymphoma cells that were then selected by neomycin resistance and for their ability to express I-A^k molecules on their plasma membrane. The translational diffusion coefficient (D_{lat}) of I-A^k molecules composed of a wild-type β chain paired with an α chain missing either 6 or 12 amino acids from the cytoplasmic domain is on the average threefold higher than the D_{lat} of wild-type I-A^k molecules as measured by fluorescence photobleaching and recovery. The removal of 12 amino acids from the cytoplasmic domain of the β chain did not change the D_{lat} value from that of wild-type I-A^k if the truncated β chain was paired with a wild-type α chain. Removing all amino acids of the cytoplasmic domains of both the α and β chains resulted in a 10-fold increase in the D_{lat}, the highest value for any of the truncated I-A^k molecules tested. These data indicate that the carboxy-terminal six amino acids of the cytoplasmic domain of the α chain and the six plasma membrane–proximal amino acids of the β chain are important in constraining the translational diffusion of I-A^k molecules in the plasma membrane.

THE cytoplasmic domains of a number of transmembrane membrane proteins are thought to be important in the membrane biology of those proteins (3, 13, 16–18, 31). Plasma membrane proteins with truncated cytoplasmic domains may not be correctly sorted or internalized and may lose signaling functions (22–24, 26). Surprisingly, the translational diffusion coefficient (D_{lat})[1] of proteins with such truncations is not found to differ from that of full-length molecules. D_{lat} of the EGF receptor (16), class I major histocompatibility complex (MHC) antigen (10), or G protein of vesicular stomatitis virus (25) is not changed when the cytoplasmic domains of these molecules are truncated by directed mutation of their genes. At the extreme, this involves a reduction from 542 to 9 residues in the EGF receptor (16). These studies imply that the amino acids removed are not associated with cytoplasmic proteins in ways that constrain their translational diffusion. There is an apparent contradiction between the results on the cell physiology of truncated molecules and the measurements of their translational diffusion. Results of endocytosis experiments indicate that the molecules are coupled to the cell cytoskeleton and that this coupling is lost with truncation since internalization is affected by truncation. On the other hand, the results of translational diffusion experiments suggest that even wild-type,

full-length molecules are not directly linked to the cytoskeleton since truncation of their cytoplasmic domains has no effect on D_{lat}. This contradiction may arise largely because translational diffusion and endocytosis of truncated molecules have been studied in different cell types. D_{lat} of the truncated class I MHC mutants has been measured in L cells, in which class I molecules are not rapidly internalized. The effect of truncation on endocytosis of the same mutants is readily shown when they are expressed in T lymphoma cells, a cell type in which there is continuous cycling of class I MHC antigens between the surface and cytoplasm (4). Similarly, D_{lat} of truncated EGF receptor was measured in COS cells, while effects of truncation on endocytosis of EGF receptor were shown in 3T3 cells (2). Here we present the first results in which D_{lat} of truncated transmembrane molecules is measured in cells in which the wild-type molecules are functional. Truncation of the cytoplasmic domain affects D_{lat} in these cells; in the extreme truncation mutant, the D_{lat} is an order of magnitude larger than that of the wild-type molecule.

Class II MHC molecules, Ia antigens in the mouse, are heterodimeric transmembrane proteins expressed on B lymphocytes, macrophages, and other antigen-presenting cells. There are two isotypes of Ia antigens in the mouse, I-A and I-E; each consists of a 34–36-kD α chain and a 27–29-kD β chain. Each chain is composed of two external domains (α_1/α_2 or β_1/β_2) and a connecting peptide that joins the ex-

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1. *Abbreviations used in this paper:* D_{lat}, translational diffusion coefficient; FPR, fluorescence photobleaching and recovery; MCF, mean channel fluorescence; MHC, major histocompatibility complex; PKC, protein kinase C.

ternal domains to the transmembrane and cytoplasmic domains. There are two asparagine-linked oligosaccharide units on each α chain and one on the β chain.

One function of Ia molecules is to present foreign peptide antigens to T lymphocytes. Antigen presentation in B lymphocytes involves facilitated internalization of native antigen by antigen-specific surface Ig (signaling aspects reviewed in reference 6) followed by processing of the foreign antigen by denaturation and/or fragmentation. Ia molecules also function as receptors that influence the differentiative or proliferative events in B lymphocytes (6, 7). Potentially, Ia molecules are coupled to the molecules of the signal-transducing cascade through the highly conserved amino acids of the transmembrane domain or cytoplasmic domain (12). Ia molecules and surface Ig (which also provides signals to B cells) are similar in their ability to associate with insoluble cytoskeletal elements after receptor cross-linking and to remain associated with the cytoskeleton during solubilization of the cells (30). These cytoskeletal associations may be important for the signaling function which Ia and Ig molecules provide.

B cell lymphomas transfected with class II genes have also been used to study one aspect of B lymphocyte signaling, the translocation of protein kinase C (PKC) from the cytoplasm to the nucleus, that is induced by perturbation of membrane Ia molecules by Ia-specific mAbs. This binding of surface Ia by mAbs presumably mimics the interaction of T cell receptors with peptide antigen-bearing Ia molecules. I-A^k molecules consisting of truncated β chains paired with either full-length α chains (αwt/βCT12) or with truncated α chains that had no cytoplasmic domain amino acids (αCT12/βCT12) had altered PKC signaling phenotypes compared with wild-type I-A^k molecules. Further, I-A^k molecules that lacked cytoplasmic domains of both α and β chains (αCT12/βCT18) were unable to signal at all as measured by the translocation of PKC from the cytoplasm to the nucleus (28).

These results collectively suggest that the cytoplasmic domain of I-A^k is functionally coupled to a signal transduction pathway and to the cytoskeleton in B lymphocytes. Given this coupling, it might be expected that deletions of all or part of the cytoplasmic domain would affect the D_{lat} of the mutant Ia molecules. Using fluorescence photobleaching and recovery (FPR) measurements, we have determined the D_{lat} of wild-type I-A^k molecules and of mutant I-A^k molecules lacking all or part of the cytoplasmic domain of one or both chains. Here we show that the D_{lat} of I-A^k molecule is a function of the length of its cytoplasmic domains.

Materials and Methods

DNA Constructs

The pSV2-Neo construct (27) was a gift from Dr. Ed Palmer (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO). The origin and generation of the α and β I-A^k genomic DNA constructs have been described (28). In brief, site-directed mutagenesis in vitro was used to introduce double-stop codons that would result in truncated α or β chains lacking 6 and 12 or 12 and 18 amino acids, respectively. The combinations of DNA constructs that were transfected and the resultant prototypic clones are (a) wild-type α with wild-type β (M12.C3-F6); (b) truncation of the carboxy-terminal six amino acids of the α chain (αCT6) with wild-type β chain (M12.C3-10-A4); (c) αCT12 with wild-type β chain (M12.C3-10-B3); (d) wild-type α chain and truncation of the carboxy-terminal 12 amino acids of the β chain (βCT12) (M12.C3-5-A2); (e) αCT12/βCT12 (M12.C3-5-B2); (f) αCT12/βCT18 (M12.C3-10-D2). Fig. 1 shows a schematic repre-

I-A^k Phenotype		Cytoplasmic Sequences	
αwt/βwt	Plasma Membrane	RSGGTSRHPGPL	– α
		RHRSQKGPRGPPPAGLLQ	– β
αCT6/βwt	Plasma Membrane	RSGGTS	– α
		RHRSQKGPRGPPPAGLLQ	– β
αCT12/βwt	Plasma Membrane		– α
		RHRSQKGPRGPPPAGLLQ	– β
αwt/βCT12	Plasma Membrane	RSGGTSRHPGPL	– α
		RHRSQK	– β
αCT12/βCT12	Plasma Membrane		– α
		RHRSQK	– β
αCT12/βCT18	Plasma Membrane		– α
			– β

Figure 1. Primary amino acid sequence in one letter code of the cytoplasmic domains of the α and β chains of I-A^k. Cytoplasmic domain representations with no amino acids shown (e.g., αCT12/βwt and αCT12/βCT18) are considered to have no cytoplasmic domain sequences left after in vitro mutagenesis. The sequence RHRSQK is the plasma membrane-proximal amino acid sequence of the β chain discussed in the text.

sentation of the mutant I-A^k molecules that demonstrates the various amino acid deletions.

Cells

Transfectants were generated by introducing pSV2-Neo DNA and α and β genomic DNA into the Ia-negative B cell lymphoma M12.C3 by electroporation. The transfected cells were selected and maintained using G418 (neomycin) at a final concentration of 300 μg/ml. Transfectants expressing membrane I-A^k were chosen based on flow cytometry using anti-I-A^k-specific mAb (Fig. 2).

mAbs, Fab Production, and Fluoresceination

mAbs were purified from tissue culture supernatants by affinity chromatography on protein A-Sepharose. The anti-I-A^k mAb, 39J, is specific for the α chain (21). The anti-I-A^k mAb, 10-3.6, is specific for the β chain (19). The anti-L^d mAb, 28-14-8, was used as a control to assess for nonspecific effect on plasma membrane protein D_{lat} values (20). 2-8 mg of mAb was digested with 1:100 (wt/wt) papain (Sigma Chemical Co., St. Louis, MO) at pH 5.2 for 10 min. The digest was fractionated on Sephadex G-100, and the first included peak was refractionated on protein A-sepharose to remove traces of IgG. The purified Fab was concentrated to 5-10 mg/ml protein, dialyzed into 0.2 M Na$_2$CO$_3$ buffer, pH 9.5, and reacted with 15 μg FITC/mg protein at 0°C for 1 h. The reaction mixture was purified by passage over Sephadex G-25. All conjugates had 3-4 mol fluorescein coupled/mol Fab.

Measurement of Translational Diffusion by FPR

In an FPR experiment, diffusion in the plane of the membrane, translational diffusion, is measured in terms of recovery of fluorescence after partly bleaching a small area on an otherwise uniformly labeled cell surface (8,
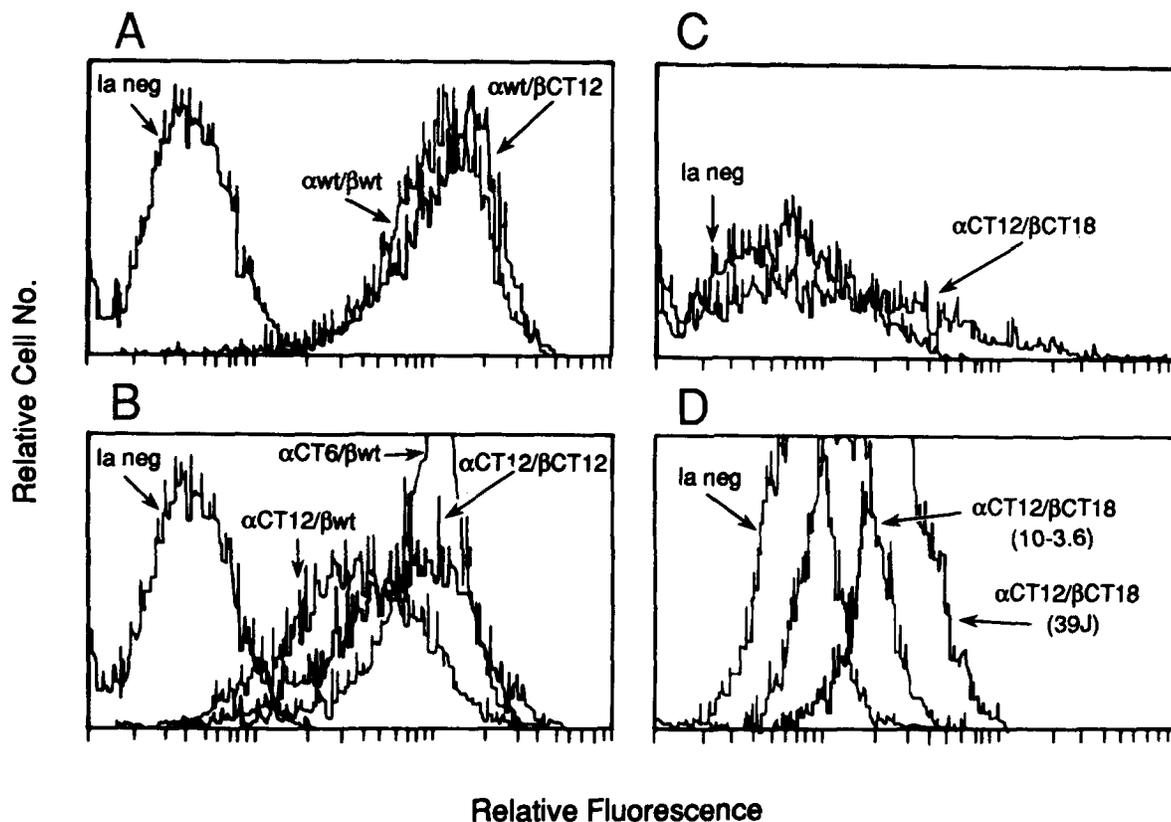
A

Ia neg

αwt/βCT12

αwt/βwt

C

Ia neg

αCT12/βCT18

B

Ia neg

αCT6/βwt →

αCT12/βCT12

αCT12/βwt

D

Ia neg

αCT12/βCT18
(10-3.6)

αCT12/βCT18
(39J)

Relative Fluorescence

Figure 2. Flow cytometric analysis of prototypic transfectants expressing either wild-type I-A^k molecules or mutant I-A^k molecules containing various combinations of truncated α or β chains. Cells were stained with 39J, an α chain-specific mAb, and then analyzed using a flow cytometer (Epics C; Coulter Electronics Inc.) with a three-decade log scale (0–256 channels); a difference in 25 channels corresponds to a twofold difference in fluorescence. (*A*) The fluorescence staining profile for transfectants M12.C3 (Ia negative; mean channel fluorescence [MCF], 51), M12.C3-F6 (αwt/βwt; MCF, 165), and M12.C3-5-A2 (αwt/βCT12; MCF, 168). (*B*) The staining of transfectants M12.C3 (Ia negative; MCF, 51), M12.C3-10-B3 (αCT12/βwt; MCF, 127), M12.C3-5-B2 (αCT12/βCT12; MCF, 151), and M12.C3-10-A4 (αCT6/βwt; MCF, 158). (*C*) The fluorescence profile of the transfectant M12.C3-10-D2 (αCT12/βCT18) stained with either D3.137 mAb, an isotype subclass–matched control that will not bind I-A^k, or with 39J mAb that is specific for the α chain of I-A^k. In general, the staining of M12.C3-10-D2 with 39J is 2.5–3-fold above the background staining with D3.137. (*D*) The fluorescence profiles of the transfectant M12.C3-10-D2-Hi. This population was generated after the parent M12.C3-10-D2 was sorted for the top 10% expression of I-A^k using an α chain–specific mAb. Shown are staining of M12.C3 (Ia negative) with 10-3.6 (MCF, 67) and the staining of M12.C3-10-D2.Hi with either 10-3.6 (MCF, 91) or 39J (MCF, 116). The staining of M12.C3 with 39J was identical to that with 10-3.6 (data not shown).

14). The recovery curve contains information about the D$_{lat}$ of the mAb-bound molecules and about the fraction, R, of the population of mAb-bound molecules that are free to diffuse, on a time scale of minutes, in the experiment. D$_{lat}$ of a given molecule is a function of the radius of the diffusing species. Hence, monovalent labels—i.e., Fab fragments (which do not cross-link bound molecules)—give more reliable estimates of D$_{lat}$ than do larger and bivalent IgG labels. The mobile fraction, R, is influenced by the intensity and specificity of binding of the Fab, as well as by the biology of the cells. Calculations and control experiments indicate that the measuring and bleaching beams do not cause detectable cross-linking of, or damage to, the low concentrations of molecules of interest in an FPR experiment (9).

In our experiments, cells were labeled with FITC-Fab for 15–30 min and washed four times in Hepes-buffered Hanks' balanced salt solution. Most measurements were made on cells in suspension and at 20°C. Our FPR machine has been described elsewhere (8). In one experiment, there was no difference between the D$_{lat}$ measured on cells in suspension and D$_{lat}$ measured on cells adherent to coverslips (data not shown). There was also no difference between D$_{lat}$ at 20°C and D$_{lat}$ at 30°C (data not shown).

In experiments with M12.C3-10-D2, the transfectant that expresses the lowest level of I-A^k, recovery of fluorescence was reduced if cells were incubated with goat anti–mouse IgG before measurement (data not shown). This demonstrates that the fluorescence measured is from mAb bound to surface I-A^k and is not cell autofluorescence.

Statistical Comparisons

The values of D$_{lat}$ typically are not a normal distribution. Accordingly, geometric mean D$_{lat}$ were calculated from normally distributed lnD. Significances of differences between mean lnD$_{lat}$ were compared in Student's *t* test. $p < 0.05$ was considered significant.

Results

Translational Diffusion of I-A^k Molecules in Transfectants Expressing α and β Chains of Different Truncation Phenotypes

Transfectants expressing various combinations of truncated α or β chains were generated, and the translational diffusion of the class II molecules was measured by FPR. Fig. 2 shows representative fluorescence profiles of the transfectants expressing either wild-type or mutant I-A^k molecules. The level of I-A^k expressed at the cell surface differs by no more than twofold between these transfectants, with the exception
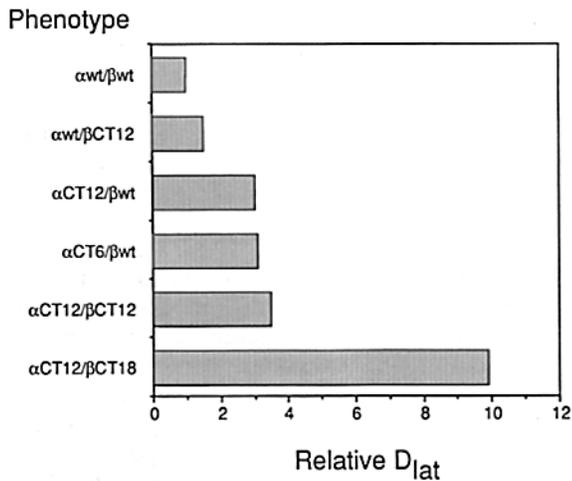
Phenotype

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αwt/βwt       ▇
αwt/βCT12     ▇▇
αCT12/βwt     ▇▇▇▇▇▇
αCT6/βwt      ▇▇▇▇▇▇
αCT12/βCT12   ▇▇▇▇▇▇▇
αCT12/βCT18   ▇▇▇▇▇▇▇▇▇▇▇▇▇▇▇▇▇▇▇▇
              0    2    4    6    8    10   12
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Relative D_{lat}

Figure 3. Fold increase in D_{lat} for truncated I-A^k antigens normalized to wild-type I-A^k antigens. The relative D_{lat} was obtained by dividing D_{lat} (obtained from Table I) of a particular transfectant by the value obtained for M12.C3-F6 (αwt/βwt) on the same day.

of the αCT12/βCT18 truncation phenotype transfectant. It can be seen from Table I that removal of the carboxy-terminal 12 (of a total of 18) amino acids of the β chain does not affect D_{lat}. Wild-type I-A^k and αwt/βCT12 I-A^k molecules have the same D_{lat}. This contrasts with the effect measured after the loss of as few as six amino acids from the α chain. D_{lat} of I-A^k molecules of the αCT6 or the αCT12 phenotypes

paired with a wild-type β chain is significantly higher than D_{lat} of wild-type I-A^k molecules. It seems that the α chain of the I-A^k molecule constrains its diffusion in the plasma membrane. This conclusion is supported by the results with the transfectant M12.C3-5-B2 with the I-A^k truncation phenotype αCT12/βCT12. D_{lat} of αCT12/βCT12 I-A^k molecules does not differ from that of transfectants of the αCT6/βwt and αCT12/βwt phenotypes.

It appears from these results that 12 of the 18 amino acids of the cytoplasmic domain of the β chain have little influence on the D_{lat} of I-A^k molecules and that the α chain is primarily responsible for constraining the translational diffusion of I-A^k. To assess the contribution of the six membrane-promixal amino acids of the cytoplasmic domain of the β chain, we generated transfectants expressing I-A^k antigen (αCT12/βCT18) lacking all cytoplasmic domain amino acids in both the α and β chains. The further truncation of the β chain resulted in a 10-fold increase in D_{lat} of the αCT12/βCT18 I-A^k molecules compared with that of wild-type I-A^k molecules. Apparently the six plasma membrane–proximal amino acids of the β chain interact with other molecules to constrain the translational diffusion of I-A^k.

For ease of comparison, the normalized fold increase of D_{lat} for individual I-A^k phenotypes is shown in Fig. 3. The order of increasing D_{lat} is αwt/βwt = αwt/βCT12 < αCT6/βwt = αCT12/βwt = αCT12/βCT12 < CT12/CT18. The fold increase from wild type describing this order is 1 ≈ 1.5 < 3.1 ≈ 3.0 ≈ 3.5 < 9.9. Statistical significance for various comparisons of the D_{lat} is summarized in the legend for Table I. All comparisons between D_{lat} of I-A^k on individual

Table I. Translational Diffusion of Wild-type and Mutant I-A^k Antigens

Experiment	Cell line	I-A^k phenotype	D_{lat}* $(10^{-10}$ cm^2/s)	Recovery	n
1	M12.C3-F6[a]	αwt/βwt	1.3 (-22.75 ± 0.78)	73 $\pm$ 14	30
	M12.C3-10-B3[b]	αCT12/βwt	3.9 (-21.67 ± 0.74)	70 $\pm$ 19	22
	M12.C3-5-B2[b]	αCT12/βCT12	4.3 (-21.57 ± 0.81)	68 $\pm$ 17	24
2	M12.C3-F6[a]	αwt/βwt	3.0 (-21.93 ± 0.67)	64 $\pm$ 12	13
	M12.C3-5-A2[a,b]	αwt/βCT12	4.5 (-21.51 ± 0.73)	63 $\pm$ 19	11
	M12.C3-10-A4[c]	αCT6/βwt	9.3 (-20.80 ± 0.64)	59 $\pm$ 13	19
	M12.C3-5-B2[b,c]	αCT12/βCT12	7.1 (-21.07 ± 0.63)	64 $\pm$ 18	13
3	M12.C3-F6[a]	αwt/βwt	1.8 (-22.44 ± 0.58)	36 $\pm$ 13	16
	M12.C3-5-A2[a]	wt/βCT12	2.3 (-22.20 ± 1.02)	37 $\pm$ 15	14
	M12.C3-F6[a]	αwt/βwt	1.0 (-23.04 ± 0.56)	37 $\pm$ 14	14
	M12.C3-10-D2[b]	αCT12/βCT18	9.9 (-20.85 ± 0.60)	54 $\pm$ 11	16
4	M12.C3-F6[a]	αwt/βwt	1.1 (-22.93 ± 0.40)	50 $\pm$ 11	9
	M12.C3-10-D2.Hi[b]	αCT12/βCT18	8.0 (-20.93 ± 0.53)	60 $\pm$ 10	26
	M12.C3-F6[a]	αwt/βwt	1.2 (-23.29 ± 0.78)	36 $\pm$ 8	7
	M12.C3-10-D2.Hi[b]	αCT12/βCT18	8.0 (-20.90 ± 0.42)	63 $\pm$ 12	14

Experiment 4 was performed using the sorted population of M12.C3-10-D2 which was enriched for the top 10% expression of I-A^k of the αCT12/βCT18 truncation. The first two measurements in this group used the Fab of the β chain–specific mAb, 10-3.6, while the last two used the Fab of the α chain–specific mAb, 39J. Mean values of D_{lat} were compared using a Student's t test to determine significance. Comparisons were made only within a single experiment. Experiments 1 and 2 were performed on different days with the same aliquot of 39J, while experiment 3 was performed on two different days with the same aliquot of 39J but that differed from that used for experiments 1 and 2. These aliquots of 39J were matched for protein concentration and for the fluorescein-to-protein conjugation ratio. Experiment 4 was done separately from the other experiments with the same 39J that was used in experiment 3. The D_{lat} of cell lines marked with the same superscript letter (e.g. *a*) are not different from each other ($p > 0.05$). Statistical comparisons for each experiment are given. Experiment 1: αwt/βwt vs. αCT12/βwt, $0.005 > p > 0.0025$; αwt/βwt vs. αCT12/βCT12, $p < 0.005$; αCT12/βCT12 vs. αCT12/βwt, $0.25 > p > 0.1$. Experiment 2: αwt/βwt vs. αwt/βCT12, $0.25 > p > 0.10$; αwt/βwt vs. αCT6/βwt; $p < 0.005$; αCT6/βwt vs. αCT12/βCT12, $0.25 > p > 0.10$; αCT6/βwt vs. αwt/βCT12, $0.025 > p > 0.01$. Experiment 3: αwt/βwt vs. αCT12/βCT18, $p < 0.005$. Experiment 4: I-A^k α chain mAb, αwt/βwt, vs. αCT12/βCT18, $p < 0.001$; I-A^k β chain mAb, αwt/βwt, vs. αCT12/βCT18, $p < 0.001$; αCT12/βCT18 vs. αCT12/βCT18, $p > 0.500$. Statistical comparisons for percent mobile fraction in experiments 3 and 4 are also given. Experiment 3: αwt/βwt vs. αCT12/βCT18, $p < 0.001$. Experiment 4: I-A^k α chain mAb, αwt/βwt, vs. αCT12/βCT18, $p < 0.001$; I-A^k β chain mAbs, αwt/βwt, vs. αCT12/βCT18, $0.025 > p > 0.01$.

* Calculated from lnD whose mean and standard deviation are shown in parentheses.
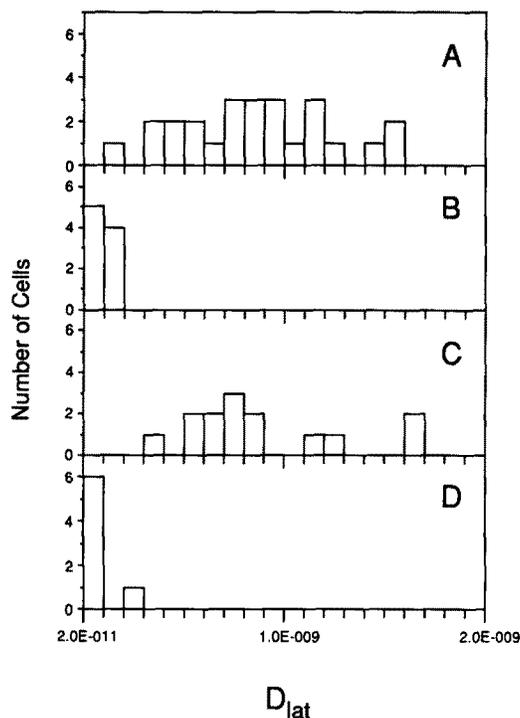
Figure 4. Distribution of lateral diffusion coefficients, D_{lat}, for I-A^k in populations of cells expressing high levels of truncated molecules of the αCT12/βCT18 phenotype. Cells were labeled with the FITC-Fab of the β chain–specific mAb 10-3.6 (*A* and *B*) or the FITC-Fab of the α chain–specific mAb 39J (*C* and *D*). The scale for the x-axis is 10^{-10} per division. (*A*) D_{lat} of M12.C3-10-D2.Hi (αCT12/βCT18) labeled with 10-3.6; (*B*) D_{lat} of M12.C3-F6 (αwt/βwt) labeled with 10-3.6; (*C*) D_{lat} of M12.C3-10-D2.Hi labeled with 39J; and (*D*) D_{lat} of M12.C3-F6 labeled with 39J. The D_{lat} measured for M12.C3-F6 stained with either 39J or 10-3.6 fall within the range of D_{lat} measured for M12.C3-F6 in Table I.

transfectants are only made within an experiment. D_{lat} were independent of the differences between experiments in the mobile fractions (see below).

Effect of Truncated I-A^k Molecules on the Mobile Fraction of I-A^k after Photobleaching

The mobile fraction of wild-type and mutant I-A^k antigens was constant within an experiment with one exception. The mobile fraction of the tailless I-A^k molecule (αCT12/βCT18) was significantly ($p < 0.05$) higher than that of the wild-type I-A^k in both experiments 3 and 4.

Translational Diffusion of αCT12/βCT18 Truncated I-A^k on a Transfectant Selected for High Expression of That Mutant I-A^k

To ensure that D_{lat} measured for the transfectant M12.C3-10-D2 (αCT12/βCT18) was in fact due to the truncation phenotype of the I-A^k molecule and not the result of the low concentration of I-A^k protein, we used cell sorting to enrich for M12.C3-10-D2 cells that expressed the highest level of the αCT12/βCT18 I-A^k molecule. A fluoresceinated α chain–specific mAb was used to label cells that were sorted on a cell sorter (Epics 751; Coulter Electronics Inc., Hialeah, FL), and the 10% of the cells expressing the highest levels of trun-

cated I-A^k were selected. The resulting sorted population is shown in Fig. 2 *D*. A normally distributed population of αCT12/βCT18 I-A^k expressing cells can be seen using either directly conjugated Fab of mAb 39J (α chain specific) or 10-3.6 (β chain specific) for staining. This sorted population, M12.C3-10-D2.Hi, was then used for FPR measurements to determine the D_{lat} of the mutant I-A^k.

Fig. 4 and Table I show the data of the D_{lat} for M12.C3-F6 and M12.C3-10-D2.Hi. The D_{lat} for the αCT12/βCT18 I-A^k molecules of the sorted population is still ∼10-fold higher than wild-type I-A^k molecules, whether an α or β chain–specific mAb is used. These results demonstrate that even for the αCT12/βCT18 truncation, using an unsorted population in which only a small number of cells clearly express the truncated I-A^k molecule above background levels produces the same D_{lat} as the use of a sorted population in which the expression of truncated I-A^k is nearly baseline resolved from the negative control.

Translational Diffusion of Class I Molecules of Wild-type and Transfectants Expressing Truncated I-A^k Molecules

The differences we measured in D_{lat} for I-A^k molecules in transfectants expressing truncated I-A^k molecules could be due to clonal variation in constraints to diffusion of plasma membrane molecules in the individual transfectants. To control for this, we measured translational diffusion of constitutively expressed class I antigens of the transfectants (Table II). D_{lat} for the L^d molecule are the same regardless of which transfectant is measured with the exception of the M12.C3-5-B2 (αCT12/βCT12) cell line. Ln D_{lat} for L^d in this clone is smaller than the others. This difference does not correlate with D_{lat} for truncated I-A^k molecules (compare Fig. 3 with Table II) and may be due to the high standard deviation of D_{lat} for the αCT12/βCT12 transfectant. Hence, the differences in the D_{lat} of I-A^k molecules in the various transfectants arise from truncation of the α or β chains and not from variations of the dynamics of the plasma membranes of individual transfectants.

Discussion

The translational diffusion of I-A^k antigens expressed in B lymphoblasts is affected by truncation of the cytoplasmic domains of these antigens. Loss of 6 of 12 cytoplasmic residues of the α chain results in an approximately threefold increase

Table II. Translational Diffusion of Ld Class I Antigen between Different Transfectants

Cell line	I-A^k phenotype	D_{lat}* (10^{-10} cm^2/s)	Percent recovery	n
M12.C3-F6	αwt/βwt	6.4 (−21.17 ± .56)‡	56 ± 10	17
M12.C3-10-B3	αCT12/βwt	6.5 (−21.16 ± .74)	52 ± 12	15
M12.C3-5-A2	αwt/βCT12	4.3 (−21.58 ± .67)	55 ± 11	11
M12.C3-5-B2	αCT12/βCT12	4.2 (−21.60 ± .89)	54 ± 16	15
M12.C3-10-D2	αCT12/βCT18	5.5 (−21.13 ± .76)	59 ± 14	15

* Calculated from lnD whose mean and standard deviation are shown in parenthesis.
‡ Student's *t* test was used to compare D_{lat} of L^d molecules expressed on αwt/βwt with D_{lat} for L^d expressed on cells with various I-A^k truncation phenotypes. None of the comparisons were significant at the $p = 0.05$ level with the exception of αCT12/βCT12.

in D_{lat} relative to wild-type I-A^k antigens. Deletion of the remaining six residues of the α chain cytoplasmic domain has no further effect on D_{lat}. This is true whether the mutant α chain is paired with a wild-type β chain or whether it is paired with a β chain lacking 12 of 18 of the amino acids of its cytoplasmic domain. Thus, the length of the β chain has seemingly little effect on D_{lat}. However, complete deletion of the β chain tail results in a further approximately threefold increase in D_{lat} when the mutant chain is paired with an α chain also completely deleted of its cytoplasmic domain. This D_{lat} is still smaller by about a factor of five than might be expected for a molecule of the size of I-A^k diffusing in a lipid bilayer. The bulky oligosaccharide units of I-A^k may impede their diffusion, as has been found for class I MHC antigens (29).

The mobile fraction of wild-type I-A^k was lower in experiments 3 and 4 than in experiments 1 and 2. Some of this variation must be due to biological rather than technical factors (such as microscope alignment or nonspecific binding of label) since in experiment 4 two different FITC-Fab labels, one for the α and one for the β chain, both report low mobile fractions of wild-type I-A^k and higher mobile fractions of tailless I-A^k (αCT12/βCT18). The mobile fraction of surface molecules on cells from dense cultures is often lower than the mobile fraction of the same molecules on cells from less dense cultures (3, 29) and it also is reduced with long times of labeling (3). The cultures used in these experiments appeared to be at about the same cell density and were labeled to the same extent. Hence, it is not clear if any of the cited factors are involved. It is evident that the mobile fractions of tailless I-A^k molecules are significantly higher than that of wild-type or αwt/βCT12 truncates (experiment 3). The complete deletion of the tails appears to uncouple the mutant molecules from the (unknown) structures that immobilize surface molecules (cf. 15).

Our previous results show that the associations of I-A^k with molecules that may be involved with the Ia-mediated signaling cascade are controlled by the β chain (18). This hypothesis is supported by the fact that I-A^k molecules containing a truncated β chain paired with a wild-type α chain still signal (albeit with an altered phenotype) but have a wild-type D_{lat}. Further, the ability to mediate PKC translocation through I-A^k can be abolished by the complete removal of all β chain cytoplasmic domain amino acids. This negative PKC signaling phenotype is correlated with the large increase in D_{lat} of I-A^k molecules of the αCT12/βCT18 phenotype, almost 10 times higher than that of full-length I-A^k molecules and 3 times higher than that of I-A^k molecules of the αCT12/βwt phenotype.

We suggest that the inability of the αCT12/βCT18 I-A^k phenotype to signal is due to the fact that an I-A^k molecule containing a β chain that is missing all its cytoplasmic domain amino acids is unable to associate with the molecules of the Ia-mediated signaling cascade (5, 6, 28). These signaling cascade molecules may be proteins responsible for the proximal events in Ia-mediated signaling: those causing an increase in cAMP and/or translocation of PKC to the nucleus.

The truncation of the α chain alone does not significantly affect signaling but does increase lateral diffusion. The fact that in these molecules PKC signaling is still intact indicates that D_{lat} may increase threefold without the I-A^k molecule

uncoupling from the signal transduction pathway. There are a number of possible explanations for this observation. One possibility is that the interaction between the Ia molecule and the molecule(s) of the signaling pathway is transient and is mediated almost exclusively by the β chain. Thus, the requisite interaction can occur with normal kinetics in spite of the fact that the Ia molecule is diffusing threefold more rapidly. Additionally, truncation of the β chain in αCT12/βCT18 I-A^k molecules removes the six plasma membrane–proximal amino acids of the β chain that are required for constitutive binding of the proteins of the Ia-mediated signaling cascade. This binding contributes to the wild-type D_{lat}, and therefore the loss of the binding results in an increase in D_{lat} as well as in inability to translocate PKC. In our view, lateral diffusion of I-A^k reflects two independent interactions: one in which the α chain interacts with cytoskeletal elements that are not necessary for PKC translocation and one in which the β chain interacts with molecules that are required for PKC translocation.

Preliminary results (Wade, W. F., E. F. Rosloniec, and J. H. Freed, unpublished observations) indicate that antigen presentation by I-A^k molecules is affected by the length of the α chain cytoplasmic domain. A published report also indicates that antigen presentation is affected by truncation of the α chain's cytoplasmic domain (13). While the mechanism underlying these observations is unknown, it is tempting to speculate that there is a causal relationship between the increased D_{lat} for I-A^k molecules with truncated α chains and their reduced ability to present antigen. Although it is possible that the increased mobility of the I-A^k molecules with truncated α chains could be directly responsible for this effect, we currently favor the alternate hypothesis that the antigen presentation defect derives from the altered transport, endocytosis, and/or reexpression of I-A^k molecules with truncated α chains.

The D_{lat} we measured and the percent mobile fraction of wild-type I-A^k is quite close to that reported for Ia in another cell line, A20.3 (Bariasas, B. G., D. A. Roess, H. M. Grey, and T. M. Jovin, unpublished observation). Another reported value for D_{lat} of I-A^k is far larger, 4×10^{-9} cm^2/s (13). The reason for this difference is not clear, but we emphasize that cell type and experimental protocols are important variables affecting measured D_{lat} and mobile fraction.

The increase in D_{lat} as a result of truncating the cytoplasmic domains of α or β chains is different from that reported by one of us for class I molecules, where truncation of the cytoplasmic domain did not alter the D_{lat} value compared with that of the full-length molecule (10). The observed differences between the behavior of truncated class I and class II molecules may be explained by the fact that the class I molecule has a single membrane domain whereas the class II molecule has two. An alternate, and not mutually exclusive, explanation may be that class I molecules may not have a signaling function in the L cells in which they were studied and thus are not associated with cytoskeletal elements (4). Additional studies will be required to provide a detailed explanation for the observed differences between the D_{lat} of truncated class I and class II molecules. Ultimately, however, studies of their diffusion should provide fundamental insights into how the physicochemical properties of the class II molecules dictate their function as signal-transducing membrane receptors.

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